

EBPH – 2025, Volume 20, Supplementary Issue 1

<https://riviste.unimi.it/index.php/ebph/index>

Volume 20

Supplementary Issue 1

XIIIth SISMEC National Congress

9-12 September 2025

Pavia, Italy

Book of Abstracts

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Milano University Press

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Vol. 20 Suppl. 1



This Book of Abstracts is a publication by
Epidemiology Biostatistics and Public Health (EBPH),
formerly Italian Journal of Public Health,

published in Diamond Open Access with CC BY-SA license

by Milano University Press.

DOI: 10.54103/ /2282-0930/sismec13

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ISSN: 2282-0930

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Table of Contents

EBPH COMMITTEE	4
XIII TH SISMEC NATIONAL CONGRESS	5
HOW TO GET TO PAVIA	6
WALKING CONGRESS	7
CONGRESS PROGRAM	8
ABSTRACTS	14
GENERAL EPIDEMIOLOGY	14
CLINICAL EPIDEMIOLOGY	139
BIostatistical METHODS	220
INDEX OF ABSTRACTS BY AUTHOR	341

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XIIIth SISMEC National Congress

INNOVATIVE DATA AND METHODOLOGIES: A LOOK AT THE FUTURE OF MEDICAL STATISTICS

9–12 SEPTEMBER 2025 – PAVIA

Artificial Intelligence has now forcefully entered the medical world. So far, data analysis has fall under the domain of Medical Statistics — but that is no longer the case.

Traditional approaches to data analysis are now facing competition from new technologies that often outperform them when it comes to managing large databases, generating data, and integrating diverse data sources.

But that is only a part of the picture.

The actors have changed, methodologies have evolved, yet the core interest remains the same: understanding the causes of diseases, evaluating treatment effectiveness, improving diagnostic strategies, and identifying new ones — working from Big Data as well as from small-scale datasets.

- How can we address methodologically the emerging challenges?
- How can we translate omics-based evidence into population-level insights?
- How should we address the issue of transportability?
- How do we integrate data from multiple sources?
- How should synthetic data be handled?
- Is it merely a matter of methods and tools, or also a question of ethics?

This Congress will offer an opportunity for professional growth, thanks to the contribution of leading experts who will help us explore these questions. It will also provide a valuable forum for discussion among professionals who work daily as statisticians and epidemiologists.



How to get to Pavia

BY TRAIN

Pavia is served by a railway station located in the city centre.

Timetables available at <http://www.trenitalia.it>.

Any train with a final destination of **Genova**, **Ventimiglia**, **Sestri Levante**, **La Spezia**, or **Voghera** will stop in Pavia.

BY PLANE

The airports closest to Pavia are **Milan Malpensa**, **Milan Linate**, and **Bergamo Orio al Serio**.

From these airports, you can reach Pavia **by train, bus, or taxi**.

From Milan Linate Airport to Pavia (served by EasyJet and several major airlines):

Take a bus to Milano Centrale railway station (approx. 25 minutes; timetables at <https://www.milano-aeroporti.it/liniate-shuttle/index.html>) or a taxi (approx. 15 minutes), then take a train to Pavia (25–40 minutes; <https://www.trenitalia.com/>).

From Milan Malpensa Airport to Pavia (served by EasyJet and several major airlines):

Direct trains to Milano Centrale (approx. 55 minutes; timetables on <https://www.trenitalia.com/>), then trains to Pavia.

Trains to Milano Bovisio (approx. every 20 minutes, 30-minute journey), then transfer at Bovisio to the S13 suburban line to Pavia (approx. 55 minutes).

Buses to Milano Centrale (approx. 1 hour; <https://www.omio.it/>), then train to Pavia (25–40 minutes; <https://www.trenitalia.com/>).

From Bergamo Orio al Serio Airport to Pavia (served by Ryanair):

Bus (approx. 1 hour; <https://www.terravision.eu/italiano/>) to Milano Centrale, then train to Pavia.

Alternatively, take a city bus to Bergamo railway station, then a train to Milan (approx. 40 minutes), and change in Milano Lambrate for a train to Pavia (approx. 20 minutes).

BY BUS

Pavia is served by several bus companies offering connections to nearby towns and cities.

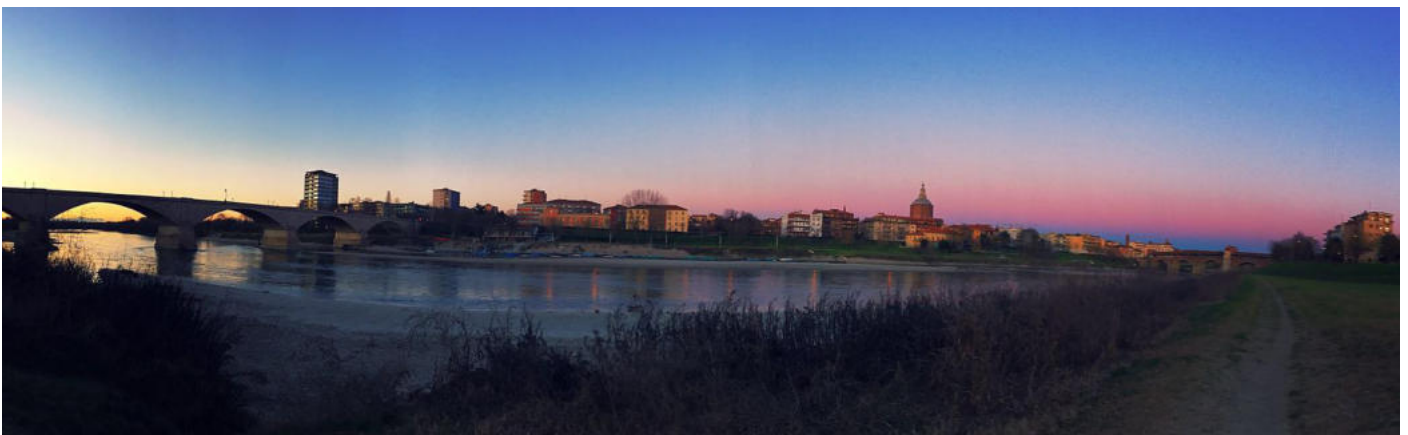
The bus station is located on Viale Trieste, just a short walk from the railway station.

BY CAR

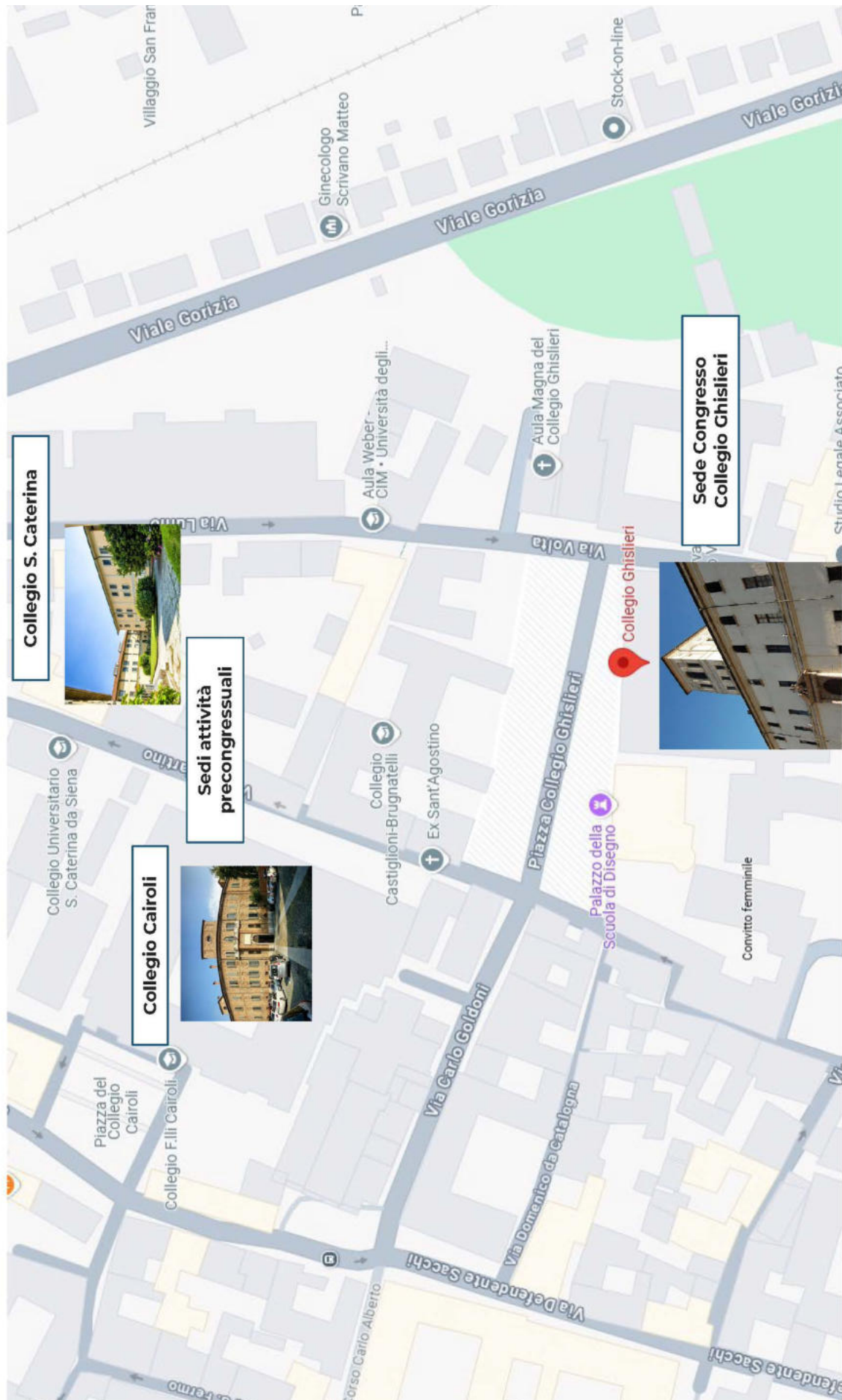
Pavia is located approximately 35 kilometres south of Milan.

To reach the city by car from Milan, take the A7 motorway towards Genoa and exit at Bereguardo-Pavia Nord.

Pavia is also accessible via the A21 motorway (Turin – Piacenza); the exit for Pavia is **Casteggio**.



Walking Congress



Congress Program

9 SEPTEMBER

10:00 INNOVATIVE DATA AND METHODOLOGIES: A 16:00 LOOK AT THE FUTURE OF MEDICAL STATISTICS

10:00-16:00

Course 1. Valutazioni di impatto sanitario in Epidemiologia Ambientale: Metodi ed Esempi. Learning by doing (Health Impact Assessment in Environmental Epidemiology: Methods and Examples. Learning by doing)

Coordinators: Prof. Veronesi - Dott.ssa Conti

Collegio Cairolì

10:00-16:00

Course 2. Dati sintetici nella ricerca biomedica (Synthetic Data in Clinical Research: Methods, Applications, and Challenges). Masterclass

Coordinators: Prof.ssa Gesuita - Prof. Bruzzese

Collegio Santa Caterina

15:30-16.30

Congress registration

Collegio Ghislieri - Aula Magna

Welcome drink

Collegio Ghislieri - Ridotto Aula Magna

CONGRESS OPENING CEREMONY 16:30 18:30

16:30-17:00

Greetings from the Authorities

17:00-17:30

25 anni di SISMEC: celebriamo il passato, modelliamo il futuro

17:30-18:30

Lectio Magistralis. Intelligenza Artificiale Investigativa: informazioni nascoste per piccole base dati

Prof. Paolo Massimo Buscema, University of Colorado (Denver, USA), Semeion Research Centre, (Roma, Italy)

Collegio Ghislieri - Aula Magna

18:45

Welcome buffet

Collegio Ghislieri - Quadriportico

Social event



Collegio Santa Caterina

10 SEPTEMBER 2025

08:30

Opening of the Conference Registration Desk

10:30

Coffee station

Collegio Ghislieri - Ridotto Aula Magna

9:00
11:30**SESSIONE PLENARIA I - CAUSAL INFERENCE:
WHICH INNOVATIVE STATISTICAL APPROACHES?**

Moderators: Prof. Lorenzo Richiardi - Prof.ssa Miriam Isola

Collegio Ghislieri - Aula Magna

9:00-9:40

Causal inference methods applied to multiomics analyses

Prof. Marc Chadeau, Imperial College (London, UK) - Ass. Prof. Dragana Vuckovic

9:40-9:45

Active break

9:50-10:30

Using triangulation to infer causality from observational data: an applied example in pregnancy

Dr. Gemma Clayton, University of Bristol (UK)

10:30-10:35

Active break

10:40-11:20

Transporting causal effects across observational studies

Prof.ssa Daniela Zugna, Università degli Studi di Torino (Italy)

11:20-11:25

Active break

PARALLEL SESSIONS11:30
17:00

11:30-11:45

Opening of parallel sessions

11:45-13:00

Parallel Sessions (presentation of free contributions)

Collegio Ghislieri - Several classrooms

13:15-14:00

Light Lunch

Collegio Ghislieri - Quadriportico

14:00-17:00

Parallel Sessions (presentation of free contributions)

Collegio Ghislieri - Several classrooms

15:30-17:00

Coffee station

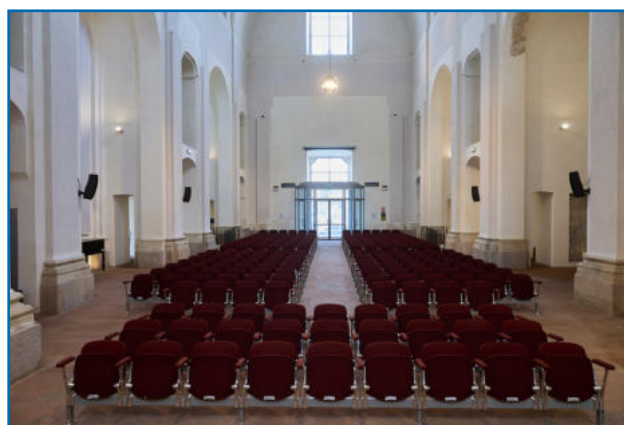
Collegio Ghislieri - Quadriportico

17:15-18:15

Riunione Ordinari MEDS-24/A

Assemblea dei Professori e Ricercatori MEDS-24/A

Collegio Ghislieri - Aula Magna

Collegio Ghislieri
Aula Magna

11 SEPTEMBER

10:30-11:30

Coffee station

Collegio Ghislieri - Ridotto Aula Magna

**9:00
11:30** **SESSIONE PLENARIA II - DATA INTEGRATION
METHODS**

Moderators: Prof. Davide Gentilini - Prof.ssa M. Cristina Monti

Collegio Ghislieri - Aula Magna

9:00-9:40

Metodologie statistiche per il data integration con applicazioni a malattie infettive

Prof.ssa Daniela de Angelis, Cambridge University (UK)

9:40-9:45

Active break

9:50-10:30

Integrazione dei dati sanitari: metodi innovativi e sfide nel Progetto Health Big Data

Prof.ssa Maria Gabriella Signorini - Prof. Pierluigi Reali, Politecnico di Milano (Italy)

10:30-10:35

Active break

10:40-11:20

Network based approaches for data integration in medicine and biology

Prof. Daniele Remondini, Università degli Studi di Bologna (Italy)

11:20-11:25

Active break

PARALLEL SESSIONS**11:30
17:00**

11:30-11:45

Opening of parallel sessions

11:45-13:00

Parallel Sessions (presentation of free contributions)

Collegio Ghislieri - Several classrooms

13:15-14:00

Light Lunch

Collegio Ghislieri - Quadriportico

14:00-16:00

Parallel Sessions (presentation of free contributions)

Collegio Ghislieri - Several classrooms

16:00-16:15

Active break

15:30-17:00

Coffee station

Collegio Ghislieri - Quadriportico

**SESSIONE PLENARIA III - SISMEC
COMMISSIONS CORNER**
**16:15
17:15**

Coordinator: Prof.ssa Antonella Zambon

Collegio Ghislieri - Aula Magna

16:15-16:30

Valutazione della qualità nella ricerca clinica: analisi e proposte della Commissione ricerca SISMEC

Dr. Vittorio Simeon (Commissione Ricerca)

11 SEPTEMBER

16:30-16:45

Ricognizione, potenzialità e spunti critici della normativa italiana ed europea sulla Protezione Dati in ambito biomedico, in particolare alla luce delle integrazioni/revisioni che si sono sviluppate nell'ultimo biennio

Dott.ssa Susanna Conti (Commissione Normativa)

16:45-17:00

La statistica medica nella didattica: spunti di riflessione e progetti concreti

Prof.ssa Margherita Fanelli (Commissione Didattica)

Coordinator: prof. Paolo Trerotoli

17:00-17:15

Un caffè con la Rete Giovani Biostatistici: appena nata e già connessa

Alessandro Fontanarosa Università Politecnica delle Marche, Giulia Gambini SSD Biostatistica e Clinical Trial Center IRCCS Policlinico San Matteo, Letizia Lorusso Scuola di Statistica Sanitaria e Biometria Università degli studi di Bari

17:15-18:00

Tempo a disposizione dei Gruppi di Studio SISMEC

Collegio Ghislieri - Several classrooms

18:00-19:30

Assemblea dei Soci SISMEC

Collegio Ghislieri - Aula Magna

20:30

Social dinner



*Collegio Ghislieri
Quadriportico*

12 SEPTEMBER

08:30

Opening of the Conference Registration Desk

11:05-11:15

Active break

9:00
12:00SESSIONE PLENARIA IV - DATI SINTETICI E AI:
QUALE FUTURO?

9.00-9:15

Opening

Coordinator: Prof. Patrizio Pasqualetti

9.15-9:30

Intelligenza artificiale, dati sintetici: una sfida per il biostatistico (dei Comitati Etici Territoriali)

Prof.ssa Annarita Vestri, Università la Sapienza (Italy)/SISMEC

9:30-9:45

Il ruolo della biostatistica nell'AI: dalla metodologia alla trasparenza dei modelli

Prof.ssa Paola Berchialla, Università di Torino (Italy)/SISMEC

9:45-10:05

AI in ambito sanitario: il punto di vista del data scientist

Prof. Giorgio Leonardi, Università del Piemonte Orientale (Italy)

10:05-10:25

Iniziative innovative per l'applicazione di Tecniche di Data Privacy Enhancement (PET) a progetti che utilizzano categorie particolari di dati - esperienze in Regione Lombardia

Dott.ssa Olivia Leoni, Regione Lombardia

10:25-10:45

Aspetti regolatori

Dott. Paolo Foggi, AIFA

10:45-11:05

Ecosistema dei dati sanitari e l'AI per gli studi di Real World Evidence

Dott. Giuseppe Seghi Recli, Farmaindustria

TAVOLA ROTONDA "IA E DATI SINTETICI
PUNTI DI FORZA E PUNTI DI DEBOLEZZA DI
QUESTE NUOVE SFIDE"9:00
12:00

Coordinator: Dr. Enrico Bucci

Collegio Ghislieri - Aula Magna

Il punto di vista del biostatistico

Prof.ssa Clelia Di Serio, Università Scienze e Vita San Raffaele (Milan, Italy)/SISMEC

Il punto di vista di un ricercatore biomedico

Prof. Damiano Baldassarre, Università degli Studi di Milano (Italy)

Il punto di vista dei Comitati Etici

Dott. Valter Torri, Presidente del CET - Lombardia 6

Il punto di vista del data scientist

Prof. Riccardo Bellazzi, Università degli Studi di Pavia (Italy)

12.00-12.30

Closing ceremony

12:30-12:45

Award ceremony

12:30

Goodbye cocktail

Collegio Ghislieri - Quadriportico



Basilica di San Michele Maggiore

Long-Term Mental Health Consequences of Road Traffic Crashes: A Scoping Review

Anelli Manuela⁽¹⁾, Ferraro Ottavia Eleonora⁽¹⁾, Celebrin Mattia⁽¹⁾, Morandi Anna⁽¹⁾, Montomoli Cristina⁽¹⁾

(1) Unit of Biostatistics and Clinical Epidemiology, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

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INTRODUCTION

The considerable number of road crashes resulting in death, injury and disability makes road safety a crucial issue worldwide [1], [2]. Preventing road deaths, addressing severe injuries in road traffic and their long-term effects, both mental and physical, remains a critical aspect of achieving a resilient and future-proof transportation system.

AIMS

To summarize findings from longitudinal studies on mental health, psychological outcomes, and mental health-related quality of life (HRQoL) following road traffic crashes (RTCs) in order to suggest implications to improve recovery and rehabilitation.

METHODS

Long-term consequences (LTCs), physical and mental health, quality of life, and road traffic crash were the keywords used to search the PubMed, Scopus, and Web of Science electronic databases. The initial search yielded 420 records, reduced to 390 after the removal of duplicates. Following the PRISMA 2020 guidelines, 50 observational studies published between 2010 and 2024 were identified as relevant, and one was found through citation searching. At the full-text reading 17 papers were excluded. As a result, 34 articles were included in the review. These studies investigated the long-term physical and psychological consequences of road crashes across all categories of adult road users. Seven papers showed a specific focus on mental health outcomes, including post-traumatic stress syndrome, pain-related psychological distress, depressive symptoms, mental HRQoL, and cognitive impairment.

RESULTS

The reviewed studies consistently show that RTCs can lead to substantial long-term mental health consequences. A considerable number of individuals experience post-traumatic stress symptoms, with higher rates observed among those with more severe injuries. Persistent pain and residual physical or psychological symptoms are common even several months after the crash, often impairing daily functions and delaying return to work.

Depression and anxiety symptoms are associated with slower recovery, and negative expectations about recovery strongly predict persistent pain and emotional distress over time. Mental HRQoL, measured by means of standardized instruments, generally improves within the first year after the crash, but often remains below pre-injury levels, especially among those with longer hospital stays or more severe injuries.

Sociodemographic and clinical factors such as female sex, middle age, higher injury severity, and low physical or mental HRQoL scores are significantly associated with poorer mental health outcomes. In contrast, higher education levels, better physical functioning, and protective behaviors (such as the use of safety equipment) are associated with improved mental recovery and reintegration into daily life and work. Overall, these findings highlight the need for targeted mental health screening and supportive interventions in the post-injury care of RTC survivors. Characteristics of the included studies are summarized in Table 1.

CONCLUSIONS

This review emphasizes the importance of early psychological screening, targeted intervention, and policy efforts to mitigate the mental health burden among RTC survivors.

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Table 1. Summary of findings from included studies

Study	Sample size	Country	Main outcomes
Hours et al., 2013	886	France	PTSD, pain, work absence
Hours et al., 2010	1168	France	PTSD, residual pain
Cassidy et al., 2014	1716	Canada	MTBI, CES-D
Edmed et al., 2018	177	Australia	PTSD; Pain expectations, anxiety
Giummarra et al., 2018	74,217	Australia	Mental health treatment, persistent pain
Doan et al., 2020	352	Vietnam	HRQoL (SF-12 MCS), return to work
Gopinath et al., 2020	1201	Australia	HRQoL (EQ-5D-3L, SF-12 MCS/PCS)

PTSD Post-traumatic stress disorder; MTBI Mild Traumatic Brain Injury; CES-D Centre for epidemiological studies-Depression Scale Symptoms; HRQoL Health-related quality of life; SF-12 Short Form-12 Health Survey; MCS Mental Component Score; PCS Physical Component Score; EQ-5D-3L EuroQol 5 Dimensions, 3 Levels

Prevention of Cervical Cancer in a Rural Primary Care Centre in Eswatini

Motsa Tengetile⁽¹⁾, Msibi Velaphi⁽¹⁾, Sikhondze Mzamo⁽¹⁾, Nhleko Bongani Alex⁽¹⁾, Niemann Nicole Rose⁽¹⁾, Fappani Clara^(2,3), Gori Maria⁽²⁾, Colzani Daniela⁽²⁾, Villa Simone^(2,3), Angelone Paola^(2,3), Baldi Sante Leandro^(2,3), Valentini Enrica⁽¹⁾, Maphalala Gugu⁽⁴⁾, Buthelezi Gcinile⁽⁴⁾, Camilla Torriani⁽⁵⁾, Assefa Mulubirhan Alemayohu⁽⁵⁾, Monti Maria Cristina⁽⁵⁾, Amendola Antonella⁽²⁾, Tanzi Elisabetta⁽²⁾, Raviglione Mario^(2,3)

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(3) Centre for Multidisciplinary Research in Health Science (MACH)

(4) Ministry of Health, Eswatini Government

(5) Università degli Studi di Pavia

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BACKGROUND

Cervical cancer is a priority public health concern in the Kingdom of Eswatini, primarily due to a high incidence rate of high-risk human papillomavirus (HR-HPV) infection [1,2]. Socio-cultural, economic, and policy-related barriers often limit women's access to essential screening and preventive services, resulting in delayed diagnoses and high mortality rates [3]. Test invasiveness and sampling modalities may further reduce women's participation in existing screening programs implemented mainly through Visual Inspection with Acetic Acid (VIA), [4, 5, 6, 7].

Since May 2023, the 4-valent (4v, HPV-6, 11, 16, 18) HPV vaccine has been offered to girls aged 9–14, with an estimated 79% of the target population vaccinated by May 2025 [8,9,10]. However, data on the prevalence and genotype distribution of HPV in Eswatini are limited, and prevention strategies do not rely on local epidemiological data that are currently missing.

OBJECTIVES

In line with WHO recommendations for HPV-DNA testing as a primary screening method, this project aims to identify and address implementation challenges by implementing a “screen, triage, and treat” prevention programme featuring non-invasive urine-based HR-HPV testing at St. Philip's Clinic, a rural primary care centre in the Lubombo region of Eswatini. Specific objectives are to assess feasibility, acceptability, effectiveness, and cost-effectiveness of urine-based HR-HPV

testing with the aim to enhance access among adolescent girls and women (AGW). Furthermore, it aims to evaluate the distribution of HPV genotypes in this area of Eswatini.

METHODS

A 12-month cross-sectional pilot study (February 2023–February 2024) was conducted at St. Philip's Clinic, Eswatini. Women aged 12–49 presenting for any reason were asked to provide a 30 mL urine sample. A 10 mL aliquot was centrifuged to concentrate viral particles and eliminate debris, and the Xpert® HPV test was performed locally to detect 14 high-risk HPV (HR-HPV) genotypes. Women aged 21–49 were additionally offered cervical brush testing for cytological analysis to be performed at Mbabane Central Pathology Laboratory. Screening-positive women underwent VIA to establish the need for local treatment or hospital referral according to the national screening programme. Concentrated urine samples were also dried on filter paper (dried urine spots, DUS) and sent to Italy for comprehensive HPV genotyping using in-house PCR, sequencing, and Ampliquality HPV-Type Express assay.

RESULTS

The study enrolled 510 AGWs (median age 29). 37% (190/510) were HIV-positive. First-time screenings accounted for 45% of women (228/510). The Xpert® HPV test provided valid results for 473 participants (93%), detecting HR-HPV in 42% (199/473). Women aged 21–25 had the highest HR-

HPV prevalence (55%, 56/101). HIV-positive participants had a 1.8-fold increased risk of HR-HPV infection compared with the HIV-negative (95%CI 1.23–2.64). Cervical brush samples were collected for 220 women consecutively recruited from the start of the project and high-grade lesions were identified in 46 women, with 7 cases of CC, including 2 in women under 30.

Overall, 333 DUS were successfully genotyped in Italy. HPV35 was the most frequently identified genotype (24%, 80/333), followed by HPV16 (18%, 61/333), and the predominant genotype among high-grade lesions (33%, 15/46) and the sole oncogenic genotype in 15% of these cases, including one CC. Among women identified with high-grade lesions, 37% (17/46) tested positive for tetravalent vaccine HR-types (HPV-16, 18), 65% (30/46) for nonavalent-vaccine HR-types (9v, HPV-16, 18, 31, 33, 45, 52, 58). Adding HPV35 to the nonavalent vaccine formulation potentially increases coverage to 80% (37/46) (100% considering only CC). Preliminary findings suggest high acceptability and good overall performance of the screening algorithm. However, several challenges emerged that may affect feasibility, including a proportion of invalid HPV test results or inconclusive outcomes in VIA assessment, and difficulties in ensuring referral compliance for women requiring further management.

CONCLUSION

This project provides fundamental evidence to enhance cervical cancer prevention efforts in Eswatini in two critical areas. The first is the support to the validity of the non-invasive urine-based screening methods. The second is the detection of a high prevalence of HPV 35 genotype and the consequent need to revisit formulation of available vaccines. Urine-based HR-HPV rapid testing proved feasible, acceptable and well-received. The study enabled the identification of critical underperforming steps and the development of corresponding solutions. A high frequency of invalid Xpert results was addressed by re-training laboratory personnel in the proper preparation of the sample. The failure of VIA to recognise presence of cervical lesions as compared with the effectiveness of the rapid molecular testing in detecting HR genotypes raises concerns about the future usefulness of VIA. The slow recruitment rate at the start could be addressed via engagement of community workers and leaders. Our findings also revealed that HPV35 is highly prevalent in high-grade lesions in Eswatini, accounting for 15% as the sole oncogenic genotype in 15% of these cases, including one CC. Current HPV vaccines do not include coverage of HPV35: such coverage could extend protection to a high proportion of AGW and further research is warranted to assess the type-specific CC burden in Sub-Saharan Africa.

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Anthropometric Indicators of Central Obesity in the Identification of General and Cancer Related Risk Mortality: Findings from the EPIC Italian Cohort

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INTRODUCTION

Obesity is a growing risk factor among adults, and its accurate identification remains a public health priority. While body mass index (BMI) is widely used, it does not account for fat distribution [1]. Combining BMI with central obesity indicators – such as waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WtHR) – has been shown to improve mortality risk prediction [2,3]. Nevertheless, these indicators have limitations, such as the absence of standardized measurement procedures [4]. Accordingly, the potential predictive role of non-traditional anthropometric indicators – such as the weight-adjusted waist index (WWI), the conicity index (C-index), and relative fat mass (RFM) – has been highlighted [5-7]. However, their clinical interpretation may be limited by the complexity of the required calculations.

AIMS

To develop a novel, clinically applicable and easily interpretable anthropometric method for the classification of central obesity, and to assess its predictive value for all-cause and cancer mortality in comparison with both traditional (BMI, WC, WHR, WtHR) and non-traditional (WWI, C-index, RFM) anthropometric indicators.

METHODS

This study included 45698 adult men and women from the Italian EPIC cohort (1993–1998). Demographic, lifestyle, and medical history data were collected enrollment using standardized questionnaires. Anthropometric measurements (height, weight, waist and hip circumferences) were used to calculate indicators. To calculate Delta Waist (ΔW) a linear regression was performed on WC as a function of height, weight, sex, age, sex/height and sex/age interaction. The model was weighted based on health risk categories defined by NICE [8]: participants with no increased risk were assigned a weighting factor of 1, those with increased risk a weighting factor of 0.5, and those with high or very high risk a weighting factor of 0. Predicted waist circumference was derived from this model by standardizing age to 35 years and reclassifying high/very high risk individuals into the nearest increased risk group stratified by waist circumference, assigning them the highest value of BMI. On this basis, weighting factor was recalculated. ΔW was calculated as the difference between observed and predicted WC. Pearson correlation coefficients were calculated between ΔW , the other non-traditional and traditional indicators. Cox proportional hazards models, with age as the primary time variable, were used to assess the association between ΔW , or other anthropometric variables, and the risk of all-cause and cancer mortality. All models were stratified by study center and adjusted for years of education, smoking status, physical activity level, diabetes, hypertension, and menopausal status for women (model 1).

All models were also adjusted for BMI (model 2). All analyses were performed separately for men and women. Hazard ratios (HRs) and 95% confidence intervals were estimated. Model performance was assessed using the Bayesian Information Criterion (BIC), with lower values indicating better fit. Statistical significance was set at $p < 0.05$.

RESULTS

During a median follow-up of 15 years, all-cause and cancer deaths were 1002 and 548 in men and 1473 and 888 in women, respectively. ΔW showed a lower correlation with BMI (men: $r=0.64$; women: $r=0.71$) than WC (men: $r=0.87$; women: $r=0.91$), WtHR (men: $r=0.84$; women: $r=0.90$) and WHR (men: $r=0.69$; women: $r=0.75$). A strong positive correlation was observed between RFM and BMI (men: $r=0.84$; women: $r=0.85$), while correlations between WWI, C-index and BMI were lower than $r=0.5$ in both sexes. WWI, C-index and RFM were positively correlated with WC, WtHR, WHR in both sexes. After adjustment for covariates, the estimated HRs of all-cause and cancer mortality according to BMI, WC, WtHR, WHR, ΔW , WWI, C-index and RFM are shown in Table 1. In both sexes ΔW was associated with an increased risk of both all-cause mortality (men: HR 1.02 (95% CI 1.01-1.03); women: HR 1.02 (95% CI 1.01-1.02)) and cancer mortality (men: HR 1.02 (95% CI 1.01-1.03); women: HR 1.01 (95% CI 1.003-1.02)) (model 1). Adjustment for BMI did not affect the association between ΔW and all-cause mortality in both sexes and cancer mortality in men, but modified the association between ΔW and cancer mortality in women (model 2). The other indicators were associated with an increased risk of all-cause mortality in men and women and with cancer mortality in men, while in women the association with cancer mortality was observed for all indicators except WHR, WWI and C-index (model 1). After adjusting for BMI no association was found between RFM and all-cause mortality in both sexes, nor between WHR and all-cause mortality in women. Furthermore, in women after adjusting for BMI, WC, WtHR, and RFM were no longer associated with cancer mortality (model 2). In the BIC analysis, the model with ΔW showed the lowest value in both men and women for all-cause mortality (men: BIC=14650; women: BIC=24329). Lower BIC values for cancer mortality were found for C-index in men (BIC=8096) and for WHR in women (BIC=15009).

CONCLUSIONS

ΔW represents a new anthropometric indicator of central obesity, easy to use and understand. In the Italian EPIC cohort it was associated with all-cause mortality risk in both sexes, with better results than other anthropometric indicators already validated and standardized.

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Table 1. Estimated HRs of all-cause and cancer mortality according to BMI, WC, WHR, WHR, ΔW , C-index, WWI and RFM.

			Men				Women			
			HR	95% CI	p	BIC	HR	95% CI	p	BIC
All-cause Mortality	Model 1	BMI	1.05	1.03-1.07	<0.001	15511.65	1.02	1.01-1.04	<0.001	24638.91
		WC	1.02	1.01-1.03	<0.001	14655.46	1.01	1.01-1.02	<0.001	24337.93
		WHR*10	1.36	1.21-1.53	<0.001	14661.52	1.2	1.11-1.30	<0.001	24342.63
		WHR	7.85	2.70-22.81	<0.001	14674.01	3.29	1.56-6.96	0.002	24337.03
		ΔW	1.02	1.01-1.03	<0.001	14650.1	1.02	1.01-1.02	<0.001	24328.64
		WWI	1.29	1.15-1.45	<0.001	14669.3	1.16	1.07-1.25	<0.001	24347.84
		C-index	12.65	4.60-34.73	<0.001	14663.43	4.55	2.30-8.97	<0.001	24343.46
		RFM	1.04	1.02-1.06	<0.001	14666.8	1.02	1.01-1.03	<0.001	24350.00
	Model 2	BMI	0.98	0.95-1.02	0.4	14661.61	0.99	0.97-1.01	0.42	24344.56
		WC	1.02	1.01-1.04	<0.001	14661.61	1.02	1.01-1.03	<0.001	24344.56
		BMI	0.99	0.96-1.04	0.85	14668.35	1.00	0.97-1.02	0.87	24349.88
		WHR*10	1.39	1.09-1.78	0.007	14668.35	1.21	1.03-1.38	0.02	24349.88
		BMI	1.03	1.01-1.05	0.002	14671.84	1.02	1.01-1.03	0.003	24335.77
		WHR	3.36	0.98-11.54	0.05	14671.84	2.21	0.98-5.00	0.06	24335.77
		BMI	1.002	0.98-1.03	0.91	14656.95	0.99	0.98-1.01	0.55	24335.55
		ΔW	1.02	1.01-1.03	<0.001	14656.95	1.02	1.01-1.03	<0.001	24335.55
		BMI	1.03	1.01-1.05	0.004	14668.01	1.02	1.002-1.03	0.02	24349.54
		WWI	1.19	1.05-1.36	0.006	14668.01	1.11	1.02-1.21	0.01	24349.54
Cancer Mortality	Model 1	BMI	1.03	1.00-1.05	0.03	8508.83	1.02	1.01-1.04	0.005	15144.87
		WC	1.02	1.01-1.02	0.001	8096.65	1.01	1.003-1.01	0.006	15025.73
		WHR	8.85	1.78-43.93	0.007	8100.19	3.64	1.28-10.25	0.01	15020.63
		WHR	1.35	1.73-31.22	0.007	8100.13	1.57	0.57-4.32	0.38	15009.79
		ΔW	1.02	1.01-1.03	0.001	8097.52	1.01	1.003-1.02	0.006	15026.04
		WWI	1.24	1.06-1.45	0.006	8099.71	1.07	0.97-1.18	0.16	15031.22
		C-index	10.32	2.64-40.28	<0.001	8095.84	2.19	0.90-5.34	0.08	15030.2
		RFM	1.03	1.01-1.06	0.01	8100.59	1.01	1.00-1.03	0.04	15029.01
	Model 2	BMI	0.96	0.91-1.004	0.07	8102.58	1.01	0.98-1.04	0.52	15032.11
		WC	1.03	1.01-1.05	0.001	8102.58	1.00	0.99-1.02	0.37	15032.11
		BMI	0.96	0.91-1.004	0.07	8105.33	1.02	0.98-1.05	0.25	15032.88
		WHR	1.03	1.01-1.05	0.001	8105.33	1.25	0.15-10.39	0.83	15032.88
		BMI	1.01	0.98-1.04	0.54	8206.32	1.02	1.01-1.04	0.01	15016.96
		WHR	5.81	1.12-30.12	0.03	8206.32	0.95	0.31-2.88	0.93	15016.96
		BMI	0.99	0.96-1.03	0.65	8103.59	1.01	0.99-1.04	0.27	15024.88
		ΔW	1.02	1.004-1.03	<0.01	8103.59	1.01	0.99-1.02	0.26	15024.88
		BMI	1.01	0.98-1.04	0.47	8105.47	1.02	1.003-1.04	0.02	15032.84
		WWI	1.21	1.02-1.44	0.03	8105.47	1.02	0.90-1.13	0.77	15032.84
		BMI	1.01	0.97-1.03	0.66	8101.91	1.02	1.00-1.04	0.03	15032.45
		C-index	9.01	2.02-40.11	0.004	8101.91	1.40	0.53-3.76	0.49	15032.45
		BMI	0.98	0.93-1.03	0.46	8106.31	1.03	0.99-1.06	0.08	15026.05
		RFM	1.05	1.00-1.10	0.05	8106.31	0.99	0.97-1.02	0.72	15026.05

Dietary Patterns, Metabolomic Profiles, and Metabolic Outcomes by Menopausal Status

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INTRODUCTION

Menopause is a critical period marked by significant metabolic and hormonal changes that impact cardiometabolic disease risk.[1] Diet plays a crucial role in lipid metabolism and visceral adiposity, key components of cardiometabolic health.[2] Metabolomics, the analysis of small molecules in biological samples, offers a tool for capturing both dietary metabolites and systemic adaptations to habitual diet.[3] In addition to provide a more objective assessment of dietary intake it can reveal different profiles between subgroups of individuals, such as women at different reproductive stages.[4] However, the underlying mechanisms by which diet affects metabolism after menopause are not yet well understood, limiting the development of targeted dietary strategies to mitigate health risks in female populations.[5]

OBJECTIVE

This investigation aimed to examine the role of menopausal status in the association between dietary patterns, metabolomic profiles associated with the dietary patterns, and lipid profile. To achieve this objective, two specific aims were pursued:

1. To determine the metabolomic profile associated with different dietary patterns according to menopausal status
2. To explore how diet influences the lipid profile and visceral fat by menopausal status, considering potential differences in the metabolome.

METHODS

We conducted a cross-sectional analysis on 1,179 women participating in the Cooperative Health Research In South Tyrol (CHRIS) study,[6] stratifying by menopausal status, defined based on an algorithm incorporating self-reported information and age. We excluded women who were pregnant, non-fasting, or in the menopause transition phase at the study visit.

To evaluate the dietary patterns, we derived three well-established healthy dietary indexes: Planetary Health Diet Index (PHDI), Plant-based Diet Index (PDI), and Alternative Healthy Eating Index (AHEI). Additionally, we included the Dietary Inflammatory Index (DII) to specifically investigate the role of inflammation. The outcomes were visceral fat (%), total cholesterol (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), and log-transformed triglycerides (log-mmol/L) concentrations.

We used elastic net regression to identify metabolites associated with each dietary index. The dataset was divided into training (70%) and testing (30%) sets. In the training set, we performed multiple iterations of 10-fold cross-validation with 100 different seeds. Optimal lambda values were selected based on the consensus mode of the number of selected features across iterations. For each dietary index, we estimated a metabolite score, calculated as the weighted sum of selected metabolite concentrations. The trained models were subsequently applied and validated in the testing set to compute final dietary metabolite scores. Associations between dietary indexes, metabolite scores, and outcomes were assessed using linear regression models. All models were adjusted for age, physical activity, smoking, self-reported diabetes and hypertension, education, following a special diet, total energy intake, and self-reported medications for lipid control. Addi-

tionally for pre-menopausal women, we adjusted for contraception use, while for post-menopausal women we adjusted for the usage of hormone replacement therapy.

Results:

In this sample of women, the pre-menopause group ($n=762$) had a median age of 35 years (interquartile range, IQR: 25-44 years) and a median BMI of 23 kg/m² (IQR: 21-27 kg/m²). The post-menopause group ($n=417$) had a median age of 62 years (IQR: 56-69 years) and a median BMI of 26 kg/m² (IQR: 23-30 kg/m²).

The selection of metabolites revealed group-specific profiles, with a modest overlap between the pre-menopause and post-menopause groups, except for the DII, which showed distinct metabolomic profiles in the two groups. Glycerophospholipids were consistently selected across almost all dietary patterns and in both groups.(Figure)

When examining associations between dietary indexes and outcomes,(Figure) the PDI and the AHEI were associated with lower visceral fat and LDL cholesterol among pre-menopausal women. In the post-menopausal group, the PHDI was positively associated with LDL cholesterol, while AHEI remained negatively associated with visceral fat.

When examining associations between metabolite scores and outcomes,(Figure) in the pre-menopausal group, all metabolite scores were associated with lower visceral fat. The PDI-metabolite score was additionally associated with lower total cholesterol. The PHDI-metabolite score was associated with lower HDL cholesterol and higher triglyceride levels. The DII-metabolic score was associated with higher levels of total, LDL, and HDL cholesterol, and with lower triglycerides. The AHEI-metabolite score was also associated with higher triglyceride levels. In post-menopausal women, the DII-metabolite score was negatively associated with total, LDL, and HDL cholesterol. The PHDI-metabolite score was associated with lower total and HDL cholesterol. The AHEI-metabolite score was positively associated with HDL cholesterol.

CONCLUSION

The metabolomic profiles associated with dietary patterns varied by menopausal status. We observed a general consistency when investigating associations between dietary indexes, their corresponding metabolomic profiles, and lipid marker concentrations. However, the associations with metabolite scores were notably stronger than those with dietary index, suggesting that while dietary patterns do influence metabolic outcomes, the specific metabolomic profiles might provide a more precise and robust measure of these associations.

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Health Literacy, Diet, and Lifestyles for the Promotion of Health and Empowerment of University Students and Staff: The “ALFADIET” Study

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INTRODUCTION

Appropriate nutrition and healthy lifestyles are key elements in the prevention of non-communicable diseases and in promoting individual well-being. In this context, health and digital literacy are essential tools to foster people’s empowerment in managing their own health. Universities, as environments with a high concentration of young people and professionals, play a strategic role in health promotion. They have the opportunity to optimize the health literacy of their students and empower them to make informed decisions for themselves and their living environments [1]. Health Literacy, meaning the ability of individuals to meet the complex demands of health in a modern society, has been associated with better health decision-making and is considered a key social determinant of healthier behaviors. Moreover, in the field of nutrition, this competency affects food selection and preparation processes and, more broadly, eating habits, thus contributing to improved health and well-being of individuals [2]. For this reason, it is important to investigate Health Literacy as well as eHealth (defined as the use of information technologies in support of health and related fields) and Digital Health Literacy, all of which are increasingly important in Public Health.

OBJECTIVES

The ALFADIET study aims to analyze eating habits, adherence to the Mediterranean Diet, lifestyles, and levels of Health and Digital Health Literacy among students, faculty, and administrative staff of the University of Catania and the University of Palermo.

METHODS

The population of the “ALFADIET” study will include students, from various degree programs and academic years, as well as teaching and non-teaching staff at the University of Catania and the University of Palermo. The study is based on an innovative real-time data collection methodology using the mobile Ecological Momentary Assessment (mEMA), aimed at capturing participants’ daily behaviors. A web-based survey will be designed to collect basic demographic data and additional information. The study will use an updated, specially designed version of the HEALTHY-UNICT web app, customized and developed at the University of Catania [3]. This application will allow for the administration of validated questionnaires assessing dietary habits, behavioral factors, and health literacy. The information collected will include anthropometric variables, lifestyles (including smoking and physical activity), emotions, and demographic characteristics. The survey will be conducted on a representative sample of students, teaching and non-teaching staff, through the use of an innovative mEMA application, specially developed by an interdisciplinary team to meet the specific objectives of the study.

RESULTS

The preliminary findings derive from a pilot study conducted on a small sample of 27 participants from the University of Catania, including students (74.1%), faculty members (18.5%), and administrative/technical staff (7.4%). The majority were female (81.5%), with a median age of 24 years. Among students, 70% were enrolled on schedule with their academic

plan, and 50% lived away from their university location. A total of 92.6% of participants reported no significant medical conditions, and 88.9% did not report any food intolerances. Regarding lifestyle habits, 81.5% reported using multivitamin and multimineral supplements, 77.8% were non-smokers, and 88.9% preferred white meat (e.g., chicken, turkey, rabbit) over red meat. Most participants (63%) slept between 7 and 9 hours per night. Psychophysical issues impacting daily activities were reported as moderately difficult by 37% of respondents, very difficult by 14.8%, and extremely difficult by one participant, while 44.4% reported no difficulties. As for digital health literacy, 55.6% stated they were very capable of finding health-related information online, 51.9% felt very capable of knowing where to look for it, and 44.4% felt very capable of evaluating the quality of such information. Additionally, 48.1% reported being very capable of distinguishing between high- and low-quality health information. However, only 29.6% felt very confident using that information to make health-related decisions.

CONCLUSIONS

This pilot study provides an initial overview of dietary habits, lifestyle behaviors, and levels of health and digital health literacy among the university population, though based on a small sample. The data collected will help identify factors associated with greater adherence to the Mediterranean Diet and higher levels of health and digital health literacy, supporting the identification of target groups for future health promotion interventions. Moreover, the study serves to test the effectiveness of innovative digital tools for real-time behavioral data collection. The future evidence will serve as a foundation for developing personalized, sustainable, and replicable strategies aimed at improving health and fostering empowerment within the academic community.

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Validity of Socioeconomic Inequality Indices over Time in Public Health Research: A Case Study on IVSM and Maternal Data

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INTRODUCTION

In recent years, studies on pregnancy outcomes and reproductive health indicators have increasingly considered the impact of socioeconomic deprivation. Area-based indices such as the Caranci Deprivation Index [1] or Synthetic Index of Socioeconomic Disadvantage (IVSM) from ISTAT [2] are commonly used as proxies to evaluate variations in fertility rates, voluntary and spontaneous abortion, and other maternal-child health outcomes. Moreover, these indices are widely used to examine how socioeconomic disadvantage influences adverse maternal and reproductive health outcomes, as well as accessibility of healthcare services [3-5]. However, both the Caranci Index and IVSM are constructed using data from the 2011 national census. Applying these outdated indicators to more recent healthcare databases risks to introduce bias and misrepresenting the current socioeconomic reality of municipalities.

OBJECTIVES

This study aims to determine a municipality-level socioeconomic score that aligns as closely as possible with the IVSM but based on individual-level health data from birth certificates (CEDAP) collected in the years 2010–2012 that are near the year of IVSM determination. The primary objectives are to estimate a predictive model of the IVSM based on aggregated CEDAP data (2010–2012), to validate the model internally within the same time period, and to test the temporal stability of the derived score by applying it to subsequent years and assessing its consistency with the IVSM.

METHODS

Municipality-level frequencies were calculated for selected sociodemographic variables present in the CEDAP records,

including maternal and paternal citizenship, marital status, educational attainment, occupational status, maternal age class, and parity. The IVSM value, available for each municipality from ISTAT (2011), was used as the dependent variable in a General Linear Model (GLM), where independent variables were the proportions of each sociodemographic category in the reference period 2010–2012. Coefficients estimated from the GLM were then used to compute a composite score (Socioeconomic Maternal Score, SMScore) for each municipality by applying the same formula to the frequencies in subsequent time periods (e.g., 2013–2015, 2016–2018).

To evaluate model performance the data of 2010–2012 were splitted into a training and validation sets. In later time periods, the SMScore was compared to the original IVSM using Pearson's correlation coefficient and Mean Squared Error (MSE). Furthermore, both the IVSM and SMScore were categorized into quartiles to simulate typical use in epidemiological studies. Linearly weighted Cohen's Kappa was calculated to assess agreement between the quartile-based classifications. This analysis rests on the assumption that, although a municipality's continuous deprivation score may fluctuate, shifts between quartiles over time are less frequent and may therefore offer a more robust indicator of socioeconomic positioning in longitudinal analyses.

RESULTS

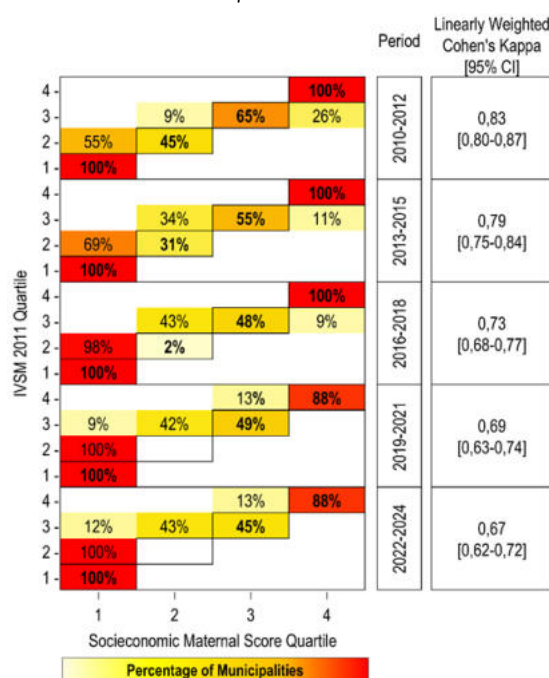
The initial validation of the SMScore against the 2011 IVSM showed a positive correlation (Pearson's $r = 0.64$, $p < 0.0001$) and a relatively low mean squared error (MSE = 3.27), confirming the validity of the constructed scoring model.

When applied retrospectively across five consecutive three-year periods (T1: 2010–2012 through T5: 2018–2020), the correlation between the calculated score and the original IVSM index progressively decreased. In the earlier periods, the relationship remained statistically significant (T1:

$r = 0.64$, $MSE = 1.87$; T2: $r = 0.60$, $MSE = 1.93$). However, the strength of association weakened in later periods, with T3 showing a low correlation ($r = 0.24$, $MSE = 4.19$), and T4 a slightly stronger but still moderate low correlation ($r = 0.34$, $MSE = 3.19$). By T5, the correlation dropped to non-significant levels ($r = 0.10$, $p = 0.0841$) with a marked increase in prediction error ($MSE = 6.44$).

The heatmap visualization (Fig.1) comparing the quartile classification of municipalities based on IVSM 2011 and the recalculated SMScore across the five periods provides further insight. In the earliest triennia (T1 and T2), the majority of municipalities remained within the same quartile or shifted only marginally, indicating good agreement between classifications. This concordance deteriorated progressively in T3 and T4, with more municipalities diverging from their original IVSM quartile. In T5, the misclassification pattern became more evident, with substantial deviation between the IVSM and SMScore classifications. This trend is quantitatively supported by the linearly weighted Cohen's Kappa, which declined from 0.83 [0.80–0.87] in T1 to 0.67 [0.62–0.72] in T5, confirming the decreasing agreement over time. Despite changes in the continuous score, quartile stability appeared more robust in the early triennia.

Figure 1. Agreement between IVSM 2011 and SMScore across periods.



CONCLUSIONS

The findings suggest that the use of area-level socioeconomic indices such as the IVSM should be temporally bounded, as their capacity to reflect current population-level vulnerability deteriorates over time. While the index performed well in the early years following its development, its predictive and classificatory coherence weakened in later periods. This divergence may stem from genuine socioeconomic transformations within municipalities or from changes in the demographic and social profiles of the women giving birth, such as age at delivery or parity.

The observed mismatch highlights the importance of regularly updating deprivation indices or developing dynamic proxies that can adapt to shifting population characteristics. Integrating information from different health service databases, which contain additional relevant variables, may refine the scoring approach and enable more robust comparisons with existing socioeconomic indices. This integration can improve area-level deprivation measures and support their effective use in public health monitoring and planning.

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Scrolling and Self: How Self-Esteem and Physical Activity Shape the Effects of Instagram Use on Eating Behavior in University Students

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INTRODUCTION

The use of Social Networking Sites (SNS) has been continuously increasing in recent years [1], contributing to the rise of significant behavioral and psychological issues, especially when SNS are used as a coping mechanism for stress and loneliness among young people. One of the most widely used SNS today is Instagram, and several studies indicate that excessive use of this platform may be associated with decreased cognitive functioning in young adults, as well as behavioral and sleep disorders [2].

In addition, the Instagram platform's focus on food imagery, fitness content, and body aesthetics often perpetuates unattainable beauty standards, potentially triggering disordered eating patterns or body dissatisfaction [3,4].

Moreover, the study by Romero-Rodríguez et al. [5], found that while problematic smartphone use negatively influences self-esteem, the intensity of Instagram use itself was not a direct predictor of self-esteem among university students. This suggests a nuanced relationship between SNS engagement and self-perception, warranting further exploration.

AIMS

This study aimed to explore the relationships between problematic social media use (PSMU), self-esteem, physical activity (PA), and eating behaviors, using a moderated-mediation model. In this study, we tested a moderated-mediation model of how explained variables may be interrelated. The model's starting point is the well-established link between PSMU and self-esteem, and PSMU and eating behaviour, with the hypothesis that higher PSMU is associated with higher levels of problematic affects and behaviors. We then

hypothesized that participants who have higher self-esteem will show better eating habits despite higher levels of PSMU (mediation analysis). Finally, we supposed that physical activity and sedentary levels could moderate the relationship between PSMU and self-esteem (moderation analysis), with a protective role of PA.

METHODS

A cross-sectional study was conducted with 1237 university students (74.4% females, mean age = 22.7 ± 5.5 years). Participants completed an online survey assessing Instagram use, enrolled in 13 different academic programs (e.g., languages, art, biology, pharmacy, sport science). The students completed an online questionnaire composed of four sections about: physical activity (International Physical Activity Questionnaire, IPAQ) [6]; Instagram usage (Social Media Use Questionnaire, SMUQ) [7]; self-esteem (Rosenberg Self-Esteem Scale) [8]; eating behaviors (Dutch Eating Behavior Questionnaire, DEBQ) [9].

A chi-square test was used to test the gender differences in the distribution of participants in the three PA categories (low, moderate, high). Non-parametric Spearman's correlations were then computed between Instagram use (minutes per day, reasons for use), SMUQ subscales (Withdrawal and Compulsion), PA (MET/min/week), sitting time (hours/day), self-esteem and eating behaviors (emotional eating, restrained eating, external eating). A multiple linear regression was then performed to test the effect of Instagram time (minutes per day) and motives of use on the two SMUQ subscales (Withdrawal and Compulsion). Finally, a moderated-mediation analysis [10] was conducted to examine the relationships between PSMU and Eating behaviors, considering PA and

sitting time, and self-esteem, as potential moderators and mediator, respectively.

RESULTS

In our sample, females reported higher levels of PSMU and more problematic eating behaviors than males, who in turn exhibited significantly higher levels of physical activity ($p < 0.001$). Regarding Instagram use, females reported significantly higher daily usage ($p < 0.001$), number of followers ($p < 0.01$), following ($p < 0.01$), and higher scores for all reasons for Instagram use, except for self-promotion, where responses were similar to males.

Two multiple linear regression analyses (one for each subscale of SMUQ) were used to investigate which of the motives for use or usage time were more influential on the problematic use. Considering Withdrawal as the dependent variable, the overall regression was statistically significant ($R^2_{adj} = 0.222$, $F(6,1165) = 56.71$, $p < 0.001$). All the independent variables were significant predictors of Withdrawal, with the exception of the use for Documenting.

The correlation analysis showed that the PSMU (both Withdrawal and Compulsion) was significantly positively correlated to the daily usage time, and to all the three typologies of eating behaviors (restrained, emotional, and external eating).

PSMU was positively correlated with restrained, emotional, and external eating behaviors and negatively correlated with PA and self-esteem. Self-esteem mediated the relationship between PSMU and eating behaviors, with higher self-esteem associated with healthier eating patterns. Sitting time moderated the relationship between PSMU and self-esteem, reinforcing the negative effects of excessive Instagram use. Gender differences were observed, with females reporting higher levels of PSMU, problematic eating behaviors and lower PA levels compared to males. This study highlights the complex interplay between PSMU, self-esteem, PA, and eating behaviors in university students.

CONCLUSION

These findings highlight the importance of addressing PSMU's impact on self-esteem and eating behaviors in young adults. PA emerged as a beneficial factor for self-esteem, but its inability to moderate the PSMU–self-esteem relationship suggests that targeted interventions should consider psychological components alongside lifestyle modifications mainly directed at reducing sedentary and sitting time. Promoting regular PA could serve as an effective strategy to enhance self-esteem and counteract the harmful effects of excessive Instagram use. Health education programs emphasizing digital literacy, self-esteem enhancement, and balanced SNS engagement may help mitigate PSMU's adverse effects. Future research should explore longitudinal relationships and intervention strategies to promote healthier social media habits and psychological well-being among university students.

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Epidemiological Approaches to Healthy Ageing Research: Using Data from the SHARE Project

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INTRODUCTION

As populations age, public health systems face the challenge of understanding and supporting healthy ageing trajectories [1]. Life-course transitions – such as retirement – affect health behaviours, psychosocial wellbeing, and cognitive functioning. Longitudinal datasets offer key opportunities to track these changes, but require rigorous analytical strategies to fully exploit their potential in epidemiology [2].

Objectives

To provide scientific evidence to support decision-making in response to the needs of an increasingly ageing population, through a Healthy Ageing and life-course research approach, by leveraging the longitudinal and multidimensional potential of SHARE data [3].

Specific objectives are:

- i) to analyse the short- and long-term impact of retirement on behavioural risk factors (e.g. smoking, alcohol use, food consumption, physical activity) and mental health (e.g. depressive symptoms and suicidality);
- ii) to evaluate individual, socioeconomic, and environmental determinants of cognitive decline using harmonised cognitive assessment protocols.

METHODS

For the specific objective i) We implemented a longitudinal cohort design using SHARE waves 1-8 (2004–2020), selecting 8,998 individuals aged 50+ employed at baseline and retired during follow-up [3,4]. Retirement timing served as a temporal exposure to construct repeated-measures analyses. Generalised estimating equations (GEE) were applied to model selected outcomes (e.g., smoking, alcohol use, food consumption, physical activity, depressive symptoms), accounting for intra-individual correlation [5]. Time was modelled in different intervals relative to the retirement year. Covariates

included sex, age, marital status, education, occupation, and chronic disease. For the specific objective ii) We adopted a retrospective design based on SHARE-HCAP Wave 9 (2021–2022), which included 2,685 individuals aged 65+ from five European countries. Respondents were classified through machine learning techniques as cognitively normal, mildly, or severely impaired based on harmonised neuropsychological protocols. To explore associations with cognitive status, we retrospectively reconstructed exposure histories using previous SHARE waves. These included educational attainment, occupational trajectories, chronic conditions, housing characteristics (e.g., presence of architectural barriers, accessibility), and perceived neighbourhood quality. Multivariable models were applied to evaluate the role of these life-course exposures in shaping late-life cognitive outcomes.

RESULTS

Retirement was associated with dynamic shifts in health behaviours and mental health. Physical activity increased during the early post-retirement period, particularly among previously inactive individuals (RR=1.49, 95%CI 1.36–1.63), but declined after a decade (RR=0.90, 95%CI 0.88–0.93). Smoking prevalence and intensity decreased substantially over time, with average daily cigarette consumption falling from 27 to 9 per day, and risk of smoking dropping by 42% a decade post-retirement (RR=0.58, 95%CI 0.46–0.74). Alcohol use patterns shifted: while daily drinking increased moderately (RR=1.28 at 10+ years), binge drinking declined (RR=0.78, 95%CI 0.66–0.93). Dietary habits showed favourable trends: intake of protein-rich foods (meat, fish, legumes, eggs) increased significantly over the long term (RR=1.09, 95%CI 1.01–1.17), while fruit, vegetable, and dairy consumption remained stable. Depressive symptoms declined in the short term (RR=0.89, 95%CI 0.81–0.99), but increased again after 10 years among non-manual workers and late

retirees (RR up to 1.37). Notably, suicidality ideation risk rose substantially in the long term, particularly among men (RR=1.78, 95%CI 1.11–2.86).

Cognitive assessment from SHARE-HCAP data showed that 63.4% of respondents were classified as cognitively normal, 25.8% as mildly impaired, and 10.7% as severely impaired. Preliminary results suggest that environmental disadvantages

Results from the SHARE HCAP wave showed that 63.4% were classified as cognitively normal, 25.8% as mildly impaired, and 10.7% as severely impaired. Retrospective reconstruction of life-course exposures showed that environmental disadvantages, including poorer perceived neighbourhood quality, may contribute to impaired cognitive impairment in later life.

CONCLUSIONS

Our findings offer a comprehensive view of behavioural and psychological changes during the transition to retirement, distinguishing short- and long-term effects across key health domains. SHARE data offer a powerful resource for life-course epidemiology with different epidemiological approaches and appropriate statistical modelling. The methodological strategy – centered on record linkage, repeated measures, and harmonised indicators – demonstrates how survey data traditionally used in economics and social sciences can inform public health. Future research should address compositional bias, improve data harmonisation, and explore other life-course transitions.

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Perioperative Nutritional Supplementation to Reduce Postoperative Infections: A Systematic Review and Meta-Analysis

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INTRODUCTION

Postoperative infections, including surgical site infections (SSIs), pneumonia, bloodstream infections (BSIs), and urinary tract infections (UTIs), remain among the most frequent and impactful complications following surgery. They contribute substantially to patient morbidity, length of stay, and healthcare costs, despite improvements in surgical techniques and perioperative care. In Europe alone, healthcare-associated infections are estimated to cause over 90,000 deaths annually and impose a burden of more than 500 DALYs per 100,000 population [1]. Despite the adoption of ERAS protocols and prophylactic antibiotics, the incidence of postoperative infections has plateaued in many settings, suggesting the need for adjunctive strategies beyond standard perioperative care. In this context, perioperative nutrition has emerged as a potentially modifiable factor capable of influencing immune responses, gut microbiota, and systemic inflammation. Immunonutrition (enriched with arginine, omega-3 fatty acids, nucleotides), probiotics, synbiotics, and protein supplementation are currently being investigated for their ability to prevent infectious complications and improve surgical outcomes [2,3]. However, the literature is fragmented and results are often inconsistent across interventions and populations [4-7].

OBJECTIVES

This study aimed to systematically evaluate the efficacy of perioperative nutritional supplementation in reducing postop-

erative infectious complications in adult surgical patients. The focus on infection stems from its clinical burden and its potential sensitivity to immune-modulating strategies. Secondary outcomes included specific infection types (SSIs, BSIs, UTIs, and pneumonia) and hospital length of stay (LOS), selected for their relevance to patient recovery and healthcare system impact. These outcomes were selected to capture both the direct impact of infections and their broader consequences on patient recovery and resource utilization.

METHODS

A systematic review and meta-analysis were conducted in accordance with PRISMA 2020 guidelines and registered in PROSPERO (CRD42024575184) [8]. PubMed, Scopus, Cochrane Library, and Google Scholar were searched without restrictions on date or language. Eligible studies included adult surgical patients receiving perioperative nutritional interventions (immunonutrition, probiotics, synbiotics, protein supplementation) compared to standard care or placebo. The research question was structured using the PICO framework to ensure structured and clinically meaningful comparisons. Both randomized controlled trials (RCTs) and observational studies were included. The primary outcome was the incidence of infectious complications. Meta-analyses were performed using STATA SE 19, with pooled odds ratios (ORs) and 95% confidence intervals (CIs). Heterogeneity was assessed via the I^2 statistic, and a random- or fixed-effects model was applied accordingly. The methodological quality of included

studies was assessed using NIH tools, with most RCTs classified as low or moderate risk of bias, and observational studies showing greater variability [9]. Risk of bias was independently assessed by multiple reviewers, and disagreements were resolved by consensus, ensuring rigorous evaluation.

RESULTS

Thirty-nine studies (27 RCTs and 12 observational) met the inclusion criteria. Immunonutrition was the most consistently effective intervention, significantly reducing the overall incidence of infectious complications in both RCTs (OR = 0.36; 95% CI: 0.21–0.62) and observational studies (OR = 0.32; 95% CI: 0.17–0.61). The protective effect was particularly evident with oral administration and in patients aged ≤65 years. Probiotics showed a borderline protective effect overall, while synbiotics appeared more effective in younger patients and when administered orally. Protein supplementation was evaluated in too few studies to allow for pooled analysis of the primary outcome.

Regarding secondary outcomes, a general trend of benefit was observed across most interventions, though with variability in effect size and consistency. Immunonutrition and protein supplementation were associated with reduced SSIs. Probiotics showed favorable effects on UTIs and pneumonia. Synbiotics were linked to a shorter LOS, though heterogeneity and limited data warrant cautious interpretation. The quality of evidence varied, with most RCTs showing low or moderate risk of bias. These findings, although promising, should be interpreted with caution due to limited data in some subgroups and moderate heterogeneity in certain analyses.

CONCLUSIONS

Perioperative nutritional supplementation, particularly immunonutrition, represents a promising and evidence-based strategy to reduce postoperative infectious complications. Secondary benefits on SSIs, UTIs, pneumonia, and LOS support the broader integration of nutritional protocols into surgical care. Immunonutrition showed the most consistent results, while probiotics were effective for UTIs and pneumonia. Synbiotics significantly reduced LOS, though limited data and heterogeneity warrant caution. The strength of evidence depends on study volume and quality; thus, interventions like immunonutrition for SSIs should be prioritized, while others—such as protein supplementation for non-SSI outcomes—require further evaluation. Clarifying differential impacts across surgical specialties and standardizing formulations, timing, and administration routes will be crucial for tailored and effective implementation.

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Hospitalizations for Mental Health in Migrants and Italian Citizens in the Marche Region between 2011 and 2023: A Population-Based Study using Healthcare Utilization Databases (MIGHTY Project P2022ASXKR)

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INTRODUCTION

International migration is considered a complex and unstoppable phenomenon. Migrant population is a heterogeneous group of people who experience migration for different reasons and that are considered to be at increased risk of developing mental disorders [1]. Scientific evidence on migrants' mental health is limited, as is knowledge of their access to and use of specific care services and treatments. As compared to natives, higher rates of involuntary hospitalization were found among migrants in most European countries [1,2].

AIM

The aim of the present study was to compare mental health hospitalization rates in Migrants and Italians in Marche Region during 2011-2023, and to investigate their differences according to demographic and clinical characteristics.

METHODS

A cross-sectional population-based study on individuals hospitalized in psychiatric departments of all ages and resident in Marche Region in the period between 2011 and 2023 was conducted, using healthcare utilization databases (Hospital Discharge and Regional Beneficiaries databases). Residents were divided into Migrants from High Migratory Pressure Country (HMPC) and Italians, according to citizenship [3].

The primary diagnosis field of hospital discharge database was used to estimate the prevalence of different types of mental disorder using ICD-9 CM codes (290.-319.).

Annual age-standardized hospitalization rates were calculated for HMPC Migrants and Italians using the 2019 Italian population [4] as standard, using the direct method. Rates were stratified by sex and the Standardized Rate Ratios (SRR) with 95% Confidence Interval (95%CI) were calculated by sex and year of observations as ratio between HMPC and Italian rates. All data were processed in compliance with the European (GDPR, EU 2016/679) and national privacy laws (D.lgs. 196/2003 and subsequent amendments).

RESULTS

A total of 59.881 hospitalizations were analyzed, 93.3% of which were of Italians. The mean age was higher in Italians than in HMPC (43.8 y versus 32.2 y). In both populations, hospitalizations most frequently referred to unmarried individuals (62% in Italians, 64% in HMPC) and to individuals with the lowest level of education (45% in Italians, 48% in HMPC).

Hospitalizations originated mainly from Emergency Departments admissions (25.4% Italians, 28.8% HMPC), medical indications (24.8% Italians 20.4% HMPC), prison (13.4% Italians, 13.7% HMPC) or hospitalization at the time of delivery (13.3% Italians 11.4% HMPC).

The most frequent diagnoses were schizophrenia and other functional psychosis (29.6% in Italians vs 31.6% HMPC), alcoholism and toxicomania (15.2% in Italians vs 21.7%

HMPC) in both populations. Hospitalizations for depression in Italians and HMPC had similar proportions (11.1% vs 11.2%, respectively) but the frequency was more than twice as high in HMPC women as in HMPC men (8.3% vs 2.9%); the proportions of hospitalizations for mania and bipolar affective disorders hospitalizations were higher in HMPC women than in HMPC men (7.0% vs 2.1%); alcoholism and toxomania were more frequent in HMPC women than in Italian women (11.5% vs 4.8%), and also than in HMPC men (11.5% vs 10.1%).

Excluding the 2020-2021 (the pandemic period) in which the lowest rates were recorded (2.5-2.5 and 2.3-2.0 for Italians men and women respectively, 1.4-1.7 and 1.6-2.2 for HMPC men and women respectively), the standardized rates (x1000 residents) of hospitalization ranged between 1.7 to 2.6 (in 2023 and 2019, respectively) for HMPC men and between 1.8 to 2.5 (in 2023 and 2017, respectively) for HMPC women; in Italians, rates ranged between 2.6 to 4.2 (2023 and 2011/2012, respectively) for men and between 2.1 to 3.5 (in 2023 and 2011, respectively) for women.

Standardized rate ratios (Figure 1) showed that HMPC reported lower hospitalizations for mental disorders than Italians over the entire study period for both genders. In females, the differences between the two populations were less pronounced than in males.

CONCLUSION

This study based on healthcare utilization databases allowed to quantify the use of inpatient psychiatric care in both Migrants and Italians in the Marche Region.

Our results showed a lower rate of hospitalization for mental disorders in Migrants than in Italians over the study period, with differences by gender and by type of mental disorder.

Considering that mental disorders are characterized by chronicity, diagnostic-therapeutic difficulties and strong family and social impact, further assessments are needed to identify the reasons for the use of hospitalization versus community-based care services in both populations.

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Figure 1. Standardized rate ratios of mental health hospitalization and 95% Confidence intervals in HMPC and Italians stratified by sex. Marche Region, 2011-2023



A Framework to Improve Data Quality and Manage Dropout in Web-Based Medical Surveys: Insights from an AI Awareness Study among Italian Physicians

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BACKGROUND

Ensuring data quality in self-reported online surveys remains a critical challenge in digital health research, particularly when targeting healthcare professionals [1,2]. Self-reported data are susceptible to multiple biases, including careless responding, social desirability bias, and dropout-related attrition, all of which may compromise the validity of findings [3,4]. In web-based surveys where researcher oversight is limited, structured quality control measures are essential to detect low-quality responses, minimise sampling bias, and enhance data reliability [5]. Previous studies have demonstrated that inadequate quality checks can lead to inflated error rates, reduced statistical power, and misleading conclusions [6]. Objective

This study presents a comprehensive methodological framework for optimising data quality in web-based medical surveys, applied to a national study on AI awareness among Italian physicians. Integrating pre-survey validation, real-time dashboards, response-time filtering, and post-hoc careless responding detection would address key challenges in digital research, while providing a replicable model for future studies.

METHODS

We conducted a national web-based survey using a validated instrument (doi:10.1101/2025.04.11.25325592) via the LimeSurvey platform. The survey incorporated two main sections: (1) a core module assessing knowledge, attitudes and practices regarding AI in medicine; (2) clinical scenarios evaluating diagnostic agreement with AI-generated proposals. Multiple quality control strategies were implemented

throughout the survey lifecycle. In terms of survey design and logic, the questionnaire employed an adaptive flow structure, whereby respondents were routed through clinical scenarios relevant to their medical speciality. To reduce the incidence of partial completions and missing data, key questions were marked as mandatory, and completion status was actively tracked. In the monitoring and recruitment phase, a real-time dashboard monitored participant distribution (gender/geographical areas/speciality); referral links were rotated to minimise snowball bias [7]. Time-based data quality checks excluded outliers (completion time <1st or >99th percentile) [8]. Completion time for the first section was analysed for all completers to assess correlations between response speed and quality indicators. Dropout patterns were analysed using Kaplan-Meier survival analysis and logistic regression, to identify systematic attrition predictors. Data quality assessments were performed on the outlier-cleaned dataset (n=587). Response quality was assessed using complementary careless responding indicators applied specifically to opinion scale items (Likert 1-5). Two detection methods were used: low response variance analysis, identifying respondents with insufficient variability (SD < 0.5), and excessive same-response detection, flagging participants using identical responses for >75% of items. Internal consistency analysis (Cronbach's α) evaluated scale reliability across different quality levels.

RESULTS

A total of 736 accesses were recorded on the survey platform. As an initial inclusion criterion, only participants who indicated current registration with the Italian Medical Council were considered eligible: 79 (10.7%) were excluded, yielding

a sample of 657 eligible participants (89.3%). Among eligible respondents, 597 completed the first section, yielding a dropout rate of 9.1% ($n=60$). A Kaplan-Meier survival analysis using total survey time revealed that most dropouts occurred early, with critical points at 45% after demographic, 51% after personal AI knowledge items, 71% after opinion items, and 100% before clinical scenarios. Logistic regression showed no significant predictors of completion (LR $\chi^2(6)=3.46$, $p=0.7497$; pseudo- $R^2=0.014$; AUC=0.60, 95%CI: 0.50–0.70). Completion time showed no correlation with response quality (Spearman's $\rho = -0.019$, $p = 0.645$). Following outlier removal, data quality assessment among 587 who completed the first section revealed two complementary patterns of careless responding: 8.52% ($n=50$) exhibited low response variance, while 32 (5.45%) demonstrated excessive same response patterns. Cross-classification analysis showed 23 participants (3.92%) flagged by both indicators, with 71.88% of excessive same responders also showing low variance. Overall, 50 participants (10.05%, 95% CI: 7.9%–12.8%) exhibited careless responding detectable by at least one indicator. Internal consistency analysis showed robust scale reliability (Cronbach's $\alpha = 0.754$) that remained stable across quality levels.

CONCLUSION

The integration of real-time monitoring, adaptive design, time-based validation, and systematic careless responding detection provides a robust methodological framework for web-based medical surveys, particularly for complex topics like AI adoption. Comprehensive data quality assessment revealed a 10.05% careless responding rate among completers, which aligns with the literature. The absence of correlation between completion time and response quality shows that careless responding could reflect attentional rather than temporal factors. Our findings suggest that both phenomena likely reflect situational or contextual factors rather than systematic participant characteristics or survey design flaws. This supports the validity and generalizability of the final dataset while providing a replicable quality control framework for future web-based medical research.

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Impact of First and Further Decompensation in Metabolic-Dysfunction Associated Compensated Advanced Chronic Liver Disease

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BACKGROUND

Metabolic dysfunction-associated steatotic liver disease (MASLD) currently stands as one of the foremost global health challenges, with a prevalence of 38% worldwide according to the most recent estimates [1] and with a concerning upward trend due to the parallel anticipated increasing of Diabetes and Obesity epidemic in the coming years [2].

There is a long-standing agreement that the first decompensation - defined as ascites, hepatic encephalopathy (HE), variceal bleeding, and jaundice- appears the pivotal event for

patients' prognosis and marks the transition from the compensated, also known as compensated advanced chronic liver disease (cACLD), to the decompensated stage of cirrhosis [3]. Although only a small fraction of patients dies following the first decompensation episode, the risk of developing further decompensation increases and the median survival dramatically decreases [4]. The occurrence of a further decompensation event - defined according to the Baveno VII Consensus [5] as either the recurrence of the initial event or the development of a second decompensation event - represents a crucial turning point in the natural history of the liver disease, markedly increasing the risk of liver-related death (LR-D) in those patients.

AIM

We assessed the cumulative incidence of first and further (acute and non-acute) decompensation and evaluated their impact on LR-D in patients with compensated advanced chronic liver disease (cACLD) due to metabolic dysfunction-associated steatotic liver disease (MASLD).

METHODS

International multicenter retrospective study (17 centers) on 6,061 consecutive patients with clinical (LSM>10 kPa) or biopsy-proven (F3-F4 fibrosis) diagnosis of cACLD due to MASLD. First and further decompensation were defined according to Baveno VII criteria. Competing risk analyses estimated the cumulative incidence of first and further decompensations, treating liver-related death (LR-D), extra-hepatic death (EH-D), and liver transplantation (LT) as competing events. Cumulative Incidence Functions (CIFs) were compared using Gray's test and stratified by decompensation type and cause of death. Time-to-event analyses were anchored at cACLD diagnosis (first decompensation) and at first decompensation (subsequent events), with 5-year CIFs reported. Cause-specific Cox models with time-dependent covariates assessed the impact of decompensations and HCC on LR-D. Multivariable models included age, sex, diabetes, and liver function markers when available.

A seven-state multistate model estimated transitions from cACLD to better assess the clinical course of cACLD due to MASLD. Analyses were conducted in R (v4.3.3) using cmprsk, mstate, and related packages.

RESULTS

The cumulative incidence of the first decompensation was 3.5% (95% C.I 3.0-4.1) at 5 years, increasing 19-fold the risk of LR-D using Cox analysis (Figure 1A); the cumulative incidence of further decompensation was 43.9% (95% C.I 37.2-50.2) at 5 years among patients with first decompensation (Figure 1A), additionally increasing 1.5-times the risk of LR-D. Ascites, followed by variceal bleeding, were the most common events in both first and further decompensation. Hepatocellular carcinoma (HCC) further independently increased the risk of LR-D by 3- and 1.4-fold in the whole cohort of cACLD due to MASLD and in those who experienced first decompensation, respectively.

CONCLUSIONS

The first and further decompensations represent tipping points in the clinical course of patients with cACLD due to MASLD, increasing 19-times and additionally 1.5-times the risk of LR-D. HCC is an independent predictor of LR-D in patients with cACLD due to MASLD, resulting in an additional risk of LR-D when associated with both first and further decompensation.

Figure 1A

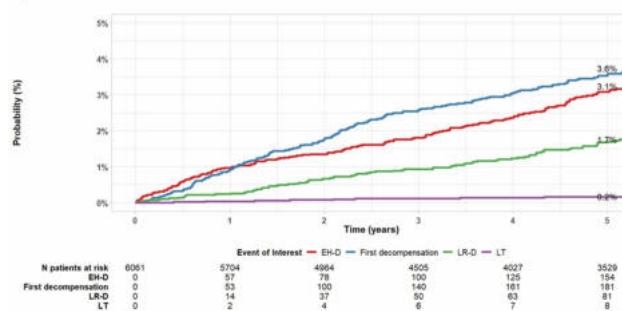


Figure 1B

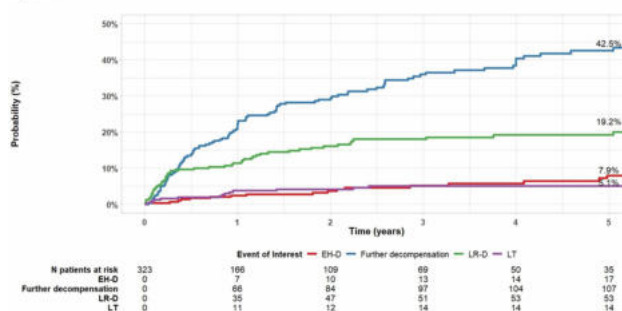


Figure 1. Five-years CIF of major events in the whole cohort of cACLD due to MASLD. (A) Five-years CIF of first decompensation, extra-hepatic death and LT being as competing events. (B) Five-years CIF of first decompensation, extra-hepatic death and LT being as competing events.

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Blood Biomarkers of Aging and Multimorbidity Prognosis in Older Adults: Preliminary Results from the BIO-SIGN Project

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INTRODUCTION

Increased life expectancy is associated with the presence of multiple chronic diseases (i.e., multimorbidity), affecting health trajectories and care demands of older adults. The identification of a pool of accessible biomarkers that reflects specific endophenotypes of physiological dysregulation, may facilitate prognostication in older adults living with multimorbidity, offering a valuable tool for clinical decision-making and personalized interventions [1-3]. The BIO-SIGN project aims to assess how a rich set of novel biomarkers, along with clinical, environmental and behavioral factors, interact in the definition of specific multimorbidity patterns and their association with negative outcomes, specifically all-cause mortality.

OBJECTIVES

First year of the project focused on two different objectives. First objective was the selection of a set of accessible and reliable biomarkers of multimorbidity, through extensive bibliographic research. Second objective was to identify homogeneous groups of multimorbid individuals (≥ 2 diseases), who share similar underlying disease patterns, using two Italian cohorts of randomly selected resident adult individuals.

METHODS

OBJECTIVE 1. A systematic review was conducted on PubMed and Web of Science to identify peer-reviewed studies assessing the association between individual biomarkers in human fluid samples and multimorbidity. Based on this review, a panel of significant blood biomarkers associated with multimorbidity was selected for further investigation in this study.

OBJECTIVE 2. The identification of multimorbidity patterns involved the two Italian cohorts of CUORE project [4] and Moli-sani project [5] (baselines 2008-2012 and 2005-2010, respectively), which share similar data collection methods. We focused on older adults aged 60-79 years. To ensure consistency and minimize data heterogeneity, a rigorous process of harmonization was applied. Population clinical characteristics were studied and homogeneous groups of individuals sharing similar underlying disease patterns were identified using Latent Class Analysis (LCA) [6]. Cox regression models were used to assess the association between multimorbidity patterns and mortality over 14 years of follow-up, with results pooled in a random-effect meta-analysis.

RESULTS

OBJECTIVE 1. SYSTEMATIC REVIEW FOR BIOMARKERS IDENTIFICATION

The systematic review identified several inflammatory and metabolic biomarkers, such as IL-6, triglycerides, LDL chole-

terol and kidney and liver markers such as CyC, Aspartate aminotransferase and Alanine aminotransferase as being directly associated with multimorbidity. Additionally, neurodegeneration related biomarkers, including NfL and p-Tau 217, showed similar associations. Plasmatic level of A β 40/42 showed a significant direct association with multimorbidity in less robust studies, while vitamin D displayed an inverse association with multimorbidity in two studies. Findings for other biomarkers such as TNF α receptor II, total cholesterol, HDL cholesterol, CRP, and Insulin-like growth factors 1 were inconsistent across studies. On the basis of the literature review, a list of blood biomarkers was selected to be assessed in relation to the aims of the BIO-SIGN project (Table 1). Furthermore, based on the researcher's expertise, standard pancreatic (insuline, C-peptide), cardiovascular (NT-proBNP), metabolic (glycemia), hepatic and renal (creatinine, albumine) biomarkers were included to the list along with emerging candidates linked to inflammatory, vascular, and hormonal dysregulation (GDF-15, I-CAM-1, V-CAM-1, leptine, MIG, GFAP) to ensure comprehensive coverage of relevant biological pathways.

OBJECTIVE 2. MULTIMORBIDITY PATTERNS IDENTIFICATION

In relation to the cohort studies, total samples of 3,695 individuals in CUORE (48% male, mean age 68.8 years [SD 5.6]) and 7,801 in Moli-sani (51% male, mean age 68.2 years [SD 5.4]) were evaluated and a total of 33 chronic diseases were considered. In both cohorts, six multimorbidity patterns were identified: hypercholesterolemia; metabolic, depression and cancer; cardiometabolic and respiratory; gastrointestinal, genitourinary and depression; respiratory; unspecific (i.e., no diseases overexpressed). A seventh pattern of multimorbidity-free participants was identified. Incidence rates of mortality were 1.7 and 1.9 per 100 person/years for CUORE and Moli-sani, respectively. When compared to participants without multimorbidity, those displaying a cardiometabolic and respiratory pattern were associated with the highest mortality (pooled HR 2.62; 95% CI 2.15-3.10), followed by unspecific (pooled HR 1.45; 95% CI 1.21-1.68), respiratory (pooled HR 1.33; 95% CI 1.01-1.64), and gastrointestinal, genitourinary, and depression (pooled HR 1.33; 95% CI 1.06-1.60).

CONCLUSIONS

During the first year of activity of BIOSIGN, we selected a panel of 25 blood biomarkers -across diverse diseases domains, as possible factors potentially able to define different endophenotypes of multimorbidity. Additionally, 6 different multimorbidity patterns were identified in two Italian cohorts and were differentially associated with survival. Further steps in the project will be to evaluate how biomarkers assessment can predict disease progressions and outcomes across these patterns. The identification of biomarkers as measurable prognosis factors for different homogeneous multimorbidity patterns in older adults may improve risk stratification, and prevent or reduce adverse health outcomes, including mortality. Such insights could enable personalized interventions and care plans and ensure a better allocation of health resources.

Biomarker	Disease domain
Interleukin-6 (IL-6)	Inflammatory
Tumor necrosis factor-alpha receptor 2 (TNF α receptor II)	
C Reactive protein (CRP)	
Monokine induced by gamma interferon γ (MIG)	
Intercellular adhesion molecule 1 (I-CAM-1)	Inflammatory/ vascular
Vascular cell adhesion molecule 1 (V-CAM-1)	
Growth Differentiation Factor 15 (GDF-15)	Cardiovascular
Pro B-type natriuretic peptide (NT-proBNP)	
High-density lipoprotein (HDL) cholesterol	Metabolic
Low-Density Lipoprotein (LDL) cholesterol	Metabolic
Total cholesterol	Metabolic
Triglycerides	Metabolic
Glycemia	Metabolic
Creatinine	Metabolic (renal)
Cystatin C (CyC)	
Albumine	Metabolic (hepatic/ renal)
Insuline	Metabolic (pancreatic)
C-Peptide	Metabolic (pancreatic)
Leptine	Metabolic/ hormonal
Vitamin D	Hormonal
Phosphorylated Tau 217 (p-Tau217)	Neurological
Neurofilament-light chain (NfL)	
Amyloid Beta aa 1-40 (A β 40)	
Amyloid Beta aa 1-42 (A β 42)	
Glial fibrillary acidic protein (GFAP)	

Table 1. Panel of selected putative blood biomarkers of multimorbidity across diverse pathophysiological domains.

ACKNOWLEDGEMENTS

Funded by the European Union - Next Generation EU - PNRR-MAD-2022-12376569 – Malattie Croniche non Trasmissibili (MCnT) ad alto impatto sui sistemi sanitari e socio-assistenziali.

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ProtAct-Us: A Study on the Long-Term Impact of Road Traffic Crashes in Europe

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INTRODUCTION

Despite advancements in emergency care and prevention, many road traffic crash (RTC) survivors suffer from enduring impairments that are insufficiently considered and registered in hospital records or existing surveillance systems. Building upon evidence from prior initiatives [1-3], this study aims to (1) assess the multidimensional long-term outcomes of RTCs, (2) identify early predictors of functional and psychological recovery, and (3) inform data-driven strategies for post-injury rehabilitation and health system planning.

AIM

The ProtAct-Us from Long-Term Consequences of Road Crashes (ProtAct-Us), a project funded by the European Union, addresses a critical, yet often underexplored, multidimensional aspect of road safety: the long-term consequences of road traffic crashes (RTCs) on individuals' physical, cognitive, psychological, and socio-economic well-being.

METHODS

This multicentre, prospective, observational longitudinal study will be conducted across Germany, Greece, and Italy. The study population comprises adults (≥ 18 years) involved in RTCs, enrolled through hospitals, trauma centres, or on the accident scene from June to December 2025. Consecutive sampling will be performed until the minimum required sample size of 120 subjects is collected. Informed consent will be obtained in compliance with national regulations. Data will be collected at two timepoints: baseline (within 30 days after the accident) and 12 months post-injury. Validated instruments will be used, covering health-related quality of life (EQ-5D),

cognitive function (MoCA), psychological status (CES-D, IES-R), social support (MOS), and economic burden (Muarc). Variables related to injury characteristics, health history, and contextual factors (e.g. access to care, social and work reintegration) will also be recorded.

STATISTICAL ANALYSIS

Descriptive analyses will summarise the sample's clinical, psychological, and socio-demographic features. Recovery trajectories and outcome prevalence at 12 months will be analyzed. Univariate analyses will explore associations between potential predictors and outcomes. Multivariate regression will identify independent predictors of poor recovery, such as persistent pain, psychological distress, or reduced participation in the daily activities.

CONCLUSION

By integrating medical, psychological, cognitive, and socio-economic data, the ProtAct-Us study will try to provide a comprehensive understanding of the long-term burden of RTCs. This multidimensional approach is expected to generate evidence-based recommendations to improve recovery pathways, tailor rehabilitation programs, and enhance policy responses. Findings will contribute to a more person-centred and sustainable management of the road traffic injury consequences for all road traffic users. Findings from ProtAct-Us will contribute to evidence-based public health and policy-making by quantifying the long-term burden of RTCs and identifying modifiable risk factors, ultimately supporting more effective post-crash care strategies.

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Psychiatric Disorders and Psychoactive Substance Use in Children and Adolescents: A Population-Based Study in Lombardy, 2016–2022

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INTRODUCTION

The World Health Organization (WHO) defines dual diagnosis (DD) as “the coexistence of psychoactive substance use and another psychiatric disorder in the same person” [1]. Clinicians, researchers, and policymakers are concerned with this issue due to the difficulties involved in managing dual diagnoses, including high relapse rates, reduced participation in treatment, and less favourable treatment results [2]. The literature indicates a higher risk of psychoactive substance use disorders in individuals who have grown up with psychiatric disorders [3]. Although psychiatric comorbidity in adults with substance abuse issues is well documented, it has been less thoroughly investigated in adolescents [4]. Nevertheless, it remains a highly relevant issue for national drug and mental health policies. Adolescents with dual diagnoses place a significant social and economic burden on the public healthcare system, as the interaction between substance use and psychiatric disorders creates a vicious cycle that further worsens later difficulties, such as family conflicts, academic problems, and criminal behavior [2]. The aim of the study is to estimate the prevalence of National Health Service users (0–18 years old) who have been diagnosed with psychiatric disorders related to substance abuse in Lombardy between 2016 and 2022.

METHODS

DATA SOURCES AND STUDY POPULATION

The data used for this study were drawn from the administrative healthcare utilization databases of the Lombardy Region, which are routinely employed for reimbursement purposes.

The observation period spanned from January 1, 2016, to December 31, 2022. The study population included all individuals aged 0–18 years as of December 31 of each year, residing in the Lombardy Region.

CASE DEFINITION

An individual was classified as a case if, at least once during a given year, they either accessed the Emergency Department or were hospitalized. Case identification was based on the presence of specific ICD-9-CM codes (291–292, 303–305), which indicate psychiatric conditions associated with psychoactive substance use.

- Emergency Department visits were included if any of the above codes were recorded as a diagnosis.

- Hospitalizations were considered when any of the specified codes appeared in one of the six diagnosis fields of the hospital discharge records.

This definition allowed for the identification of adolescents

(aged 0–18 years) who had contact with healthcare services for issues related to the comorbidity of psychiatric disorders and psychoactive substance use.

STATISTICAL ANALYSIS

Annual and monthly prevalence rates (per 1,000 individuals) were calculated by gender, by age group (0–2, 3–5, 6–10, 11–13, and 14–18 years) and observation year, using the number of identified cases as the numerator and the total population aged 0–18 in Lombardy as the denominator.

A negative binomial regression model was applied to estimate the monthly number of prevalent cases, stratified by gender and age group. The model included month as a categorical variable, year as a continuous variable, and period as a categorical variable (January 2016–December 2019, January 2020–December 2021, and January 2022–December 2022). The natural logarithm of the population count was included as an offset term.

RESULTS

In 2022, 87 cases involving hospital admissions or Emergency Department visits for psychiatric disorders related to substance and alcohol abuse were recorded. From 2016 to 2022, prevalence showed variable trends: an increase from 2016 to 2018, a decline until 2020, followed by fluctuations in the last two years, without returning to 2016 levels.

Stratification by sex revealed fluctuating prevalence before the pandemic, with females peaking in 2018 and males in 2017 and 2019. Both sexes reached their lowest levels in 2020, with a more pronounced rebound in females during the final two years.

By age group, the highest prevalence was consistently observed among adolescents aged 14–18, peaking in 2018, dipping sharply in 2020, and partially recovering in 2021. Younger age groups (0–2 and 3–5 years) showed stable or gradually decreasing prevalence, while the 6–10 and 11–13 groups maintained very low rates throughout the period.

CONCLUSIONS

The rise in prevalence from 2016 to 2018 may reflect increased adolescent substance use, growing attention to youth mental health, and the impact of social media and cyberbullying [5]. The 2020 decline likely relates to COVID-19, with a rebound in 2021 and slight drop in 2022 due to case re-emergence and service adjustments [6,7]. By sex, females peaked in 2018, possibly due to binge drinking and psychotropic use; males peaked in 2017 and 2019, linked to social media and cyberbullying. Post-COVID sex differences may stem from different coping strategies. Adolescents are the most affected age group, followed by infants (0–2), possibly due to prenatal exposure [8]. Children aged 6–13 show low prevalence, likely due to limited exposure. Further investigations are currently underway to assess the relationship between case distribution and socio-economic factors across Lombardy's provinces.

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Cross-Country External Validation of a Multisource Comorbidity Score

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BACKGROUND

The increasing impact of multimorbidity is escalating clinical and economic demands on healthcare systems, underscoring the necessity for effective tools to assess clinical complexity and enhance management strategies [1-3]. The Multisource Comorbidity Score (MCS) is a population-based index based on regional healthcare Utilization databases - hospitalizations and drug prescriptions - of beneficiaries with age equal or greater than 50 years. The MCS was developed in the framework of the Monitoring and Assessing Care Pathways working group of the Italian Ministry of Health, and validated in four Italian regions, showing good performance in predicting mortality, hospitalizations, and healthcare costs [4].

OBJECTIVE

This study aimed to externally validate and adapt the MCS within the Catalan healthcare system, assessing its predictive capability outside the original Italian setting. This validation seeks to determine whether the MCS can serve as transferable tool in other healthcare systems with different data availability.

METHODS

An observational longitudinal study was performed on subjects aged 50 years or older, residing in the health district of Barcelona-Esquerri (ES) continuously during 2014-2015 and followed between January 1st, 2016, and December 31st, 2019.

Data were obtained from the Catalan Health Surveillance System [5] which integrates demographic, clinical and healthcare utilization information from several healthcare databases. For this study, we used Catalan healthcare system beneficiary's, primary care, hospitalization, and pharmacy dispensation databases.

First, the MCS with Italian weights (MCS-1) was applied in the Catalonia setting using the same data sources (hospitalization and pharmacy dispensation databases) as in the original Italian version. Second, new MCS weights (MCS-2) were estimated in predicting one-year mortality (primary outcome) in the Catalonia setting using the methodology and data sources as described in [4]. Finally, a third MCS version (MCS-3) was developed estimating specific weights based on hospitalization, pharmacy dispensation and primary care databases to predict the primary outcome.

Secondary outcomes considered were four-year mortality, one- and four-year hospitalizations (≥ 1) and one- and four-year hyperfrequency primary care utilization (≥ 10 visits). To assess the performance of the three MCS versions, generalized linear models (GLMs) with a binomial distribution were used for each outcome. ROC curves and Area Under the Curve (AUC) with 95% Confidence Intervals (95% CI) were estimated to assess the discrimination ability of the three MCS versions. De Long's method was used to compare the AUCs [6].

Net Reclassification Improvement (NRI) [7] was also calculated to assess improvements in risk classification by comparing new MCS versions with the MCS-1. The predicted GLM values, for each outcomes, of the three MCS versions were estimated and a threshold of 0.5 was used to distinguish between high and low risk individuals. The NRI estimates the percentage of individuals who were correctly reclassified into

a higher risk category (according to the threshold) if they experienced the outcome, or into a lower risk category if they did not, minus those who were incorrectly reclassified when comparing the two models.

RESULTS

As of January 1st, 2016, a total of 440,790 individuals had resided in the health district of ES for at least two years. Among them, 198,753 (45%) were aged 50 or older and formed the study cohort. They were mostly women (57%), with a median age of 66 years (IQR: 57–76).

Table 1 shows the MCS versions performances according to one- and four-year outcomes. All MCS versions demonstrated good discriminatory performance in primary and secondary outcomes.

For one-year mortality, the MCS-1 achieved an AUC of 0.742 (95% CI: 0.734–0.750) similarly to MCS-2 (AUC=0.756, 95% CI: 0.744–0.768), while MCS-3 version showed a significant improved AUC respect to MCS-1 (AUC=0.771, 95% CI: 0.760–0.783, $p<0.001$). Both new MCS versions performed better in predicting four-year mortality compared to MCS-1 ($p=0.012$; $p<0.001$, respectively).

On the contrary, the MCS-1 showed better performance in predicting all secondary outcomes except one-year hospitalizations with respect to MCS-3.

In addition, significant improvements in risk reclassification for both one-year and four-year mortality were observed with the MCS-2 and MCS-3 compared to the MCS-1. For one-year mortality, the NRI increased by 0.63% (95% CI: 0.14–1.17) in MCS-2 and by 2.17% (95% CI: 1.39–2.97) in MCS-3. Similarly, for four-year mortality, the NRI increased by 1.8% (95% CI: 1.33–2.25) and 2.9% (95% CI: 2.35–3.45), respectively.

An increment in risk reclassification was found in four-year hospitalizations and hyper-frequency when comparing MCS-3 to MCS-1; the reclassification worsened in all secondary outcomes comparing MCS-2 to MCS-1.

Conclusions

The study supports the external validity of the Multisource Comorbidity Score in other healthcare systems with different data availability, such as Catalonia. The local adaptation slightly improved the ability of the score in predicting mortality, however this advantage was not maintained in the secondary outcomes, highlighting the importance of contextual adaptation of such tools.

These findings provide a basis for expanding the use of the score and refining it in different health systems and population segments.

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Table 1. Comparison of discriminant power of MCS scores version in predicting in mortality, hospitalization and primary care hyperfrequency: AUC, NRI and 95% Confidence Intervals.

	All-cause Mortality		All-cause hospitalization		Primary care hyperfrequency [#]	
	1 Years	4 Years	1 Year	4 Years	1 Year	4 Years
AUC (95%CI)						
MCS-1	0.742 (0.734;0.750)	0.732 (0.728;0.736)	0.705 (0.701;0.708)	0.681 (0.678;0.683)	0.749 (0.747;0.751)	0.767 (0.754;0.769)
MCS-2	0.756 (0.744;0.768)	0.742 (0.735;0.748)	0.689 (0.684;0.695)	0.661 (0.658;0.665)	0.717 (0.713;0.721)	0.734 (0.731;0.738)
MCS-3	0.771 (0.760;0.783)	0.757 (0.750;0.763)	0.700 (0.695 ; 0.706)	0.670 (0.667;0.674)	0.729 (0.725;0.733)	0.747 (0.743;0.751)
NRI (95%CI)						
MCS-2 vs MCS-1	0.63 (0.14;1.17)	1.8 (1.33;2.25)	-0.43 (-0.72;-0.15)	-0.47 (-0.89;-0.05)	-1.45 (-1.9;-0.99)	-1.47 (-1.97;-1.00)
MCS-3 vs MCS-1	2.17 (1.39;2.97)	2.9 (2.35;3.45)	0.19 (-0.2;0.55)	1.08 (0.65;1.58)	-1.37 (-1.91;-0.85)	1.18 (0.64;1.69)

AUC: Area Under the Curve; CI: Confidence Intervals; NRI: Net Reclassification Improvement;

[#] ≥10 visits per year; Result to DeLong methods to compare AUC to MCS ($p < 0.05$)

MCS-1: original Italian version; MCS-2: Catalonia version; MCS-3: Catalonia version with enhanced data-sources.

Evaluation of an Educational Intervention Program Designed to Increase Vaccination Attitude in Prison Setting: Results from the RISE-Vac Project

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INTRODUCTION

Prison population is particularly vulnerable to communicable diseases, including vaccine-preventable infections [1], due to various factors such as low social distancing, high turnover rates, and a high proportion of migrants [2]. However, vaccination coverage among people living in prison (PLP) remains low, largely due to limited access to healthcare services, low levels of vaccine literacy and general health literacy [3]. Additional challenges include persistent vaccine hesitancy and widespread distrust in institutions.

OBJECTIVES

As part of the RISE-Vac project—co-funded by the 3rd EU Health Program—we developed and implemented an evidence-based educational program aimed at boosting vaccination rates in both PLP and prison staff (PS) and evaluated the effect of the intervention on vaccine hesitancy, vaccine literacy and vaccine attitude.

METHODS

We conducted a cross-sectional non-randomised study in 24 prisons of 6 European countries. The sample included 782 PLP and 717 PS members. In participant prisons, an educational program about vaccination for preventable infection diseases was implemented; for PLP, the program consisted in the distribution of educational material (leaflet and a short video) and/or an educational event; for PS, it consisted in a 4-hours online course. Within the study sample, 387 from PLP (49%) and 285 from PS (40%) undertook the intervention.

Vaccine hesitancy was measured through a scale previously validated in a sub-cohort of participants – in the follow-

ing referred to as pre-test [4]. A structured questionnaire was administered to measure vaccine literacy, general health literacy and socio-demographic characteristics of participants. Vaccine attitude was determined as the willingness to accept a vaccine if offered. Linear regression model was applied to assess the effect of the intervention and pre-test on vaccine and general health literacy and vaccine hesitancy. Logistic regression was applied to assess the association between the intervention and pre-test on vaccine attitude. All analyses were stratified by group (PLP and PS) and adjusted for socio-demographic variables. Mediation analysis was conducted to quantify the proportion of the effect of the intervention on vaccine attitude mediated by vaccine hesitancy, adjusting for pre-test and socio-demographic variables.

RESULTS

In both PLP and PS, the intervention was associated with higher levels of vaccine literacy and stronger associations were observed among those who undertook the pre-test (PLP: interaction between pre-test and intervention, $p=0.03$; intervention, $p=0.005$ and $p<0.001$ in the non-pre-test and pre-test group respectively; PS: interaction, $p<0.001$; intervention, $p=0.02$ and $p<0.001$ in the non-pre-test and pre-test group respectively).

In PLP, the intervention was inversely associated with vaccine hesitancy only in those who did not take part of the pre-test but not in the others (interaction, $p=0.02$; intervention, $p<0.001$ and $p=0.22$ in the non-pre-test and pre-test group respectively). In PS, no significant interaction was observed between the intervention and pre-test ($p=0.11$); the intervention was significantly associated with lower vaccine hesitancy ($p=0.002$).

In both PLP and PS, the intervention was significantly as-

sociated with a positive vaccine attitude toward vaccination with no significant interaction with pre-test: in PLP, OR=5.21 (95% CI: 2.74 to 9.91); in PS OR=2.52 (95% CI: 1.54 to 4.13).

Finally, mediation analysis showed that less than 30% of the effect of the intervention on vaccination attitude was mediated by a reduction in vaccine hesitancy (24% with $p<0.001$ and 28% with $p=0.002$ in PLP and PS respectively).

CONCLUSION

Evidence-based educational interventions are effective in improving vaccine hesitancy and vaccine literacy among PLP and PS. Also, they enhance the willingness of participants to be vaccinated, through a mechanism that is only partially explained by their effect on vaccine hesitancy. Further research should be conducted to quantify the real impact of this kind of intervention on vaccine uptake in prison population.

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Time Windows Used when Identifying Current Drug Use and Polypharmacy

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BACKGROUND

There is no consensus on the definition of the prevalence of drug use, including polypharmacy, regarding the length of the time window and the number of required concomitant medications.

OBJECTIVES

We aim to explore how the estimated prevalence of drug use in general, and of polypharmacy in particular, is affected by the applied definition.

METHODS

We conducted a drug-utilization study divided into two parts: in the first part, we focused on estimation of current use, corresponding to 'baseline drug use' in a cohort study. Using population-based registries from Denmark, we identified a cohort of individuals aged ≥ 18 years during 2020-2022, assigned them a random index date and considered 'current use' of the following drugs: statins, glucose-lowering drugs (GLDs), selective serotonin reuptake inhibitors (SSRIs), opioids, and non-steroidal anti-inflammatory drugs (NSAIDs). The second part of the study focused on polypharmacy, defined according to five different definitions, with estimations of its prevalence using population-based registries from Denmark. We identified a cohort of individuals older than 65 years in 2022, and we considered all drugs available in the registries except for anti-infectives for systemic use. We also evaluated the accuracy of different criteria for predicting polypharmacy using simulations.

RESULTS

Evaluating baseline drug use, we observed that the proportion of individuals classified as exposed increased with use of time-windows up to the first 90 days before the index date, reaching a plateau using windows around 120-150 days for statins, GLDs, and SSRIs, and around 180-300 days for opioids, whereas it was not reached for NSAIDs within 360 days. The prevalence of polypharmacy ranged from 21.1% (10 different 4th level Anatomical Therapeutic Chemical (ATC) groups in one year) to 92.3% (two different 4th level ATC groups in one year) depending on the applied definition, varying with the number of different ATC groups and time periods. In the simulation, the best criterion for identifying polypharmacy required at least two dispensations for each of at least five drugs, with sensitivity ranging between 0.93 and 1.00, and specificity between 0.72 and 1.00.

CONCLUSIONS

Time windows up to 90 days are too short to identify baseline drug use in the Danish setting. How polypharmacy is defined significantly influences its estimate, suggesting a need to use multiple definitions in each study.

Data Harmonization of Psychosocial Questionnaires across Population Cohorts: A Differential Item Functioning Analysis

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INTRODUCTION

Data harmonization is the process of achieving comparability of similar measures collected by separate cohorts by aligning their definitions and measurement formats [1]. In epidemiologic research, and in particular in predictive modelling for chronic non-communicable diseases, it may facilitate the identification of derivation and external validation cohorts [2]. This process may be particularly challenging when psychosocial variables are amongst the predictors of interest, as different self-report questionnaires measuring the same psychosocial variable might capture different aspects, and even small differences in item wording affect responses [3]. Therefore, measurement invariance; i.e., whether respondents from different populations (e.g.; at different time periods, or different cultures) with the same latent trait (e.g. depression) level respond similarly, should be preliminarily established to avoid bias in subsequent analyses [4]. Currently, there is little guidance on how to assess this property for data harmonization purposes. A promising framework is Item Response Theory (IRT), a probabilistic approach for modelling the relationship between a latent trait and observed item responses [5].

We designed an international project pooling Italian and German cohorts with recruitment time spanning over a 10-year period, with the aim to identify a restricted set of psychosocial items able to increase the predictive power of established risk prediction models for Cardiovascular Diseases (CVD). Here, we leverage IRT to assess Differential Item Functioning (DIF) in harmonised items, thereby testing whether item characteristics are invariant across the Italian cohorts.

AIMS

To harmonise two questionnaires used in different cohorts to assess depressive symptoms and to evaluate the measurement invariance of the harmonised items across cohorts.

METHODS

The MONICA Brianza study includes three independent cohorts recruited from the Brianza population, Lombardy region, over a 10-year period (MONICA87: 1986–1987; MONICA90: 1989–1990; MONICA93: 1993–1994). Each cohort includes a 10-year age- and gender-stratified random sample of the target 25- to 64-years old population. Overall, 4932 individuals participated to the study (69% of invited). Depressive symptoms were measured in the MONICA87 cohort using the Beck Depression Inventory (BDI) [6], a 22-item instrument with a 0 (absence of symptom) to 4 (severe symptom) response format, and in the MONICA90-93 cohorts using a 14-item version of the Maastricht Vital Exhaustion Questionnaire (EX) [7], coded on a scale of 0 ("No") – 1 ("Not sure") – 2 ("Yes").

PROTOCOL STEPS FOR DATA HARMONIZATION AND ANALYSIS

We developed an a priori protocol for data harmonization based on the following steps. First, the content of the BDI and EX items were analysed to select item pairs measuring the same depressive symptoms. Second, for each item pair, the response format was dichotomised ensuring that the collapsed response categories have the most similar possible frequencies of endorsement across cohorts. Third, the resulting scale was analysed through a unidimensional Confirmatory Factor Analysis and, in case of departures from unidimensionality, a minimum residual exploratory factor analysis (EFA) to identify subscales. Fourth, a 2-parameter logistic IRT model was fitted on the resulting subscales to estimate for each item a discrimination parameter (ability to differentiate between individuals with close levels of the latent trait), and a difficulty parameter (level of the latent trait at which the item has a 50% probability of endorsement). Item fit was evaluated through S-X2 statistics [8]. Finally, DIF analyses were conducted to evaluate whether

item parameters were invariant across cohorts through Likelihood ratio tests comparing nested models with increasing equality constraints on discrimination and difficulty parameters.

RESULTS

525 individuals in the MONICA87 and 48 individuals in the MONICA90-93 cohorts were excluded due to unavailability of the questionnaires at baseline date. The final sample (51% female, mean age $\pm$ SD: 45.9 $\pm$ 11.3) included 1134 subjects from MONICA87 and 3225 from MONICA90-93.

The item content analysis led to the selection of 10 item pairs, which were used to form a harmonised scale of depressive symptoms (Dep). One item pair was discarded due to overlap with another item pair, both assessing sleep disturbance (tetrachoric correlation: 0.78). Standard fit indices from the CFA suggested departures from unidimensionality. EFA identified two latent variables, one underpinning a "Neurovegetative and arousal disturbance" subscale (4 items; e.g., sleep problems; loss of energy), and one an "Affective and cognitive disturbance" subscale (5 items; e.g., suicidal thoughts). Both subscales met the assumptions for the IRT model and all items showed adequate model fit. DIF analysis (Figure 1) confirmed measurement invariance for six items (e.g.; Dep03, panel A). Items Dep02 (panel B) and Dep05 (panel C) exhibited different discrimination and difficulty parameters across cohorts, suggesting non-uniform DIF. Finally, the different difficulty parameters for Dep10 (panel D), indicating uniform DIF, suggests that people from different cohorts with the same latent trait have different probability to endorse the item.

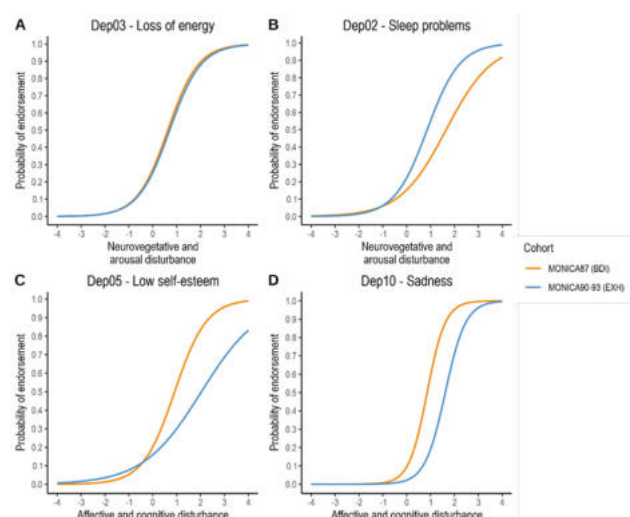


Figure 1. Item Characteristic Curves (ICC) representing the probability to endorse items across the relevant latent trait (mean=0, SD=1) across cohorts. Panel A: Example of the ICC of an item without DIF. Panel B, C, D: ICC of items showing DIF

CONCLUSIONS

The harmonization of two depression questionnaires identified 6 items with measurement invariance and revealed DIF in items that could have biased subsequent analyses if undetected. In epidemiological research, measurement invariance of psychosocial questionnaires should be thoroughly checked using comprehensive methods. Analysis of DIF within an IRT framework offers a promising approach, and can be further evaluated for cross-cultural evaluations.

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Disability-Adjusted Life Years due to Asbestos-Related Diseases in Italy, 2010–2020

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INTRODUCTION

Estimates of Disability-Adjusted Life Years (DALYs) are a key tool for quantifying the global burden of diseases and risk factors, and are essential for health policy planning [1]. Asbestos is a certain carcinogen, known to cause mesothelioma (M), as well as cancers of the lung, ovary, and larynx, in addition to asbestosis and pleural thickening. In 2019, globally, 4,189,000 DALYs were attributed to diseases resulting from occupational exposure to asbestos. In Italy, 16,993 deaths due to M were recorded between 2010 and 2020, while the National Mesothelioma Registry (ReNaM) reported over 35,000 incident cases between 1993 and 2021.

OBJECTIVES

To estimate the DALYs attributable to asbestosis and mesothelioma—diseases with a high etiologic fraction linked to asbestos exposure (80% and 100%, respectively)—in Italy over the decade 2010–2020.

METHODS

The analysis considered deaths from M and asbestosis, hospital admissions for asbestosis, and incident cases of M. DALYs were calculated as the sum of Potential Years of Life Lost due to premature death (PYLLs) and Years Lived with Disability (YLDs). Data on deaths from M (ICD-10: C45) and asbestosis (ICD-10: J61), as well as hospitalizations for asbestosis (ICD-9-CM 501), were obtained from databases curated by the Istituto Superiore di Sanità (ISS), using sources from ISTAT and the Ministry of Health. Incident M cases were estimated from ReNaM data. All information was stratified by sex, age group, and calendar year. PYLLs were calculated

both in absolute terms and as age-standardized rates (per 100,000 population) at national and regional levels, using ISTAT life expectancy data disaggregated by sex, age, and year. YLDs were estimated at the national level, based on hospitalizations for asbestosis and incident M cases, using a disability weight of 0.217 and a duration of 20–30 years for asbestosis, and a weight of 0.540 with a one-year duration for M [2, 3].

RESULTS

Between 2010 and 2020, an estimated 204,232 DALYs were attributable to asbestos-related diseases among men and 72,625 among women in Italy, assuming a 30-year duration for asbestosis. PYLLs accounted for 161,300 in males (96.7% due to M) and 67,311 in females (99.1% due to M). Northern regions—particularly Liguria, Piedmont, and Friuli-Venezia Giulia—showed the highest PYLL rates from M in both sexes. YLDs amounted to 7,075 for M and 35,857 for asbestosis in men, and 2,710 for M and 2,604 for asbestosis in women.

CONCLUSIONS

This is the first estimate of the total burden of mesothelioma and asbestosis in Italy expressed in terms of DALYs. These data, which include years lived with reduced functioning, provide significant support for healthcare planning and the implementation of appropriate welfare measures.

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Adherence to Diabetes Care Pathways and Risk of Diabetes-Related Complications by Citizenship: A Population-Based Study from The MIGHTY Project (Cup P2022ASXKR)

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INTRODUCTION

National and international guidelines on diabetes recommend continuous monitoring of this condition to prevent short- and long-term complications [1–3]. Adherence to the Diagnostic Therapeutic Care Pathway (DTCP) is evaluated by the Italian Ministry of Health using annual indicators based on five key recommendations [4], to ensure uniformity of care across all regions. However, equitable access to these services remains a public health challenge, particularly for migrant populations, who may face cultural, linguistic, or systemic barriers.

OBJECTIVE

Within the MIGRants' Health and healthcare access in Italy (MIGHTY) project, this population-based study aimed to compare adherence to DTCP in subjects with new diagnosis of diabetes between migrant and Italian populations, and to evaluate the association between citizenship and diabetes complications in the Marche Region, between 2013–2023.

METHODS

A population-based cohort study was conducted using Healthcare Utilization Databases from the Marche Region, including Regional Beneficiary, Hospital Discharge, Drug Prescription, Outpatient Care, and Exemption databases.

The cohort of new cases of diabetes included adults (≥ 18 years) with a first diabetes-related event between 2013 and 2017, defined as: ≥ 2 prescriptions of glucose-lowering drugs (ATC: A10) within one year, ≥ 1 hospitalization with diabetes as primary/secondary diagnosis (ICD-9-CM: 250.), or ≥ 1 diabetes exemption (code: 013); the date of the first event was defined as the index date. Exclusion criteria were being resident in Marche Region for less than two years prior to index date, any diabetes-related events in the two preceding years, and childbirth-related hospitalizations (Major Diagnostic Category 14) during the year of inclusion in the cohort or the two prior years.

Subjects were classified as Italian or migrants from High Migratory Pressure Countries (HMPC) based on citizenship [5].

The five recommendations monitored by the Ministry of Health [4] were assessed: ≥ 2 HbA1c tests (PDTA-05.1), ≥ 1 lipid profile (PDTA-05.2), ≥ 1 microalbuminuria test (PDTA-05.3), ≥ 1 renal function test (PDTA-05.4), and ≥ 1 eye exam (PDTA-05.5) each per year. The adherence to each recommendation and the overall adherence, i.e. meeting at least 4 out of 5 recommendations (PDTA-05), was annually evaluated over six years from the index date for each subject.

Mixed-effects logistic models for repeated measures were used to evaluate adherence to recommendations considering as independent variables citizenship (Italian vs. HMPC), year of evaluation, sex, age groups (65–74 vs. 18–44, 45–54, 55–64, 75+ years), and Multisource Comorbidity Score (MCS) [6] classes (≤ 4 vs. 5–9, 10–14, 15–19, ≥ 20).

Diabetes-related complications were defined as a hospi-

talization with a primary diagnosis of short- or long-term diabetes complications, or uncontrolled diabetes, or non-traumatic lower limb amputation [7]. Subjects with at least one year of follow-up, were followed up from index date to a diabetes-related complication, all-cause death, emigration out of the region, or December 31, 2023, whichever came first. Cox proportional hazards model was used to estimate the association between citizenship and risk of complications, adjusting for age groups, sex, MCS classes, and including annual adherence to each DTCP's recommendation as time-dependent covariates. Results are reported with 95% Confidence Interval (95% CI). All data were processed in compliance with the European (GDPR, EU 2016/679) and national privacy laws (D.lgs. 196/2003 and subsequent amendments).

RESULTS

The study cohort comprised 28,674 adults with newly diagnosed diabetes, of whom 1,529 (5.3%) were migrants from HMPC. At index date, migrants from HMPC were younger (mean age 50 vs. 66 years), more frequently female (59% vs. 48%), and had lower comorbidity scores (MCS median: 2 vs. 6) compared to Italians.

Migrants from HMPC compared to Italians showed lower observed adherence to all DTCP recommendations: HbA1c (26.1%, 95%CI 25.1-27.1 vs. 36.0%, 95%CI 35.7-36.2), lipids (43.4%, 95%CI 42.2-44.5 vs. 61.3%, 95%CI 61.1-61.6), microalbuminuria (27.0%, 95%CI 25.9-28.0 vs. 31.3%, 95%CI 31.0-31.5), renal function (46.8%, 95%CI 45.7-47.9 vs. 66.2%, 95%CI 66.0-66.5), eye exam (6.7%, 95%CI 6.1-7.3 vs. 8.4%, 95%CI 8.3-8.6), and overall adherence (15.6%, 95%CI 14.7-16.4 vs. 20.3%, 95%CI 20.1-20.6). Mixed-effect models confirmed lower adherence among migrants, except for eye exams (Figure 1).

Of 24,992 with ≥ 1 year follow-up, 3,229 experienced complications (3,161 Italians, 68 migrants). Six-year complication-free survival was 85.7% (Italians) vs. 94.1% (migrants). In the adjusted Cox regression model, citizenship was not significantly associated with the risk of developing diabetes complications (HR=0.98; 95% CI: 0.77–1.26; $p=0.896$).

CONCLUSIONS

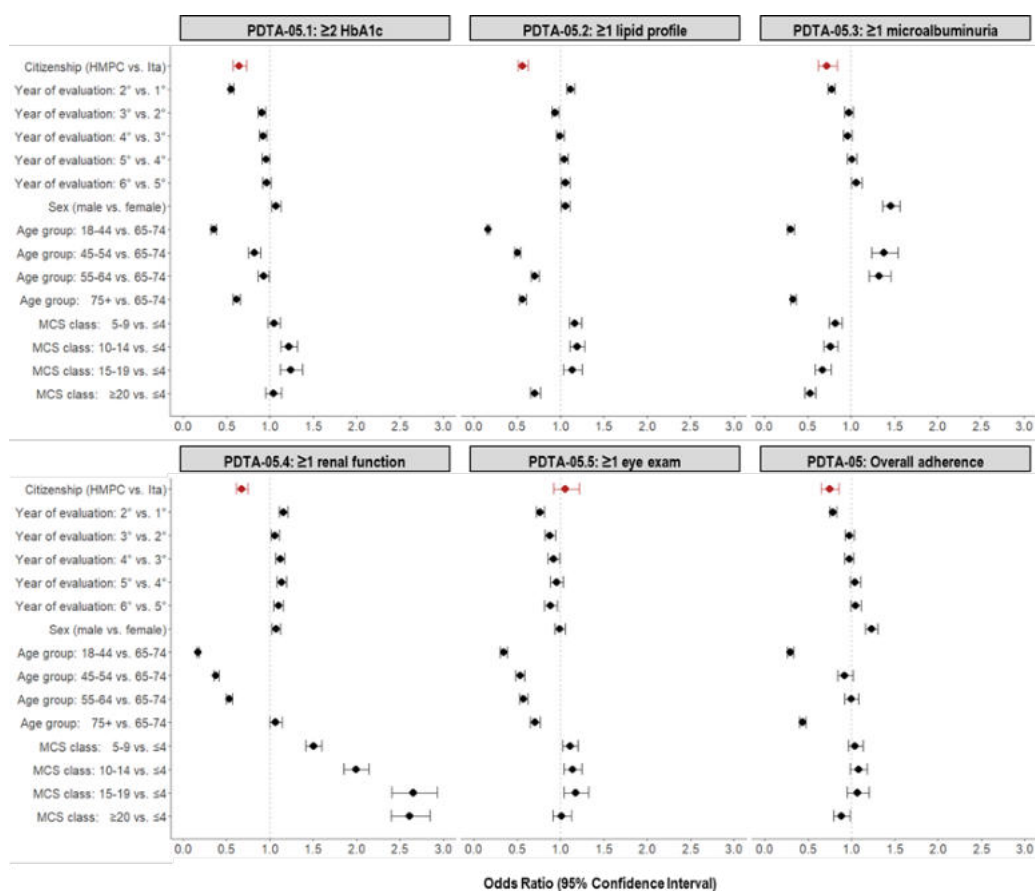
This study highlights differences in adherence to recommended diabetes care pathways between Italians and migrant populations. However, these differences did not translate into a higher risk of developing diabetes-related complications. Longer period of observation might be required to evaluate the impact of citizenship on adverse diabetes-related outcomes. Sustained monitoring and culturally tailored interventions remain essential to ensure equitable access and prevent future health inequalities.

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Figure 1. Probability of adherence to the DTCP's recommendations according to citizenship (red dots), adjusted for year of evaluation, sex, age group, and Multisource Comorbidity Score. Results from the mixed-effects logistic models for repeated measures



Evaluation of Vaccination Status of Patients Diagnosis of Multiple Myeloma or Monoclonal Gammopathy of Uncertain Significance: Analysis of Coverage in the Province of Catania

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INTRODUCTION

The patient diagnosed with multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS) is considered a fragile patient, at risk of severe post-infectious outcomes [1] for which adequate vaccination prevention against pneumococcus and meningococcus, Herpes Zoster virus, influenza virus, SARS-CoV2 is recommended [2].

Despite existing recommendations, there is limited evidence regarding actual vaccination coverage of these patients [3].

OBJECTIVES

This study aims to evaluate the vaccination status in a cohort of haemato-oncology patients and to propose strategies to improve their vaccination coverage.

METHODS

We conducted a retrospective cohort study on 737 patients with multiple myeloma or MGUS diagnosed in the province of Catania in the period 2000-2023, coded by the Integrated Cancer Registry. Vaccination status was retrieved by cross-referencing cases with data in the Provincial Vaccination Registry. Medical records and vaccination data collected during outpatient visits, hospital admissions for possible hospitalization for infectious disease were examined and the

presence of infectious disease diagnoses among the causes of death was verified. The data will be presented in aggregate form. The variables considered were: tumor diagnosis, last follow-up date, date of death, date and components of vaccinations performed.

Relative risk with 95% confidence intervals on deaths with respect to the vaccination stratum analyzed by type of disease was calculated. Analyses were performed with Stata 17. For all statistical tests, the significance level was set at a p value <0.05.

RESULTS

Preliminary results indicate poor adherence to recommended vaccinations. Frequency distribution of variables of vaccination status, post-diagnosis vaccination, cycle completeness and timing of vaccinations were calculated.

The observed coverage is 30.9% for influenza, 6% for Varicella Zoster, 39% for SARS-CoV2, 1.5% for meningococcus and 10% for pneumococcus of vaccineable subjects.

The observed all-cause mortality rate among never-vaccinated subjects is 45.4%; among subjects with at least one dose of vaccine, it is 18.4%. Delays in meeting recommended intervals and incomplete cycles have been observed.

Relative risk (RR) analysis shows that vaccination is significantly associated with a reduction in mortality in the overall cohort (including patients with MGUS and multiple myeloma). In particular, subjects who have received at least one of the recommended vaccinations have a reduction in the risk

of death of approximately 60% compared to unvaccinated subjects.

In detail:

- In patients with MGUS, vaccination is associated with an approximately 74% reduction in the risk of death.

- In patients with multiple myeloma, the risk reduction is around 42.7%.

Focusing the analysis on individual vaccines:

- Vaccination against COVID-19 is associated with an approximately 63% reduction in the risk of death.

- The one against the flu shows a reduction of 59.2%.

For pneumococcal, meningococcal, and Herpes Zoster vaccines, no statistically significant association with mortality was observed, either in the overall cohort or in subgroups. However, potentially relevant trends emerge:

- In patients with MGUS, meningococcal vaccine showed a RR of 1.65 (95% CI: 0.91–3.02; $p = 0.10$), while herpes zoster vaccine showed a RR of 0.65 (95% CI: 0.35–1.21; $p = 0.18$). The wide confidence intervals suggest a possible lack of statistical power to detect significant effects.

CONCLUSIONS

Protection provided by vaccination is present in both subgroups, but is more marked in MGUS patients ($RR \approx 0.26$) than in those with myeloma ($RR \approx 0.57$). The study provides an overview of the vaccination status in haemato-oncology patients, highlighting the need for personalized strategies: vaccination recommendations in the discharge letter, promotion of training of hospital staff and the attending physician. The organization of vaccination sessions in the hospital outpatient setting has been activated in conjunction with periodic or follow-up checks.

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Determinants of Carbon and Water Footprints of Food Consumption in an Italian Population

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INTRODUCTION

The environmental impacts of food consumption, specifically carbon footprint (CF) and water footprint (WF), are gaining increasing importance in the context of global sustainability and public health. As global food demand is expected to increase by 35% to 56% between 2010 and 2050 [1] substantial shifts in dietary habits are required to sustain a growing global population while aligning with the One Health approach [2], and the 2030 agenda for the accomplishment of Sustainable Development Goals (SDGs) established by the Food and Agriculture Organization (FAO) [3]. However, dietary choices are influenced by a complex interplay of factors, including socioeconomic status, cultural traditions, and individual preferences [4]. This study evaluates the contribution of different food groups to environmental footprints and examines how health determinants such as gender, age, BMI, geographic location, and adherence to the Mediterranean diet (MD) influence these impacts. The goal is to better understand how dietary habits affect environmental sustainability and to provide data-driven recommendations for policy development.

OBJECTIVES

To estimate the average daily CF and WF of the study sample, leveraging estimates from the SU-EATABLE LIFE (SEL) Database [5]. Furthermore, the study will identify the food groups with the highest and lowest environmental impact and explore how dietary impact varies based on gender, age, Body Mass Index (BMI), education level, geographic region, and adherence to the MD measured through the Mediterranean Adequacy Index (MAI).

METHODS

This cross-sectional study utilised data from 2,831 participants in the third INRAN-SCAI Survey, conducted across various regions of Italy in 2005-2006. We calculated the environmental footprints by multiplying the daily consumption of each food item ($n = 878$), classified using the European Food Safety Authority (EFSA) FoodEx2 system, by the footprint coefficients from the SEL database. For items without direct matches (e.g., canned beer), we assigned median values from related food commodity subcategories as proxy conversion factors. We excluded approximately 35% of items lacking reliable conversion data, leaving 617 food items with assigned footprint values. In addition to dietary data, we considered sociodemographic and anthropometric variables including age, sex, BMI, education level, and geographical area of residence. We evaluated adherence to the MD using the MAI score [6,7]. We excluded foods incompatible with the MAI framework (e.g., alcoholic beverages other than wine, mixed processed dishes, coffee), representing less than 7% of total energy intake in the population. We performed the comparisons of CF and WF across sociodemographic variables using two-sided t-tests and one-way analysis of variance (ANOVA) to examine differences by sex, BMI, geographical area of residence, and education level. We used multiple linear regression models to investigate associations between environmental impact indicators and individual characteristics, with CF and WF as the dependent variables in separate models. Independent variables included age, sex, BMI, educational level, geographical area, total energy intake, MAI score, and energy density of food consumed (kcal/kg).

RESULTS

The average CF was 3.53 kg CO₂eq/day, and the average WF was 3,330.96 L/day. The mean daily CO₂ emissions per kcal consumed were 1.88 g CO₂eq (SD = 0.05), while the average WF per kcal was 177.12 L (SD = 34.87). Red meats (1.08 kg CO₂eq; 657.24 L) and dairy products (0.28 kg CO₂eq; 708.62 L), were the largest contributors to CF and WF. Overall, meat accounted for 68.7% of the total CF and 27.5% of the total WF, and dairy products, contributing 20.0% to CF and 21.3% to WF. Moreover, subjects with the highest adherence to MD (above the population mean) showed reduced CF (9.84 vs. 11.01 kg CO₂eq) and WF (9,356.0 vs. 10,348.3 L) compared to lower adherence. Males had higher environmental impacts (3.92 kg CO₂eq; 3,691 L) than females (3.21 kg CO₂eq; 3,037 L) ($p < 0.001$), as well as younger individuals (3.76 kg CO₂eq; 3,491 L) versus older adults (3.12 kg CO₂eq; 2,948 L) ($p < 0.001$). In multiple linear analysis, beyond sex, age, education and geographic area of residence, subjects with higher adherence to the MD were linked to a lower environmental impact both in term of CF ($\beta = -0.239$) and WF ($\beta = -206.4$), independently on kcal intake.

CONCLUSIONS

Animal-based foods, particularly red meat and dairy products are the primary drivers of the environmental impact of diets in Italy. Health determinants, including gender, age, and adherence to the MD, significantly influence individuals' environmental footprints. Specifically, males and younger individuals exhibit higher environmental impacts, whereas stronger adherence to the MD is associated with lower environmental burdens. Transitioning towards more plant-based, sustainable dietary patterns is crucial not only for reducing the environmental burden of diets but also for addressing pressing global challenges such as climate change and water scarcity. These findings further highlight the complexity of environmental impacts linked to dietary behaviours and emphasises the importance of considering multiple factors, including further socio-demographic and lifestyle variable. Dietary shifts, when combined with targeted policy intervention aimed at specific populations and strategic modifications in food systems, have the potential to promote more sustainable diets and address the urgent environmental challenges facing our planet.

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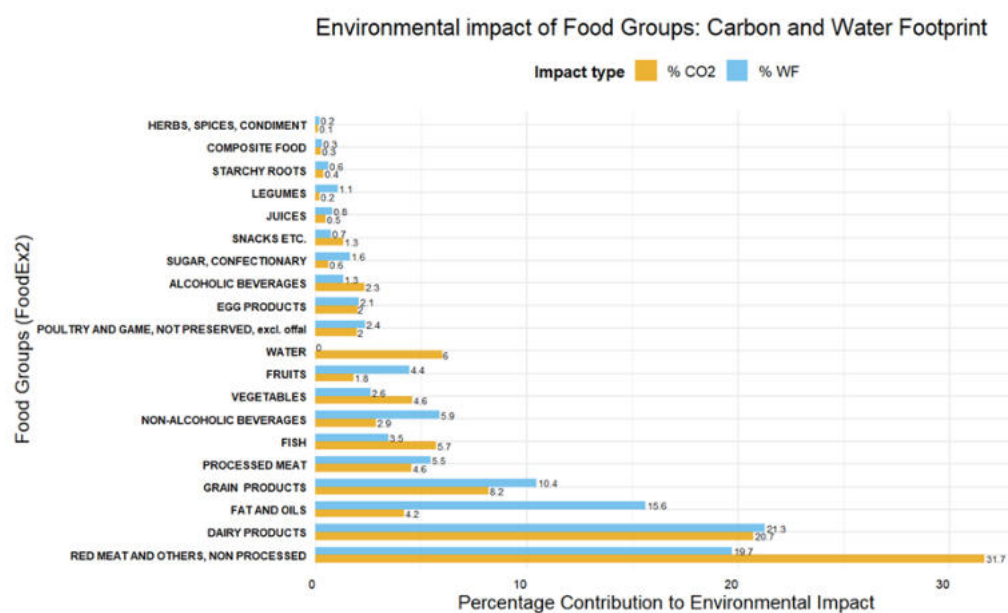


Figure 1. Contribution of food groups according to a modified FoodEx2 classification

Clinical Impact of Molecular Tumor Boards on Patient Care: A Systematic Review and Meta-Analysis

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INTRODUCTION

Precision oncology has transformed cancer treatment by enabling personalized strategies based on tumor molecular profiling. Molecular Tumor Boards (MTBs), which are multi-disciplinary teams of specialists, interpret genomic alterations and provide evidence-based treatment recommendations. These teams play a pivotal role in translating complex molecular data into clinical practice. Despite their growing adoption, the medical community continues to debate the clinical effectiveness of MTBs, as outcomes vary considerably across study designs, tumor types, and healthcare settings

OBJECTIVES

This systematic review aimed to evaluate the clinical impact of therapies recommended by MTBs in oncology practice.

METHODS

We conducted this systematic review and meta-analysis in accordance with PRISMA 2020 guidelines. We searched PubMed, Embase, Scopus, and CENTRAL comprehensively up to March 2025. We included studies that reported clinical outcomes for cancer patients evaluated by an MTB, including overall survival (OS), progression-free survival (PFS), the proportion of patients achieving a progression-free survival ratio (PFSr) ≥ 1.3 , objective response rate (ORR), and disease control rate (DCR). We included all study designs: randomized controlled trials (RCTs), non-randomized clinical trials, prospective and retrospective observational studies.

We assessed the risk of bias using the RoB 2.0 tool for RCTs and the ROBINS-I tool for non-randomized studies. We

stratified all analysis by study design. We calculated pooled estimates and 95% confidence intervals (CIs) using a random-effects model with inverse-variance weighting method. We estimated between-study variance with the REML method and quantified heterogeneity using the I^2 statistic. To test the robustness of our findings, we performed sensitivity analysis that included leave-one-out analysis and excluded studies with serious or critical risk of bias. We assessed publication bias through funnel plots, Egger's test, and excess significance tests. We conducted meta-regression to explore sources of heterogeneity, reporting R^2 values to indicate the proportion of variance explained.

RESULTS

After screening 6,846 records, we included 78 studies in our analysis. We classified them as RCTs ($n = 7$, 9.0%), non-randomized clinical trials ($n = 16$, 20.5%), prospective observational studies ($n = 20$, 25.6%), and retrospective observational studies ($n = 35$, 44.9%).

For OS, we observed a reduction in the risk of death ranging from 12% to 43%, depending on study design. Specifically, the meta-analysis of four RCTs showed a pooled hazard ratio (HR) of 0.88 (95% CI: 0.75–1.04; $I^2 = 0.0\%$). For PFS, we found a 27% to 37% reduction in the risk of disease progression, with a significant pooled HR of 0.73 (95% CI: 0.64–0.84; $I^2 = 0.0\%$) in the meta-analysis of four RCTs. Regarding the proportion of patients achieving a PFSr ≥ 1.3 , pooled estimates ranged from 33.1% to 43%, depending on study design. The only RCT included reported a PFSr ≥ 1.3 in 36.8% of patients (95% CI: 24.6–48.6).

In the meta-analysis of relative risk (RR) for ORR, patients treated according to MTB recommendations showed a significantly higher likelihood of achieving an objective response,

with RRs ranging from 1.72 to 3.32 across study designs. The meta-analysis of five RCTs produced a significant pooled RR of 1.72 (95% CI: 1.23–2.42; $I^2 = 0.0\%$). For DCR, pooled RR estimates ranged from 1.20 to 1.65 depending on study design. The meta-analysis of three RCTs showed a significant pooled RR of 1.20 (95% CI: 1.03–1.40; $I^2 = 19.9\%$).

Meta-regression analysis that considered study design and cancer type focus of the MTB (single cancer vs. multiple cancers) explained a substantial portion of the between-study variance, particularly for OS ($R^2 = 58.2\%$) and ORR ($R^2 = 60.8\%$). Funnel plots, Egger's test, and tests for excess significance did not indicate any publication bias or selective reporting. Excluding studies with serious or critical risk of bias did not substantially change the direction or magnitude of the pooled estimates, except for OS in non-randomized clinical trial and retrospective observational studies.

Among RCTs, the overall risk of bias was low, with only one rated as having some concerns. In contrast, the risk of bias was predominantly serious for non-randomized clinical trials ($n = 9$, 56.3%), prospective observational studies ($n = 18$, 90.0%), and retrospective observational studies ($n = 27$, 77.1%). Additionally, four retrospective studies (11.4%) were rated as having a critical risk of bias, while the remaining 13 studies were considered to have a moderate risk.

CONCLUSIONS

Therapies guided by MTBs are associated with improved clinical outcomes in cancer patients, including reductions in disease progression and increases in response and disease control rates. While RCTs provide the most robust and reliable evidence, findings from observational studies, despite their inherent risk of bias, largely corroborate these benefits. The clinical effectiveness of MTBs is influenced by factors such as timely access to targeted treatments, the accuracy and comprehensiveness of molecular profiling, and the expertise and resources available within healthcare institutions. To fully establish the role of MTBs in precision oncology, there is a critical need for additional well-designed, large-scale RCTs that also address cost-effectiveness, implementation feasibility, and strategies for sustainable integration into diverse clinical settings. These efforts will be essential to optimize MTB-driven personalized therapies into routine oncology practice and maximize patient benefit across cancer types.

Sleep Disorders, Smartphone Use and Mental Health: A Cross-Sectional Study on a Sample of Students from the University of Palermo – MORPHEO

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INTRODUCTION

Sleep disorders constitute a significant public health concern recognized by the World Health Organization (WHO) in the ICD (International Classification of Diseases), with notable implications for young populations. Research demonstrates that disrupted sleep patterns significantly impair mental recovery processes and emotional stability [1]. Poor sleep quality contributes to mental health deterioration through disruption of emotional regulation and neurobiological mechanisms. Inadequate sleep compromises hypothalamic-pituitary-adrenal axis function, increasing cortisol production and stress perception, potentially leading to depressive symptoms [2]. Young adults represent the population stratum with the highest smartphone and electronic device usage rates, sometimes developing behavioural dependencies. Studies show that light exposure to these devices before falling asleep significantly disrupts sleep quality [3]. Moreover, excessive smartphone use is associated with reduced cognitive performance, negatively affecting work efficiency and academic achievement [4].

This study investigates the interactions between sleep disorders, mental health, electronic device usage, and academic performance among university students. We specifically examine how sleep quality and quantity influence students' psychological functioning, with particular attention to psychological distress.

METHODS

The Pittsburgh Sleep Quality Index (PSQI) [5], the Kessler Psychological Distress Scale (K10) [6] and the Smartphone Application-Based Addiction Scale (SABAS) [7] were used

to assess sleep quality, mental distress and problematic smartphone use, respectively. Descriptive statistics were expressed as Mean (SD), for continuous variables, and as count/percentages for categorical variables. "Good sleepers" and "Poor sleepers" were compared using Chi-square test or Fisher's exact test for categorical variables, and Student's t-test or the Wilcoxon-Mann-Whitney test for continuous variables, with significance at $P < 0.05$. Logistic regression identified independent predictors of poor sleep quality (PSQI > 5). Variables with significant univariate association ($p < 0.05$) were included in the multivariate model, with results expressed as odds ratios (OR) with 95% confidence intervals (95% CI).

RESULTS

This cross-sectional study involved 208 students from the University of Palermo, with 58.7% ($n=122$) enrolled in medical degree programs. The average age of the sample was 22 ± 1.99 years, and 71.6% were female.

The analysis revealed that 61.54% ($n=128$) of students had inadequate sleep quality. Univariate analysis showed that their exam completion rate (80.1%) was lower than that one reported for good sleepers (83.5%) ($p < 0.05$). On average, daily smartphone use was higher among poor sleepers (6.46 ± 3.03 vs 5.57 ± 2.22 hour/day, $p < 0.05$), and a significant association was found between poor sleep quality and the risk of problematic smartphone use (OR=2.83, 95%CI [1.27-7.00], $p < 0.05$). Furthermore, results from K10 revealed that reporting severe psychological distress was significantly associated to poor sleep quality (OR=13.25, 95%CI= [5.34-37.28], $p < 0.001$).

The multivariate analysis confirmed that higher daily smartphone usage, measured in hours, is associated with poor

sleep quality (AdjOR=1.21; 95% CI [1.02-1.45]) and, notably, subjects with high probability of severe psychological distress have significantly higher likelihood of being classified as poor sleepers (AdjOR = 9.59, 95% CI = [3.57-28.82]).

DISCUSSION

Our analysis revealed a strong association between psychological distress (K10 scale) and poor sleep quality among university students. Students experiencing significant psychological distress showed markedly higher likelihood of being poor sleepers, confirming bidirectional relationships between mental health and sleep, as documented in previous research. Daily smartphone uses also emerged as a significant predictor of poor sleep quality, aligning with literature on electronic devices' detrimental effects on sleep hygiene. Smartphone light emissions, particularly blue light, suppress melatonin production and disrupt circadian rhythms [8]. These findings emphasize the importance of addressing sleep health within university mental health and academic support initiatives. The strong psychological distress-sleep quality association suggests interventions targeting either aspect may benefit the other. Universities should consider implementing screening programs to identify students at risk of sleep disorders, especially those reporting psychological distress symptoms. Additionally, digital hygiene education should be incorporated into student wellness programs to mitigate electronic devices' negative impact on sleep.

CONCLUSIONS

The study highlights the link between psychological distress, smartphone use, and sleep quality in university students. The strong connection between mental health struggles and sleep issues underscores the need to integrate sleep health into mental health services. Universities should promote well-being and responsible technology use to enhance academic performance and overall student health.

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Evaluating the Use of Cluster Analysis to Detect and Correct Selection Bias in Single-Center Observational Research on Voluntary Terminations of Pregnancy

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INTRODUCTION

Voluntary termination of pregnancy (VTP) has always been debated within the general population. The circumstances leading women to choose VTP are multifaceted, and it remains challenging to identify patterns leading women to opt for this procedure [1, 2]. Up to now, numerous studies have focused on characterizing demographic or psychological profiles of women within specific geographic regions [3, 4]. Due to the sensitive nature of the decision and privacy requirements, studying population clusters in VTP presents inherent methodological limitations. While these challenges can be partially mitigated, they risk a potential selection bias during cohort formation. In these cases, a monocentric study may introduce biases that could arise primarily from the selection of the study centre and secondarily from the difficulty of properly recruiting a sample representative of the entire population.

In this context, cluster analysis conducted first on the general population and subsequently applied to the selected sample could clarify the nature of the selection process that occurred, thereby enabling better estimation of the final conclusions [1, 2]. In general, clustering demonstrated efficiency and feasibility to handle large datasets [5, 6]. In particular, the use of hierarchical clustering procedure is able to choose the optimal number of clusters in the population with a readily available stopping rules, without a priori selection of number of clusters [7]. This approach aligns with contemporary efforts to leverage data-driven methodologies in reproductive health research while addressing ethical considerations inherent to studies of private medical decisions.

OBJECTIVE

The aim is to evaluate the effectiveness of a clustering method in detecting selection bias within a sample from a monocentric observational study, to generalize the findings from the small sample to the broader population and draw valid inferences from the results.

METHODS

We used a retrospective observational database of 6559 women that carried out VTP in Apulia, from January 2023 to January 2024. Data was collected according to the regulations established by the National Surveillance System and were taken from the Health Information System of the Apulia region. The second database was collected, as a cross-sectional, observational study, conducted on 122 women > 18 years who underwent VTP in the Family Planning Unit of the "Di Venere-Fallacara" hospital, between November 2023 and January 2024. The assumption made were that the women of the selected sample are drawn from the same population.

Listwise deletion was applied, to handle missing value. A Multiple Correspondence Analysis (MCA) was first conducted on the population sample to identify the structure of relationships among variables, reducing dimensionality to two principal dimensions. The socio-demographic characteristics and the characteristic of the VTP event were chosen as variables.

We did a hierarchical cluster analysis using Hierarchical Clustering on Principle Components (HCPC) procedure [8, 9]. We use the Euclidean metric for calculating distances between observations and the Ward's method. The procedure was applied only on the population dataset. When the cluster has been defined, we predicted the MCA output on the selected sample [11, 12].

We selected 10 casual sample of the 111 women from the entire population to compare differences in percentage among clusters. Chi-square tests were employed to compare the proportion of women in each cluster in both samples. We use the z-tests to account the difference in proportions among clusters between the selected sample of women and the population sample [13].

Results with a two-sided p-value < 0.05 were considered statistically significant. The statistical comparisons were done using SAS/STAT® Statistics version 9.4. The cluster analyses were developed using R software version 4.5 [8, 9, 14, 15].

RESULTS

Following listwise deletion, MCA was conducted on a total of 6353 women. The total women in the selected sample with no missing value were 111. The optimal number of clusters was determined using hierarchical clustering procedures implemented through built-in package functions. The p-values across all examined variables are highly significant ($p < 0.001$), providing robust evidence that the HCPC analysis has identified clinically meaningful clusters, representing authentic subgroups within the population. Table 1 shows the percentage distribution of women across cluster.

Cluster 1 is overrepresented in both samples and subsamples (38% vs. 24%, median 41%). Cluster 2 shows minimal deviation between population and sample (8% vs. 9%), but the median percentage in 10 random subsamples deviates substantially (26%). Cluster 3 is significantly overrepresented in the sample selected (53% vs. 28%, median 27%). Cluster 4 is severely underrepresented in both the samples (0.9% vs. 39%, median 8%).

CONCLUSIONS

The selected sample overrepresents Cluster 3 (53% vs. 28%) and nearly excludes Cluster 4 (0.9% vs. 39%). Random subsamples did not align with population proportions, suggesting possible random deviations in the selected sample or from sample size. The observed selection bias might originate from the a priori selection in the monocentric study design. This methodological limitation introduces systematic differences between the sampled cohort and the target population, particularly affecting the generalizability of findings to underrepresented clusters. Beyond ensuring transparency regarding the sample's generalizability, a solution involves implementing covariate balancing techniques [16, 17], balancing alongside synthetic oversampling methods [19] to address overrepresentation of specific variables. This dual approach aligns with causal inference frameworks while mitigating selection bias inherent in monocentric observational designs [20, 21].

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Table 1. Cluster Distribution Comparison: Population, Selected Sample, and Random Subsamples

	GENERAL POPULATION (N=6353)	SAMPLE SELECTED (N=111)	MEDIAN PERCENTAGE OF 10 RANDOMLY GENERATED SUBSAMPLES (N=111)
Cluster			
1	1527 (24.0%)	42 (37.8%)	41.0%
2	540 (8.5%)	9 (8.1%)	26.0%
3	1786 (28.1%)	59 (53.2%)	28.0%
4	2500 (39.4%)	1 (0.9%)	8.1%

Trajectories of Adherence to Biologics in Patients with Rheumatoid Arthritis and Risk of a Secondary Immuno-Mediated Inflammatory Disease: A Large Multi-Database Italian Study

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BACKGROUND

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease (IMID) with a global prevalence of approximately 1%. The therapeutic strategy, aiming at achieving low disease activity, includes both conventional and biologic disease-modifying anti-rheumatic drugs (bDMARDs). Evidence from the literature suggests that patients with one IMID are at higher risk of developing another. However, data are lacking on the association between the occurrence of secondary IMIDs and longitudinal adherence to bDMARDs.

AIM

To evaluate the association between adherence trajectories to bDMARDs and the occurrence of secondary IMIDs in patients with RA.

METHODS

We conducted a population-based retrospective observational cohort study using administrative data [1]. We included residents of the participating regions from 2010 to 2023 who had at least one biologic dispensing approved for RA, and a diagnosis of RA identified through a validated algorithm. We excluded individuals younger than 18 years, had less than one year of continuous enrolment (look-back), were prevalent users of RA bDMARDs, were treated with rituximab

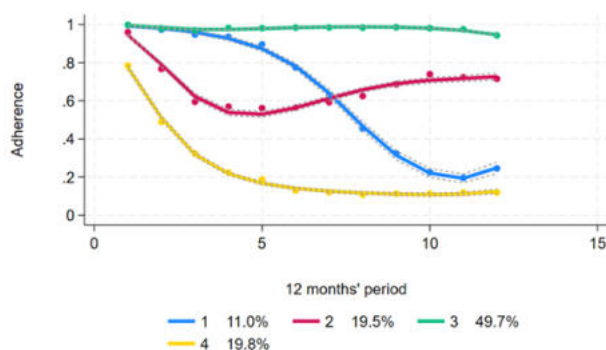
as index drug, or had a diagnosis of any IMID other than RA during the look-back period. The first dispensing date of a bDMARD was defined as the index date. Patients were observed over a two-year period: the first year (exposure period) to assess adherence, and the second year (follow-up period) to monitor the occurrence of secondary IMIDs, death, or end of the study period—whichever came first. During the exposure period, patients were censored if they developed cancer, became pregnant, or died. Treatment coverage was estimated assuming a daily intake of one Defined Daily Dose. We assessed adherence to bDMARDs monthly over the exposure period using the Medication Possession Ratio. Adherence trajectories were identified using Group-Based Trajectory Modeling (GBTM) [2,3]. We used a Cox proportional hazards model to estimate the hazard ratio, and corresponding 95% confidence interval, for developing a secondary IMIDs.

RESULTS

We identified a cohort of 35,600 individuals, with a higher proportion of females (78%), and a mean age of 56.5 (standard deviation: 14.0). We identified four distinct adherence trajectories over a 12-month period. Group 3, labeled as the High Adherent group and comprising 49.7% of participants, maintains nearly 100% adherence consistently throughout the entire follow-up period, indicating stable and optimal adherence. Group 1, referred to as the Declining Adherent group and accounting for 11.0% of the sample, starts with high adherence but shows a gradual and marked de-

cline, particularly after the fifth month, reaching levels around 0.2 by month 12. Group 2, named the Moderate Adherent group and comprising 19.5% of the sample, experiences an initial drop in adherence in the early months, followed by an increase and stabilization around 0.6 to 0.7. Group 4, described as the Low Adherent group and representing 19.8% of participants, demonstrates a steep and continuous decline in adherence from the beginning, falling below 0.2 within the first few months and remaining low for the rest of the follow-up period, highlighting significant variability in adherence behaviors across the groups.

During the one-year of follow-up, 205 events of secondary IMIDs were observed. The Cox proportional hazards model did not reveal statistically significant differences in the risk of developing a secondary IMID across the adherence trajectory groups (overall $p = 0.20$). Compared to the High Adherent group (Group 3, reference), the Declining Adherent group (Group 1) had a hazard ratio (HR) of 1.28 (95% CI: 0.83–1.99), the Moderate Adherent group (Group 2) had an HR of 0.67 (95% CI: 0.43–1.04), and the Low Adherent group (Group 4) showed a non-significant increase in risk with an HR of 1.11 (95% CI: 0.78–1.59).



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CONCLUSION

The study identified four distinct trajectories of bDMARD adherence, showing marked heterogeneity in patients' behaviors. However, there were no statistically significant differences in the risk of developing a second IMID between the groups. These findings suggest that factors other than adherence may influence the occurrence of autoimmune comorbidities.

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A Randomized Controlled Field Trial: A Gamified Training Course on Workplace Health and Safety Prevention for Middle School Students – “Let’s Play 81!”

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INTRODUCTION

Injury prevention and the promotion of health in the workplace are priority objectives starting from school age. Early and effective training can help develop a safety culture in future workers. However, traditional methods often fail to engage students. Gamification, the integration of game elements into educational contexts, has emerged as a promising strategy to increase engagement, improve learning, and enhance working memory [1-5].

OBJECTIVES

To evaluate the effectiveness of the gamified training course “Let’s Play 81!” (“Giochiamo a 81!”) in promoting and improving knowledge of workplace health and safety, in reference to the contents of Italian Legislative Decree 81/2008 [6], among middle school students.

METHODS

A teaching kit was created, consisting of a box (30×20×10 cm) containing an operational manual and the materials necessary for three different types of games: a role-playing game (“81 si gira!”), a board game (“Riduci il premio”) and a card game (“Mettici la mano”) [7]. The games were designed to reinforce the key concepts of Legislative Decree 81/2008, with particular attention to the duties and responsibilities of

key figures in prevention (company owner, safety executive, operator in charge, Prevention and Protection Service Manager, security officer, inspector, occupational health physician, workers’ safety representative and a simple worker).

The study was conducted following CONSORT guidelines [8]. The study was set in third-year classes from three middle schools of Rome. A 15-question quiz was administered to both the intervention group (IG) and the control group (CG) at the beginning (T0) and at the end of the training day (T1). “Score81” was calculated (range 0–15), based on the number of correct answers of the quiz. The quiz, validated in a previous study with an adult population (Cronbach’s $\alpha > 0.70$), required one correct answer among four alternatives, including the “don’t know” option.

The intervention included a 1-hour lecture, with materials (slides and theoretical content) available in the manual included in the kit, followed by 2.5 hours of playful-formative activities with the three games. Students were divided into groups of 7–9 participants, with about 40 minutes dedicated to each game. The activities were led by graduates in Prevention Techniques, specializing in Occupational Medicine, competent doctors, and university professors.

The project received ethical approval from the Universitas Mercatorum Ethics Committee on March 13, 2025.

Qualitative variables were described using absolute frequencies and percentages, while quantitative ones were described using means and standard deviation (SD). The comparison between IG and CG was performed using chi-square test for qualitative variables and independent t-test or Mann-Whitney test in the case of non-normality. The pre-

post Score81 change was analyzed with paired t-tests or the equivalent non-parametric test. Normality was checked using skewness and kurtosis coefficients and the Kolmogorov-Smirnov test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Ten classes ($n = 226$ students) were invited to participate (100% response rate). On the day of the intervention, 36 students were absent, resulting in 190 students participating (mean age 13.08 ± 0.36 years; 49.5% female), evenly distributed between the intervention (IG: $n = 97$) and the control groups (CG: $n = 93$). Three students from the CG were excluded from the analysis due to missing the T1 questionnaire. The groups were comparable for age, gender, and school of origin ($p > 0.05$).

Based on the sample size obtained of both groups, it was possible to define the hypothesis of being able to observe a mean difference in the Score81 between CG and IG at time T1 of at least 3.96, with 80% power and a 95% level of significance, assuming $SD=2$.

At the start of the study (T0), there were no significant differences in the Score81 between the groups (IG: 4.87 ± 2.41 ; CG: 4.69 ± 2.14 ; $p=0.597$). By the end (T1), the IG showed a significant increase in the mean score (9.30 ± 2.89), while the CG maintained values similar to T0 (4.60 ± 2.41) (Table 1). The difference within groups was highly significant ($p < 0.001$) only in the IG.

When analyzing the change ($\Delta = \text{post-pre}$) within the groups, the IG showed an average increase of $\Delta = +4.43$ points ($SD=2.98$; $p < 0.001$), whereas the CG showed no significant change ($\Delta = +0.09$ points; $SD=1.68$; $p=0.617$).

CONCLUSIONS

The gamified training course "Let's Play 81!" proved effective in significantly improving knowledge of workplace health and safety among middle school students. Students in the intervention group achieved a significant average increase in Score81 of more than 4 points, while no changes were observed in the control group.

The teaching kit "Let's Play 81!" was found to be easy to manage and use by trainers. The manual allows for the independent delivery of the theoretical lesson by teachers and trainers. The integration of theoretical content and gamified activities represents a replicable and sustainable strategy to promote a prevention culture among young people.

Despite encouraging results, the study has limitations. Group assignment at the class level may have introduced selection bias, and the sample was limited to Roman schools, affecting generalizability. The absence of long-term follow-up prevented assessment of knowledge retention over time.

The project is currently being extended to high school students to evaluate effectiveness in older age groups and broader educational contexts.

Table 1. Analysis of Score81: comparison between the IG and CG (T0 and T1), and within the two groups pre-post

Group	Score 81 Mean $\pm$ SD		P (T0 vs T1) ^a
	T0	T1	
GC ($n=90$)	4.69 ± 2.14	4.60 ± 2.41	0.617
GI ($n=97$)	4.87 ± 2.41	9.30 ± 2.89	<0.001
P (GC vs GI) ^b	0.597	<0.001	-

Legend:

T0 = pre-intervention time;

T1 = post-intervention time;

a: p-value of the paired t-test;

b: p-value of the independent t-test.

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Assessment of Thermal Regulation before Urban Tree Cover Restoration in Verona, Italy

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BACKGROUND

Green spaces offer various essential regulating ecosystem services, with trees playing a significant role in mitigating urban heat island effects. In the éVRgreen project, we evaluated the potential of greenness to reduce air temperatures in Verona, Northern Italy, to serve as a baseline assessment before implementing an intervention of tree-cover restoration.

METHODS

In the municipality area, we assessed blue/green cover (water, grass/agriculture, trees) and grey cover (bare soil, impervious surfaces) at >200,000 random locations (points) using i-Tree Canopy software. We installed 50 stations that monitored temperatures 2.5 m above ground. We estimated the percentage of blue/green spaces in 200-m radial buffers around each station by determining the proportion of sampled locations in the corresponding land cover classes. We retrieved data of land surface temperatures (LST) from the Landsat 8 satellite and spatially associated it with the sampled locations. We analysed whether land cover can mitigate summer LST (16 July 2024) and winter air temperatures (January 2025).

RESULTS

On 16 July 2024, the median (1st-3rd quartiles) LST was 37 (35-40) degrees Celsius in 61,335 locations covered by trees and 45 (43-47) degrees Celsius in 44,458 locations covered by impervious surfaces ($p<0.001$) (Table 1). In January 2025, daily temperatures were on average 0.49 degrees Celsius lower in areas with a percentage of blue/green cover within 200-m buffers greater than 49% (the median across 50 stations), compared to areas with lower blue/green cover ($p<0.001$).

CONCLUSIONS

The results showed that urban vegetation has a substantial cooling potential. Median ground-level temperature differences between vegetated and impervious surfaces reached 8 degrees Celsius on a typical hot summer day in Northern Italy. Smaller, consistent differences were also observed for winter air temperatures. Therefore, tree cover restoration can be an effective public health strategy for thermal regulation in cities facing climate change.

Table 1. Descriptive statistics of Land Surface Temperature (°C) at the sampling locations by land cover class

Land cover class	n	Median (Q1-Q3)
Water	3163	33 (31-37)
Tree/Shrub	61335	37 (35-40)
Grass/Herbaceous	85739	40 (38-42)
Soil/Bare Ground	8472	42 (38-44)
Impervious surfaces	44458	45 (43-47)

n = number of sampling locations; Q1: first quartile; Q3: third quartile

Validation Protocol of the Italian Version of the Internet Related Measures (IRM) Questionnaire and Assessment of Internet Use Among High School Students in Italy: A Multicenter Study

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INTRODUCTION

Internet use has become an integral part of adolescents' daily lives, a trend that intensified during and after the COVID-19 pandemic [1]. Alongside the increase in online activity, adolescents are now more exposed to digital threats such as harmful content (e.g., self-harm, radicalization), sexual exploitation, and cyberviolence [2]. Scientific literature highlights that some groups of adolescents—particularly those with psychological, familial, or social vulnerabilities—are more likely to encounter these risks [3,4].

In the Italian context, however, validated tools to assess Internet use and related risks are often outdated and lack cultural and technological relevance. This gap hinders effective screening and prevention efforts in schools. The present study addresses this need by validating an internationally adopted tool—the Internet Related Measures (IRM) questionnaire—for use with Italian adolescents, ensuring both scientific accuracy and contextual relevance.

OBJECTIVES

The study aims to:

1. Validate the Italian version of the Internet Related Measures (IRM) questionnaire through linguistic, cultural, and psychometric adaptation;
2. Explore and describe patterns of Internet use and perceived online risks among high school students in Lombardy.

METHODS

This study will adopt a multicenter design and will involve students attending upper secondary schools (scuole secondarie di secondo grado) across the Lombardy region in Northern Italy. Participants will complete a structured questionnaire comprising sociodemographic items (age, gender, ethnicity, school attended) along with the Italian version of the IRM questionnaire.

Descriptive statistics will summarize participant characteristics and Internet use patterns. Means and standard deviations will be used for normally distributed variables; medians and interquartile ranges for non-normally distributed data; and frequencies and percentages for categorical variables.

Psychometric properties of the IRM will be evaluated through both exploratory and confirmatory factor analyses. Internal consistency will be assessed using Cronbach's alpha and corrected item-total correlations. To explore associations between IRM responses and sociodemographic variables, generalized additive models for location, scale, and shape (GAMLSS) will be employed. All statistical analyses will be performed using R software (version 4.4.1), with significance set at $p < 0.05$.

EXPECTED RESULTS

The Italian version of the IRM is expected to show robust psychometric properties, including high internal consistency and structural validity in line with its original version. The study

is also expected to reveal a relevant prevalence of problematic Internet use among high school students in Lombardy.

Additionally, the analysis is anticipated to identify associations between certain sociodemographic characteristics—such as age and gender—and increased perceptions of digital risk. These may include exposure to harmful or inappropriate content, compulsive or excessive use of the Internet, and experiences of cybervictimization. The findings will offer valuable insights for designing evidence-based digital health promotion strategies in the educational setting.

CONCLUSIONS

This study will provide an updated and in-depth picture of Internet use and digital risk perception among Italian adolescents. By validating the Italian version of the Internet Related Measures (IRM) questionnaire, it will offer a reliable and culturally adapted tool for use in educational and public health contexts. The results are expected to support the development of targeted prevention programs, digital literacy initiatives, and evidence-based policies aimed at promoting safer and healthier online behaviors among youth.

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Case Study: Influence of Climatic Condition on the Seasonal Trends of Gastrointestinal Nematode Infections in Sheep

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KEYWORDS: gastrointestinal nematodes, sheep, monitoring, epidemiology, datalogger.

INTRODUCTION

Gastrointestinal nematodes (GINs) represent a major health challenge in grazing ruminants, significantly affecting animal productivity and farm sustainability [1]. The epidemiology of GIN infections is influenced by multiple factors, including farm management system, breed susceptibility, and climatic conditions [2]. The latter play a crucial role in the survival and transmission of GIN infective larvae in pastures, affecting the seasonal dynamics of infestations [3].

OBJECTIVES

This study investigates the epidemiology of gastrointestinal nematodes (GIN) infections in a semi-extensive dairy sheep farm located in the Campania region (Southern Italy), where two breeds, Lacaune and Bagnolese, are raised under identical management conditions. The Lacaune, a high-yielding cosmopolitan French dairy breed, and the Bagnolese, a hardy Italian native breed, differ in morphology, productivity, and adaptability to pasture environments factors that may influence their susceptibility to helminth infections. The study aims to evaluate breed and category related differences in parasitic load (EPG – eggs per gram of faeces) over time using a mixed-effects modeling. Furthermore, it explores the association between climatic trends and parasite dynamics, with a specific focus on assessing whether temperature and humidity are significant predictors of parasitic load.

METHODS

This observational study was conducted from April 2024 to March 2025 on a group of 44 selected sheep (22 animals per breed). Animals were stratified by physiological category to assess helminth burden across different life stages. Monthly faecal sampling was performed, and individual copromicroscopic analyses were carried out at the laboratories of the Regional Center for Monitoring Parasitic Infections (CREMOPAR) using the Mini-FLOTAC technique. To investigate the influence of climatic conditions on the epidemiology of GINs, temperature and relative humidity were continuously recorded in two distinct grazing areas (shaded perimeter and fully sun-exposed field) using dataloggers. A mixed-design repeated measures ANOVA was performed to evaluate the effects of breed (Bagnolese vs. Lacaune), physiological category, and time (monthly sampling) on parasite load. The dependent variable was the logarithmically transformed egg count per gram of faeces (log EPG) to meet the assumptions of normality and homogeneity of variance. Normality of residuals was tested using the Shapiro–Wilk test, while Levene’s and Mauchly’s tests were used to assess homogeneity of variance and sphericity, respectively. When sphericity was violated, Greenhouse–Geisser or Huynh–Feldt corrections were applied. To investigate the influence of climatic conditions, a lagged linear regression model was applied to monthly aggregated parasitic load and environmental variables (temperature and humidity in shaded and sun-exposed areas). The model accounted for the temporal structure of the data

using one-month lags for each predictor ($t-1$), implemented with the `dynlm()` function. All analyses were performed using RStudio (version 2024.12.0, Build 467).

RESULTS

Preliminary results showed that the average parasitic load followed a seasonal trend, with the lowest values in spring and a sharp increase in summer (Bagnolese: 310 EPG, Lacaune: 214 EPG, difference: 96.5). Bagnolese sheep consistently showed higher values than Lacaune sheep, particularly in summer, when the average load reached 262 EPG. The greatest differences were observed in the warmest months, suggesting a breed-related variation in susceptibility to gastrointestinal parasites. Parasitic load also varied across animal categories. Lambs had generally lower values, though some individuals showed high values in summer. Primiparous ewes exhibited a gradual increase, with peaks during the warm season. Adult sheep had the highest helminth infections, suggesting greater long-term exposure or reduced parasite resistance with age. These patterns were statistically confirmed by the mixed ANOVA, which revealed a highly significant effect of season on parasitic load ($p < 0.05$), indicating substantial intra-annual variation. A significant Group $\times$ Season interaction ($p < 0.05$) suggested that seasonal patterns differed across experimental groups. In contrast, the main effects of Group ($p > 0.05$) and Breed ($p > 0.05$) were not significant. However, the Breed $\times$ Group interaction approached significance ($p = 0.082$), pointing to a possible differential breed response depending on group conditions. Additionally, the dynamic regression analysis showed a significant effect of temperature in the previous period (lag 1) on parasitic load. A temperature increase was associated with a significant rise in the average EPG ($p < 0.05$), while humidity had no significant effect ($p \approx 1$). The model explained 64.5% of the variance in parasitic load (adjusted $R^2 = 0.556$), reinforcing the role of temperature as a key environmental driver and aligning with the observed seasonal peak.

CONCLUSIONS

The mixed ANOVA and dynamic regression analyses together demonstrated that temperature is a key environmental driver influencing parasitic load, with significant seasonal variations and differential responses among groups. The study aims to provide insights into the adaptation of different sheep breeds to helminth infections and environmental conditions, supporting the implementation of targeted helminth management programs in extensive and semi-extensive farming systems. This allowed for the evaluation of climatic influences on larval survival and helminth transmission risk. Understanding how climate and management strategies interact with breed-specific resistance to GINs is essential for developing predictive models and sustainable helminth control strategies.

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Is Data Collection Harmonized Across Italian Clinical Cohorts on Alzheimer's and Other Dementias? A Systematic Review

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INTRODUCTION

Dementia, particularly Alzheimer's disease, is a progressive neurodegenerative condition that severely affects patients' quality of life and imposes a substantial burden on healthcare and social systems. Recent estimates indicate that over 57 million people worldwide are living with dementia, and this number is expected to triple by 2050. In Italy, current estimates exceed 1.2 million cases. The increasing incidence highlights the urgent need to enhance prevention strategies, especially considering that approximately 40% of cases can be attributed to modifiable risk factors, as noted by the Lancet Commission in 2020 report (1)

Despite scientific recognition of the importance of prevention, Italy's involvement in international dementia research networks (e.g., GAAIN, DPUK, ADDI) remains marginal. A major barrier is the fragmentation and heterogeneity of data collected across different Italian observational studies. The lack of standardized methodologies for data collection, classification, and risk factor analysis hampers comparability between studies and limits their integration into meta-analyses or cohort inclusion in existing networks (2).

OBJECTIVES

As part of the PREV-ITA-DEM project (PN-RR-MAD-2022-12375822), funded by the Italian Ministry of Health, we conducted a systematic review to identify, map, and evaluate observational studies carried out in Italy between 2019 and 2024 focusing on dementia, Alzheimer's disease, and modifiable risk factors.

Specifically, the review aimed to: assess the methodologies and the tools used; develop data harmonization rules,

based on internationally recognized standards (3) to support integration of the Italian cohorts into global research networks; propose validated protocols and tools to improve the data quality and comparability, suggesting a methodology that can be transferred also to non-modifiable factors such as biomarkers and genetic data.

METHODS

A systematic review of observational studies of Italian clinical cohorts was conducted using MEDLINE, Embase, and Scopus, covering the period from January 2019 to December 2024. Studies were included if published in English and focused on at least one of the eight modifiable risk factors for dementia (BMI, hypertension, diabetes mellitus, dietary habits, alcohol consumption, depressive symptoms, physical inactivity, and smoking).

Data were extracted on measurement methods, units used, assessment tools (e.g., questionnaires, clinical tests), diagnostic criteria, and classification categories.

Definitions and international criteria were used to assess compatibility and the DataSHaPER (Data Schema and Harmonization Platform for Epidemiological Research) methodology was applied to evaluate the degree of variables' harmonization (3). Variables were classified as completely harmonizable when they strictly adhered to standard definitions and formats, allowing direct comparison without any loss of information; partially harmonizable when some discrepancies or incomplete data could result in information loss; and impossible to harmonize when insufficient or incompatible data prevented meaningful alignment.

This rigorous approach facilitates the identification of gaps and inconsistencies in data collection methods.

RESULTS

Of the 365 articles initially identified, 18 Italian observational studies met the inclusion criteria. Study designs included cross-sectional, longitudinal, and case-control approaches, with sample sizes ranging from fewer than 100 to more than 5.000 participants.

The analysis revealed significant heterogeneity in data collection protocols. Obesity, assessed via BMI, was the best documented factor: 38% of the studies showed complete harmonization with international standards, while 33% showed partial harmonization. Dietary habits, assessed through FFQ questionnaires, also showed reasonable harmonization (30% complete).

Smoking (28% complete harmonization), physical inactivity (using IPAQ), and alcohol consumption were addressed with variable tools and categorizations, while depression (measured with GDS or CES-D) and diabetes (through fasting blood glucose) showed only 22% complete harmonization. Variability in tools and their application emerged as a major obstacle to data comparability. Moreover, in many cases, key methodological details such as diagnostic thresholds or data collection frequency were missing.

DISCUSSION

The results of the review highlight methodological fragmentation that undermines the ability to integrate Italian data into large international consortia. The systematic adoption of recognized tools and validated protocols for the assessment of risk factors will enable the generation of more robust evidence, enhance the statistical power of analyses, and support the inclusion of Italian cohorts in international consortia. This represents a critical step toward making a meaningful contribution to the fight against dementia, with direct benefits for public health and the sustainability of the national healthcare system (2).

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An Early Warning System Based on Temperature for West Nile Neuroinvasive Disease in Northern Italy

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INTRODUCTION

In recent years, Northern Italy has experienced a rise in cases of West Nile Neuroinvasive Disease (WNND), the most severe manifestation of West Nile Virus (WNV) infection. Temperature, along with other meteorological factors, plays a crucial role in WNV transmission by influencing mosquito density and virus prevalence [1].

AIM

This study aims to investigate whether meteorological data—such as temperature and precipitation—can be used in an early warning system to predict the likelihood of WNND outbreaks in Northern Italy.

METHODS

We developed a spatial-temporal Bayesian hierarchical model using monthly confirmed WNND cases from 2014 to 2021 across 36 provinces in Northern Italy. The model assessed the relationship between WNND incidence, average temperature, and cumulative precipitation recorded in the three months preceding case reports. Spatial random effects were included using a Besag-York-Mollié model, while temporal random effects were modeled with an autoregressive structure, assuming a negative binomial distribution for observed cases. Using 2022 data, we evaluated the model's out-of-sample predictive ability with a 2-month lead time. An expanding window approach was adopted, progressively incorporating observed monthly meteorological inputs and WNND cases. To estimate the probability of WNND occurrence during the 2022 season, 1,000 samples were drawn from the posterior predictive distribution for each province and target month. The probability of WNND occurrence was calculated as the proportion of these samples in which the predicted number of cases was greater than or equal to 1. Model fitting was performed using the R-INLA software [2].

RESULTS

Between 2014 and 2022, 793 WNND cases were reported, with notable increases in 2018 and 2022. Among the meteorological variables, temperature recorded 1–2 months prior to case occurrence was strongly associated with WNND incidence, showing a clear linear exposure–response relationship, while no clear association was observed with cumulative precipitation. Thus, we specifically selected the model including temperature observed 2 months in advance as a predictor, aiming to forecast WNND occurrence using only observed meteorological inputs. The predictive ability of the selected model was evaluated using the Area Under the Curve (AUC); the temperature-based model achieved an AUC of 0.81, indicating good predictive accuracy.

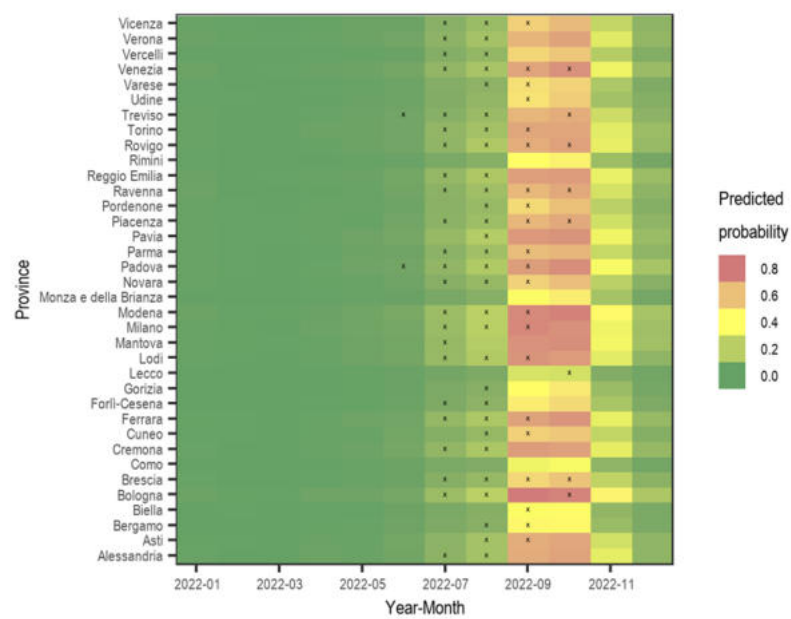
CONCLUSIONS

Our results demonstrate that temperature recorded in the preceding months can be used to predict the occurrence of WNND cases with a 2-month lead time on a monthly basis. This modeling framework could be applied to predict WNND onset in other settings, in order to support decision-making processes for outbreak mitigation and prevention.

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Figure 1. Probability of observing at least one WNND case for each province and month of 2022



Probability of observing at least one WNND case for each province and month of 2022. Months in which WNND cases occurred are marked with an "x".

A Retrospective Analysis of Severe Injury Patients in the Hub and Spoke Centres in the Province of Alessandria

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INTRODUCTION

Trauma is one of the leading causes of death in Western countries and results in severe health, economic, and social consequences due to residual disabilities. To improve the management of major trauma cases, integrated trauma care systems (SIAT) have been implemented in Italy, inspired by North American models. The goal is to centralize severe cases in the most equipped facilities (trauma centers), thereby reducing avoidable mortality [1]. These systems are based on a “Hub and Spoke” network, which includes a Hub center with a Level II Emergency Department (DEA) and several Spoke centers (with Level I DEAs), such as peripheral hospital facilities. In the Province of Alessandria, in Piedmont region, the Hub center is the hospital of Azienda Ospedaliero-Universitaria of Alessandria (AOU AL), and there are four Spoke centers representing the hospitals of the Local Health Authority of Alessandria (ASL AL), located in the cities of Acqui Terme, Casale Monferrato, Novi Ligure, and Tortona. Despite the public health relevance, epidemiological data on trauma in the Italian territory are scarce and inconsistent in the literature.

OBJECTIVE

This study aims to give an epidemiological description of hospitalized severe trauma cases in the Spoke and Hub centers of the Province of Alessandria during the period 2017–2021, also analyzing potential differences between the pre-pandemic and pandemic periods. Additionally, the study aims to assess in-hospital and 30-day mortality for this patient category in both Spoke and Hub centers.

METHODS

Subjects admitted to the Hub and Spoke centers were identified based on hospitalizations (ICD9-CM codes: 800.xx–904.xx; 925.xx–939.xx; 950.xx–959.xx) with discharge dates between 01/01/2017 and 31/12/2021. Trauma severity was assessed using the Injury Severity Score (ISS), based on ICD9-CM codes for principal and secondary diagnosis [2,3,4]: only admissions with ISS > 15 (severe trauma) were included. In cases of multiple hospitalizations for the same patient, only the first was considered. Mann-Whitney and Chi-square tests were used to evaluate associations between variables. In order to find determinants of in-hospital and 30-day mortality, Odds Ratios (ORs) with 95% Confidence Interval (95% CI), estimated from multivariate logistic regression models, were used; gender, age, ISS, length of stay, access via Emergency Department, being hospitalized in Intensive Care Unit, being hospitalized during Covid-19 pandemic period (after 01/03/2020) and being hospitalized in Hub/Spoke center were put as covariates. The probability of in-hospital death for each subject was also calculated using the Trauma Mortality Prediction Model (TMPM) [5,6,7]. The predictive capacity of the model was tested on the study sample using ROC (Receiver Operating Characteristic) curves and the corresponding area under the curve (AUC) [8].

RESULTS

In this study, a total of 1,337 patients were included: 705 hospitalized in the Hub center and 632 in the Spoke centers. Most patients in the whole sample were male (59.6%) and the median age was 76 (IQR: 60–85) years. The median length

of hospital stay was 8 (IQR: 4–14) days, significantly longer in the Hub center: 9 (IQR: 5–16) days in the Hub center, 7 (IQR: 4–12) days in the Spoke center ($p<0.001$). In-hospital mortality was 10.3% in ASL AL centers and 6.4% in AOU AL ($p=0.01$). The emergency department was the primary point of access (79.4%) for these hospitalizations. The median ISS was 16 (IQR: 16–22). There were no significant differences in ISS between the hospitalizations during Covid-19 pandemic period and the ones that occurred before ($p=0.95$), nor in the distribution of in-hospital ($p=0.48$) and 30-day ($p=0.90$) deaths. TPMC ROC curves showed an AUC of 0.74 for in-hospital mortality and 0.71 for 30-day mortality. The results of the multivariate logistic regressions indicate that increasing of age (OR=1.08; 95% CI: 1.07–1.10), being male (OR=1.50; 95% CI: 1.06–2.14), higher ISS (OR=1.03; 95% CI: 1.02–1.05), and admission to Intensive Care Unit (OR=3.17; 95% CI: 1.74–5.70) are associated with increased odds of 30-day mortality. Furthermore, increasing of age (OR=1.06; 95% CI: 1.04–1.09), shorter length of hospital stay (OR=0.96; 95% CI: 0.94–0.99), higher ISS (OR=1.02; 95% CI: 1.00–1.03), admission to Intensive Care Unit (OR=5.72; 95% CI: 2.93–11.10), and being hospitalized in Spoke centers (OR=1.74; 95% CI: 1.10–2.80) are associated with increased odds of in-hospital mortality. Being hospitalized during the Covid-19 pandemic period was not significantly associated with increased odds of either in-hospital mortality (OR=0.83; 95% CI: 0.52–1.29) or 30-day mortality (OR=0.96; 95% CI: 0.66–1.37).

CONCLUSIONS

The TPMCs showed good discriminatory power in predicting mortality, supporting their reliability in clinical and operational settings. The absence of statistically significant differences in outcomes between the pre-pandemic and pandemic periods suggests that the Covid-19 pandemic had a limited impact on the care of polytrauma patients. The analysis of in-hospital mortality highlights the need to optimize the management of severely injured patients within the “Hub and Spoke” network. In 2024, the urgent transfer protocol for patients from ASL AL centers to the AOU AL center was revised; a future perspective will therefore be the evaluation of in-hospital mortality in the coming years.

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Investigating the post-COVID-19 Spike in Bronchiolitis Emergency Admission among Infants: A Cohort Study from Northern Italy

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INTRODUCTION

Bronchiolitis is the leading cause of Emergency Room (ER) visits and hospitalizations in infants, primarily due to Respiratory Syncytial Virus (RSV) infection [1,2]. The COVID-19 pandemic, along with the associated non-pharmaceutical interventions, led to a marked decline in bronchiolitis cases during the 2020–2021 season [3]. However, in 2021, a resurgence of RSV resulted in a double increase in bronchiolitis-related hospital admissions in several countries [4]. In Italy, data on bronchiolitis trends after COVID-19 remain limited. A multicentre study from 27 hospitals in Lombardy [5] and a single-centre study from Genoa [6] both suggest increased hospitalizations and ER visits in recent seasons but relying on data limits a comprehensive national understanding.

OBJECTIVES

This study aims to analyse the impact of the COVID-19 pandemic on bronchiolitis-related Emergency Room (ER) visits in infants aged 0–6 months in Lombardy, Italy. It also explores potential factors underlying any observed changes, including shifts in population demographics, variations in disease severity, alterations in age distribution, and the effects of COVID-19 on infant susceptibility.

METHODS

We used linked administrative data on healthcare utilization covering the entire population of residents in Lombardy. Two distinct cohorts were analysed: a paediatric cohort of one million births for descriptive trend analyses to assess the indirect effects of COVID-19, and a maternal-paediatric cohort

for hypothesis-driven analyses on the potential direct effects. We calculated incidence rates (IRs) of bronchiolitis-related ER visits between 2012 and 2023, using hospital admissions as a proxy for more severe disease. Due to the lack of swab testing in newborns, we used maternal COVID-19 vaccination as a proxy to investigate direct effects. Multivariable-adjusted logistic regression was employed to assess the association between COVID-19 vaccination before or during pregnancy and bronchiolitis-related ER visits in the first six months of life. To evaluate whether any observed associations could be explained by underlying family-level factors, we applied a negative control design, comparing ER visit rates in older siblings born to mothers who were later vaccinated with those born to mothers who remained unvaccinated.

RESULTS

Bronchiolitis incidence rates (IRs) showed a marked shift after 2020, with rates dropping to near zero in 2020–2021, then doubling in 2021–2023 compared to pre-pandemic levels, increasing from 2 to ≥ 4 per 100 person-time, as shown in Figure 1. In exploring potential explanations for this change, we ruled out shifts in population demographics, disease severity (hospital admissions remained consistently at half the IR of ER visits, as illustrated in Figure 1), and age distribution at the time of visits. Maternal COVID-19 vaccination before or during pregnancy was associated with a reduced risk of bronchiolitis-related ER visits in infants (adjusted OR = 0.59 [95% CI: 0.50–0.69]). The negative control analysis suggested that this association is unlikely to be fully explained by family-level confounding, as no similar association was observed in older siblings (adjusted OR = 1.50 [0.93–2.47]).

CONCLUSIONS

Consistent with previous studies, our findings confirm the absence of bronchiolitis ER visits during the first COVID-19 lockdown and highlight the subsequent rise in cases during 2021–2023. The protective role of maternal COVID-19 vaccination on infant bronchiolitis strongly suggests that (pre- or post-natal) SARS-CoV-2 infection contributes to explaining the large increase in incidence post-pandemic.

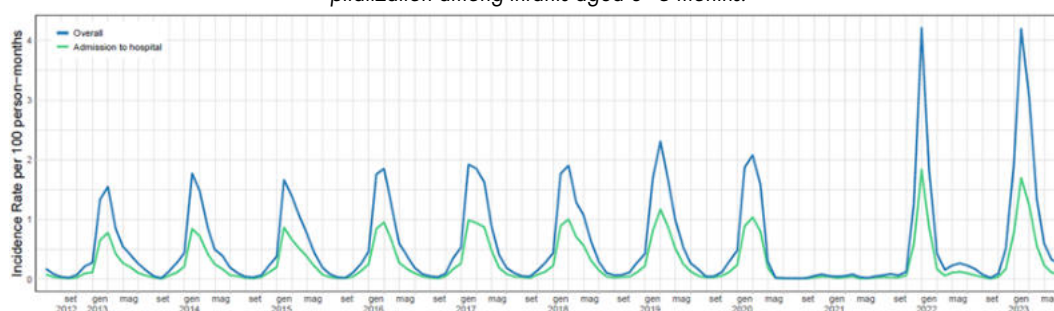
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Figure 1. Monthly incidence rates of Emergency Room visits for bronchiolitis and of visits resulting in hospitalization among infants aged 0–6 months.



Investigating the Timing and Predictive Value of Clinical Conditions Preceding Multiple Sclerosis in the UK Biobank's Population-Based Cohort

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INTRODUCTION

Multiple Sclerosis (MS) is a complex autoimmune disease[1]. Growing evidence suggests a prodromal phase marked by increased healthcare use and various clinical symptoms before diagnosis, such as pain, fatigue, urinary issues, and mental health conditions[2]. These objectives are particularly relevant due to the recently highlighted increase in the age at MS onset due to a higher incidence in late-onset MS (LOMS) cases, which may result from the accumulation of different conditions[3]. However, prior studies often relied on limited time windows (5 years prior the diagnosis), introducing temporal bias by over-emphasizing conditions occurring closer to MS diagnosis, other than failing to discover associated early conditions occurring many years prior the diagnosis[4]. Moreover, the predictive value of MS prodromes and their temporal trajectories have not been explored yet.

OBJECTIVES

To investigate these aspects, we used the large UK Biobank's population-based cohort, which provided the clinical history for each individual through ICD-10 diagnosis codes and diagnoses dates. Specifically, using time-to-event analyses, we aimed to (i) identify early conditions associated with later MS diagnosis, (ii) assess their predictive value, and (iii) map disease trajectories leading to MS.

METHODS

We assessed associations between 600 clinical conditions and MS risk in 477,421 individuals using Cox models

adjusted for demographics, smoking, and MS polygenic risk score (MS-PRS). To account for multiple testing, we applied a False Discovery Rate (FDR) correction at 0.05. Statistically significant conditions were classified based on their timing of appearance in relation to MS diagnosis, i.e., >5 years, 3-5 years, 1-3 years, or within 1 year, as well as based on their clinical relationship with MS, i.e., onset symptoms, prodromal conditions, risk/protective factors, or unknown relationship. We then included these significant conditions in a LASSO Cox regression (5-fold cross-validation on 70% training-validation set) to identify key predictors, with performance assessed by the C-index and age-dependent Area Under the Curve (AUC) in the 30% test set[5]. To rank the most important conditions based on their predictive value, we used permutations. Lastly, temporal trajectories of MS-associated conditions were analyzed in MS cases using conditional logistic regression models[6].

RESULTS

We identified 192 conditions associated with MS, of which only ~20% were onset symptoms. Integrating these conditions into a predictive model already including demographics and smoking improved the C-index from 0.65 to 0.71. Among the thirty model-selected best predictors, ~25% were prodromal conditions, including neuromuscular diseases, thromboembolism, and depression which typically occurred more than five years before MS diagnosis. Including MS-PRS further increased the C-index to 0.78, with an age-dependent AUC exceeding 0.80 in individuals over 50 years. Trajectory analysis highlighted migraine as a common early diagnosis, often followed by hypertension, depression, and dorsalgia.

CONCLUSIONS

Our findings highlight early conditions and diagnostic trajectories of MS, supporting the existence of a prodromal phase. Specifically, while genetic risk represented the strongest predictor in adulthood, clinical history represented the strongest predictor in individuals over 50 years of age[7]. Importantly, the identified disease trajectories showed that MS onset symptoms occurring closer to diagnosis were themselves predictable by earlier prodromal conditions. These insights could improve MS prediction and facilitate earlier detection, particularly for late-onset cases. Future research should validate these results in independent cohorts, and explore how integrating subtle signs and symptoms, lifestyle factors and biomarkers could further refine and enhance the accuracy for an MS prediction tool to be implemented and tested in the clinical practice.

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Assessing Lifestyle Risk Profiles in Older Adults via Latent Class Analysis: A Cross-Sectional Analysis of the Association with Metabolic Biomarkers Using ELSA Data

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INTRODUCTION

The global prevalence of metabolic diseases such as diabetes continues to rise, particularly among aging populations [1]. Among risk factors, lifestyle behaviors are modifiable and play a central role in the prevention and progression of metabolic diseases [2]. These risks do not act alone, making it important to examine how lifestyle behaviors cluster and interact with one another and how their joint classification is associated with biological markers of metabolic diseases. ELSA [3] is a longitudinal study of over-50 years old subjects, specifically designed to study ageing population in Britain.

OBJECTIVES

The main objective was to identify distinct behaviorally defined subgroups among older adults from Wave 4 (2008/2009) of the ELSA through latent class analysis and characterize their associated biomarker profiles[4]. By linking these lifestyle patterns to early biological risk markers, this study seeks to enhance understanding of the mechanisms connecting lifestyle behaviors to metabolic health, ultimately informing more targeted interventions for the prevention of diabetes and related metabolic conditions in later life.

METHODS

This study utilized data from ELSA Wave 4 specifically for its inclusion of extensive biomarker data, which are critical for analyzing metabolic and diabetes-related health risks.

For downstream analysis, several data transformations and recoding steps were performed. Each blood biomarker (white blood cells, haemoglobin, insulin growth factor 1, HbA1c, fasting glucose, triglycerides, DHEAS) was recoded

into a binary variable using cutoff values defined through clinical practice/literature specific to the British population. The initial sample size including data on these biomarkers was $n=3147$.

Latent Class Analysis (LCA) was performed using the `poLCA` package in R, incorporating categorized lifestyle factors such as sleep quality, comorbidities, smoking habits, nutrition (fruit and vegetable intake), alcohol consumption, physical activity, and obesity as manifest variables. To determine the optimal number of latent classes, multiple models were estimated and compared using model fit indices, including the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and significance of predicted class memberships, to ensure meaningful interpretation. A complete case LCA approach was chosen as this resulted the best model fit compared to analysis with inclusion of missing values.

Depending on the nature of each biomarker, appropriate regression methods were applied: linear regression for continuous biomarkers, binary logistic regression for those with strict cutoff thresholds, and multinomial regression for biomarkers with multiple healthy/unhealthy categories. All regression models were adjusted stepwise, starting with unadjusted models and progressing to fully adjusted ones, incorporating confounders grouped as demographic/biological (age, sex, ethnicity), socioeconomic (education, net worth, deprivation score), health-related (comorbidities, depression), and, finally, household composition.

RESULTS

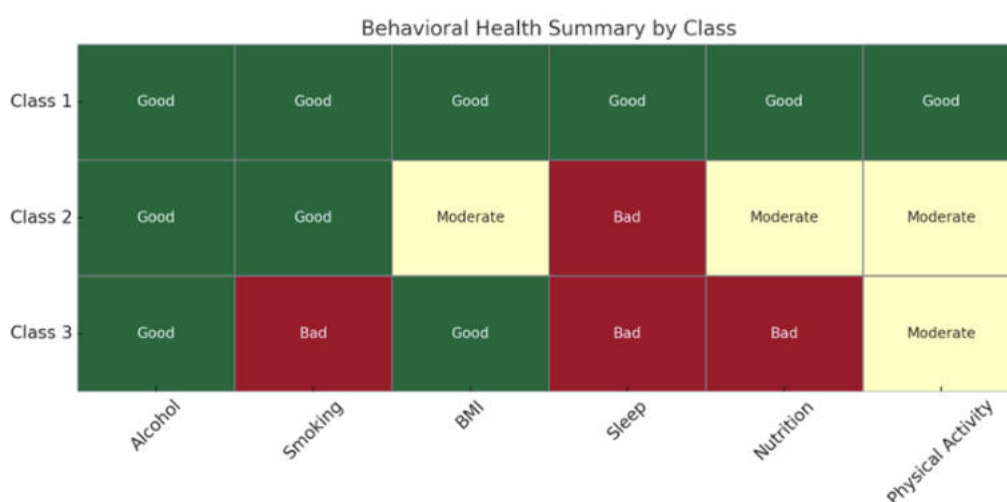
Latent Class Analysis identified three distinct lifestyle behavior profiles: Class 1—characterized by overall healthy behaviors; Class 2—marked primarily by poor sleep and Class 3—defined by a combination of poor sleep, unhealthy nutrition, smoking and borderline levels of physical activity (Figure

1). Compared to individuals with healthy behaviors (Class 1), those in Class 2 had higher odds of elevated triglycerides (OR = 1.73; 95% CI: 1.43-2.08), and very similar odds for Class 3 (OR = 1.74; 95% CI: 1.31-2.29). Elevated triglycerides were also significantly associated with male sex. These findings were supported by linear regression results, which showed that Classes 2 and 3 had significantly higher average triglyceride levels—an increase of 0.17 mmol/L (95% CI: 0.09 to 0.24) and 0.25 mmol/L (95% CI: 0.13 to 0.36), respectively—approaching borderline unhealthy levels.

For HbA1c, individuals in Class 2 and Class 3 had higher odds of elevated levels (OR = 1.30; 95% CI: 1.11-1.60 and OR = 1.87; 95% CI: 1.41-2.51, respectively), compared to Class 1. In linear regression, the baseline HbA1c level (intercept) was 5.64 (95% CI: 5.57 to 5.70), and both Class 2 (+0.11, 95% CI: 0.06 to 0.15) and Class 3 (+0.09, 95% CI: 0.03 to 0.16) showed significant increases—pushing average levels to or just above the 5.7% threshold. Elevated HbA1c was also significantly associated with older age, particularly higher in those in their 70s.

In the fully adjusted multinomial regression, participants in Class 2 had significantly higher odds of low DHEAS levels compared to Class 1 (OR = 2.02, 95% CI: 1.51 to 2.70), suggesting a strong association between poor sleep and reduced DHEAS.

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CONCLUSIONS

This study highlights that poor sleep alone (Class 2) is associated with significant increases in metabolic risk markers such as HbA1c and triglycerides, pushing levels toward borderline or unhealthy ranges. When combined with other unhealthy lifestyle factors such as unhealthy diet and smoking (Class 3), there is an additional negative effect for several biomarkers. Furthermore, poor sleep was also strongly related to low DHEAS, which is in turn a known factor linked to the hormonal processes of aging[5]. Overall, these findings highlight the importance of addressing specific patterns of behavior in order to increase awareness of and protection against early metabolic dysregulation and enhance healthy aging.

Figure 1. Behavioral profiles of latent classes using a ≥ 0.50 probability cutoff for unhealthy behaviors (red) and a 0.35–0.49 range to denote moderate probability of unhealthy behaviour (yellow); <0.35 indicates lower probability of unhealthy behaviour

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Application of Logistic Regression and Random Forests to Assess the Relevance of Chrononutrition Information for Prediction of Overweight in the INRAN-SCAI 2005-2006 Nutrition Survey

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INTRODUCTION

Obesity is a world wide crisis nowadays it contributes to a number of serious diseases like type 2 diabetes[1] and coronary heart disease. Chrononutrition and in particular timing of eating, has been shown to have an influence on obesity in studies with animal models[2]. Previous human epidemiological studies have already investigated the association between timing and regularity of eating with BMI [3,4]. However to date no study specifically assessed the added value of using data on the timing and regularity of calorie intake for predicting obesity, on top of total calorie intake across the day, when this information is available in nutritional epidemiological studies like surveys with diet diaries.

OBJECTIVES

The target population of this study are Italian adults aged 18 to 64 years old from a past cross sectional nutrition survey INRAN-SCAI[5]. The aim of the study is to compare the performance, in predicting overweight (BMI>25), of models based on 6 day time intervals of calorie intake, to models based on the total calorie intake across the day and comparing prediction performance of logistic regression and random forests using ROC curves.

METHODS

Data collected during the Italian nationally representative food consumption survey INRAN-SCAI in the years 2005-2006 was used to make the predictive models. The data used for our analysis was collected from 2312 adults aged

18-64, in the form of a diet diary over 3 consecutive days as well as sociodemographic questionnaire. The days were divided into 6 time intervals, which correspond to the times of 3 main meals and the intervals between them (6am-9am; 9am-12pm; 12pm-3pm; 3pm-7pm; 7pm-10pm; 10pm-6am).

Three different types of models were compared in this study: (i) models trained on the mean energy intake and irregularity of the 6 time intervals, (ii) models trained on the 6 time intervals but using repeated measures from the 3 days and (iii) models trained using the mean energy intake and irregularity for the whole day.

Logistic regression models were built using the whole data sample as well as separating the training(70%) and testing(30%) set for the model, with overweight (chosen due to the small number of strict obesity cases) as outcome and including as predictors also several available sociodemographic variables (including physical activity) chosen by a preliminary application of a forward variable selection algorithm. Sensitivity, specificity, negative predictive value, positive predictive value and error rate were calculated for two different cut off points, one being 0.5 and the second being the optimal cutoff point maximizing the specificity and sensitivity simultaneously. Cross validated models (10 fold) were also generated. The ROC curves corresponding to all the models were compared

For all 3 conditions random forest models were also generated. Their ROC curves were compared and metrics such as specificity, sensitivity NPV, PPV and error rate were in turn calculated.

RESULTS

When logistic regression models were trained on 100% of the data sample (including 34.6% overweight subjects), the

models using time intervals repeated measures or means both performed better than the model using a day mean. The AUC for repeated measures was 0.7664, very similar to AUC for interval means (0.7639), the AUC for the mean of the day model was 0.7520. The difference between them was statistically significant both when tested across the whole ROC curve and when tested for optimal cutoff point and 0.5 cutoff point. At the optimal cutoff point specificity, NPV, PPV and error rate performed better in the models using time intervals, whereas sensitivity was higher in the model using day mean. For the 0.5 cutoff the models with intervals performed better in all values except for specificity, which was roughly the same for all models.

After separating the training set and test set, the differences in AUC between the models were no longer statistically significant. Similar result was obtained for cross validated models, the differences in AUCs were slightly larger than when separating the test and training sets by hand but the confidence intervals were overlapping. All the metrics (sensitivity, specificity, PPV, NPV and error rate) for training and test sets for all 3 models were very close, at both mentioned cutoff points, the only one which stood out was sensitivity in the repeated measures at 0.5 cutoff (40.7% vs. 37.1%, 35.5%).

The random forest models also did not show a significant difference between the AUC corresponding to the 3 conditions. The sensitivity for the random forest models was generally low (38% for mean of the day, 32.9% for means of intervals, 36.1% for repeated measures). NPV was much higher for the mean of the day model at 86.3%, compared to around 70% for others while the rest of the metrics, also except for sensitivity, performed better in the models using time intervals. Comparison of sensitivity/specificity/ROC for random forests and 0.5 cut-off of logistic models are shown in Figure 1.

CONCLUSIONS

The study provides an insight into how chrononutritional data based on repeated diet diaries summarized by calories consumed in 6 time intervals (as defined in previous work [3,4]) compares in terms of overweight prediction with using just mean total calorie intake in the day, while also comparing the performance of a machine learning technique like random forest with a classical method like logistic regression. The area under ROC was significantly higher when using time intervals (repeated or averaged) compared to whole day only when they were trained on 100% of the data sample, and this result became nonsignificant after a 30% test set was randomly taken from the sample, both within the training and test sample.

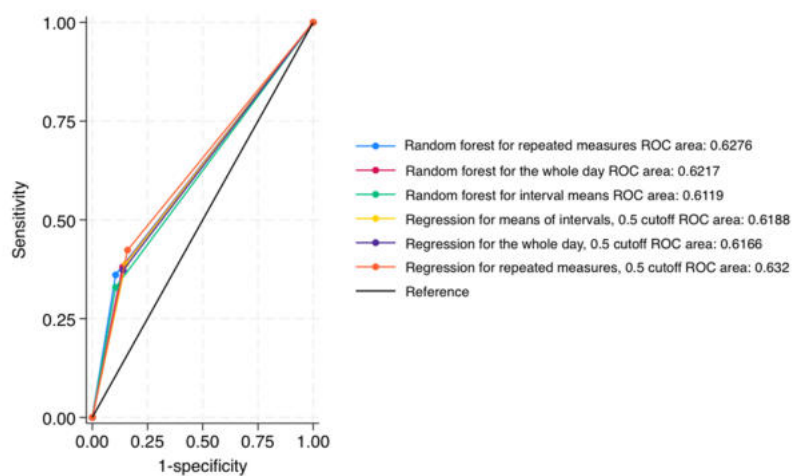
Chrononutrition information in this case allowed for a significantly better prediction of overweight only when testing on the same data as the model was trained on and may thus be attributable to overfitting. Hence the results, despite being based on a nationally representative sample, don't seem to be generalizable for public health purposes. On the other hand, population heterogeneity may also be hindering prediction. Another limitation is the cross-sectional nature of the INRAN-SCAI nutrition survey. In conclusion, timing/regularity of eating may still capture useful information to study overweight/obesity but a properly powered prospective study is

warranted to truly assess its potential different impact/relevance also for subgroups of the population.

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Figure 1. The area under ROC obtained from random forest and logistic regression models across 3 different set of nutritional intake predictors: the repeated measures across time intervals; the means and irregularities of time intervals; the mean and irregularity of the whole day



Road Traffic Accidents Epidemiology: Evaluations on a 15 Years Surveillance Program from an Emergency Care Unit (ECU) in Northern Italy

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INTRODUCTION

Road accidents cause 20-50 millions injuries every year and 2.35 millions deaths, representing the eighth cause of death in the general population and the first in people between 5 and 29 years of age [1]. Human and health care costs of the phenomenon have a significant impact on the population and the institutions, with about 280 billions of euros spent in Europe each year [2]. Legal regulations, educational interventions and new safety technologies have the potential of lowering the number and severity of road accidents [3]

AIMS

To evaluate the prevalence, characteristics and severity of road accidents and their changes through the 16 years of observation and to estimate the impact of recent lifestyle and legislation changes on road accident epidemiology.

METHODS

We retrospectively evaluated data from the DATIS/SINI-ACA registry considering all ECU admissions to one of the main hospitals in Genoa due to road accidents from January 1st, 2008 to December 31th, 2023. The observation period was divided into subgroups considering legal regulation changes for further studies. We used the data software STATA 14.2 SE® for data analysis. We described the events using absolute and percentage frequencies. Chi-squared test (χ^2) had been used to evaluate differences between categorical

variables, p-values <0.05 were considered as statistically significant.

RESULTS

During the 16 years of the study (2008-2023) we documented a total of 33.708 road accidents, with an overall lowering trend and a dramatic deflection in 2020 along with the COVID pandemic. Our local data followed a similar trend of national registries but with a greater percentage decline during the years. The majority of the accidents involved motorbikes (16.779) followed by cars (6.379), public transportation services (2.467), pedestrians (1.620), bicycles (1542), microcars (685), and heavy vehicles (225). Nearly half of the victims (49,78%) were motorcyclists and the drivers were the most frequently involved (55,08%); in total 19.104 (56,67%) of injured people were males and 14.604 (44,33%) females, most commonly in the 20-29 years (7041 patients, 20,89%) and e 40-49 years (6550 patients,19,43%) age-range. Triage codes had been classified as "urgent" in 20,71% of the events; female victims were more likely to show lower priority injuries compared with males. Accidents with bicycles, microcars and pedestrians had a greater chance of receiving higher priority triage codes. Pandemic effects on people's habits and both national and local policies about sustainable mobility caused interesting changes in road accidents epidemiology for what concerns the kind of involved vehicles and the severity of the events.

CONCLUSIONS

Road safety is a crucial issue in public health. Collecting and interpreting data on road accidents is an important tool for evaluating and implementing preventive strategies such as changes in legal regulations. In the next future we are planning to analyze data on drugs and alcohol-related events aiming to quantify the effect of the recently approved changes in traffic laws.

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Abuse in Individuals with Multiple Sclerosis: The SocialMS Italian Study

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INTRODUCTION

Abuse is a social determinant of health (SDH) which consists in behaviors used to gain or maintain power and control, including physical, sexual, financial, emotional or psychological actions [1]. In general, individuals with disabilities are at higher risk of abuse; and diseases like Multiple Sclerosis (MS) can cause both physical and cognitive disability [2,3]. However, no studies have explored abuse prevalence and risk factors in Italian individuals with MS [4].

AIMS

To estimate the prevalence of abuse in individuals with MS in Italy exploring a wide range of other SDH.

METHODS

The SocialMS is a cross-sectional observational study based on anonymous surveys submitted to patients followed up in 67 MS centers. Disability was self-assessed by the Patient Determined Disease Steps (PDDS) scale. Descriptive statistics, parametric and non-parametric tests and cluster analysis were performed; Gradient Boosting Machine (GBM) was used as an exploratory screening and logistic regression models were performed to quantify the impact of MS itself on increased risk of abuse.

RESULTS

We included 1,004 patients (mean (sd) age: 44.1 (11.6), 68.8% females, median (IQR) PDDS: 1.0 (0.0, 3.0)). A total of 235 individuals (23.4%) were victim of at least one form of abuse over the course of life: emotional or psychological (n=203, 20.2%, mainly in the workplace, in the relationship with the partner and within family), physical (n=56, 5.6%, mainly in the relationship with the partner and within family), financial (n=42, 4.2%, mainly in the relationship with the partner and in the workplace) and sexual (n=25, 2.5%, mainly in the relationship with the partner, within family and in other contexts) (Figure 1). Victims of abuse were younger ($p<0.001$), lived alone ($p=0.001$), were smokers ($p=0.018$), with higher BMI ($p<0.001$) and comorbidities ($p=0.036$), younger at diagnosis ($p=0.001$) and more disabled ($p=0.029$). Abuse was more frequent in individuals who were females ($p=0.048$), non-heterosexual ($p<0.001$), with lower literacy ($p=0.001$) and with financial difficulties ($p<0.001$) but victims of abuse received more tangible support ($p=0.004$). Geographical area (South and Center vs North), lower literacy or educational level and financial difficulties had a stronger impact on financial abuse compared to other forms. According to GBM, the characteristics with higher relative influence in characterizing the risk of any form of abuse were household income, BMI, age and age at diagnosis, personal income, living alone or only with children, sexual orientation and health literacy. A total of 539 individuals (54%) believed that MS itself could increase the risk of any forms of abuse, of whom 29 for per-

sonal experience, and factors associated in univariable analysis were progressive phenotype (OR=1.69 (95%CI=1.16; 2.46)), longer disease duration (OR=1.02 (1.00; 1.03)), number of treatments (OR=1.11 (1.00; 1.23)) and greater PDDS (1.19 (1.11; 1.27)). However, PDDS was the only factor which was statistically significant in the multivariable model (OR=1.18 (1.09; 1.27)). Cluster analysis identified two subgroups of participants: in cluster 2 (n=157) all patients were victims of at least one form of abuse and experienced a greater number of forms of abuse compared to cluster 1 (median(IQR): 1.0 [1.0, 2.0] vs 0.0 [0.0, 0.0], $p<0.001$) and all the forms of abuse were more common (emotional or psychological abuse: 99.4% vs 5.5%; sexual abuse: 12.7% vs 0.6%; physical abuse: 23.6% vs 2.2%; financial abuse: 13.4% vs 2.5%, $p<0.001$). Moreover, 68.8% of participants in cluster 2 perceived the potential impact of MS itself on the increased risk of abuse, of whom 13.4% for personal experience.

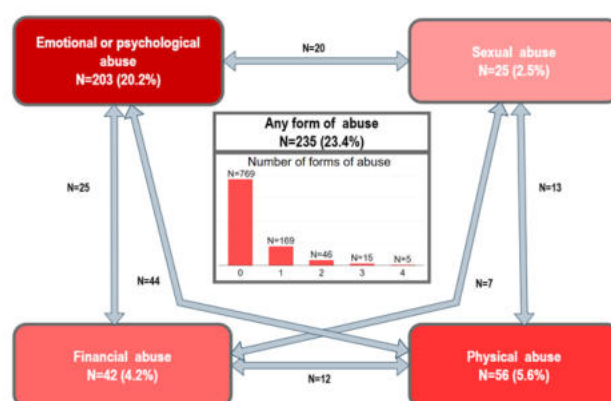
CONCLUSION

We characterized abuse phenotypes from clustering, we conducted robust identification of independent predictors from high-dimensional ML-based screening of predictors, and we observed the impact of MS disability on risk of abuse. Findings show the necessity of 1) identifying actionable targeted interventions to address modifiable SDH and to support victims of abuse (e.g., training of health-care providers, social and psychological services), 2) treating the Person with MS, not only MS, 3) increasing the awareness among the health-care practitioners and the general population that preventing MS-related disability can also have an impact on many hidden aspects of patients' lives. This work emphasizes the ethical and social importance of protecting the most vulnerable individuals.

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Figure 1. Prevalence of abuse in a sample of 1,004 individuals with multiple sclerosis in Italy. Arrows show numbers of individuals reporting more forms of abuse



Cluster Analysis to Identify Frailty Profiles in Individuals with Chronic Neurological Diseases: A Strategy Focused on Access to Health and Social Care Services in People with Multiple Sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune neurological disease, and the leading cause of neurological disability in adults. In Italy, 140,000 people were affected in 2024, with 3,600 new cases each year [1]. MS impacts patients' physical, psychological, social, and economic well-being, leading to multifaceted care that includes disease-modifying drugs (DMDs), symptomatic therapies, and rehabilitation [2]. The most common form is relapsing-remitting MS, which can progress to secondary progressive MS over time. Around 15% of patients have primary progressive MS from the onset, for which treatment options are limited. Initially, patients are referred to neurological services for diagnosis and DMD treatment. Still, many, especially those in the progression phase, are no longer followed by clinical centers as the disease progresses and DMDs become less effective [3]. These subjects often present severe forms of the disease and have a significant need for personal care and support, which the Italian system generally struggles to provide [4]. They are at risk of receiving suboptimal health care (sporadic access to a neurologist), and little, if any, multidisciplinary management is provided for their symptoms.

Knowing the accessibility of health and social health services that can meet the needs of people with MS is an important element because it is an indirect indicator of the health system's ability to coordinate and personalize care for people with MS for all phases of their disease.

This study aimed to use cluster analysis to identify relevant subgroups based on the accessibility and satisfaction of health and social care services that meet the needs.

METHODS

Data were obtained from a national cross-sectional study conducted by the Italian Multiple Sclerosis Foundation in 2024, which investigated health and social care needs, including the use and satisfaction of local services.

The following variables were used to study the cluster profiles in the sample: Integrated Care Experience Scale; distance from health, basic necessities and transport services; adequacy of health services on your territory for your needs; completeness of care of symptoms and comorbidities by health specialists; knowledge of MS by non-specialised MS health professionals; MS knowledge by social service workers; level of knowledge of the functioning of and access to social and health services; ability of the clinical centre to refer to specialists for MS symptom management and comorbidities; capacity of the clinical centre to orientate towards rehabilitation services. All variables are based on a Likert scale. Min-max normalization was applied to rescale continuous variables to a 0–1 range. All observations with missing values were excluded, resulting in a final sample of $N = 7,419$.

To reduce dimensionality, we applied ISOMAP, as the data were not standardized, and to capture potential non-linear structures. We selected 5 components, as they jointly explained 85% of the total variance, based on a preliminary Principal Component Analysis (PCA).

Subsequently, we performed clustering using the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) algorithm [5]. This method is suitable for identifying non-spherical clusters, automatically estimates the optimal number of clusters, and handles outliers effectively.

We performed a grid search to optimize the neighborhood radius (ϵ) and the minimum number of points (minPts) for clustering. We selected configurations with a silhouette score > 0.5 , 2–5 clusters for meaningful and interpretable segmentation, and $< 25\%$ outliers to prevent excessive data loss.

The final parameters were $\epsilon=0.54$ and minPts=19.

Differences between profiles in terms of sociodemographic and clinical characteristics were analyzed using the χ^2 test for categorical variables and the ANOVA test for continuous variables. Statistical significance was set at $\alpha = 0.05$. When ANOVA test yielded significant results, Bonferroni post-hoc comparisons were conducted. Cluster analysis was performed with MATLAB 2024b.

RESULTS

Five clusters, including 7,188 subjects, were identified; DBSCAN failed to assign 231 individuals to any dense region, and they were therefore categorized as outliers (cluster -1) and removed.

These five distinct profiles reflected varying levels of access and satisfaction with health and social care services in relation to individual needs. They were categorized as follows (Fig.1):

- Low access and poor satisfaction with services received (19.6%);
- Low to moderate access, with more complete health services received for the management of symptoms and comorbidities (32.7%);
- Moderate access, with better perceived adequacy of health services (3.6%);
- Fair access, with good perceived adequacy and completeness of services for managing symptoms and comorbidities (7.6%);
- Sufficient access, meeting basic health and social care needs (36.6%).

Each cluster demonstrated different demographic and clinical patterns, delineating distinct types of service access. Notably, profile 1 included individuals with MS who were older, lived mainly in the south/islands, had a lower educational level, a longer disease duration, higher levels of disability, less use of DMD, and reported more comorbidities. This finding lends support to the hypothesis that individuals affected by more severe forms of the disease and greater care demands frequently face unmet needs, in part due to systemic limitations within the Italian healthcare system.

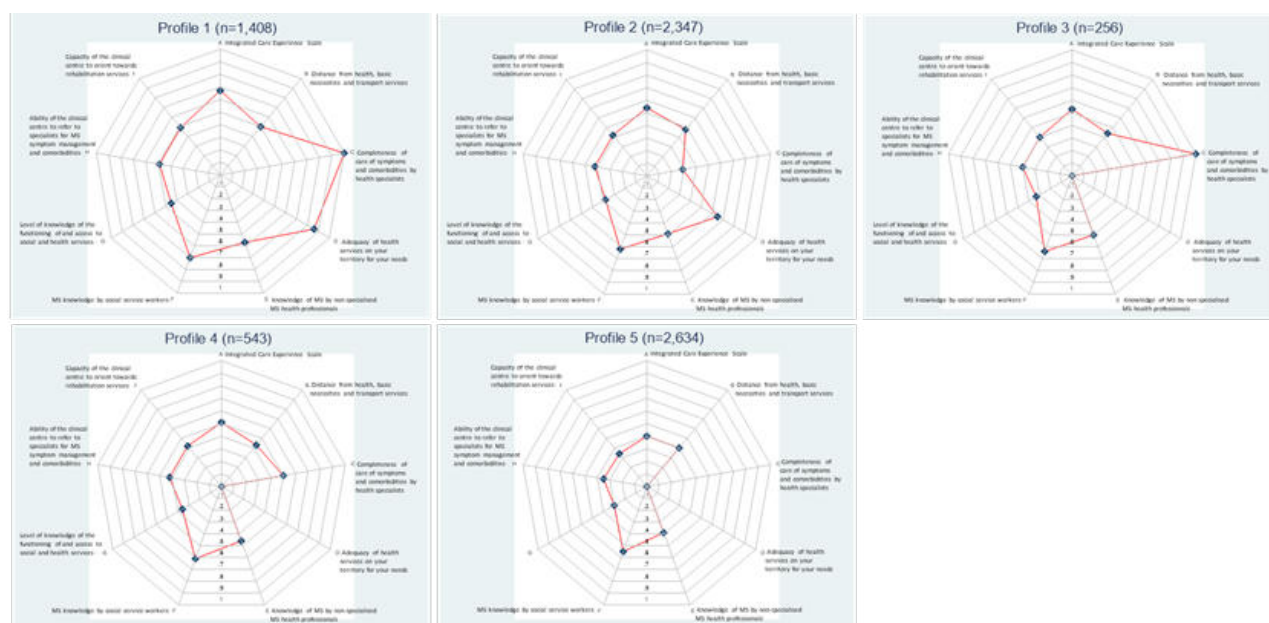
CONCLUSION

Identifying the accessibility profile of services is useful because it could address the health system across targeted care management strategies. This is important in order to save costs and improve the effectiveness of services for groups with different care needs.

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Figure 1. Radar plots displaying rescaled mean values for each profile. Each plot illustrates the mean values of variables within a profile, with all axes scaled to the [0, 1] interval



A) Integrated Care Experience Scale; B) Distance from health, basic necessities and transport services; C) Completeness of care of symptoms and comorbidities by health specialists; D) Adequacy of health services on your territory for your needs; E) Knowledge of MS by non-specialised MS health professionals; F) MS knowledge by social service workers; G) Level of knowledge of the functioning of and access to social and health services; H) Ability of the clinical centre to refer to specialists for MS symptom management and comorbidities; I) Capacity of the clinical centre to orient towards rehabilitation services

Canine Lymphoma: Retrospective Analysis of the Histopathological Registry of the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"

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INTRODUCTION

Neoplasms of the lymphatic system, and in particular lymphoma, represent the most common hematopoietic tumors in dogs and share many similarities with human non-Hodgkin lymphomas (NHLs), both in terms of clinical behavior and cyto-histopathological and immunophenotypic characteristics [1]. Epidemiological data on the incidence of NHLs place them among the top ten cancers by frequency in Italy, equally for men and women, equal to about 3% of all neoplasms. The analysis of incidence trends confirms a substantial stabilization of the increase in new cases in recent years.

In veterinary medicine, unfortunately, the acquisition of reliable epidemiological data is difficult, due to the few animal tumor registries present throughout the national territory. The scientific literature on the subject provides fragmentary values showing that lymphoma is, even in the canine species, a very represented neoplastic disease, with incidence rates of 22.9 in females and 19.9 in males [2].

AIMS

The aims of our observational retrospective study were to analyze the territorial distribution of canine lymphomas in Umbria region and to identify potential risk factors.

METHODS

A ten-year dataset of canine lymphoma cases examined at the histopathological diagnostic laboratory of the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" was evaluated. The study cohort included 4946 dogs, divided into two groups: 202 dogs diagnosed with lymphoma (group K) and 4744 dogs without neoplastic conditions (group NT). Dogs diagnosed with neoplasms other than lymphoma were excluded. Data were collected on individual characteristics including age, sex, breed, body size, geographical location, housing conditions, and dietary habits. Descriptive analysis was carried out using mean and standard deviation ($\pm$ sd) or median and interquartile range (IQR) and n (%). Normality distribution for quantitative variables was assessed by the Shapiro-Wilk Test. The association between categorical data was investigated by Pearson χ^2 or Fisher's exact test and the Student's t-test for independent data or analogue non-parametric test (Wilcoxon rank sum test). Complementary log-log (cloglog) model was applied to identify the mutually adjusted effect among K/NT groups and the independent variables. A statistical significance was set at the level of ≤ 0.05 . All analyses were performed using Stata software v18.0 MP (StataCorp, College Station, USA), GraphPad Prism 10 and geospatial mapping conducted with QGIS 3.40.4.

RESULTS

Among the 4946 dogs, females were 51.3%, and the mean age was 8.7 ± 3.5 years. A significant lower age was revealed in K group (8.7 ± 3.5 years) vs NT group (9.4 ± 3.3 years), with $p=0.004$. A statistically association was found between dietary habits and lymphoma group ($p<0.001$). No significant relationships were observed regarding sex, breed, housing conditions, or urban versus rural habitat. Cloglog model shows that the occurrence of lymphoma diagnosis increased, independently of other variables, for each increment of 1 year of age (OR 1.05, 95%CI: 1.01-1.09, $p=0.017$), with a mixed diet (OR 2.83, 95%CI 1.04-7.70, $p=0.041$) and be included in the risk breeds (OR 1.36, 95% CI 1.00-1.86, $p=0.05$). Among diagnosed cases, the most frequent topographical sites were spleen (27.2%), and lymph nodes (26.2%), followed by gastrointestinal tract (20.3%) and skin (20.8%). Geospatial analysis revealed case clusters within several municipalities in the Umbria region, which could be suggestive of environmental exposure patterns requiring further investigation.

CONCLUSIONS

Previous studies have shown that specific chemical exposures are associated with the risk of contracting lymphoma in dogs, such as commercially applied herbicides, the domestic use of paints and some solvents. Environmental exposures have also been associated, including proximity to industrial areas, waste incinerators, polluted sites, exposure to radiation, electromagnetic fields and secondhand smoke. As with dogs, exposure to these factors is associated with an increased risk of lymphoma in humans [3]. The study of the behavior of spontaneous dog tumors and the possible role played by intrinsic risk factors (e.g. sex, breed, etc.) and extrinsic risk factors (e.g. environmental factors) in the determinism of the tumors themselves, can therefore provide useful indications for the prevention of neoplasms affecting humans and constitute an integrated system of permanent epidemiological surveillance.

This study stresses the value of veterinary tumor registries and the potential of dogs as spontaneous models for human cancer epidemiology. It also confirms the importance of dietary and environmental risk factors in the development of lymphoma in dogs, reinforcing the need for targeted preventive strategies and more structured surveillance systems.

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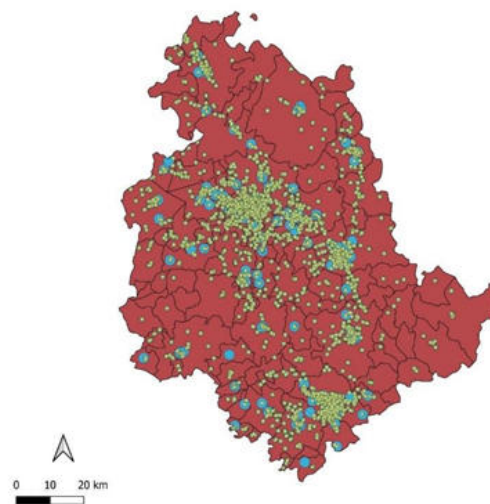


Fig. 1 Distribution of dogs diagnosed with lymphoma (group K, blue dots) and dogs without neoplastic conditions (group NT, green dots).

Access to Emergency Department in the Marche Region During 2011-2023: Exploring Differences between Italian and Migrant Residents (MIGHTY PROJECT P2022ASXKR)

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INTRODUCTION

Scientific literature reports high geographical variability in migrants' health status and access to healthcare services in Europe and inconsistent evidence on their access to Emergency Department (ED) respect to natives [1-3]. Nevertheless, migrants' accesses to ED for non-urgent conditions, presenting as "walk-ins", and during unsocial hours seems to be higher than natives [4-5].

AIM

We want to investigate whether people coming from High Migratory Pressure Country access to the ED differently than Italians and to explore differences according to their demographic characteristics and clinical presentation, in Marche Region between 2011 and 2023, using healthcare utilization databases.

METHODS

In this cross-sectional study, we used the Emergency Department and the Regional Beneficiary databases of the Regional Healthcare System as data sources. In this study, residents in the Marche Region were distinguished by citizenship

as Migrants, considering only those who came from High Migration Pressure Countries (HMPC) [6], and Italians. Sex, age, arrival mode (walk-in, ambulance, other emergency medical services), access time (daytime: 8:00-20:00; nighttime: 20:00-8:00), and distribution of main access diagnosis (first three digits of ICD-9 CM code) were analyzed by citizenship.

ED admission rates per 1,000 person-years (py) were standardized by age and stratified by sex using the direct method and the Italian ISTAT population as of January 1, 2019, as the standard population [7]. The standardized rate ratios (SRRs) were calculated as the ratio of HMPC to Italian rates and estimated with a 95% confidence interval (95%CI). SRRs were also stratified by three age groups (0-19, 20-65, over 65 years) and by triage categories based on priority: emergent (red), urgent (yellow, orange, blu), less urgent (green), non-urgent (white), according to the new Regional Guidelines, DGR n. 1457/2019. All data were processed in compliance with the European (GDPR, EU 2016/679) and national privacy laws (D.lgs. 196/2003 and subsequent amendments).

RESULTS

In the period 2011-2023, there were 5,189,603 visits to the ED for Italians and 442,514 for HMPCs, corresponding

to 1,253,820 and 114,503 subjects, respectively. In the entire period, HMPC subjects accessing the ED were younger (mean age, SD: 33, 18 years) and more frequently female (54%) than Italians (mean age, SD: 50, 27 years; female: 49%). Both HMPCs and Italians presented to the ED more frequently as “walk-ins” (79% vs 73% respectively), in ambulance (12% vs 17% respectively), and at night (66% and 64% respectively). The most frequent main access diagnoses were “trauma or poisoning” (27% Italians vs. 21% HMPC), followed by “symptoms, signs, ill-defined conditions and unknown causes of morbidity” (16% Italians vs. 18% HMPC), in both populations.

Overall standardized ER access rates for Italians and HMPCs were 279.1 per 1,000 py (95%CI 279.1 - 279.2) and 275.6 per 1,000 py (95%CI 275.5 - 275.6), respectively. Excluding the pandemic years, the lowest values were observed in 2012 (266 per 1,000 py) in Italians and in 2015 (250 per 1,000 py) in HMPCs, while the highest values were observed in 2019 (321 and 317 per 1,000 py, respectively) for both populations. During the pandemic years, as expected, a decrease in access rates was observed in both populations (213 and 240 for Italians versus 212 and 249 for HMPCs in 2020-2021, respectively).

SRRs showed that HMPC men had lower access rates than Italian men between 2012 and 2020, while HMPC women had lower access rates than Italian women between 2014 and 2018; SRRs were above 1 in other years (Figure 1).

In the stratified analysis by age group, the standardized ED access rates and SRRs comparing the HMPC with Italians were: 341.7 versus 314.7 in the 0-19 age group (SRR 1.085, 95%CI 1.085-1.086); 272.6 versus 231.8 in the 20-65 age group (SRR 1.176, 95%CI 1.176-1.176); 243.0 versus 381.9 in the over 65 age group (SRR 0.636, 95%CI 0.636-0.637).

Overall, ED admission rates standardized by HMPC and Italian triage categories were, respectively: emergent 4.9 vs 6.4 per 1000 py (SRR 0.757, 95%CI 0.756-0.758); urgent 68.6 vs 75.1 per 1000 py (SRR 0.913, 95%CI 0.913-0.914); less urgent 169.7 vs 171.3 per 1000 py (SRR 0.991, 95%CI 0.991-0.991); non-urgent 24.9 vs 18.9 per 1000 py (SRR 1.317, 95%CI 1.316-1.318).

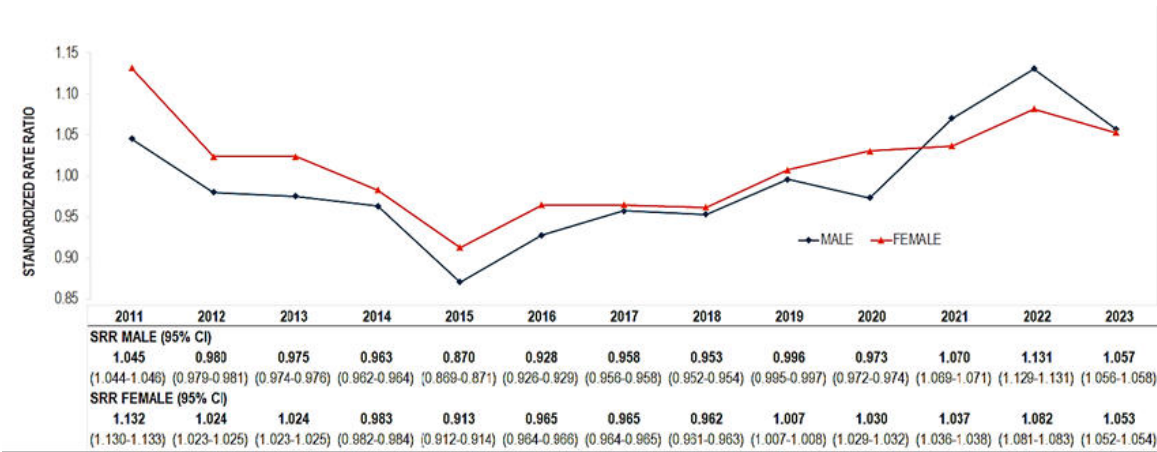
CONCLUSIONS

The use of healthcare utilization databases has allowed us to assess the heterogeneity of emergency department access in Migrant and Italian populations. However, analyses stratified by age and triage highlighted a higher emergency department access in Migrants younger than 65 years and for non-urgent conditions compared to Italian citizens. Further analyses are needed to identify factors associated with different emergency department access based on citizenship, in order to promote healthcare strategies for appropriate access to emergency care.

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Figure 1 - Standardized Rate Ratios (95% CI) between emergency department access rates of Migrants from High Migratory Pressure Country and Italians in the Marche Region (2011-2023)



Intensity and Type of Physical Activity and Semen Quality in Young Healthy Men Living in Brescia, North Italy

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INTRODUCTION

A decline in semen quality has been documented over recent decades, possibly linked to rising rates of obesity, unhealthy diets, and exposure to environmental toxins, although the exact causes remains a topic of debate [1]. Recreational physical activity (PA) has been associated with numerous health benefits, including the prevention of chronic diseases and the promotion of overall well-being, and it is strongly recommended by the World Health Organization (WHO) and various Scientific Societies.

Physical activity is hypothesized to have a positive effect on semen quality due to its favorable impact on metabolic and endocrine functions [2]. However, excessive physical activity may have the opposite effect, potentially leading to reduced semen quality and fertility. This may be due to impaired function of the hypothalamic-pituitary-gonadal axis, increased oxidative stress, and chronic inflammation [3–5].

AIMS

To investigate this topic and add evidence, we conducted a cohort study aimed to assess the relationship between PA intensity and semen quality – measured by sperm concentration, total and progressive motility, and proportion of normal morphology cells – in a population of healthy young men living in Brescia, North Italy.

MATERIALS AND METHODS

A prospective study within the FAST randomized trial was conducted between April 2018 and June 2019 [6]. Semen quality parameters were assessed at the enrollment (baseline) and again after 4 and 8 months. Each semen sample was collected in a sterile container via masturbation after a period of sexual abstinence lasting at least 3 days and no more than 5 days. Samples were delivered to the laboratory within 30–40 minutes of collection, and a portion of each sample (<50 µl) was immediately processed for semen analysis (spermogram). Additionally, a 20 ml blood sample was collected from each participant under fasting conditions.

PA was assessed at baseline and after 4 and 8 months using the International Physical Activity Questionnaire (IPAQ), which evaluates various types of activity – including walking, moderate-intensity, and vigorous-intensity activities – and estimates the total energy expenditure expressed in Metabolic Equivalent of Tasks (METs).

Due to the longitudinal nature of the data, a linear mixed model with robust variance estimation was used to assess the association between PA with sperm concentration. A generalized linear mixed model with a Poisson distribution was applied to evaluate total, progressive motility and normal morphology cell counts, using the total number of cells as an offset.

Restricted spline regression models were fitted to model the potential nonlinear shape of the associations between total PA and semen parameters.

RESULTS

A total of 143 young healthy men (median age 20 years, IQR 19-21 years) participated in the study. The majority were engaged in moderate (45%) or high (43%) recreational PA, with a median expenditure of 1,960 (95% confidence interval, 1,055–3,182) Metabolic Equivalent of Tasks in min/wk.

The main results are presented in Table 1. An increase in total sperm motility (IRR 1.11, 95% CI, 1.05-1.17) and normal morphology (IRR 1.18, 95% CI, 1.03-1.35) was observed among participants engaged in moderate PA. Conversely, an inverse association was observed for walking and vigorous-intensity PA. No association was observed between PA and sperm concentration.

An inverse U-shape relationship was identified, with the highest values of total sperm motility and normal morphology occurring at intermediate levels of PA. No statistically significant trend was found for sperm concentration, although a U-shaped relationship association was suggested by the restricted cubic spline model.

CONCLUSIONS

Our findings are consistent with several studies previously conducted on healthy young men from the general population, as well as on male partners of infertile couples, which have shown that individuals engaging in moderate-to-high levels of physical activity tend to exhibit better semen quality compared to those with sedentary lifestyles or very high levels of activity. The results support current recommendations to engage in moderate physical activity to promote overall health, including improvement in semen quality [7]. Future research should investigate the mediating role of DNA methylation in the relationship between physical activity and semen quality.

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Table 1. Multivariable association estimates between physical activity levels and semen quality parameters

	Sperm concentration (10 ⁶ /ml)	Total motility (%)	Cell with normal morphology (%)
Physical activity	(95% CI)	IRR (95% CI)	IRR (95% CI)
IPAQ (x1000 MET)	0.52 (-0.83,1.9)	0.98 (0.97,0.99)	0.99 (0.97,1.00)
IPAQ category			
Low	Ref	Ref	Ref
Moderate	-8.40 (-17,-0.53)	1.11 (1.05,1.17)	1.18 (1.03,1.35)
Intense	-3.30 (-13,6.7)	1.01 (0.94,1.07)	1.09 (0.95,1.26)
Walking METs (x1000 METs)	-0.73 (-3.8,2.3)	0.95 (0.93,0.98)	0.94 (0.89,0.99)
Moderate METs (x1000 METs)	1.80 (-0.85,4.4)	1.00 (0.99,1.02)	1.00 (0.97,1.04)
Vigorous METs (x1000 METs)	0.54 (-1.4,2.5)	0.97 (0.96,0.98)	0.99 (0.97- 1.01)

Statistically significant at $\alpha=0.05$

Developing a Framework for Assessing the Applicability of the Target Condition in Diagnostic Research

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KEYWORDS: Applicability, Systematic review, QUADAS-2, Reference standard, Target condition

INTRODUCTION

Assessment of the applicability of primary studies to the review questions is an essential but often challenging aspect of systematic reviews of diagnostic test accuracy studies (DTA reviews).

OBJECTIVES

To explore review authors' applicability assessments for the QUADAS-2 reference standard domain within Cochrane DTA reviews, by highlighting applicability concerns, identifying potential issues with assessments. Using the findings to develop a framework for assessing the applicability of the target condition as defined by the reference standard.

METHODS

Methodological review. DTA reviews in the Cochrane Library that used QUADAS-2 and judged applicability for the reference standard domain as "high concern" for at least one study, were eligible. One reviewer extracted the rationale for the "high concern", this was checked by a second reviewer. Two reviewers categorized the rationale inductively into themes, a third reviewer verified these. Discussions in the QUADAS development group (steering group and expert panel) regarding the extracted information informed framework development.

RESULTS

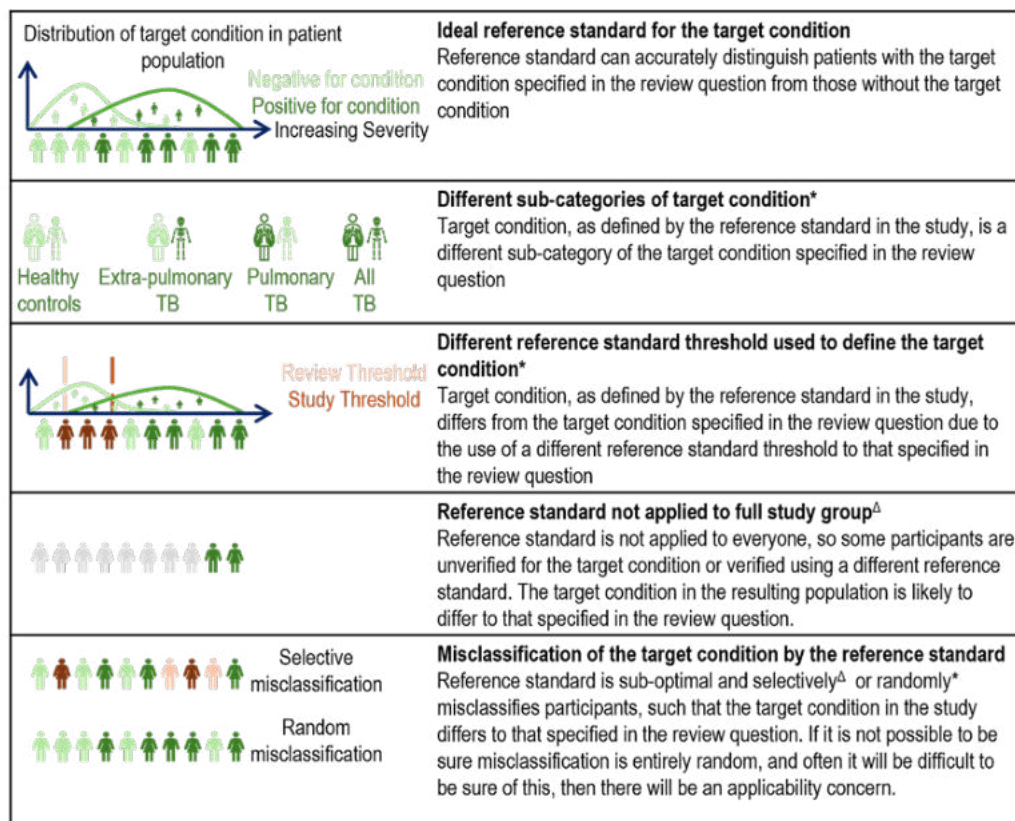
We identified 50 eligible reviews. We identified potential issues over half ($n=28$) of the included reviews. In 7, applicability assessments deviated from QUADAS-2 definitions and in 21 applicability concerns were insufficiently described. Five themes emerged in the remainder: study uses different reference standard threshold to define the target condition (6 reviews), misclassification by the reference standard in the study such that the target condition in the study does not match the review question (11 reviews), reference standard could not be applied to all participants resulting in a different target condition (5 reviews), misunderstanding QUADAS-2 applicability (7 reviews), and insufficient information (21 reviews).

The Figure shows the framework that was informed by our findings and discussions. Our framework outlines four potential applicability concerns for the assessment of the target condition as defined by the reference standard: different sub-categories of the target condition, different threshold used to define the target condition, reference standard not applied to full study group, and misclassification of the target condition by the reference standard (Figure).

CONCLUSION

Clear sources of applicability concerns are identifiable, but several Cochrane review authors struggle to adequately identify and report them. We have developed the first applicability framework to guide review authors in their assessment

of applicability concerns for the QUADAS reference standard domain. The development of a framework for the QUADAS-2 domains Patient selection and Index Test are ongoing.



^ΔApplicability issue linked to risk of bias (RoB) judgement (if high applicability concerns applicability, then high RoB); *Applicability issue separate from RoB judgement

Figure 1. Framework for assessing the applicability of the target condition in diagnostic research

QUADAS-3: Updated Tool to Evaluate Risk of Bias and Applicability Concerns in Diagnostic Test Accuracy Studies

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KEYWORDS: Risk of bias assessment, applicability, diagnostic test accuracy, systematic review

INTRODUCTION

The QUADAS-2 tool, published in 2011, was designed to evaluate the risk of bias and applicability of diagnostic test accuracy (DTA) studies. The publication reporting QUADAS-2 has been cited over 12, 000 times and it is the recommended tool to assess risk of bias and applicability of studies for major HTA organizations. Although feedback on QUADAS-2 has generally been positive, some signaling questions have been identified as problematic and the tool could be improved based on features included in more recently developed tools.

OBJECTIVES

To update QUADAS-2 to develop the new QUADAS-3 tool.

METHODS

We established a core-group of methodological experts to lead the development of the QUADAS-3 tool supported by a wider steering group.

We followed the following steps:

- Summarised modifications made to QUADAS-2 for the Cochrane Handbook
- Web-based survey of reviewers that have used QUADAS-2
- Considered developments from more recent tools in

terms of tool structure and implementation

- Undertook a review of methodological studies that had evaluated QUADAS-2

- Undertook a review of 50 Cochrane DTA reviews to highlight challenges with the assessment of applicability

We have produced a draft tool which has undergone piloting. The results of the piloting, which also included a comparison of the use of signalling questions with signalling statements, was used to inform the final version of the tool.

RESULTS

The new tool follows a similar structure to the QUADAS-2 tool but with some major updates. Key changes include:

- An option to define separate synthesis questions rather than just a single review question
- A new section on defining the ideal test accuracy trial for each synthesis question
- Assessment of risk of bias and applicability at the accuracy estimate level rather than the study level
- A change in answers to signaling questions to include options of "probably yes" and "probably no" and to replace "unclear" with "no information"
- Replacement of "Flow and Timing domain" with new "Analysis" domain
- Changes to some signaling questions
- Inclusion of a section for judging overall risk of bias and applicability (across domains)

CONCLUSIONS

The QUADAS-3 tool incorporates several changes compared to the previous version (QUADAS-2) which we hope will improve its validity, usability, and usefulness. QUADAS-3 will be introduced at the conference and the results of piloting discussed.

Understanding the Difference between Risk of Bias and Concerns Regarding Applicability Using QUADAS-2: A Methodological Review

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KEYWORDS: Risk of bias assessment, applicability, diagnostic test accuracy, systematic review

INTRODUCTION

Diagnostic test accuracy (DTA) systematic reviews bring together findings from DTA studies to summarise the accuracy of a diagnostic test. Studies included in a DTA review should be assessed for risk of bias and applicability concerns, because a review of biased studies, or studies that do not apply directly to the review question, could result in misleading conclusions. Studies are most commonly assessed with the QUADAS-2 tool. Anecdotal evidence has suggested that researchers sometimes struggle to differentiate between risk of bias and applicability. Here, we investigate this distinction for the patient selection and index test domains.

OBJECTIVES

To develop a framework for assessing the applicability of the study target population and the study index test to the review defined target populations and index test(s). We aimed to explore review authors' applicability assessments for the QUADAS-2 patient selection and index test domains, to inform the framework.

METHODS

DTA reviews were eligible for inclusion if they were published in the Cochrane Library, had used QUADAS-2, and had at least one study rated as "high concerns" for appli-

cability of the patient selection or index test domain. Review selection was checked by a second reviewer. From each review, we extracted article identifiers such as title, authors and publication date, extracted the primary objectives and elements of review questions: population, index test(s), target condition, reference standard. For each review, we extracted the rationale provided by the authors for "high concerns" applicability judgements for the respective domains. We also extracted author's rules how to assess applicability concern with QUADAS-2, whenever it was tailored to the review topic. One reviewer assessed these rationales and rules, which were verified by another. We also recorded any other issues that arose as part of the applicability assessment, such as sub-optimal reporting or erroneous applicability assessment. Two reviewers categorized the rationales inductively into themes, which will be discussed in the QUADAS steering group. The final framework will be informed by the identified themes and thorough group discussions.

RESULTS

This review is in progress. Of the 186 available Cochrane DTA reviews, 123 met our inclusion criteria: 110 for the patient selection domain, 75 the index test domain. The data extraction process is ongoing and final results will be presented at the conference. The majority of the more recent Cochrane DTA reviews include guidance tailored to the review's topic. Review authors typically have a broad review question, whereas signaling questions tailored to the review's topic are

more restrictive. Several themes emerged in the patient selection domain. These include: study setting not matching the review question, study unit not matching the review study unit (lesion level versus patient level); study's target condition not meeting the review's target condition (in-vitro versus in-vivo); study's disease spectrum not covering the review's targeted disease spectrum (i.e. due to sampling methods, choice of selection criteria; inappropriate exclusions) and study's indication for testing not sufficiently matching the reviews indication. In the index test domain, themes identified so far include a mismatch in the study and the review with respect to: index test technology; test protocol; thresholds to define the target condition; clinical background of the examiner; experience of the examiner; use of consensus test interpretation rather than use of the interpretation of a single examiner, use of clinical information. In both domains, a number of reviews misunderstood QUADAS-2 applicability or provided insufficient information on the rationale for high concerns. Once all themes are identified, the framework will be developed and presented as SISMEC.

CONCLUSIONS

Clear sources of applicability concerns are identifiable, but several Cochrane review authors struggle to adequately identify and report them. At SISMEC, we will present the applicability framework to guide review authors in their assessment of applicability concerns for the QUADAS patient selection and index test domains

Developing a Framework for Assessing the Applicability of the Target Population and Index Test in Diagnostic Research

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KEYWORDS: Risk of bias assessment, applicability, diagnostic test accuracy, systematic review

INTRODUCTION

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OBJECTIVES

To develop a framework for assessing the applicability of the study target population and the study index test to the review defined target populations and index test(s). We aimed to explore review authors' applicability assessments for the QUADAS-2 patient selection and index test domains, to inform the framework.

METHODS

DTA reviews were eligible for inclusion if they were published in the Cochrane Library, had used QUADAS-2, and had at least one study rated as "high concerns" for appli-

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more restrictive. Several themes emerged in the patient selection domain. These include: study setting not matching the review question, study unit not matching the review study unit (lesion level versus patient level); study's target condition not meeting the review's target condition (in-vitro versus in-vivo); study's disease spectrum not covering the review's targeted disease spectrum (i.e. due to sampling methods, choice of selection criteria; inappropriate exclusions) and study's indication for testing not sufficiently matching the reviews indication. In the index test domain, themes identified so far include a mismatch in the study and the review with respect to: index test technology; test protocol; thresholds to define the target condition; clinical background of the examiner; experience of the examiner; use of consensus test interpretation rather than use of the interpretation of a single examiner, use of clinical information. In both domains, a number of reviews misunderstood QUADAS-2 applicability or provided insufficient information on the rationale for high concerns. Once all themes are identified, the framework will be developed and presented as SISMEC.

CONCLUSIONS

Clear sources of applicability concerns are identifiable, but several Cochrane review authors struggle to adequately identify and report them. At SISMEC, we will present the applicability framework to guide review authors in their assessment of applicability concerns for the QUADAS patient selection and index test domains.

A Comparative Analysis of High and Low Physical Activity Levels among Children from 2023 “OKkio alla SALUTE” Survey

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INTRODUCTION

Physical activity (PA) in childhood is widely recognized as a critical determinant of long-term health, contributing to cardiovascular function [1], cognitive development [2], and overall quality of life [3], [4]. However, insufficient PA during childhood is a significant public health concern, associated with increased risk of obesity [5], cardiovascular disorders [6], and type 2 diabetes [7]. The World Health Organization (WHO) recommends that children aged 5-17 years engage in at least 60 minutes of moderate-to-vigorous physical activity (MVPA) daily and vigorous physical activity (VPA) at least three times per week [8]. Despite these guidelines, a considerable proportion of children fail to achieve these targets [9]. Understanding the factors influencing children's PA patterns, including socio-demographic, parental, and lifestyle characteristics, is essential for developing effective public health interventions [10].

OBJECTIVES

This study aimed to assess the levels of PA among Italian children aged 8-9 years, evaluating their compliance with the 2020 WHO recommendations for MVPA and VPA [8]. Additionally, it aimed to explore potential statistical associations between PA levels and socio-demographic, familial, and lifestyle factors.

METHODS

A cross-sectional, population-based study was conducted using data from the 2023 round of the “OKkio alla SALUTE” surveillance system, involving 42,873 third-grade chil-

dren (aged 8-9 years) across Italy. Data were collected from March to June 2023 through stratified cluster sampling, with classes as primary sampling units. Information on PA, dietary habits, screen time, and socio-demographic characteristics was collected via parent, teacher, and student questionnaires. PA levels were categorized as follows: MVPA (≥ 5 days a week) and VPA (≥ 3 days per week). Logistic regression models were used to evaluate the association between PA levels and independent variables, adjusting for the complex survey design, including dietary habits [11] (such as fruit and/or vegetables daily consumption, breakfast daily consumption), sedentary behaviour, parental education [12], and financial status [13], using a nationally representative sample.

RESULTS

Of the 42,873 children surveyed, 51.3% were boys, and 48.7% were girls. Among these, 63.5% were aged 8 years, and 36.5% were aged 9 years. Regionally, 50.6% resided in the North, 20.5% in the Centre, and 28.9% in the South. Nutritional habits showed that 59.8% consumed fewer than two servings of fruit and/or vegetables daily, while 68.8% had breakfast every day. In terms of sedentary behavior, 55.3% spent less than two hours per day in front of screens. Parental education varied, with 33.2% of mothers and 21.4% of fathers holding high qualifications. Financial status was good for 49.6% of families, while 5.0% faced significant economic difficulties.

PA data revealed that 30.2% engaged in VPA at least three days per week, while 32.9% met the MVPA criteria for at least five days per week. Boys were more likely to engage in both VPA (63.7%) and MVPA (54.1%) than girls. Higher parental education was positively associated with VPA but negatively related to MVPA: for example, children with

highly educated mothers (33.2%) and fathers (21.4%) were more likely to engage in VPA at least three days per week, while children with parents with lower education levels were less active. In contrast, a higher proportion of children with medium or low parental education engaged in MVPA for at least five days per week, indicating a complex relationship between educational level and PA. Children with better financial status were also more likely to be active, aligning with the descriptive statistics.

Logistic regression models confirmed these descriptive findings, indicating that gender, parental education, dietary habits, and lower sedentary behavior were consistent predictors of PA. Overall, these models validate the observed descriptive trends, confirming that socio-demographic and behavioral factors are critical determinants of PA.

CONCLUSIONS

This study confirms significant socio-demographic and behavioral disparities in PA levels among Italian children, with gender, parental education, and dietary habits emerging as critical determinants. These findings highlight the importance of tailored public health strategies to promote PA in early childhood, addressing socio-economic barriers and encouraging healthy lifestyle choices to reduce the long-term burden of physical inactivity [14], [15], [9], [16].

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The Evolution of Telemedicine in Pre-Post COVID-19 Period

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INTRODUCTION

In recent years, telemedicine has become increasingly widespread, with a marked rise in adoption during the COVID-19 pandemic. During the period of restrictions, telemedicine resulted to be particularly useful for managing various types of outpatient visits, both initial and follow-up. Several clinical studies have assessed the impact of telemedicine on patient compliance during the COVID-19 pandemic [1]. However, it remains unclear whether its use continued to grow in the post-pandemic period. This may depend on factors such as the efficiency of the telemedicine tools provided, the presence of a supportive organizational structure or patient's attitudes toward telemedicine [2].

OBJECTIVES

This study aimed to identify the typical patient using online monitoring, analysing demographics such as age, gender, and region, along with physician characteristics. It also examined which clinical units achieved the largest growth in telemedicine use. A secondary goal was to explore how patient preferences evolved over time. The analysis focused on telemedicine data from San Raffaele Hospital in Milan. The study period spanned from March 2020 to December 2024.

METHODS

Longitudinal data on visit frequency for patients aged over 18 years were analyzed, focusing on individuals with at least two visits during the study period. Generalized linear mixed models (GLMMs) with a random intercept for patient were used to account for within-subject correlation [3]. The outcome variable (visit count) was modeled using a negative binomial distribution due to overdispersion [4]. Two separate models were estimated to evaluate the characteristics of the visit and the ones of the patient. For the first case, the fixed effects included clinical unit, visit type, physician characteristics, and semester.

For the second case, fixed effects included patient characteristics, region, and semester. To further investigate the variability in the number of visits, aggregated visit counts were calculated by clinical unit, gender, geographical area, and year. A GLMM was fitted, incorporating a random intercept for clinical unit to account for repeated measures within each statistical unit. In this analysis, the number of visits was modeled using a Poisson distribution, given the absence of overdispersion.

RESULTS

In general, the clinical unit with the highest number of visits was psychology, likely due to the nature of the visits themselves. Consequently, it was selected as the reference category in subsequent analyses. Results from the GLMM focusing on visit characteristics revealed the highest number of visits per patient in diabetology unit compared to psychology unit ($p=0.013$). The semester variable was always positively significant, indicating a constant increase in visits over time compared to the reference category, i.e. the first semester of 2021 (all $p<0.001$). The only negative estimate was observed for the first semester of 2020 ($p<0.001$), reflecting the impact of the first wave of the COVID-19 pandemic.

In the analysis on patient characteristics, overall Generation X showed the highest number of online visits. This trend was confirmed by the GLMM results, in which all other generational groups (i.e., Greatest and Silent Generations, Boomers, Millennial, and Generation Z) had significantly fewer visits compared to Generation X. Again, the semester variable was positively associated with visit frequency, indicating a continuous increase over time respected to the first semester of 2021 (all $p<0.001$), except for the first semester of 2020 ($p<0.001$). Male patients had significantly fewer visits than female patients ($p<0.001$).

Finally, analyzing the visits per unit, the number of follow-up visits increased across all semesters, but declined significantly in the second semester of 2021 with respect to the first semester of 2021. Male patients had significantly fewer follow-up visits compared to female patients.

CONCLUSION

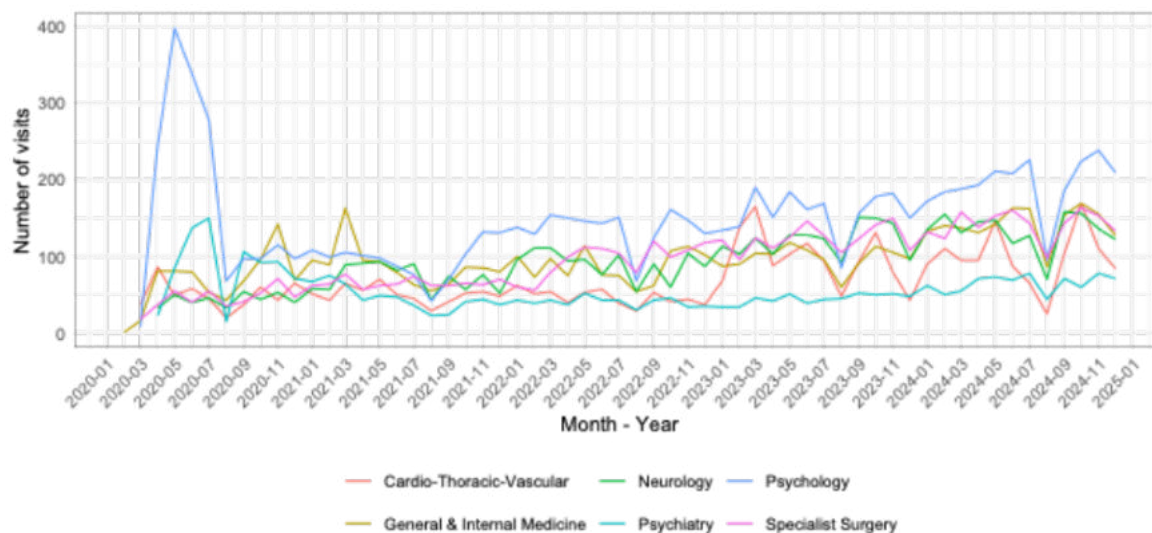
Our study highlighted a significant increase in the use of telemedicine from its initial implementation through 2024. Specifically, our data showed that psychology was the clinical unit with the highest overall number of telemedicine visits, likely due to its natural compatibility with remote care, but diabetology showed the highest number of visits per patient. From a generational perspective, the greatest users of telemedicine were the Generation X cohort, and the women were the major users with respect to men. Identifying such characteristics may be crucial for tailoring public health strategies and improving access to and the quality of telemedicine services across diverse patient populations.

Further insights may be gained through analysis of more specific visit-related characteristics and reasons for visit.

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Figure 1. Trend of monthly visits per patient across the most required clinical units in total sample



Do COVID-19 Pandemic Increase the Risk of Non-COVID-19 Mortality in Frail Elderly? A Real-World Retrospective Cohort Study

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INTRODUCTION

During the SARS-Cov2 pandemic, urgent measures were carried out to face the increasing healthcare demand due to the pandemic, including, among others, the suspension of non-urgent hospital activities and specialist consultations unrelated to COVID-19 disease [1]. These measures, however, significantly restricted access to hospital facilities for patients with chronic conditions, including cancer patients and frail elderly, suggesting a potential increased risk of non-COVID-19 mortality due to a lack of care of these vulnerable populations.

AIMS

The aim of the study was to compare the risk of non-COVID-19 mortality during the pandemic period with the pre-pandemic period specifically among elderly individuals with at least one chronic disease (vulnerable subjects) exploiting the data available in the healthcare utilization data from the Local Health Authority (LHA) of Vercelli province, Piedmont, Italy.

METHODS

This cohort study was conducted using the data available in the healthcare utilization databases of the LHA of Vercelli including: i) the registry of healthcare beneficiaries assisted by the LHA of Vercelli ii) the drug prescription database, iii) the hospital discharge database, iv) the exemptions databases and v) the mortality registry. The information included in the different databases belonging to the same subject were linked through an anonymized individual identification code which prevented the identification of the people included in the databases.

Two cohorts of subjects were selected: the pre-pandemic cohort included all subjects assisted by the LHA of Vercelli alive the 1st January 2018, aged 65 years or older and affected by at least one chronic condition in 2017. The pandemic cohort was made of all subjects alive the 1st January 2020, aged 65 years or older and affected by at least one chronic condition in 2019.

The outcome of interest was death due to causes other than COVID-19. The subjects of both cohorts identified in the mortality registry with a cause of death different from COVID-19 were considered as events.

The information regarding age, sex, vulnerability and the chronic disease from which cohorts' members were affected were retrieved for all subjects. The vulnerability level was defined according to the severity index, an index assigning to each chronic condition the corresponding severity score (from 1 not severe condition to 3 severe condition) according to the Italian Ministry of Health chronic diseases classification available in the COVID-19 vaccination plan [2]. Subjects were classified according to the most severe disease as non-vulnerable (severity index 0-1), vulnerable (severity index 2) and extremely vulnerable (severity index 3), further detail on the severity index are reported elsewhere [3].

Descriptive statistics were used to summarize the demographic and clinical information collected on study subjects overall and according to cohort. Categorical variables were reported as absolute frequencies and percentages by cohort and overall. The Chi square test was used to compare the distribution of subjects' characteristics between cohorts. Multivariable Fine and Gray [4] model was used to calculate the adjusted sub-distribution hazard ratios (asHR) and the corresponding 95%CI to evaluate the relationship between time periods and non-COVID-19 mortality accounting for COVID-19 mortality as competing risk. The rule-out approach was used to assess the impact of the unmeasured confounder seasonal flu on the association estimate.

RESULTS

91777 elderly subjects with at least one chronic disease were included in the study, 46048 belong to the cohort 2018 and 45729 to the cohort 2020. Overall, the proportion of females were slightly more frequent than males (57.58% vs 42.42%) and the mean age was 76.64 years. 44.63% of subjects were classified as vulnerable. Overall, the proportion of deaths tends to increase from 2018 to 2021 varying from 4.62% in 2018 to 5.40% in 2021, however, considering only non-COVID-19 mortality, the proportions of death in 2020 and 2021 were lower than 2019 (4.06% and 4.85% respectively).

Table 1 reports asHR and the corresponding 95%CI derived from multivariable Fine and Gray models for the association between time periods and non-COVID-19 mortality and the p-value of the trend test for age and vulnerability classes.

Table 1. Adjusted subdistribution hazard ratios (asHR), the corresponding 95% confidence intervals (95%CI), for the association between time periods and non-COVID-19 mortality and the p-value of the trend test

	asHR (95%CI)	trend
Pandemic vs pre-pandemic period	0.90 (0.87-0.95)	
Sex		
F vs M	0.73 (0.70-0.76)	
Age classes		
70-74 vs 65-69	1.55 (1.38-1.74)	<0.0001
75-79 vs 65-69	2.63 (2.36-2.93)	
80-84 vs 65-69	4.93 (4.46-5.46)	
85-89 vs 65-69	9.52 (8.62-10.51)	
≥90 vs 65-69	20.47 (18.53-22.61)	
Vulnerability classes		
Vulnerable vs non-vulnerable	1.20 (1.12-1.26)	<0.0001
Extremely vulnerable vs non-vulnerable	2.00 (1.88-2.13)	

The results of Fine and Gray model show that, among subjects that did not die due to COVID-19, the risk of non-COVID-19 mortality was 10% lower during the pandemic period compared to the pre-pandemic one. The results of the rule-out analysis show that part of the decreased risk of non-COVID-19 mortality observed in the pandemic period could be explained by decreased mortality for flu.

CONCLUSIONS

A decreased risk of non-COVID-19 mortality was observed during the pandemic period compared to the pre-pandemic one. An explanation may be that during the pandemic, many frail individuals died from COVID-19, resulting in a chronically ill elderly population that was, on average, healthier and had a lower mortality rate than in the pre-pandemic period, also due to the absence of flu-related deaths. Lack of care did not seem to affect non-COVID-19 mortality during the pandemic period in frail elderly.

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Differences in Gestational Diabetes by Migrant Status in Marche Region, 2013 – 2020. A Population-Based Study from THE MIGHTY PROJECT (CUP P2022ASXKR)

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INTRODUCTION

Gestational Diabetes (GD), defined as glucose intolerance first detected during pregnancy [1], is a common complication of pregnancy which impacts both maternal and child's health. Monitoring and characterizing its occurrence, particularly in high-risk groups, can guide GD prevention and reduce the burden of the disease.

OBJECTIVE

The study aimed to compare the risk of Gestational Diabetes between Migrant and Italian women at their first pregnancy during 2013 and 2020 in the Marche Region.

METHODS

This population-based study was based on the healthcare utilization databases of Marche Region. Using the Certificates of Delivery Assistance database (CeDAP), a cohort of women at their first singleton delivery during 2013-2020, aged 15 to 50 years at delivery, was selected. Women with at least one diabetes-related event during the year before pregnancy or within the 12th gestational week (gw), defined as glucose-lowering drug prescription (Drug prescriptions database: ATC A10), hospitalization with a primary/secondary diagnosis of diabetes (Hospital Discharge database: ICD-9-CM 250.), diabetes exemption (Exemption database: code

013), or residing in the Region for less than 1 year before pregnancy (Regional Beneficiary Database), with pregnancy terminating before the 20th gw (CeDAP), were excluded.

Women with GD were defined as those with an hospitalization for diabetes (Hospital Discharge: ICD-9-CM 250., 648.8), or ≥ 1 glucose-lowering drug prescription, or included in an educational program for people with diabetes and obesity (Outpatients care database 93.82.1, 93.82.2), or an exemption for GD/diabetes (code 013, RM013T) activated during pregnancy [2]. To detect women not traced for diabetes in healthcare services during pregnancy, those undergoing at least an OGTT or HbA1c control (Outpatients care database 92.26.4, 90.26.5, 90.28.1), or prescription for glucose-lowering drugs, within one year from delivery, were considered to have GD.

Women were classified as Italians or Migrants from High Migratory Pressure Countries, HMPC [3] based on their citizenship. The HMPC group was distinguished according to the geographical area: East Europe, Africa, West-Center-South Asia, East Asia and Latin America.

Adherence to the recommended AnteNatal Care (ANC) was assessed in terms of promptness and appropriateness of the number and timing of ANC, including gynaecological visits (1st visit within the 12th gw, at least 4 visits during pregnancy), ultrasounds (at least 2 examinations during pregnancy), and laboratory tests (tests appropriately performed during the pregnancy trimesters [4]). Information was retrieved from the outpatient care database. The cumulative number of ANC recommendations followed during the pregnancy was also evaluated.

The association between GD and citizenship was evaluated using a multiple logistic regression adjusted for age (≥ 35 vs. < 35 years), year of delivery and adherence to ANC recommendations. Potential interactions between age, year of delivery, and adherence to ANC recommendations with citizenship were tested. Results were expressed as Odds Ratios (OR) and 95% Confidence Intervals (95%CI). All data were processed in compliance with the European (GDPR, EU 2016/679) and national privacy laws (D.lgs. 196/2003 and subsequent amendments).

RESULTS

Of 31786 women included in the study cohort, 28331 (89%) had Italian citizenship and 3455 (11%) were migrant women, most of whom were from East Europe (58%) and 13% from populations considered at high risk of GD (South Asia, Middle East). Migrant women were younger than Italian women (mean age, standard deviation: 29, 5.6 vs. 32, 5.3 years).

In both groups, adherence was over 90% in all ANC recommendations referring to gynaecological visits and ultrasounds, and over 65% for laboratory tests appropriateness. Women adherent to at least 3 recommendations were 67.4% and 75.4% in the Migrant and Italian group, respectively. For each of the considered recommendations, Migrant women had lower observed level of adherence compared to Italian women ($p < 0.001$).

During pregnancy, almost 30% of women underwent an OGTT assessment, with comparable proportions between the two population groups.

GD occurrence during the study period was 13.7% (95%CI: 12.6-14.9) and 8.7% (95%CI: 8.4-9.1) among Migrants and Italians, respectively; higher use of glucose-lowering drugs was found among Migrant women with GD compared to Italians (24.7% vs. 20.3%).

In the multiple logistic regression model, citizenship was associated to GD, with an OR of 1.8 (1.6-2.0) when comparing Migrant to Italian women. In the model that distinguished Migrant women according to their geographical area of origin (Figure 1), the higher GD risk was confirmed in all areas and not only for those known to be at higher risk. The risk of GD increased of 78% (OR=1.78, 95%CI: 1.64-1.93) in women ≥ 35 years old with respect to those < 35 years. No association between GD and ANC recommendations was found except for laboratory tests appropriateness. Furthermore, an average increase in GD of about 12% was found during the study period (OR=1.12, 95%CI: 1.01-1.14). No significant interactions between the evaluated factors were found.

CONCLUSIONS

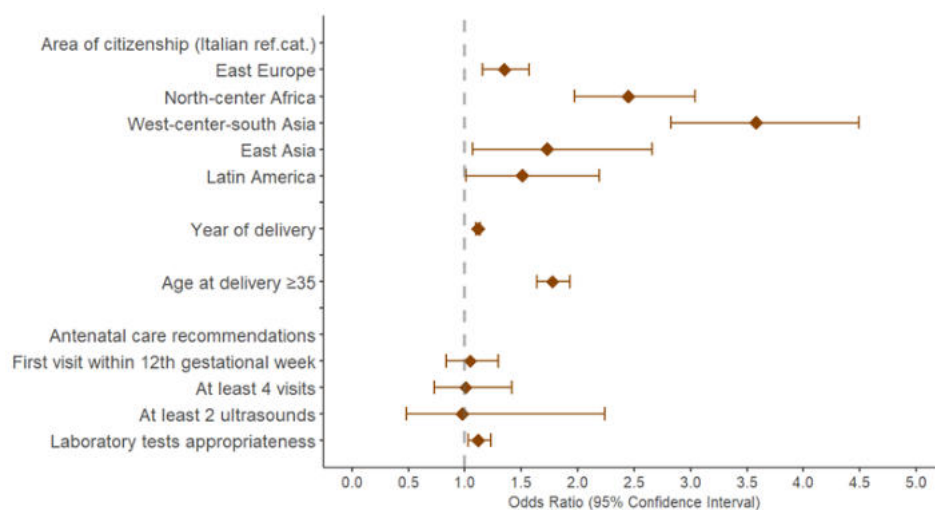
The study showed that Migrant women from different geographical areas were at higher risk of Gestational Diabetes, confirming citizenship an important factor to consider when monitoring women's health in pregnancy. Although almost high levels of adherence to ANC standards were found, the lower levels of adherence characterizing Migrant women with respect to Italian citizens underline the need of tailored

prevention strategies, culturally responsive, to mitigate differences in women's health and access to healthcare during pregnancy.

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Figure 1. Factors associated to Gestational Diabetes risk in women at their first pregnancy between 2013-2020. Results of the multiple logistic regression model



Hosmer and Lemeshow test: $X^2 = 6.65$, $df = 8$, $p = 0.575$

Likelihood Ratio test: $X^2 = 378.6$, $df = 6$, $p < 0.001$

The Association between Individual and Area-Level Socioeconomic Indicators with Mortality and Health Outcomes in a Female Cohort Living in the Metropolitan Area of Naples (PROGETTO ATENA)

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INTRODUCTION

Unfavorable socioeconomic conditions are associated with an increased risk of death and the onset of various diseases in the general population across all countries worldwide [1]. The Metropolitan Area of Naples (ANM), characterized by high deprivation, social polarization, and mortality, represents an urban context with high social heterogeneity, offering the opportunity to analyze health inequalities in relation to socioeconomic position (SEP). The relationship between socioeconomic position and health, analyzed through individual and geographical indicators, is crucial to guide effective prevention strategies.

OBJECTIVES

We present here a study whose objectives were (1) to assess the association between socioeconomic position and life-style-related risk factors and (2) to estimate the extent to which socioeconomic inequalities, measured at both individual and area levels, influence mortality, breast cancer incidence and cardiovascular diseases in a female cohort from the Progetto ATENA.

METHODS

The Progetto ATENA is a prospective cohort study whose main goal was to investigate the causes of certain chronic diseases that affect the female population more significantly at dif-

ferent life stages. Between 1993 and 1996, the project enrolled 5.062 women aged between 30 and 70 years, residing in the ANM. To estimate incidence and mortality rates, two follow-ups on the participants' health status were conducted (in 2010 and 2016) [2].

Socioeconomic position was measured both at the individual and area levels. At the individual level, the Relative Index of Inequality (RII) was used, divided into tertiles representing decreasing levels of education. At the area level, the 2001 Italian Deprivation Index (DI) [3] was used, calculated by census block, georeferenced according to the residential address, and classified into population quintiles.

Associations with the endpoints (overall mortality, breast cancer, cardiovascular events and coronary heart disease) were studied using Cox proportional hazards regression models [4] adjusted for age, treated as the underlying time variable. For each endpoint, four multivariate models were built: Model 1 included RII tertiles and DI quintiles as covariates; Model 2 included Model 1 covariates plus clinical risk factors (systolic blood pressure, total cholesterol, and diabetes); Model 3 included Model 1 covariates plus lifestyle-related risk factors (smoking, physical activity, BMI, waist circumference, alcohol consumption, Mediterranean diet score, and energy intake); Model 4 included the covariates from both previous models. For breast cancer incidence, Model 2 and Model 4 were developed considering clinical variables such as age at menarche and number of children (instead of total cholesterol and systolic blood pressure). Participants with missing values for the mentioned variables were excluded from the analysis. Trend analyses were also performed.

RESULTS

Among the 4.814 women included, follow-up recorded 411 deaths, 225 breast cancer diagnoses, 241 cardiovascular events (CVD), and 150 coronary heart disease events (CHD). At baseline, a positive association between the two SEP indices was observed ($r=0.318$). Both DI and RII were positively associated with systolic blood pressure, BMI, waist circumference, and waist-to-height ratio. Total and HDL cholesterol were negatively associated with both SEP indices. Age and energy intake were positively associated with RII and negatively with DI.

In the mortality analysis, a significant association with DI was observed, with hazard ratios (HR) of 1.56 (Q5 vs Q1, 95%CI 1.13–2.13, $p=0.006$) in Model 1 and 1.45 (Q5 vs Q1, 95%CI 1.05–2.02, $p=0.026$) in Model 4; significant trends ($p=0.014$, $p=0.046$). Similar results were found for both Model 2 and Model 3.

Similarly, an association between breast cancer incidence and DI was observed in Model 1 (Q5 vs Q1, HR 1.57, 95%CI 1.04–2.38, $p=0.032$); a significant trend was also observed ($p=0.042$). Model 2 showed comparable outcomes. In none of the models was RII associated with mortality or breast cancer.

Survival analysis revealed, in all models, a significant association between RII and CVD, with HRs of 1.62 (Q5 vs Q1, 95%CI 1.18–2.21, $p=0.003$) and 1.44 (Q5 vs Q1, 95%CI 1.03–2.01, $p=0.034$) in Model 1 and Model 4, respectively. The trends were also significant ($p=0.003$, $p=0.043$). Comparable results were observed in Model 2 and Model 3.

Likewise, an association between CHD and RII was observed in all models with HRs of 1.94 (Q5 vs Q1, 95%CI 1.31–2.87, $p=0.001$) and 1.62 (Q5 vs Q1, 95%CI 1.07–2.48, $p=0.024$) and significant trends ($p=0.001$, $p=0.025$). Analysis revealed similar outcomes for Model 2 and Model 3. In contrast, no significant association between CVD and CHD with DI was found in any of the models.

CONCLUSIONS

The analysis highlights that the local socioeconomic environment influences mortality and breast cancer more than individual education. Conversely, individual education seems to have a greater impact on the risk of cardiovascular events and coronary heart disease than area-level deprivation. In the comparison between Q5 and Q1, an increase of up to 56% in mortality risk and up to 62% in breast cancer risk was observed; in the comparison between RII 3 and RII 1, an increase of up to 62% in CVD risk and up to 94% in CHD risk was observed.

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Table 1. Hazard Ratios (HR) and 95% Confidence Intervals (CI) for Mortality, Breast Cancer, CVD, and CHD by Socioeconomic Position

		Model 1		Model 4	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Mortality	Deprivation index				
	Q5 vs Q1	1.56 (1.13-2.13)	0.006	1.45 (1.05-2.02)	0.026
	p-value for trend		0.014		0.046
	Education				
	RII 3 vs RII 1	1.19 (0.93-1.52)	0.170	1.03 (0.79-1.34)	0.847
	p-value for trend		0.200		0.914
Breast cancer	Deprivation index				
	Q5 vs Q1	1.57 (1.04-2.38)	0.032	1.41 (0.92-2.16)	0.114
	p-value for trend		0.042		0.116
	Education				
	RII 3 vs RII 1	0.77 (0.54-1.09)	0.136	0.80 (0.55-1.16)	0.236
	p-value for trend		0.154		0.308
CVD	Deprivation index				
	Q5 vs Q1	1.13 (0.76-1.68)	0.533	1.20 (0.79-1.80)	0.392
	p-value for trend		0.722		0.653
	Education				
	RII 3 vs RII 1	1.62 (1.18-2.21)	0.003	1.44 (1.03-2.01)	0.034
	p-value for trend		0.003		0.043
CHD	Deprivation index				
	Q5 vs Q1	1.08 (0.64-1.80)	0.782	1.15 (0.67-1.99)	0.611
	p-value for trend		0.824		0.716
	Education				
	RII 3 vs RII 1	1.94 (1.31-2.87)	0.001	1.62 (1.07-2.48)	0.024
	p-value for trend		0.001		0.025

Note. Results for Models 2 and 3 are not shown due to space constraints. Model 1: adjusted by age; Model 4: adjusted by age, total cholesterol, diabetes, systolic blood pressure, BMI (adjusted by age, number of daughters, menarche age, diabetes for breast cancer) and smoking status, physical activity, waist/height ratio, alcohol, Mediterranean Index, energy intake.

Climate-driven Patterns of West Nile Virus in Lombardy: A Spatio-Temporal Analysis (2013-2022)

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INTRODUCTION

Climate change is one of the most pressing global health threats of the 21st century, driving shifts in infectious disease patterns and facilitating the spread of mosquito-borne viruses like West Nile Virus (WNV). Lombardy, in northern Italy, is a high-risk area for WNV circulation and now represents an endemic zone in the Po River Valley. Despite the well-established impact of temperature and precipitation on mosquito population dynamics and WNV transmission, current surveillance does not incorporate climatic parameters to monitor WNV epidemiology.

OBJECTIVE

This study aims to describe the epidemiology of WNV in Lombardy Po Valley from 2013 to 2022 and to evaluate the association between climatic factors – specifically temperature and precipitations – and the occurrence and distribution of WNV cases, in order to determine whether climate data can support more effective surveillance and warning systems.

METHODS

A retrospective observational study using human WNV case data collected through the national surveillance system in Lombardy from 2013 to 2022 was conducted. Weekly provincial case counts were matched with meteorological data from ARPA Lombardy, including weekly average temperature and total precipitation. In the descriptive phase, we created choropleth maps showing the geographical distribution of WNV cases across across the period in Lombardy provinces.

Moreover we plotted weekly time series of case prevalence per 10000 inhabitants, temperature, and precipitation to assess their temporal co-occurrence and seasonal patterns. For inferential analysis, we fitted a hurdle model with two components: (1) a logistic regression modeling the probability of WNV case occurrence, and (2) a zero-truncated Poisson regression for the count of cases, conditional on occurrence. Models included a two-week lag for temperature and a one-week lag for precipitation and fixed effects for province and year, and offset terms for population size.

RESULTS

Between 2013 and 2022, 311 WNV cases were recorded in six Lombardy provinces, with a prevalence in males (74%) and individuals over 65 years (58%). Among the 164 cases with clinical classification (available from 2019 onward), 53% were neuroinvasive (WNND), while 25% were identified through blood/tissue donation. The majority of cases occurred between July and October, peaking in August (52.4%). The most affected years were 2018, 2020 and 2022. Spatially, the highest prevalence was observed in southern provinces (Cremona, Mantua, Pavia, Lodi), forming a south-north gradient. Time-series plots highlighted a recurrent seasonal alignment between rising temperatures and WNV prevalence, while precipitation showed less consistent patterns. The hurdle model confirmed a significant role of temperature: a 1°C increase (lagged two weeks) raised the odds of case occurrence by 27% (OR=1.27) and the number of cases by 11% (IRR=1.11). Precipitation (lagged one week) had no effect on outbreak onset but increased case counts by 13% (IRR=1.13) once circulation began. Provincial and in-

ter-annual differences were significant in predicting virus occurrence but not outbreak intensity.

CONCLUSIONS

Temperature is the most influential factor for both triggering and amplifying WNV transmission, while precipitation acts as a secondary amplifier. This study demonstrates the critical role of short-term climatic variables, particularly temperature, in shaping WNV dynamics in Lombardy. Rising temperatures significantly increase both the probability of outbreak initiation and the intensity of transmission. While precipitation alone does not appear to initiate outbreaks, it contributes to amplifying transmission once WNV is circulating. These findings reinforce the importance of integrating climate indicators into surveillance systems to enhance early warning capacity and timely public health responses. The identification of provincial-level risk differentials and temperature thresholds can inform geographically tailored interventions. In the context of ongoing climate change, such predictive models could become essential tools to mitigate the impact of future arboviral epidemics.

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Epidemiology of Acute Cardiovascular and Cerebrovascular Events in the Lombardy Region: A Population-Based Study (2015–2021)

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INTRODUCTION

Cardiovascular and cerebrovascular diseases represent one of the leading causes of morbidity and mortality in Italy [1]. Acute events such as myocardial infarction and ischemic stroke have a significant clinical, economic, and social impact. Each year, approximately 150,000 new cases of myocardial infarction and 200,000 strokes are registered in Italy, with a substantial healthcare burden especially among the elderly [2,3]. In Lombardy, in 2020, diseases of the circulatory system were the leading cause of death, accounting for about 34,000 deaths, corresponding to 25% of the total [4].

Despite advances in prevention and treatment strategies, territorial and sociodemographic disparities in the distribution of events persist [5]. Temporal and spatial analysis of acute event incidence, particularly at the sub-regional level, represents an essential tool to guide healthcare planning and to evaluate the effectiveness of public health policies.

The analysis of acute event incidence is therefore a key instrument to monitor the effectiveness of prevention strategies and healthcare responses. Observing trends over an extended time frame allows detection of significant trends and potential inequalities in access to care or in the distribution of risk factors [6].

OBJECTIVE

This study aims to describe the epidemiology of acute cardiovascular and cerebrovascular events in the Lombardy Region between 2015 and 2021, analysing temporal trends and differences by sex, age, and Local Health Protection Agencies (ATS). Additionally, it seeks to assess the presence of significant differences between ATS in terms of in-hospital mortality for these events.

METHODS

The study population includes all residents of Lombardy aged ≥ 45 years who were hospitalized for an acute cardiovascular or cerebrovascular event between 2015 and 2021.

To identify incident events, new cases were selected for each year by excluding individuals who had experienced the same type of event in the five years prior to the hospitalization date.

Events were identified using regional administrative healthcare databases, specifically hospital discharge records (SDO), based on selected ICD-9-CM codes for myocardial infarction, unstable angina, acute heart failure, ischemic and haemorrhagic stroke, and transient ischemic attack.

Annual incidence was estimated by calculating crude rates, stratified by sex and age group, using person-time denominators.

Temporal trends were analysed using Poisson regression models to estimate the annual rate variation and assess its statistical significance.

To compare geographic differences, age- and sex-standardized incidence rates were calculated for each ATS through direct standardization, using the Lombardy population as the standard.

Results are presented as rates per 100,000 population with 95% confidence intervals (CIs).

To explore geographic heterogeneity in in-hospital mortality for the studied acute events, a multilevel logistic regression model was implemented, adjusted for age, sex, and comorbidities. Subsequently, a fixed-effects model was used to assess whether there were statistically significant differences in in-hospital mortality across the different ATS.

RESULTS

A total of 260,725 residents of Lombardy aged ≥ 45 years who experienced an acute cardiovascular or cerebrovascular event were included. Of these, 42.5% were female, and the overall median age was 76 years (IQR: 65–83).

The average annual rate was higher in men than in women across all age groups. In the ≥ 75 age group, the rate was 2,162 per 100,000 population (95% CI: 2,138–2,187) in men and 1,236 per 100,000 (95% CI: 1,222–1,231) in women ($p < .001$). Marked differences ($p < .001$) were also observed in the 45–59 age group: 293 per 100,000 (95% CI: 289–297) in men vs. 167 (95% CI: 165–170) in women.

Age and sex standardized incidence rates varied across ATS from a minimum of 622 per 100,000 population (95% CI: 600–663) to a maximum of 771 (95% CI: 739–783), highlighting geographic differences.

Finally, the multilevel logistic model showed a random-effect variance between ATS of 0.007, indicating limited geographic heterogeneity in in-hospital mortality. However, based on the fixed-effects model, only one ATS showed a significantly lower in-hospital mortality probability compared to the others.

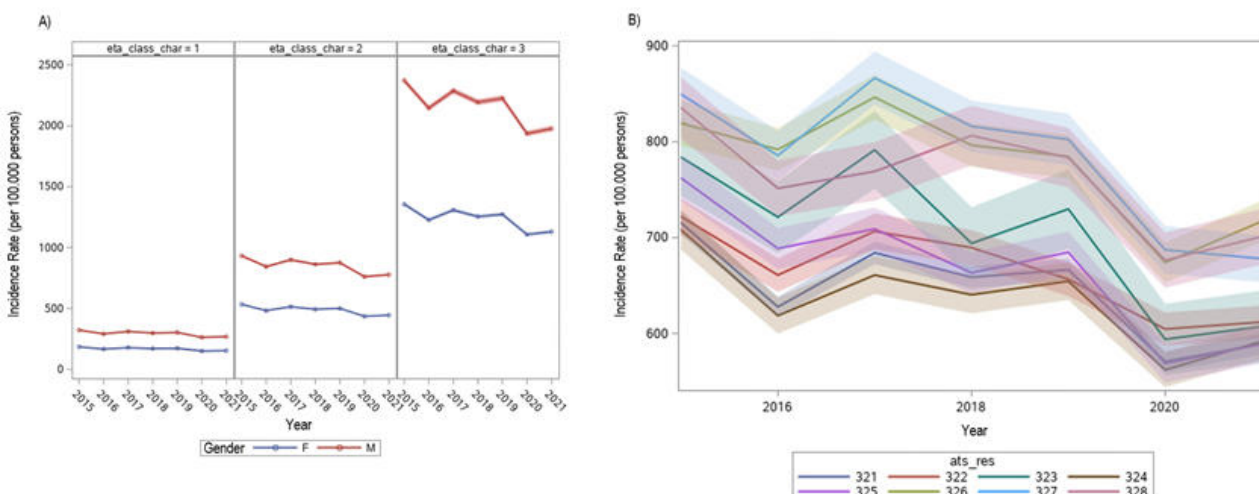
CONCLUSIONS

The study showed a significant temporal reduction in the incidence of acute cardiovascular and cerebrovascular events in Lombardy between 2015 and 2021, with higher rates in men and older age groups. Although substantial territorial variability in in-hospital mortality at the ATS level was not observed, some localized differences point to the need for targeted investigations and interventions to address potential territorial health inequalities.

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Figure 1. A) Temporal trend of incidence rates stratified by sex and age group (per 100,000 population); B) Temporal trend of incidence rates by Health Protection Agency (ATS), standardized by sex and age (per 100,000 population).



Association Between Indices of Social Disadvantage and Rate of COVID-19 Vaccination Booster Dose

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INTRODUCTION

Adherence to COVID-19 vaccination has declined with the decrease in epidemic waves, despite the clearly higher risk of infection and hospitalization among the unvaccinated. Misinformation, cognitive biases, and other not always measurable factors have contributed to vaccine refusal. Although some studies have explored the causes of vaccine hesitancy, the knowledge of the effect of socio-cultural and economic conditions are still limited.

OBJECTIVE

This analysis aims to evaluate the association between adherence to the first COVID-19 vaccine booster dose (3rd dose) and available deprivation indices (Caranci index and ISTAT's social and material vulnerability index), to understand whether the social context influences the population's vaccination behaviour.

METHODS

A retrospective observational study was conducted on the population residing in Apulia eligible for the first COVID-19 vaccine booster dose between January 1, 2021, and December 31, 2022. Demographic data were obtained from ISTAT [1], while vaccination status information was retrieved from the regional vaccination registry (GIAVA) in aggregated form (number of vaccinated individuals) by municipality of residence, sex, and age. The social and material vulnerability index (ISMV), provided by ISTAT [2], consists of a score with a reference value of 100 (higher values indicate greater vulnerability) determined by seven vulnerability indicators (single-parent households, large household size, illiteracy, elderly members, unemployment, economic hardship). The Caranci

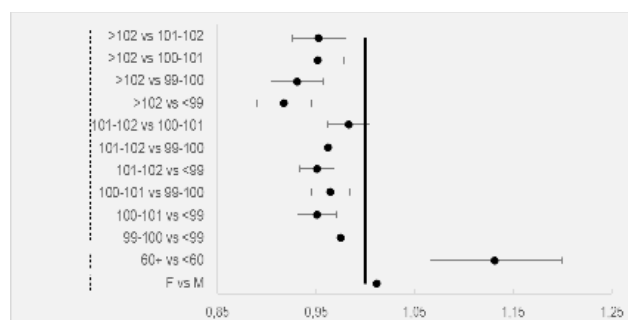
deprivation index (ID) [3] is also calculated using census data and is based on five indicators: poor education, job shortages, poor housing, and family conditions.

The applied model is a multivariable Poisson regression, with the number of individuals vaccinated with the booster dose as the dependent variable, and ISMV (or ID), sex, and age group as independent variables. Additionally, the COVID-19 case rate was considered as an adjustment variable.

RESULTS

The percentage of residents in Apulia who received the booster dose progressively decreases from 75% among individuals with ISMV <99 to 70% among those with ISMV >102, with no significant differences by sex. The age-stratified analysis (<60 and ≥60 years) has shown, among individuals aged ≥60, a slight reduction in the percentage who received the booster dose from 81% (ISMV <99) to 74% (ISMV >102), while the decline is more pronounced in the population <60 years, dropping from 70% with ISMV <99 to 63% with ISMV >102. All factors included in the model are statistically significant, in both model with the ID or the ISMV. The model with ISMV (Figure 1) has shown that in the ISMV >102 class the acceptance of the vaccination is lower compared to classes with lower vulnerability, with rate ratios of 0.97 (0.95–0.99), 0.97 (0.95–0.99), 0.95 (0.93–0.97), and 0.94 (0.92–0.96) for the 101–102, 100–101, 99–100, and <99 classes, respectively.

Figure 1. Estimated Rate Ratios and corresponding 95% confidence intervals from the multivariable Poisson regression model for the Social and Material Vulnerability Index (ISVM), age, and sex. The confidence interval for the variable Sex is not visible due to scale



CONCLUSIONS

The analysis revealed a significant association between socioeconomic deprivation and lower adherence to the third dose of the COVID-19 vaccine. The decline is more pronounced among individuals under 60 with high levels of vulnerability, while no significant differences emerged with respect to gender.

A limitation of the study is the use of aggregated data, which does not allow for in-depth individual-level analysis and may lead to a generalization of the results (a possible “ecological fallacy”). Nevertheless, the findings suggest that in specific social contexts, there are groups—defined by age and vulnerability status—that could benefit from targeted communication strategies aimed at increasing awareness and addressing vaccine hesitancy.

FUNDING

Funded by the European Union – Next Generation EU, Mission 4 Component 2 CUP H93C22000640007, Project PE_0000007.

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Outdoor Particulate Matter, Cardiovascular Health and Incidence of Coronary or Ischemic Stroke Events: A Population-Based Study

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INTRODUCTION

Long-term exposure to outdoor particulate matter of size <2.5 microns (PM_{2.5}) has been associated with increased risk of cardiovascular diseases (CVD) incidence [1], although with substantial between-study heterogeneity. In addition, pathways and factors of individual susceptibility to PM_{2.5} have been poorly characterized so far. The American Heart Association (AHA) proposed the Life's Simple 7 (LS7) metric of cardiovascular health by combining lifestyle (not smoking, healthy diet, engaging in sufficient physical activity) and health (normal body weight, maintaining low values of blood pressure, total cholesterol and glucose) components [2]. The mediating role of LS7, and whether the risk excess due to PM_{2.5} is exacerbated in individuals with a "poor" cardiovascular health, remain to be ascertained.

AIMS

To investigate the association between long-term exposure to PM_{2.5} and the incidence of first coronary heart disease or ischemic stroke event, and the contribution of LS7 on explaining the risk excess, in a population-based cohort in Northern Italy. Furthermore, to assess the role of LS7 as an effect modifier for the association.

METHODS

STUDY POPULATION

The RoCAV study [3] is a population-based cohort of n=3777 50+ years old residents in the city of Varese (Lombardy region, northern Italy) at the time of recruitment (2013-2016; 64% participation rate). For the aims of these analyses, we retained individuals free of coronary heart disease and stroke at baseline (n=3313, 62% men).

CARDIOVASCULAR HEALTH

Participants underwent a comprehensive baseline examination, assessing cigarette smoking (MONICA questionnaire), dietary intake (EPIC food frequency questionnaire), habitual physical activity (Baecke questionnaire), as well as clinical and laboratory parameters (fasting blood lipids and glucose, blood pressure and anthropometric measures). From these, we calculated the LS7 metric on a 0-to-14-points scale [2], further categorized as poor (0-4 points), intermediate (5-9 points) and ideal (10-14 points) [4]. We categorized the two LS7 components of lifestyle (LS7-ls) and health (LS7-h) in a similar way.

PM EXPOSURE

Monthly concentrations for PM_{2.5} over the period 2000-2019 were retrieved from the EXPANSE project models [5], at a spatial resolution of 25mt. Individuals' concentrations were attributed from spatial linkage at the residential address at baseline, geo-referenced; the exposure metric was the average concentration in the 12 months before the month of baseline visit.

STUDY ENDPOINTS

Individuals were followed-up through record linkage with Electronic Health Records (hospital discharge and mortality) provided by the Local Health Agency. We selected discharge codes suggestive of myocardial infarction and unstable angina (ICD-IX codes: 410-411) or elective coronary revascularization; cerebrovascular infarction (ICD-IX 433 or 434) or endarterectomy with stenosis at cerebral or pre-cerebral arteries (ICD-IX 433.1, 433.3, 434.0). Selected records were then reviewed to identify and retain the first index case. Fatal cases were identified from underlying causes of deaths suggestive of coronary deaths (ICD-X codes: I21-I25) or ischemic stroke (I63). Censorship occurred at the date of death from

other causes or emigration outside the study Region, as ascertained by contacting the municipality of residency. The study endpoint is the occurrence of first coronary heart disease or ischemic stroke, fatal or non-fatal, before Dec 31st, 2022.

STATISTICAL ANALYSES

Due to the low number of events in women, the analyses were carried out on men and women combined, and on men only. We first estimated the rate ratios for the LS7 categories ("ideal" as reference) from Poisson regression models, adjusting for age and sex. Then, we estimated the Hazard Ratios (HR) and 95%CI for 1 interquartile range (1.93 $\mu\text{g}/\text{m}^3$) increase in PM2.5 using nested Cox models with attained age during follow-up on the time scale, and adjusting for: sex and education (Model1), and further for LS7 (Model2). We computed the percent explained by LS7 on the log scale as $100[\log(\text{HRM2}) - \log(\text{HRM1})] / [\log(\text{HRM1})]$. Finally, we investigated individual susceptibility by adding to Model2 a LS7PM2.5 interaction and by reporting the corresponding p-value from a Wald chi-square test (2df). These analyses were repeated for LS7-ls and LS7-h components.

RESULTS

Mean $\pm$ SD PM2.5 concentrations were $18.6 \pm 1.8 \mu\text{g}/\text{m}^3$. During 7.1 years of median follow-up time, we observed $n=196$ CVD events for a rate of 8.6 per 1,000 person-years (men: $n=150$ events, 10.8 per 1,000 py). "Poor" vs. "ideal" LS7 resulted in a 2.34-fold (95%CI: 1.33-4.10) increased event rate (men: 2.13, 1.12-4.06). In Model1, PM2.5 was associated with increased CVD risk, in the overall sample (HR=1.11, 95%CI: 0.95-1.29) and in men (HR=1.19, 95%CI: 1.00-1.43). Further adjustment for LS7 explained 13.4% (men and women) and 7.2% (men) of the risk excess, respectively, mainly due to the LS7-ls component. We found evidence of interaction between PM2.5 and the LS7-ls component (Wald test p-values: 0.03 [men and women] and 0.007 [men]). In men, the HRs for PM2.5 in the "poor" (40% of the sample) and in the "ideal" (15% of the sample) LS7-ls categories were 1.49 (95%CI: 1.11-1.98) and 0.67 (0.45-1.00), respectively. Conversely, no interaction was observed for LS7, nor for the LS7-h component.

CONCLUSIONS

In our cohort, representative of contemporary outdoor PM2.5 levels in a North Italian population, long-term exposure to PM2.5 was associated with an increased risk of first major CVD event, especially in men. AHA LS7 explained only a small proportion of the association. Adults engaging poor lifestyles are more susceptible to the detrimental effect of PM, and can benefit the most from policies reducing pollution levels.

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Risk of Hospitalizations for Infectious and Parasitic Diseases in Native and Migrant Populations of the Lombardy Region

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INTRODUCTION

Migrant populations mainly move from low- and middle-income countries in Europe, Asia, Africa, and South America. Compared to host populations, they show different patterns in the prevalence of infectious and chronic diseases that change over time and across generations. Upon arrival, migrants tend to be healthier due to the “healthy migrant effect”, but their health deteriorates the longer they reside in the host country [1]. In Italy, foreign residents encompass 8.6% of the total population (about 5 million people), highlighting the need to monitor their health, especially infectious and parasitic diseases (IPDs), which are a critical growing issue for public health [2]. The availability of population-based data is essential for developing prevention and control strategies to reduce this burden.

OBJECTIVES

The main objectives of this cohort study were to examine the occurrence of the first hospitalization due to infectious and parasitic diseases among the assisted population in the Lombardy Region and to compare the probability of such an event between individuals from foreign birth countries, regarded as migrants, and Italian natives, adjusting for sex and age effects. Since only birth countries rather than citizenships were available, in our study, we considered the individuals born abroad as migrants.

METHODS

We obtained the cohort from the Health Service database of the Lombardy Region. We included individuals aged 18 to

65 years in the period 2010–2019 who began to be assisted before 2010. Each participant was monitored to identify any hospitalization due to IPDs according to ICD-9 codes 001–139. We excluded any hospitalizations that occurred before 2011 to ensure that only incident hospitalizations entered the study.

We classified each individual based on his/her birth country to distinguish migrants from Italian natives and, among migrants, those coming from countries with higher migratory pressure. To this end, we combined the ISTAT classification of foreign territorial units [3] with the one considered in [4] that distinguishes High Migratory Pressure Countries (HMPC) from Highly Developed Countries (HDC). This way, we obtained the following seven areas: Italy, regarded as the reference area for the analyses; five HMPC areas, each corresponding to Africa, Central-South America, Asia, the European Union (EU) (with Bulgaria, Croatia, Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, Slovenia), and Eastern Europe with countries outside EU (e.g., Moldova and Ukraine); one HDC area comprising countries both in Europe (e.g., Germany and the United Kingdom) and outside Europe (e.g., Israel, Japan, and U.S.A.).

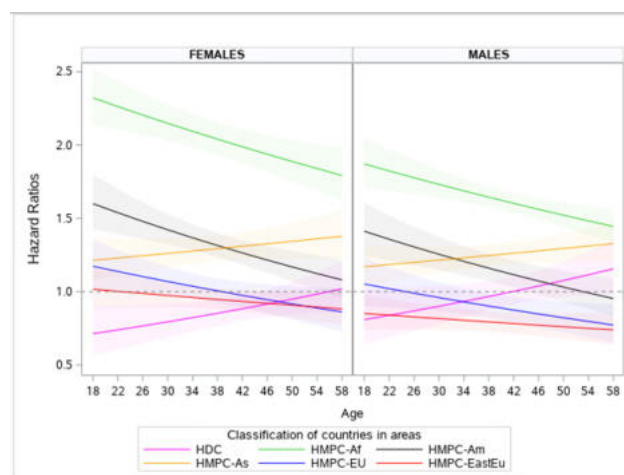
To meet our study objectives, we performed a survival analysis using the Cox proportional hazard regression model, assuming the first hospitalization for IPDs as the outcome and the deceased individuals or emigrants from the Lombardy Region during the follow-up as censoring. As independent variables, we included the classification in seven areas of the birth countries, sex, and age as main effects and first- and second-order interactions. The results are presented as hazard ratios (HRs) with 95% Wald confidence intervals (CIs). All the statistical analyses were carried out using the SAS Studio software version 9.4.

RESULTS

Our study set comprised 5,098,372 individuals: 84.97% Italian natives, 13.56% from the HMPC areas (Africa: 3.81%, Central-South America: 2.37%, Asia: 2.80%, EU: 2.12%, and Eastern Europe: 2.46%), and 1.48% from the HDC area; 49.23% were females (mean age in 2010: 38.28 ± 10.13 years), and 50.77% were males (mean age in 2010: 38.16 ± 10.09 years). A total of 66,934 individuals (1.31% out of 5,098,372) reported new hospitalizations for IPDs (the event of interest) during follow-up, who represented 1.29% in Italy; in HMPC areas: 2.07% for Africa, 1.39% for Central-South America, 1.45% for Asia, 1.04% for EU, 1.00% for Eastern Europe; in HDC area: 1.17%. The most occurring diseases were those in ICD-9 codes 030–041 (other bacterial diseases such as leprosy, diphtheria, and scarlet fever), amounting to 45.28% of the 66,934 individuals with the event.

The final Cox model, which did not include the non-significant second-order interaction, revealed significant differences affecting certain areas compared to the Italian natives. Figure 1 depicts the HRs of females and males for HMPC and HDC areas against age, using Italian females and males as references. Given the absence of the second-order interaction, the HRs of females have the same trend for each area as those of males. Nevertheless, regardless of age, African females have significantly higher HRs than males. For instance, with age fixed at 18, the HR of HMPC-Africa vs. Italy is equal to 2.32 (CI: (2.14, 2.52)) in females and 1.87 (CI: (1.71, 2.05)) in males; with age fixed at 54, the HR of HMPC-Africa vs. Italy is equal to 1.84 (CI: (1.69, 2.01)) in females and 1.48 (CI: (1.39, 1.58)) in males.

Moreover, in both panels, the gap in terms of risk of Africa, Central-South America, EU, and Eastern Europe compared to Italy decreases with age, while that of Asia and HDC increases. The risk of the event occurring is always significantly higher in African and Asian migrants than in Italians, regardless of sex, while it is significantly lower in Eastern European males than in Italian males. In Central-South American females, the risk is significantly higher than in Italian females up to 54 years, while in males, it is up to 44 years. Finally, HDC females have a significantly lower risk than Italian females up to 44 years, while no significant difference is detected for males.



approach an effective prevention and/or monitoring plan.

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Evaluating The Real-World Effectiveness of A New Class of Drugs For Cystic Fibrosis: A Study Based On European Cystic Fibrosis Society Patient Registry Data

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INTRODUCTION

Cystic fibrosis (CF) is a heterogeneous multisystemic disease. Despite considerable improvement in survival, CF remains a life-shortening disease with respiratory failure as the main cause of death. In 2020, elexacaftor/tezacaftor/ivacaftor (ETI) became available as a new highly effective CFTR-modulator therapy targeting the basic protein defect for people with a specific gene variant, and it was shown to improve lung function respiratory symptoms, and other clinical outcomes, including pulmonary exacerbations (PEx) [1]. However, despite the reduced frequency of PEx, these events remain an important driver of morbidity and mortality in people with CF (pwCF) [2]: in 2023, 16% of adults with CF in Europe experienced at least one PEx during the year [3]. The full extent of CFTR modulators effect on chronic airway infections and the recurrent PEx, especially in the long-term, remains uncertain with some studies reporting inconsistent results [4].

OBJECTIVES

This study aimed to evaluate the real-world effectiveness of ETI therapy on PEx frequency in pwCF followed up between 2018 and 2023, using data from the European Cystic Fibrosis Society Patient Registry (ECFSPR). Specifically, a longitudinal comparison was conducted to evaluate the effectiveness of the new CFTR-modulator therapy on the number of PEx and the prevalence of chronic infections over time.

METHODS

Data were obtained from the ECFSPR, a population-based Registry that collects clinical and demographic data on an annual basis according to agreed definitions of a common set of variables from Centres and national Registries in Europe and neighbouring countries from 2008 to 2023 [9].

To determine the effectiveness of ETI on PEx and chronic infections, a retrospective and longitudinal comparison from 2018 to 2023 was implemented. The analysis included the following variables: total number of days of intravenous antibiotics (IV Abx), including those performed both in hospital and at home (as a proxy for PEx), number of days of IV Abx performed exclusively in hospital, total number of days in hospital for any cause and the presence of chronic infections, including *Pseudomonas aeruginosa*, *Burkholderia Cepacia* Complex and *Staphylococcus aureus*. The first three variables were analysed both as continuous and as categorical (≥ 1 day vs. 0 days). The chronic infections were instead categorized as the presence of at least one infection versus no infection. To assess whether there were significant differences between the paired periods before and after treatment, the Wilcoxon signed-rank test and McNemar's test for paired data were used, as the same pwCF were evaluated at two time points.

All analyses were performed using R software.

RESULTS

The study included pwCF aged 12-60 years; pwCF who underwent solid organ transplants were excluded. Partici-

pants had either an F508del homozygous genotype or were F508del heterozygous with a minimal function variant. The analyses were restricted to those who started ETI modulator therapy in 2020 (N= 6,628). Only people with available data on the number of days on IV Abx therapy administered at home and in hospital were considered, resulting in a final study population of 4,602 pwCF.

Comparison of two years before and two years after starting ETI showed that the percentage of pwCF with at least one day on IV Abx decreased significantly from 64% to 23% ($p<0.001$), the percentage of pwCF with at least one day on IV Abx in hospital decreased from 50% to 16% ($p<0.001$) and, in parallel, that the percentage of pwCF hospitalized dropped from 56% to 24% ($p<0.001$). Also considering the corresponding variables as continuous, all the differences remain statistically significant. Notably, these improvements measured by total days on IV antibiotics and related variables persisted through three years post-ETI initiation.

The prevalence of at least one chronic infection significantly decreased from 67.8% to 36.8% after 2 years of ETI therapy ($p<0.001$). Number of chronic infections further decreased to 34.5% after 3 years of treatment.

CONCLUSIONS

This analysis of the ECFSPR data demonstrates a marked reduction in PEx after the introduction of the new highly effective CFTR-modulator therapy. The introduction of ETI significantly reduced the clinical burden in pwCF, with decreases of days on IV Abx, hospitalizations and chronic infections. These benefits were observed consistently after 3 years of therapy.

Further steps will include the implementation of statistical models to study changes in days on IV Abx as well as in other clinical outcomes before and after ETI.

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Insights from the EXPOSITION Study: Exposome-related microRNA Expression and Clinical Outcomes in People with Multiple Sclerosis

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INTRODUCTION

MicroRNAs (miRNAs) are emerging as promising biomarkers of neuroinflammation and may capture the influence of lifestyle and environmental exposures in people with multiple sclerosis (pwMS). The EXPOSITION study[1] aims to elucidate relationships between internal exposome markers including miRNA profiles and clinical, demographic, and lifestyle factors in pwMS.

OBJECTIVE(S)

To assess the associations between the relative expression of five candidate miRNAs and clinical, demographic, and lifestyle variables, with a particular focus on the exposome and functional and psychological outcomes in pwMS.

METHODS

In this cross-sectional analysis, we included 139 pwMS (median age 45 years [IQR: 35–56], 65% female) from the provinces of Pavia and Milan in the Lombardy region of Italy.

Relative expression levels of five candidate miRNAs (mir30, mir146, mir330, mir574, mir664) were quantified and compared across clinical and lifestyle categorical variables groups using the Mann-Whitney U test (for binary variables) or the Kruskal-Wallis test (for variables with more than two categories). Spearman correlation analyses were conducted to assess the relationship between each miRNA and continuous variables, including age, BMI, EDSS, dietary inflammatory index, and quality of life scores.

RESULTS

There were no statistically significant differences in microRNA expression between EDSS disability groups or across most clinical or lifestyle variables. Notably, mir146 expression was significantly higher in participants with a pro-inflammatory dietary pattern compared to those with an anti-inflammatory pattern ($p = 0.0187$), and mir146 was positively correlated with the mental health component of the quality of life MSQoL-29 questionnaire ($\rho = 0.336$, $p = 0.0174$) [Table 1]. In contrast, higher disability status (EDSS >4) was significantly associated with older age (median 51 vs. 44 years, $p = 0.047$), more frequent prior relapses (94% vs. 69%, p

= 0.040), and lower physical ($p = 0.039$) and mental ($p = 0.034$) quality of life. Significant group differences were also observed for MS type ($p = 0.008$), MS stage ($p = 0.034$), and occupational status ($p = 0.063$, trend) between the two EDSS groups. No significant associations were identified between disability status and microRNA expression, diet category, physical activity, or MRI lesion status.

Table 1 Summary of MicroRNA Expression by Exposome and Clinical Variables using preliminary data from EXPOSITION exposome study

	MicroRNA Expression				
	mir30	mir146	mir330	mir574	mir664
Dietary Inflammatory Index (DII)[#]: DII < 0 anti-inflammatory vs DII > 0 proinflammatory	anti-inflammatory: 0.00395 (0.00314, 0.00504) vs. proinflammatory: 0.00914 (0.00484, 0.0187); $p=0.0821$	anti-inflammatory: 0.0155 (0.0129, 0.023) vs. proinflammatory: 0.0802 (0.0287, 0.153); $p=0.0187$	anti-inflammatory: 0.00298 (0.00229, 0.00624) vs. proinflammatory: 0.00752 (0.00308, 0.01); $p=0.486$	anti-inflammatory: 0.0314 (0.0176, 0.057) vs. proinflammatory: 0.0427 (0.0295, 0.0591); $p=0.505$	anti-inflammatory: 0.00223 (0.00186, 0.0026) vs. proinflammatory: 0.00328 (0.00253, 0.005); $p=0.414$
Expanded Disability Status Scale Score (EDSS)[#]: EDSS ≤ 4 vs. EDSS > 4	EDSS ≤ 4: 0.00838 (0.00406, 0.0163) vs. EDSS > 4: 0.00832 (0.00623, 0.00914); $p=0.841$	EDSS ≤ 4: 0.0778 (0.019, 0.151) vs. EDSS > 4: 0.0218 (0.0161, 0.0549); $p=0.181$	EDSS ≤ 4: 0.00542 (0.00268, 0.00958) vs. EDSS > 4: 0.00752 (0.00575, 0.00851); $p=0.818$	EDSS ≤ 4: 0.0423 (0.0289, 0.0618) vs. EDSS > 4: 0.0352 (0.0326, 0.0477); $p=0.835$	EDSS ≤ 4: 0.0035 (0.00266, 0.00515) vs. EDSS > 4: 0.00224 (0.00185, 0.00264); $p=0.286$
MSQOL-29 Mental[§]	$\rho=0.274$; $p=0.0599$	$\rho=0.336$; $p=0.0174$	$\rho=0.141$; $p=0.424$	$\rho=0.137$; $p=0.327$	$\rho=0.139$; $p=0.462$
Age (in years)[§]	$\rho=-0.0143$; $p=0.917$	$\rho=0.0382$; $p=0.778$	$\rho=0.146$; $p=0.356$	$\rho=0.0906$; $p=0.48$	$\rho=-0.0301$; $p=0.861$

MSQOL - Multiple Sclerosis Quality of Life, [#] Median (IQR), [§] Spearman rank correlation

CONCLUSIONS

In this preliminary analysis, higher disability among pwMS was more strongly linked to clinical history, age, and quality of life than to lifestyle factors or circulating miRNA levels. Mir 146 may act as a molecular intermediary between dietary inflammation, mental health, and neuroinflammatory processes in MS. These findings highlight the need for replication and longitudinal validation in larger, independent cohorts.

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A Random Forest Algorithm for Identifying Risk Factors for Multimorbidity in the UK Biobank Cohort

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INTRODUCTION

High-income countries are undergoing significant demographic shifts, characterized by population decline and progressive aging. These transformations are associated with an increase in the prevalence of chronic diseases, which often coexist, worsening individuals' quality of life and increasing healthcare costs. Identifying the factors that contribute to the onset of multimorbidity is particularly complex, as these factors often interact with each other and cause multiple effects across different diseases.

OBJECTIVES

This study aimed to identify the main risk factors for multimorbidity within a large UK cohort using a fully nonparametric ensemble method. This approach makes no assumptions about the underlying relationships between variables and allow managing high-dimensional data while preventing overfitting.

METHODS

We analyzed data from the UK Biobank cohort, which includes detailed information on socioeconomic status, lifestyle, anthropometric measures, and environmental exposures collected at recruitment, along with disease occurrence obtained through linkage with hospital admissions (primary and secondary diagnoses), death records, and cancer registries. Multimorbidity was defined as the presence of at least two chronic conditions from a list developed through an international consensus using a modified Delphi method [1]. To assess the role of 18 candidate variables in predicting the onset of multimorbidity over a five-year follow-up, we applied a random forest algorithm adapted for survival analysis within

a competing risk framework [2], considering two competing events: the development of multimorbidity and death prior to its onset. The candidate variables included: white British/Irish ethnicity (Yes/No), qualification level, average total household income before tax (adjusted for household size and categorized into quintiles), area-level index of multiple deprivation (deciles), body mass index (kg/m²), waist circumference (cm), pack-years of smoking, alcohol drinking (g/day), healthy diet score (ranging from 0 to 5, based on the intake of fruit, vegetables, fish, whole grains, processed and red meat), walking (at least 10 min, number of times a week), moderate physical activity (at least 10 min, number of times a week), vigorous physical activity (at least 10 min, number of times a week), particulate matter air pollution 2.5 (PM_{2.5}) (µg/m³), PM_{2.5-10} (µg/m³), PM₁₀ (µg/m³), NO₂ (µg/m³), average exposure to evening (7:00 pm – 11:00 pm) or night noise (11:00 pm – 7:00 am) (dB). Results were summarised using out-of-bag partial dependence plots and variable importance (VIMP) metrics.

RESULTS

Of the 422,344 individuals included in the cohort, aged between 39 and 73 years, we selected 137,565 participants who were free from the conditions included in the definition of multimorbidity at the time of recruitment and for whom risk factor information was available. During the five-year follow-up, 4384 individuals developed multimorbidity (2740 males, 1644 females). The five-year cumulative incidence was 3.9% in males and 2.6% in females. Among individuals who developed multimorbidity during follow-up, the main conditions observed were cancer (52.4% of males and 52.1% of females), arrhythmias (44.7% of males and 28.5% of females) and coronary artery disease (42.1% of males and 24.8% of females). Based on VIMP metrics, the strongest predictors in men were smoking, waist circumference, and sleep duration; in women

alcohol, smoking, and waist circumference. Five-year cumulative incidence was higher for heavy smokers (sex-specific 95th percentile of pack-years) (males: 6.3%, females: 4.0%) compared to non-smokers (males: 3.5%, females: 2.4%); for individuals with elevated waist circumference (sex-specific 95th percentile) (males: 6.1%, females: 5.2%) versus those with median values (males: 3.9%, females: 2.6%); for heavy alcohol drinkers (sex-specific 95th percentile) (males: 4.6%, females: 4.0%) versus median intake (males: 3.8%, females: 2.4%); for those sleeping 4 hours/day (males: 6.3%, females: 4.2%) or 10 hours/day (males: 6.5%, females: 4.5%) versus 7 hours/day (males: 3.7%, females: 2.5%). Diet, physical activity, and air pollution had smaller impacts.

CONCLUSIONS

Preventive interventions targeting smoking, abdominal obesity, and heavy alcohol consumption among middle-aged adults in the UK and likely in other high-income countries, may substantially reduce the incidence of multimorbidity. Such interventions could improve the health trajectory and burden of disease of future older populations. In addition, promoting adequate sleep duration appears to be beneficial and should be integrated into public health recommendations.

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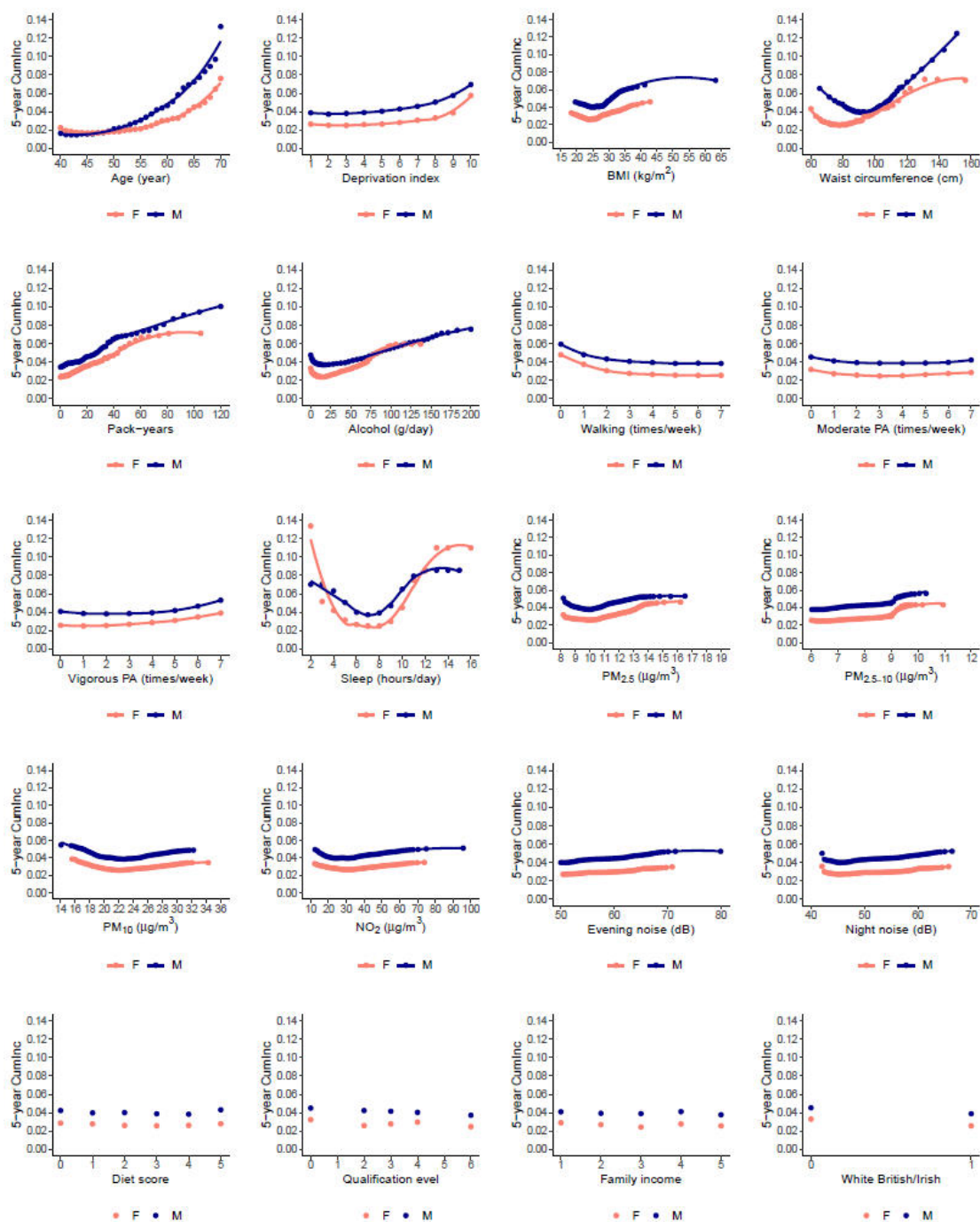


Figure 1. Partial dependence plots displaying the predicted five-year cumulative incidence of multimorbidity across different levels of socioeconomic, lifestyle and environmental exposures, stratified by sex. Family income before tax was adjusted for household size and categorized into quintiles. Diet score, ranging from 0 to 5 (with 5 indicating the healthiest diet), was based on intake of fruit, vegetables, fish, whole grains, and consumption of processed and red meat. Qualification levels are based on the UK Regulated Qualifications Framework (RQF), which classifies education attainment from Entry Level through Level 8 [Level 0: No qualification; Level 1: General Certificate of Secondary Education (GCSE) or equivalent functional skills; Level 2: Higher attainment (e.g. GCSE grades A–C); Level 3: A-levels and access diplomas; Levels 4–5: Sub-degree higher education qualifications (e.g. HNC, foundation degrees); Level 6: Undergraduate degrees; Level 7: Postgraduate qualifications (e.g. Master's, PGCE); Level 8: Doctoral-level education (e.g. PhD)].

Variations in Plasma Proteome across the Menstrual Cycle

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INTRODUCTION

Menstrual cycles are a fundamental aspect of female reproductive health. However, variations in the plasma proteome across the menstrual cycle remain largely unknown. Previous studies suggest that these changes, along with menstrual irregularities associated with cycle length, may hold substantial diagnostic and prognostic value, not only for reproductive health conditions but also for broader health outcomes, including cardiovascular diseases [1, 2, 3, 4, 5].

OBJECTIVES

1. Do plasma proteins show phase-specific variation across the menstrual cycle?
2. Do trajectories differ by protein type or by women's characteristics?

METHODS

We analysed socio-demographic, health and proteomic data from a representative sample of 1,284 non-pregnant pre-menopausal women from a cohort of South Asian ancestry (2016-2019). Plasma proteins were measured with SomaScanAssay (v4.1, N=7k). To test for differences in protein levels, participants were categorized into eight groups based on the date of their last menstruation. A subset of 60 proteins was selected based on their association with menopause, divided into three groups: high association with menopause, medium association with menopause and low association with menopause. Statistical analyses were conducted accordingly:

Welch's ANOVA was applied to the first set (20 proteins) due to unequal variances, followed by post-hoc analysis using the Games-Howell test to adjust for multiple comparisons; standard one-way ANOVA followed by Tukey's test was used for the second and third sets, where homogeneity of variance was met.

RESULTS

The first research question, which examined whether plasma proteins exhibit phase-specific variations during the menstrual cycle, identified significant changes for nine proteins in the first set (LHB, FSHB, CGA, CGB7, SFRP4, TFPI, HAMP, FTL, FCGRT), one protein in the second set (OXT), and four proteins in the third set (CHRD12, BIN1, CREBBP, and SLC9A3R1).

Of these, twelve proteins (LHB, FSHB, CGA, CGB7, HAMP, FTL, FCGRT, SFRP4, TFPI in the first set; OXT in the second set; CHRD12, and CREBBP in the third and last set) are linked to menstrual physiology or reproductive health through their function, tissue expression (e.g., endometrium, cervix, ovaries, placenta), or association with reproductive-related conditions or pregnancy. No significant differences were found when stratified by age, BMI, chewing tobacco use, type 2 diabetes, or oral contraceptive use.

CONCLUSIONS

Understanding how menstrual cycles influence circulating proteins could improve our knowledge of biomarker fluctuations and their role in predicting disease. This insight may help identify potential diagnostic and prognostic markers. The next

step is to expand the analysis to all 7,000 available proteins to uncover associations with the menstrual cycle and validate significant variations across its phases.

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Observational Retrospective Study On Dialytic Clearance Parameters Obtained During Hemodialytic Sessions in Condition of Normal and Suboptimal Blood Flow

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INTRODUCTION

Hemodialysis is a life-saving procedure that is required by patients with end-stage chronic kidney disease. This procedure, based on diffusive and convective mechanisms, uses a large amount of treated water (dialysate) to capture patient's blood toxins using a semi-permeable membrane (dialysis filter). The volume of blood cleared from toxins per minute of treatment is defined dialytic clearance and it is indicated by the letter K [1]. K is modulated by 3 different factors: patient blood flow (Qb), dialysate flow (Qd), filter-specific diffusive coefficient (KoA). During standard practice, these factors are adjusted in order to achieve an appropriate toxins clearance: KoA can be controlled by physician that uses different filter type, meanwhile the dialysis machine uses an autoflow mechanism (AF) to adjust Qd accordingly to Qb [2, 3]. However, in a real case scenario, some patients cannot withstand normal Qb (Qb < 300 ml/min) then AF need to increase Qd to achieve similar purification results but consequently leading to higher Qd usage.

Since most information on dialysis filter KoA is based on in vitro data, we decided to take advantage of a novel dialysis machine (Fresenius 5008®) capable of measuring real-time K value and we used this information to quantify in vivo KoA.

OBJECTIVES

Our study aimed to describe, in real case scenario, the clearance performance of five different dialytic filters working

on subjects with suboptimal Qb and to measure how different Qd can influence in vivo KoA.

METHODS

We conducted a retrospective descriptive study on Nephrology and Dialysis Unit hemodialyzed population (AUSL-IRCCS di Reggio Emilia). A cohort of 70 subjects was screened for presence of at least 6 hemodialytic sessions (all of them with the same filter type) in a time span of 90 days and starting from a first session with suboptimal Qb. For each session, K values were collected for different combination of Qd (500, 300 ml/min) and Qb (300, 250, 200 ml/min). KoA in vivo was calculated according to Alayoud et al. work [4] and compared with manufacturer in vitro KoA using one sample Wilcoxon signed-rank test. Then, using only suboptimal Qb condition, it was investigated the effect of different Qd on in vivo KoA using a paired samples Wilcoxon signed-rank test. Finally, for each dialysis filter, in vivo KoA values were used to generate a nomogram to visualize the relation between K and Qb at the end of a standard dialytic session. Nomogram calculations were done according to already published method [4] and assuming a target of $K=1.2V/t$ for the minimal adequate dialytic dose [5].

RESULTS

Our data showed that in vivo KoA of Fx80®, Fx100®, Fx1000®, Solacea 19H® and Filtryzer BG2.1® dialysis fil-

ters are all significantly lower ($p < 0.001$) than their respective in vitro KoA values reported in the manufacturer datasheet. Comparison between KoA values, among different dialysis filters, in condition of suboptimal Qb, showed a non-significant difference among groups, suggesting that usually higher Qd can only increase marginally in vivo KoA. Lastly, we produced a nomogram that compared dialysis filter performance side by side. From top performer to the lowest: Fx® filters groups showed similar in vivo K for all Qb modeled, followed by Solacea 19H® filter, then Filtryzer BG2.1®.

CONCLUSIONS

Our data confirmed the discrepancy between in vitro and in vivo KoA values for each dialysis filter analysis. Our results showed a percent decrease spanning from 48% to 61% among all filters, and these results are in line with the model developed by Daugirdas and colleagues [6] that estimates a 57% lower in vivo/in vitro KoA. Unfortunately, due to low patient enrollment and data availability, the statistical test comparing distinct filters in condition of suboptimal Qb was underpowered so it cannot be considered conclusive. Lastly, produced nomogram is helpful to visually describe the link between Qb and K among distinct dialysis filters. Understanding which are the real in vivo clearance performance of these filters, in both normal and suboptimal Qb, is an important knowledge to achieve and it can lead to further improvement in patient care and resources allocation for the health care system.

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Beyond the Nutrition5k Project: Data Curation and Deep Learning Algorithms to Predict the Nutritional Composition of Dishes from Food Images

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INTRODUCTION

In recent years, artificial intelligence (AI) has emerged as a powerful tool to overcome limitations of traditional dietary assessment methods such as 24-hour recalls, food frequency questionnaires, and dietary records [1, 2]. Nevertheless, the success of AI models heavily depends on high quality, well-curated data. Pre-processing—handling missing values, outliers, and inconsistencies—is essential to ensure reliable model performance [3, 4]. The Nutrition5k project [5] is the first to adopt Deep Convolutional Neural Networks for the 2D direct prediction of mass and nutritional composition of dishes.

AIMS

We used the US-based Nutrition5k project to evaluate the performance of various deep learning (DL) algorithms, and to compare them in predicting mass, energy, and the macronutrient content from food images. We explored different ground truth configurations (by combining data curation with two country-specific food composition databases—FCDBs) and checked if there were specific dishes consistently mispredicted by most algorithms, and what common features they shared.

METHODS

Within the Nutrition5k project, mass (grams), energy (kcal), protein, fat, and carbohydrates (grams) contents were provided for each of the 5006 dishes as sum of nutritional

values of single ingredients derived from the US-FCDB. In a previous publication [6], we have matched the US dishes with their Italian nutritional composition. This gave birth to four versions of the Nutrition5k dataset, specifically obtained as ground truths by crossing country-specific FCDBs with ingredient-mass correction of outlier dishes.

We chose Inception_V3_IMAGENET1K_V1 (IncV3, the updated version of the IncV2 proposed in [5]), ResNet101_IMAGENET1K_V2, ResNet50_IMAGENET1K_V2, ViT_B_16_IMAGENET1K_SWAG_E2E_V1 (ViT-B-16), built in two variants (2+1 and 2+2), and pretrained via the open-source ImageNet. IncV3_2+2 was our benchmark algorithm as in [5]. To ensure reproducibility, we adopted the same pipeline as in the Nutrition5k project for train/test split of dishes, loss function, frame preprocessing, and performance metrics (root mean squared error, mean absolute error – MAE – and its percentage – MAPE).

Dish-specific (raw, absolute) differences between predicted and observed values of the target variables on the test set ($n=676$) were evaluated across datasets and algorithms (160 predictions per dish), by considering: (1) percentages of perfect, adjacent, and opposite agreement among quartile-based categories, and unweighted Cohen's kappa statistics, and 2) Bland-Altman plots.

We defined "incorrectly predicted dishes" dishes as those that for 7 or 8 DL algorithms (1) exceeded the 95% limits of agreement in the Bland-Altman plots and (2) had the highest 5% of absolute differences across target variables and datasets. Their dish frames were manually inspected and further removed when needed. The "incorrectly predicted dishes" were then grouped based on similarity in content.

A sensitivity analysis was carried out to study whether energy content should be directly predicted by DL algorithms or deterministically calculated by summing up predicted macronutrients multiplied by the corresponding conversion factor. This led to three scenarios: the 5-task predicted energy content (main analysis), the 5-task computed energy content (energy calculated based on macronutrients predicted together with energy), and the 4-task computed energy content (no energy prediction potentially improving macronutrient prediction).

RESULTS

The median dish to be predicted on the test set had a mass of 142 g, energy content of 164.5 kcal, 8.3 g of protein, 6.9 g of fat, and 11.3 g of carbohydrates. When dishes showed ingredients with extreme weight or composition, algorithms tended to pull their predictions toward the center of the distribution.

For the same dataset, IncV3s consistently showed the worst percentages of perfect agreement across all target variables. For a given algorithm, perfect agreement was generally higher in the corrected datasets, with the exception of protein. Similarly, Cohen's kappa values were lower for the IncV3s and higher for the corrected datasets.

Globally, mass and energy content had more similar and lower error metrics, followed by protein, carbohydrates, and fat (Figure 1). By dataset, IncV3s generally exhibited the worst performances. Ingredient-mass correction strongly improved performance metrics.

The incorrectly predicted dishes were 80, of which 12 were discarded (7 for discrepancies between ingredient names and images and 5 for image-related issues for all images). Beyond the corrected-portion-size group (5%), Salad-based (44%), Chicken-based (25%), Eggs-based (13%), and the Western-inspired breakfast foods (13%) groups were identified. From this list we removed a median of 60% of the original frames, which led to a slight reduction in MAPE values.

While comparing our three scenarios, we observed a gradient: performance was the highest in the 5-task predicted, then the 5-task computed and finally the 4-task computed energy content scenario, advancing that energy prediction may partially compensate for macronutrient prediction errors, particularly those arising from image grounding issues. The ViT-B-16's showed minimal differences ($\sim 7\%$) across scenarios.

CONCLUSIONS

We investigated the use of the Nutrition5k dataset for directly predicting the nutritional composition of dishes (including mass) using 2D images. All six selected algorithms outperformed the benchmark IncV3_2+2, as well as the lighter IncV3_2+1. Data curation, especially ingredient-mass correction, is critical in influencing algorithm performance.

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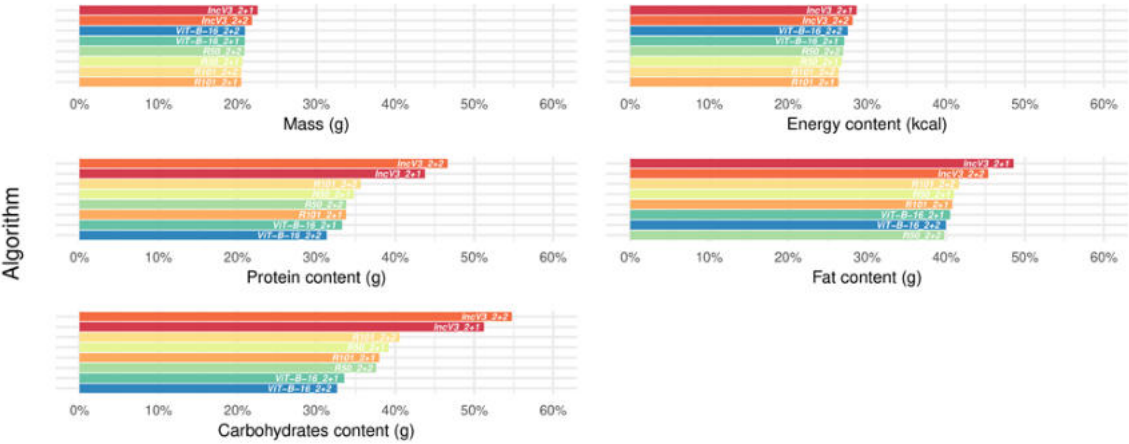


Figure 1. Median error, as measured by mean absolute percentage error, for single target variables and algorithms across datasets, before frame filtering. Abbreviations: MAPE, Mean Absolute Percentage Error.

Predicting Acute Biliary Pancreatitis Relapse using CNN: The MINERVA Multicentric Study

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INTRODUCTION

Acute pancreatitis (AP) is the main pancreatic disease diagnosed in the world [1]. The etiology of AP is commonly alcoholic or related to biliary events [2,3]. Current guidelines recommend performing early cholecystectomy (EC), as surgery significantly reduces the risks of subsequent recurrence [4,5,6,7,8]. Recurrence of acute biliary pancreatitis (RBAP) is defined as a syndrome of multiple distinct acute inflammatory responses originating in individuals with genetic, environmental, traumatic, metabolic, who experienced a second episode of AP after at least 3 months [9]. To date, RBAP is a dangerous clinical complication of the pancreas, requiring emergency surgery and it can cause death if not operated on within 24 hours of onset [9]. However, due to particular patient frailties, medical-surgical conditions, or logistic problems, EC is not always performed [10]. The early identification of patients at high risk of recurrence could lead to better clinical and logistics management and provide practical recommendations for cholecystectomy priority [11,12,13,14]. Predicting and preventing RBAP can reduce costs of hospitalization and medical care and, more importantly, promote the management and prioritization of cases hospitalized with AP and potentially subject to relapse. Our recent systematic review [15] confirmed that there are no prospective studies that tried to model the prediction of RBAP. All evidence emerged through monocenter, retrospective data, was inconclusive and contradictory. The aim of the MINERVA study is 2-fold: on one hand, it aims to gather prospective data about RBAP from XX centers in Italy; on the other, it aims to develop and validate the first machine learning-based predictive model to identify patients at risk of RBAP [16].

OBJECTIVE

The MINERVA (Machine learnINg for the rElapse Risk eValuation in Acute Biliary Pancreatitis) project is the first observational multicenter prospective trial designed to investigate the predictive factors of relapse in acute biliary pancreatitis using artificial intelligence (AI) and by collecting outcomes at 3 months, 6 months, and 1 year follow-up. The aim of this project is to develop a predictive model of acute biliary pancreatitis recurrence based on a convolutional neural network (CNN), using images generated from tabular clinical data from both prospective and retrospective multi-centric sources.

Methods Clinical tabular data from the retrospective MANCTRA [17] and prospective MINERVA [16] datasets were merged to obtain 2413 instances appropriately pre-processed to manage missing values, normalizations and prevent errors. A strong imbalance was regulated applying the adasyn algorithm [18], to increase the minority class (initially 8% of the total) with artificial instances. The final dataset was made by 3630 subjects divided in 1441 with relapse and 2189 without it. The selected predictors were divided in numerical: the patient's age, BMI, white blood cell (per mm³), neutrophil (per mm³), platelet (per mm³), international normalized ratio, protein c-valuesreactive, aspartate aminotransferase (units/liter), alanine aminotransferase (unit/liter), total bilirubin (mg/dl), direct conjugated bilirubin, gamma glutamyl transpeptidase (units/liter), serum amylase (units/liter), lipase (units/liter), the lactate dehydrogenase (units/liter); and categorical: sex of the patient, previous episodes of pancreatitis, clinical history of diabetes, clinical history of chronic lung disease, hypertension, atrial fibrillation, chronic kidney disease, disease of the hematopoietic system, immunosuppressive drugs at the time of admission, cholelithiasis, acute cholangitis, department of hospitalization and Endoscopic Retrograde Colangiography-Pancreatography. Then, using the PCA-based Deepinsight algorithm [19], the

features have been mapped to pixels with a grid size equal to 128; To process the images, we designed a CNN consisting of 3 reduction blocks with ReLU and MaxPooling activation, followed by 2 fully connected layers: the first with ReLU and Dropout, the second with sigmoid activation for binary classification (RBAP: yes/no). we used Adam optimizer (L2 adjustment) and binary cross-entropy loss. Data were divided into training (70%), validation (15%) and test sets (15%), and AUC, F1-score and accuracy were used for evaluation. We used an adaptive variable learning rate (LR) starting from 0.001, a batch size (BS) between 16 and 32, the number of periods incrementally validated according to a trial and error approach, and then fixed by early stopping (ES) technique. A classical non-parametric bootstrap approach, based on 50-iterations, was adopted to estimate the variability of performance metrics by evaluating greater robustness and reliability of the predictive capabilities compared to fluctuations in baseline data. The python code took about 30 minutes to run on a PC with Processor 12th Gen Intel(R) Core(TM) i5-12400F (12 CPUs) 2.5 GHz, Operating System Microsoft Windows 11 Pro and RAM from 32GB, equipped with NVIDIA GeForce RTX 4060.

RESULTS

The model showed good predictive performance in validation phases with an AUC of $(84.38 \pm 1.76)\%$, while on test AUC is 82.25%. Parameters as follows: BS=32, LR=0.001, ES at 60 epochs and weight decay equal to 0.0001.

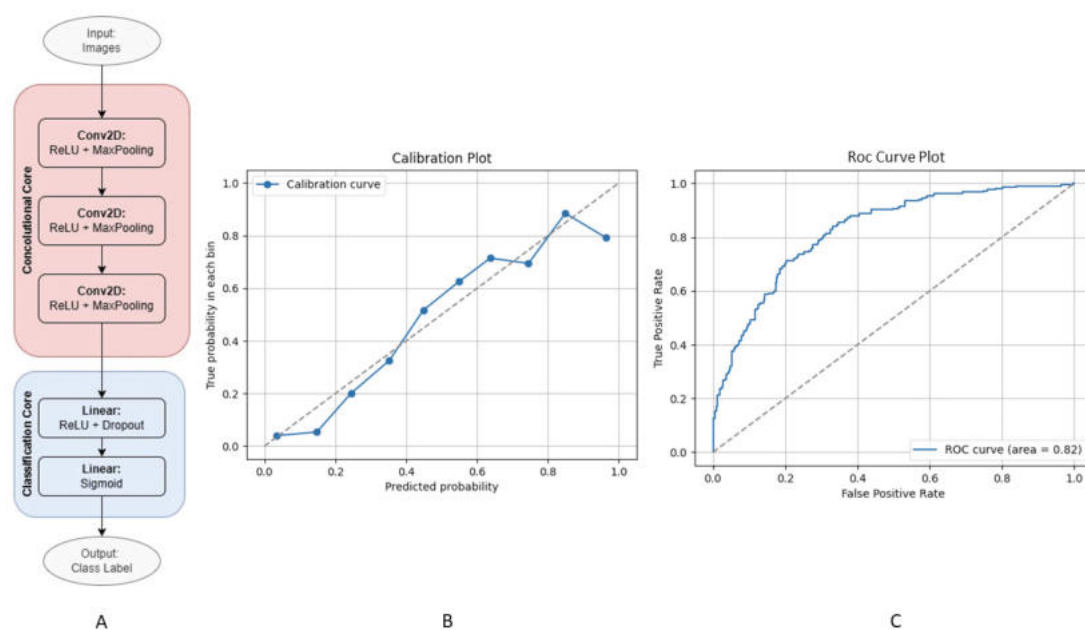


Figure 1. Structure of the implemented CNN (A), Calibration Plot (B) and Roc Curve Plot (C) of the model.

CONCLUSION

This study presents the first AI model developed specifically for the prediction of RBAP. The integration of clinical data from complementary sources and the application of CNN techniques demonstrate the feasibility and clinical potential of this approach in an area that has been little explored so far. The results of this work are promising and the testing of AI to support the management of cases of relapse of acute biliary pancreatitis can prove a beneficial factor in supporting the medical clinic.

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Treatment Persistence of Lacosamide, Perampanel and Brivaracetam: An Extended Real-World Analysis from the COMPARE Italian Cohort

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OBJECTIVES

Antiseizure medication (ASM) discontinuation is a frequent and clinically meaningful outcome in the treatment of epilepsy, often reflecting inadequate tolerability, limited effectiveness, or both [1, 2]. In this study, we reanalyzed data from the COMPARE Italian multicenter cohort previously published by Roberti et al [3], to investigate real-world outcomes in patients treated with the most recent add-on ASMs. Our aim was to compare treatment persistence among lacosamide, brivaracetam, and perampanel over time, and to assess how adverse events and clinical response influence the discontinuation process. In contrast to the original multivariable regression approach, we applied a propensity score weighting framework to better approximate causal effects and address residual confounding.

METHODS

We estimated stabilized inverse probability of treatment weights (IPTW) using a multinomial propensity score model based on age, sex, epilepsy duration, etiology, seizure type, prior ASM exposure, epilepsy surgery, and baseline polytherapy [4, 5]. Time to treatment discontinuation was analyzed through log-logistic accelerated failure time (AFT) models, which allow direct estimation of time ratios (TRs) [6]. Clinical response and adverse events were added as covariates to explore their potential mediating role. Interaction terms with log-transformed time were introduced to assess how treatment effects evolve longitudinally. Robustness was evaluated through sensitivity analyses using cluster-robust standard errors and symmetric weight trimming to exclude individuals with extreme propensity scores.

RESULTS

The analysis included 828 patients (250 lacosamide, 234 brivaracetam, 344 perampanel), with a maximum follow-up of 36 months. In unadjusted AFT models, brivaracetam (TR = 0.48, 95% CI: 0.27–0.84) and perampanel (TR = 0.46, 95% CI: 0.27–0.76) were associated with shorter persistence than lacosamide. After adjusting for adverse events and response, the association was attenuated for brivaracetam (TR = 0.68, 95% CI: 0.42–1.14), while perampanel remained significant (TR = 0.61, 95% CI: 0.38–0.97). Both adverse events (TR = 0.44, 95% CI: 0.30–0.66) and clinical response (TR = 4.25, 95% CI: 2.97–6.07) were independently associated with time to discontinuation, supporting their potential mediating role. Time-dependent models revealed that the initial disadvantage of brivaracetam (interaction TR = 2.50, 95% CI: 2.21–2.83) and perampanel (TR = 2.60, 95% CI: 2.34–2.88) diminished over time. Clinical response became increasingly protective, while the impact of adverse events waned. Sensitivity analyses confirmed the robustness of these findings.

CONCLUSIONS

The risk of treatment discontinuation evolves dynamically and differs among ASMs. Lacosamide showed greater early persistence, whereas brivaracetam and perampanel, despite being associated with earlier dropout, demonstrated improved retention among patients who tolerated them beyond the initial period. These findings suggest that the first weeks of treatment may be critical for identifying and managing tolerability issues, especially with brivaracetam and perampanel. Our results underscore the importance of adopting a dynamic, patient-centered approach to ASM selection, considering not only baseline characteristics but also longitudinal

response and tolerability. Propensity score-based methods, when applied appropriately, can enhance causal inference in observational studies and provide clinically meaningful insights to guide therapeutic decisions in epilepsy care.

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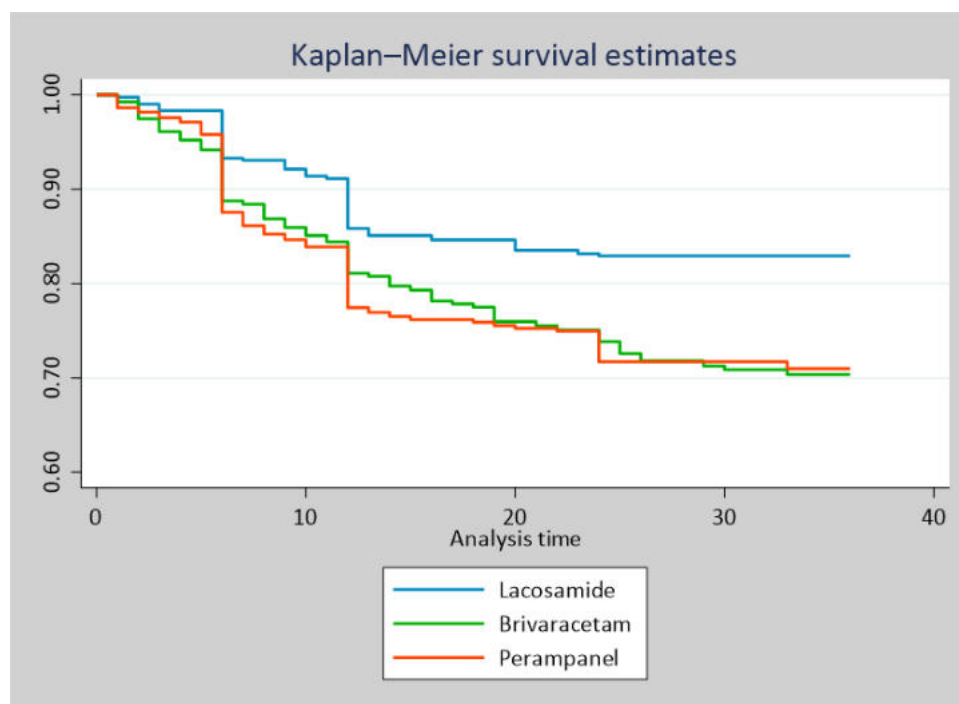


Figure 1. Kaplan-Meier survival curves showing time to treatment discontinuation for lacosamide, brivaracetam, and perampanel over a follow-up of up to 36 months. Lacosamide was associated with higher early treatment persistence.

Effectiveness and Safety of Control-IQ Technology in Preschool and School-Aged Children with Type 1 Diabetes: A Real-World Multicenter Study

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INTRODUCTION

Achieving and maintaining optimal glycemic control from the onset of type 1 diabetes (T1D) is crucial in pediatric care, especially in early childhood when the developing brain is highly vulnerable to both hypo- and hyperglycemia [1-3]. Hyperglycemia during early childhood increases the risk of long-term vascular complications, while severe hypoglycemia may impair neurocognitive development, causes family anxiety, and complicates social integration [4-5]. Although automated insulin delivery (AID) systems have demonstrated efficacy in controlled trials, real-world evidence in children under six years of age, particularly involving off-label use, remains limited. The Control-IQ (CIQ) algorithm, integrated into the Tandem t:slim X2 insulin pump, has shown benefits in adolescents and school-aged children [6-10]. However, few studies have evaluated its long-term use in children under six in routine clinical practice.

OBJECTIVE

This study aimed to compare the real-world effectiveness and safety of the CIQ system in two pediatric age groups—children aged 0.5–5 years and children aged 6–10 years—over an 18-month follow-up period. We evaluated effectiveness in terms of glycemic control (% of time in glucose range 70–180 mg/dL [TIR], % of time in glucose range 70–140 mg/dL [TITR], and HbA1c) and safety in term of adverse events (diabetic ketoacidosis [DKA], hyperglycemia and severe hypoglycemia).

METHODS

This prospective, multicenter observational study used retrospective data from 32 Italian pediatric diabetes centers. Eligible participants had T1D diagnosed ≥ 6 months, were < 11 years old at CIQ initiation, and had continuous glucose monitoring (CGM) data available via Glooko® or Clarity® software at least every 6 months during the 18-month follow-up. Children with non-T1D or aged > 10 years at CIQ start were excluded. Participants were stratified by age at CIQ initiation (0.5–5 and 6–10 years). At CIQ initiation (baseline) sex, presence of celiac disease or thyroiditis and parents' age, nationality and education, were collected. HbA1c, BMI z-score, CGM-derived data (TIR, TITR, % of time spent in glucose ranges: < 54 mg/dL, 54– < 70 mg/dL, 180– < 250 mg/dL, > 250 mg/dL, Glucose Monitoring Indicators and coefficient of variation of glucose), Glycemia, Standard Deviation of Glycemia [SD] and DKA episodes were assessed at baseline, 6, 12, and 18 months. Descriptive statistics were used for baseline comparisons. Chi-square or t tests evaluated group differences. Trend over time points in TIR, TITR, and HbA1c were analysed using mixed-effects models for repeated measures, adjusted by age group, sex, time from diagnosis to CIQ initiation, DKA at onset and parents' socio-economic characteristics (at least one non-Italian parent, parents' education). A sequential difference contrast was used to model time; interaction between time and age groups was evaluated. Only children with complete data on the outcomes at all four time points were included in these models.

Safety outcomes included the proportions of DKA and severe hypoglycaemias occurring during 18-month follow-up.

RESULTS

Of the 334 children enrolled, 253 (106 aged 0.5–5; 147 aged 6–10) had complete data on the outcomes and were included in longitudinal analyses. At T1D diagnosis, a higher prevalence of thyroiditis in the older group was found, and no significant sociodemographic differences. At CIQ initiation, younger children had a significantly shorter time from diagnosis to CIQ initiation (1.36 vs 2.61 years, $p < 0.001$), higher HbA1c (8.3% vs 7.7%, $p = 0.020$) and higher glycaemic variability (SD 63.3 mg/dL vs 58.3 mg/dL, $p = 0.023$) while TIR, TITR, and the other CGM-derived data were comparable.

Longitudinal analysis (Figure 1) showed significant improvement in both groups 6 months after CIQ initiation: TIR increased by 6.62% (95% CI: 4.89–8.36) and TITR by 5.63% (95% CI: 3.61–7.66), corresponding to over 80 additional minutes/day spent in target ranges. These improvements were sustained at 12 and 18 months. HbA1c decreased by an average of 0.82% (95% CI: –1.01 to –0.62) in the first 6 months, remaining stable thereafter. No significant interaction between time and age groups was observed, indicating similar trends in both cohorts. Having at least one non-Italian parent was significantly associated with lower TIR (–5.82%, 95% CI: –10.33 to –1.31) and higher HbA1c levels (0.31%, 95% CI: 0.01 to 0.63). A high parental education level (university or higher vs. up to lower secondary education) was associated with higher TIR (8.61%, 95% CI: 3.03–14.18) and lower HbA1c levels (–0.42%, 95% CI: –0.78 to –0.06). Age at CIQ initiation, time from diagnosis to CIQ initiation, DKA at diagnosis, and sex were not significant predictor.

Regarding safety, no severe hypoglycaemia episodes were reported in the younger group, and only one occurred in the older group after 12 months. A single DKA episode was recorded in a child under six. Moreover, CGM-derived data indicated that time spent in hypoglycaemia (<54 and 54–69 mg/dL) remained consistently below clinically relevant thresholds (<1% and <3%, respectively).

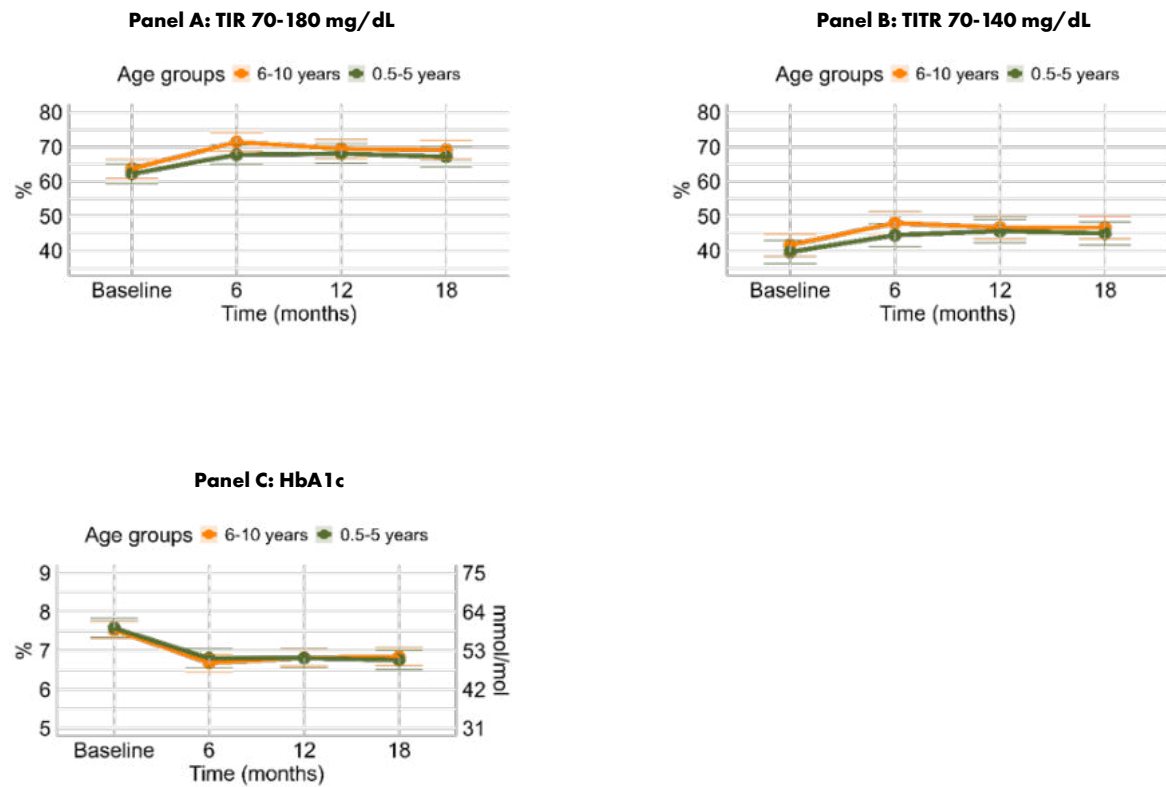
CONCLUSION

In this large real-world cohort of young children with T1D, the CIQ system demonstrated consistent and sustained improvements in glycaemic outcomes over 18 months, with minimal adverse events. Significant gains in TIR, TITR, and HbA1c were observed in both age groups, particularly in the first 6 months after CIQ initiation. These benefits were maintained long-term, regardless of initial glycaemic status and presence of DKA at diagnosis. The system proved safe even in children under six, supporting its current use in off-label settings with appropriate clinical oversight. Our findings reinforce the value of early AID adoption to optimize long-term metabolic outcomes in pediatric T1D.

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Figure 1. Effect of time on Time In Range, Time In Tight Range and HbA1c between age groups.



A Bidirectional Two-Sample Cis-Mendelian Randomization in the MHC Region of Immune Responses against Epstein-Barr Virus Nuclear Antigen 1 and Multiple Sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system with a complex etiology involving both genetic and environmental factors [1]. Epstein-Barr virus (EBV), a ubiquitous herpesvirus infecting over 90% of the global population, has consistently been identified as one of the strongest environmental risk factors for MS. Observational studies have shown increased MS risk following infectious mononucleosis and elevated EBV antibody titers. Proposed mechanisms include molecular mimicry and impaired immune control of latent EBV infection [2]. A notable longitudinal study in U.S. military personnel demonstrated a >30-fold increase in MS risk following EBV seroconversion [3]. However, such findings remain susceptible to residual confounding, selection bias, and reverse causation, particularly considering emerging evidence suggesting that MS-related immune dysregulation may begin years before diagnosis.

OBJECTIVES

To address limitations in observational research and strengthen causal inference, we applied two-sample Mendelian Randomization (MR) to investigate whether humoral immune IgG responses to EBV antigen EBNA-1 is causally associated with MS risk specifically considering the MHC region, where the majority of genetic signals associated with EBNA-1 antibody levels are located [4]. To address the extensive linkage disequilibrium (LD) and pleiotropy in the MHC region, we applied a cis-MR framework centered on the constrained maximum likelihood method (cis-MR-cML), a methods allowing for correlated instrumental variables (IVs) and robust to IV assumption violations [5].

METHODS

We conducted a two-sample MR analysis using summary statistics from genome-wide association studies of antibody responses to EBV antigen EBNA-1 in 8,477 white British participants from the UK Biobank, and of MS risk in 115,803 individuals of European ancestry from the International MS Genetics Consortium. SNPs were selected using GCTA-COJO with UK Biobank cohort as LD reference panel [6,7]. To account for the extensive LD in the MHC region, it was treated as a single LD block. A stepwise regression approach was used, with p-value thresholds of 5×10^{-3} for SNPs associated to EBNA-1 levels (I_x, set of candidate IVs) and of 5×10^{-8} for SNPs associated to MS (I_y). Then, conditional effect of each candidate IV with both EBNA-1 and MS was estimated conditioning for all the other SNPs in I_x and in I_y in LD with it (considering an $R^2 > 0.005$). This approach aims to mitigate LD-driven horizontal pleiotropy. Instrument strength and directionality were then assessed using F-statistics and Steiger filtering to obtain the final set of IV to be used in the MR analysis. To obtain estimates for causal effect, we applied cis-MR-cML [5] and, as sensitivity analyses, additional MR methods (GSMR and LDA-Egger) [8,9] for robustness checks. We also conducted reverse MR analyses to evaluate whether MS genetic susceptibility may influence EBV antibody levels, reflecting potential bidirectional or disease-driven effects.

RESULTS

GCTA-COJO identified 19 SNPs, candidate IVs, jointly associated with EBNA-1 IgG levels. After conditioning the effect of this potential IVs for the other SNPs in I_x and I_y, we obtained a set of 6 IVs with F-statistic > 10 and passed the Steiger directionality test ($p < 0.05$). Cis-MR-cML identified

and removed one invalid IVs identified as outliers, leading to a final set of five IVs with global F-statistic = 24.6 and a significant correct causal directionality ($p < 0.001$). The MR results indicated that a 1 SD increase in genetically predicted EBNA-1 IgG levels was associated with a two-fold increase in MS risk ($OR = 2.42$ [95%CI: 1.87;3.14], $p < 0.001$). GSMR provided further support for this significant causal association with similar effect estimate ($OR \approx 2$). LDA-Egger did not detect significant directional pleiotropy ($p > 0.05$), and the slope estimate further supported a significant risk association. Reverse MR analysis did not find a bidirectional relationship, as the estimated association between MS and EBNA-1 levels was not statistically significant ($p > 0.05$).

CONCLUSIONS

Our findings support a causal role of increased humoral immune responses to EBV antigen EBNA-1 in increasing MS risk. Using a robust cis-MR framework and accounting for extensive MHC region LD, we showed that genetically predicted EBNA-1 IgG levels are significantly causally associated with MS unidirectionally. These results strengthen the hypothesis that impaired immune control of EBV may be a key mechanism in MS pathogenesis and highlight the potential of EBV-targeted prevention strategies.

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Clinical Outcomes after Transcatheter Aortic Valve Implantation in Nonagenarian Patients: A Retrospective Population-Based Cohort Study

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INTRODUCTION

Transcatheter aortic valve implantation (TAVI) has emerged as first choice for severe aortic stenosis (AS) in patients older than 75 years for all surgical risks [1], as well as in inoperable or high surgical risk patients [2], including nonagenarians [3]. Invasive treatments of these very elderly raise several ethical, financial, and procedural issues. Current data on the effectiveness and clinical outcomes of TAVI in this aged group remain limited, with unclear benefits and potential futility of the procedure in nonagenarians.

OBJECTIVES

To compare the overall survival and the risk of all-cause and heart failure-specific hospitalization in nonagenarian patients hospitalized for symptomatic severe aortic stenosis (AS) who underwent transcatheter aortic valve implantation (TAVI) or conservative treatment in the Lombardy Region.

METHODS

This population-based retrospective cohort study was entirely based on healthcare utilization databases of the Italian region of Lombardy. The cohort included all nonagenarians hospitalized for AS between 2017 and 2021, who underwent TAVI within 90 days from first diagnosis or conservative treatment. The association between TAVI and clinical outcomes was assessed through Cox or Fine&Grey regression models. Given that patients exposed to TAVI are likely to have a more

favorable clinical profile compared to unexposed patients, two different comparative analyses were conducted. First, since the baseline characteristics were restricted to specific measurable variables (age, sex, comorbidities and co-treatments summarized in the MCS [4]), the comparison could have been influenced by residual unmeasured confounding. To address this potential bias, a semi-automated data-adaptive high-dimensional propensity score (HDPS) approach was employed to reduce potential founding by indication [5]. Second, administrative data may not fully capture the clinical heterogeneity between exposed and unexposed patients, and unexposed patients are likely to have a higher mortality rate within the first months after the diagnosis of severe aortic valve stenosis. Thus, an additional analysis was conducted on patients who survived the first six months of follow-up.

RESULTS

Overall, 16,848 nonagenarians hospitalized for AS were identified. Among these, 320 patients underwent TAVI. In the unmatched cohort, patients who underwent TAVI were younger, more frequently males, and exhibited a more favorable clinical profile compared to those who were unexposed to TAVI. In the HDPS analysis, 193 patients exposed to TAVI were matched to as many control patients. The 2-year survival rates were 76.0 % and 37.7 %, respectively, in TAVI and control patients, corresponding to an HR of 0.24 (95% CI 0.15–0.37). When excluding patients who died within the first 6 months of follow-up, a significantly higher survival was still observed among TAVI patients (HR 0.27, 95% CI 0.16–0.47). The 2-year cumulative incidence of all cause re-hospitaliza-

tion was higher during the first six months, being 47.4 % and 34.7 %, respectively. Rehospitalization for heart failure was 11.1 % and 26.5 %, respectively, corresponding to an HR of 0.64 (95% CI 0.40–0.99).

CONCLUSIONS

This study further supports the usefulness of TAVI in nonagenarians, as it showed to improve their survival rate, reduce their risk of rehospitalization, and likely increase their quality of life.

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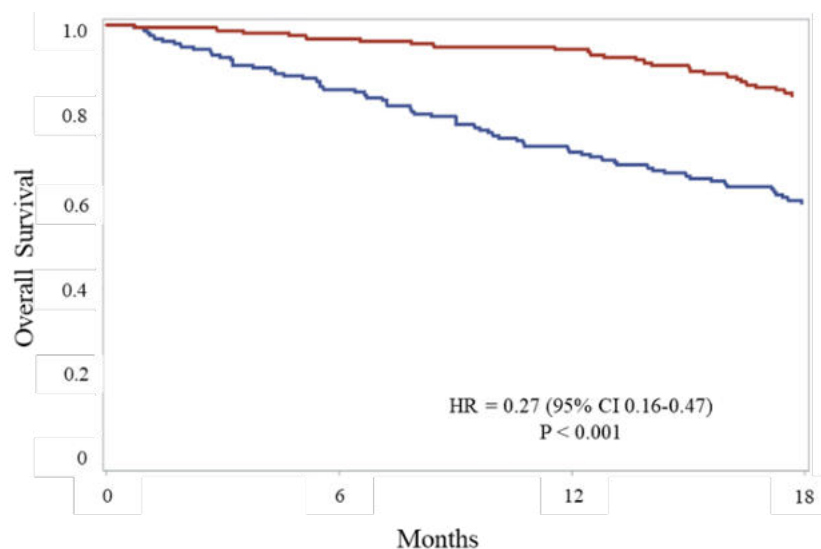


Figure 1. Overall survival of patients who did (red line) or did not (blue line) undergo transcatheter aortic valve implantation (TAVI) in the high-dimensional propensity score (HDPS) the matched cohort that only included patients who survived the first six months of follow-up.

Predicting Methodological Quality in No Profit Clinical Trial

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INTRODUCTION

The SPIRIT 2013 Statement [1] has long represented the international gold standard for the content of clinical trial protocols, providing a comprehensive framework to ensure transparency, methodological rigor, and ethical soundness. However, the rapid evolution of trial methodologies, regulatory landscapes, and data-sharing practices has created the need for a revised standard. Just days ago, SPIRIT 2025 [2] was released, introducing updated and expanded guidance that reflects contemporary challenges and expectations, particularly in areas such as adaptive designs, patient involvement, and statistical analysis. Despite these efforts, numerous studies continue to document suboptimal adherence to SPIRIT recommendations [3], especially concerning trial design and statistical methods. Evaluating real-world adherence to SPIRIT guidelines [4] offers valuable insights into common shortcomings, systemic barriers, and areas requiring targeted support or training [5].

OBJECTIVES

The aim of this study was to assess whether it is possible to predict which clinical trial protocols are likely to show high adherence to SPIRIT guidelines, with a specific focus on methodological items. We sought to identify study-level characteristics that may act as potential predictors of adherence, to better understand structural drivers of protocol quality and support future improvement strategies.

METHODS

We retrieved information on design and methodological features of clinical trial protocols submitted between 2021 to 2025 to the local Ethics Committee and recorded them in a centralized REDCap registry. Adherence to SPIRIT 2013 items

related to methodology, study design, data collection, management, and analysis (items 9–21 b) was assessed. The 2013 version was used because all studies included were submitted prior to the release of Spirit 2025. Each item was scored as fulfilled or not, and an individual adherence score was computed as the total number of satisfied items. We described the distribution of adherence scores using median and interquartile range (IQR). Since adherence scores were not normally distributed, we dichotomized the scores at the median value, classifying them into higher adherence (“good”) and lower adherence (“poor”) categories. To address the predictive objective, we implemented a set of machine learning algorithms (e.g. Random Forest, eXtreme Gradient Boosting, and Boosted Logistic Regression) applied to a pool of candidate predictors selected for their potential relevance to the outcome. Model performance was evaluated using accuracy, area under the ROC curve (AUC), and F1 score. Variable importance was then assessed across models, and the most influential predictors were subsequently incorporated into a multivariable logistic regression model to evaluate their independent association with the outcome. Covariates included study characteristics related to sponsorship, methodological features, submission timing, and thematic focus. Odds ratios and corresponding 95% confidence intervals will be reported to evaluate the direction and strength of the association. Analyses were conducted using Stata software, release 19 and R version 4.4.3.

RESULTS

All 132 protocols included in the analysis were no profit interventional. Of these, 28% were monocentric, 59% multicentric within Italy, and 13% involved international sites. Overall, 32.6% of the studies were promoted by Italian sponsors with structured biostatistical support, 62.9% by other Italian sponsors for whom the availability of such support is unknown, and 4.5% by international institutions, also with unknown bi-

ostatistical support. Randomization was used in 59% of protocols. Blinding was reported in 12% of protocols: 9% were double-blinded and 3% single-blinded. Median adherence to the overall SPIRIT checklist was 72% (IQR 52.3%–85.7%). In the methods section, adherence was 80% (IQR 60%–90%) for items 9–15, 100% (IQR 66%–100%) for items 16–17, and 62.5% (IQR 37.5%–87.5%) for items 18–21b. The outcome was defined as a summary score representing the number of methodological items checked and was dichotomized at the median value. The presence of a biostatistics unit was significantly associated with higher methodological quality (OR = 3.44; 95% CI: 1.08–10.99; $p = 0.037$). Protocols involving pediatric populations were less likely to meet high-quality criteria (OR = 0.085; 95% CI: 0.007–1.048; $p = 0.054$), as were those in the Oncology/Infectious Diseases area (OR = 0.20; 95% CI: 0.045–0.870; $p = 0.032$). The model demonstrated good discriminative ability (AUC = 0.792) and excellent calibration ($p = 0.91$).

CONCLUSIONS

By combining traditional statistical approaches with innovative machine learning models, we gained a clearer understanding of which protocol features are predictive of high adherence to SPIRIT guidelines. Our findings suggest that the involvement of a multidisciplinary team, including biostatisticians, is strongly associated with better methodological quality. Protocols submitted by IRCCS institutions showed higher adherence. In contrast, trials involving special populations, particularly pediatric studies, were more likely to exhibit lower adherence, highlighting a need for targeted guidance and support in these contexts. Future analyses should include a larger sample and protocols evaluated by multiple Ethics Committees to enhance the generalizability of these findings.

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Exploring the Genetic Link between Clonal Hematopoiesis and Dilated Cardiomyopathy: Insights from a Polygenic Risk Score Analysis

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INTRODUCTION

Clonal hematopoiesis (CH) refers to the expansion of a blood stem cell and its descendants, driven by somatic driver mutations, and includes clinically relevant subsets such as clonal hematopoiesis of indeterminate potential (CHIP). CHIP, in particular, is increasingly recognized for its role in lymphoid malignancies. Although CH is a relatively common phenomenon—affecting over one-third of individuals and becoming more prevalent with age—it is linked to a heightened risk of hematological cancers, various non-hematological conditions and inflammation. Inflammation responses play a central role in cardiovascular diseases and heart failure and recent studies suggested CH as an important trigger for dilated cardiomyopathy (DCM) [1].

AIMS

Thanks to recent findings of a genome-wide association study (GWAS) that identified 42 independent genetic variants associated with the risk of developing CH [2], we conducted a study aimed at evaluating whether a polygenic risk score (PRS) for CH risk is related to the diagnosis and prognosis of DCM.

METHODS

The study analyzed a DCM cohort of 315 patients recruited in the Heart Muscle Disease Registry of Trieste (IT) and 718 healthy individuals from the same region. A PRS was derived based on 27 GWAS loci was calculated using imputed SNP-array data. PRS standardized levels were compared across groups using a generalized linear mixed-model that

included a genomic relatedness matrix as random effect to account for familial relationships. As for the analysis of disease progression in the DCM cohort, two primary outcomes were investigated: (1) life-threatening arrhythmic events, and (2) heart failure-related events. Time-to-event analysis was performed using cause-specific Cox mixed-models.

RESULTS

The PRS was significantly higher in healthy individuals compared to DCM patients (OR=0.82 95% CI [0.69, 0.97] per SD increase, $p=0.005$). When differentiating between DCM patients who were carriers and non-carriers of pathogenic/likely pathogenic variants, the observed difference was primarily driven by the carrier group (mean difference=-0.22, 95% CI [-0.41, -0.04]). During a median follow-up of 109 months (IQR=[24,194]), 80 (25%) individuals experienced life-threatening arrhythmic events, 43 (14%) experienced heart failure-related events and 57 (18%) died. No association was observed between PRS and either arrhythmic outcome, neither with heart failure outcome.

CONCLUSIONS

These findings contribute to expanding the knowledge on the relationship between clonal hematopoiesis (CH) and cardiovascular diseases, specifically dilated cardiomyopathy (DCM), where current understanding remains limited. The observed association between lower CH PRS levels and higher DCM risk was unexpected. Further studies are needed to confirm these results and clarify their implications.

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MMR-Colon Study: First Attempt at Quantifying MMR Protein Expression as a Prognostic Factor in Colorectal Cancer

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INTRODUCTION

Colorectal cancer (CRC) represents one of the most important public health issues worldwide and the third most diagnosed cancer globally. International Agency for Research on Cancer (IARC) data reported a global incidence of more than 1.9 million in 2022, and at least 900,000 deaths, representing the second leading cause of cancer death[1]. Molecular events CRC include genetic and epigenetic anomalies, such as DNA Mismatch Repair (MMR) system instability. The loss of MMR repair activity results in microsatellite instability[2]. Mismatch repair system deficit (dMMR) is observed in about 15% of all CRCs[3]. While in an undamaged and efficient repair system, MMR proteins identify and repair DNA mismatches that occur during replication, when microsatellites are unstable mismatches that result from DNA replication cannot be repaired [4]. It is of fundamental importance to distinguish MSI CRC from CRC with microsatellite stability (MSS), both from a prognostic point of view, as the former show a better overall survival (OS) and disease-free survival (DFS), but also predictively as MSI tumors respond poorly to adjuvant treatment with 5- fluorouracil (5-FU) in the early stages [5].

OBJECTIVE

Evaluate whether an increase or decrease in the expression of MMR proteins is quantitatively associated with morphological patterns and the impact this has on survival.

METHODS

All patients with CRC diagnosed from 2020 to 2021 carried out at the Surgical Pathology Unit of S. Spirito Hospital in Casale Monferrato (Alessandria, Italy) were included. dMMR immunohistochemical assessment was performed with ROCHE's automated VENTANA BenchMark Ultra platform and the following antibodies: VENTANA anti-MLH1, VENTANA anti-PMS2, VENTANA anti-MSH6. IHC-marked cells for all four MMR proteins are evaluated for presence or loss of expression based on the signal occurrence or absence released by the diaminobenzidine chromogen (DAB) of the detection system. MMR protein expression quantification, mainly MLH1 and MSH6, was performed by optical microscopy observation. MMR quantification was assessed in an independent evaluation by two observers. Any glandular structure was considered as expressed when more than half of the cells were stained by IHC assay. Further to the independent evaluation of the observers, samples without quantification agreement were jointly assessed and re-estimated to reach a quantitative evaluation mutually agreeable to the investigators, with a 5% variation range between both observations. Specific quantification was then provided for all samples, with a lowest of 5% and a highest of 100%. The resulting expression was lastly subdivided into two scores, high and low, according to the median value that was calculated on the whole sample. Univariate and multivariate logistic regression models were used to find determinants of low or no expression of MSH6 and MLH1 separately. The estimated Odds Ratio (OR) and 95% confidence interval (CI) were considered to this aim. Variable selection for multivariable logistic models was performed with a forward stepwise process. In order to assess the effect of MMR expression on overall survival (OS), Kaplan-Meier

curves with log-rank test were used. Hazard Ratio (HR) and 95% CI, estimated by semi-parametric proportional hazard Cox regression models, were then also used. Variable selection for the multivariate Cox regression model was performed by considering those variables resulted statistically significant in the logistic regression models and those considered clinically meaningful. For survival analyses, the last episode recorded in the hospital repository was considered as the last follow-up date for patients with a lifetime status.

RESULTS

In our study 73 patients were included. Male gender was the most represented (63.0%), the median age of patients was 74 years [IQR 66-80], with a range of 52-89 years. Most patients were affected by CRC with the following morphological features: histologic grade G2 (68.5%), high tumor budding (71.2%) and infiltrating growth margin (75.3%). The not otherwise specified (NOS) histotype was the most frequently diagnosed (82.2%). Multivariate analysis showed that high-grade budding is associated with increased odds of low MSH6 expression (OR = 11.20; 95% CI: 2.34-53.72), as well as, for MLH1 more aggressive histotype (OR=4.81; 95% CI: 1.25-18.51) and perineural invasion (OR=3.61; 95% CI: 1.20-10.87). Neither MSH6 nor MLH1 expression resulted to have an effect on survival.

CONCLUSIONS

MMR expression quantification in CRCs could be a valuable tool for prognostic patient stratification, as patients with low expression may show a response more similar to that of unstable tumors. Although some aggressive features are associated with lower MMR expression, this does not seem to have an effect on survival. Given the high variability in the resulting estimates, further studies on larger sample are needed to confirm the relationships found.

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Evaluating Inflammation as a Mediator between Adiposity and Coronary Artery Diseases Using Mediation Models within the Epicor Study

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INTRODUCTION

Obesity and overweight are globally recognized as major modifiable risk factors for coronary artery disease (CAD), yet the mechanisms underlying this association are complex and multifaceted ([1],[2]). Beyond mechanical and metabolic alterations, a growing body of evidence suggests that chronic low-grade inflammation plays a central role among indirect factors increasing cardiovascular risk in obese individuals. This systemic inflammation is typically quantified by circulating biomarkers, including C-reactive protein (CRP) and Plasminogen Activator Inhibitor-1 (PAI-1), both of which have known pro-atherogenic properties. However, few studies have formally quantified the indirect role of inflammation in mediating the effect of excess weight on coronary outcomes using robust causal frameworks.

AIMS

The main objective of this study was to quantify the extent to which chronic low-grade inflammation, measured prospectively via CRP and PAI-1, mediates the relationship between body mass index (BMI) and the risk of CAD. By applying a formal mediation model adapted for time-to-event data, we aimed to provide a nuanced understanding of this relationship disentangling the direct and indirect components and considering their possible evolution over time.

METHODS

Data were derived from the EPICOR study ([3]), the cardiovascular branch of the EPIC Italy study (European Pro-

spective Investigation into Cancer and Nutrition). Here 1416 participants were involved in a case-cohort design with 622 incident CAD cases (major coronary events or myocardial infarction). Baseline measures included BMI, CRP, and PAI-1. We estimated the total causal effect (TCE) of BMI categories on CAD and decomposed it into the pure direct effect (PDE) and the natural indirect effect (NIE) via inflammation, using a counterfactual-based weighting approach developed for survival outcomes ([4],[5]). This methodology allows valid mediation estimation even in the presence of non-rare outcomes, under the key assumptions of no unmeasured confounding of the exposure-outcome, exposure-mediator, and mediator-outcome relationships; and no mediator-outcome confounders affected by the exposure ([6]).

Flexible parametric Royston-Parmar survival models ([7]) were used to estimate hazard ratios (HRs), adjusting for age, sex, relative index of inequality ([8]), total physical activity, smoking status, and geographic area. CRP and PAI-1 levels were treated as continuous mediators. Sensitivity analyses excluded early events to reduce reverse causality risk. Mediation effects were computed at 1, 5, and 10 years post-enrollment, and stratified analyses were conducted by sex. The positivity assumption was verified, and no violations of identifiability conditions were detected.

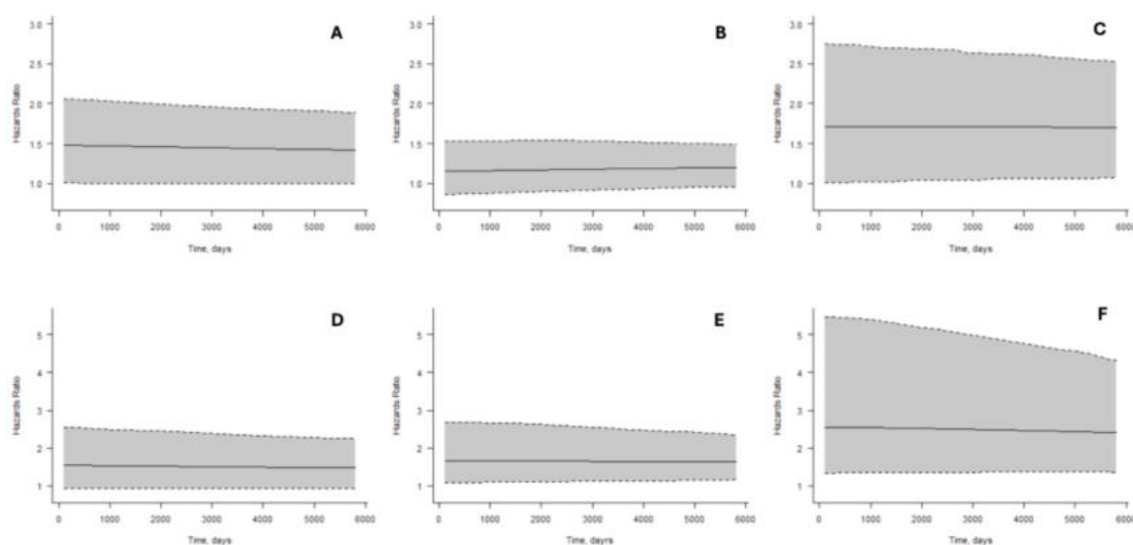
RESULTS

The causal effects estimated as a function of time in terms of HRs are shown in the figure below for the intermediate BMI category (upper panels) and for the highest BMI category (lower panels) with respect to the normal weight one. A direct effect (panels A and D) was detected over the whole follow-up time. There was also evidence for an indirect effect (panels B

and E), more relevant for the third BMI category than for the second one with respect to the first one. Only in the first case these effects slightly increased with time. The TCEs (panels C and F) remained always significant with a slowly decreasing pattern for the highest BMI category. Point estimations of the effects at different time epochs confirmed this interpretation: at 10 years, the NIE for the intermediate BMI category was 1.19 [95% CI: 0.93–1.52], and for the highest one was 1.65 [95% CI: 1.14–2.48]. These indirect effects represent relevant portions of the total effect (TCE = 1.71 and 2.47, respectively), indicating that inflammation explains a meaningful share of the increased CAD risk in higher BMI categories. Notably, the

NIE for obese individuals remained significant across all time points, with relatively stable HRs over time. The PDEs, while also statistically significant, showed smaller magnitudes, particularly at longer follow-up.

Stratified analyses confirmed the robustness of these findings. In both sexes, similar patterns of mediation were observed, although confidence intervals widened slightly. Similar analyses using waist-to-hip ratio WHR instead of BMI as a measure for obesity yielded consistent results, reinforcing the biological plausibility of the causal pathway.



Pure direct effects, natural indirect effects and total causal effects in the hazard ratio scale as a function of time for the intermediate category and for the highest BMI category (upper and lower panels respectively) versus the lowest one

CONCLUSIONS

Our findings provide strong epidemiological and methodological evidence that chronic low-grade inflammation, as measured by CRP and PAI-1, mediates a portion of the effect of excess adiposity on CAD. By adopting a formal mediation framework compatible with survival analysis, we disentangled the pathways linking BMI and cardiovascular outcomes, highlighting inflammation as a biologically plausible and statistically significant intermediate mechanism. These results underscore the importance of incorporating inflammatory biomarkers into cardiovascular risk prediction and may inform future prevention strategies. The study also illustrates how mediation analysis, when carefully implemented, can yield insights into the causal architecture of complex epidemiological relationships. This methodological approach may prove valuable in evaluating the effects of novel anti-inflammatory or weight-reducing therapies on cardiovascular outcomes through their impact and have potential implications for a better selection of patients for treatment on systemic inflammation

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Effectiveness of Laser Therapy in Adults with Knee Osteoarthritis: A Bivariate Meta-Analysis of Placebo-Controlled Randomized Controlled Trials

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BACKGROUND

Knee pain represents the second most common musculoskeletal disorder after low back pain [1]. Knee osteoarthritis (OA) represents the leading cause of knee pain, as well as the most common site for OA, with a projected increase of 74.9% [uncertainty interval 59.4-89.9%] in the number of cases by 2050 compared to 2020 [2]. Laser therapy (LT) represents a non-invasive treatment modality that is frequently provided to adults with knee OA due to its anti-inflammatory effects, despite not being recommended in major OA treatment guidelines [3]. Trials usually report measures of function and pain in this population, which are correlated when measured on the same participants. Nevertheless, meta-analyses typically ignore such correlations and perform univariate meta-analyses on the two outcomes independently, which may impact the estimates and their precision [4]. Conversely, multivariate meta-analyses consider the correlation between different outcomes and have the potential for the estimate of one effect to borrow strength from the data on other effects of interest [5].

OBJECTIVES

To assess the effectiveness of LT compared to sham LT on function and pain in adults with knee OA, taking advantage of the correlation between the two outcomes.

METHODS

PubMed and Embase were systematically searched from inception to May 6th, 2025 for placebo-controlled randomized controlled trials (RCTs) comparing LT to sham LT, alone or in addition to other conservative interventions (e.g., physio-

therapy, exercise), in adults with knee OA. Studies were included if they measured function and pain with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Physical Function and Pain subscales, respectively. In case of multiple relevant study groups, the sample size of the control group was split accordingly. Within-group mean changes and corresponding standard deviations of change (SDs) were extracted. When not reported, mean changes were computed and the SD of change was calculated following the Cochrane Handbook guideline [6] and assuming a correlation of 0.5 between baseline and post-treatment values.

A frequentist random-effect bivariate meta-analysis was performed on function and pain at the end of treatment, assuming a common correlation between outcomes ($r=0.824$, from external reference [7]) for all included studies. Sensitivity analyses assuming different correlation values (i.e., 0.2, 0.4, 0.6) were also performed. Univariate random-effects meta-analyses for the two outcomes disjointly were performed estimating the between-study heterogeneity using the restricted maximum likelihood estimator (REML) and computing the 95% confidence interval (95%CI) using the Hartung-Knapp (HK) method. Mean differences (MD) and 95%CI were calculated for both univariate and bivariate meta-analysis. Bivariate estimates were compared with univariate estimates and the impact of bivariate meta-analysis was assessed using the Borrowing of Strength (BoS) index [5] and the estimated number of additional studies using correlated evidence [8]. All the analysis were performed in R version 4.4.1.

RESULTS

From 233 individual records identified, 14 RCTs (16 effect sizes) involving 728 adults with knee OA were included. Main bivariate meta-analyses supported the effectiveness of

LT compared to sham LT (function: MD -3.57, 95%CI -5.27 to -1.86; pain: MD -1.37, 95%CI -2.12 to -0.61). Similar estimates have been computed in univariate meta-analyses and in sensitivity analyses (Figure 1).

Compared to univariate meta-analyses, the main model improved the precision of the estimates (i.e., reduced standard error), with a BoS of 15.5% and 9.5% for function and pain, respectively. The extra information gained by using correlated evidence is similar to finding direct evidence from approximately three and two additional studies, respectively. When considering sensitivity analyses, bivariate models improved the precision of the estimate for function for every assumed correlation, while bivariate models with lower correlations (i.e., $r < 0.4$) resulted in reduced precision and, consequently, larger confidence intervals compared to the univariate model for pain. Heterogeneity statistics were similar in univariate and bivariate models.

CONCLUSIONS

Compared to sham LT, the current findings support that LT improves function and pain in adults with knee OA, irrespective of the meta-analytic model considered. Despite univariate and multivariate models provide very similar results, with apparently little information gained from considering the correlation between the two outcomes, the latter may improve the precision of the estimates. Nevertheless, the extent to which multivariate meta-analyses may provide more precise estimates and thus narrower confidence intervals depends on the assumed correlation and the considered outcome. Future research, considering situations where studies do not provide information on all outcomes or provide different measures of selected outcomes (thus requiring standardization of the effect estimates), or extending the bivariate meta-analysis to more than two outcomes, may shed light on the impact of a multivariate meta-analytic approach compared to separate univariate meta-analyses in the field.

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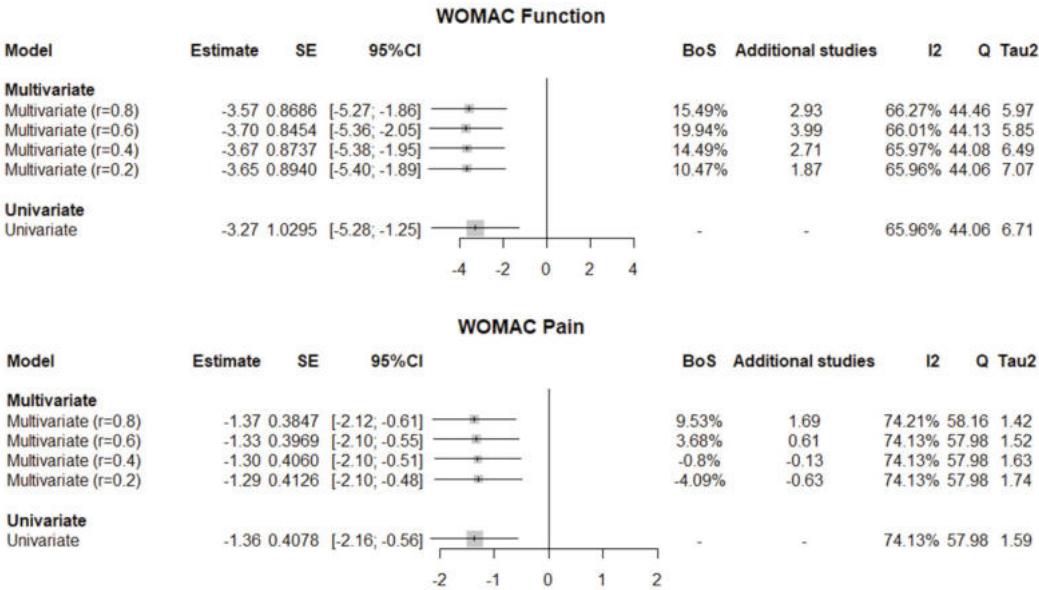


Figure 1. Comparison between multivariate and univariate meta-analyses

Belimumab in Lupus Nephritis Patients with Impaired Renal Function: A Post-Hoc Analysis of the Bliss-LN Trial

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with multiorgan involvement. Lupus Nephritis (LN) is a common severe manifestation of SLE which occurs in up to 60% of SLE¹. Despite immunosuppressive therapies, long-term outcomes remain poor, with a significant proportion progressing to end-stage kidney disease. Belimumab, a monoclonal antibody targeting B-cell activating factor, has shown promise in SLE treatment. The BLISS-LN trial², a 104-week, phase 3, randomized, placebo-controlled study, demonstrated improved renal responses with belimumab added to standard therapy in patients with active LN. However, a key limitation of most trials, including BLISS-LN, is the underrepresentation of patients with impaired kidney function (eGFR <60 ml/min/1.73m²). Considering the prognostic relevance of renal impairment at lupus nephritis onset, this study evaluates the effects of belimumab in the subgroup of patients with impaired kidney function from the BLISS-LN trial.

OBJECTIVES

To assess renal response and eGFR recovery in LN patients with impaired renal function treated with belimumab versus placebo at 52 and 104 weeks.

METHODS

Patients with baseline eGFR between 30–60 ml/min/1.73 m² (N=74) were included. Participants received belimumab or placebo in combination with corticosteroids and either mycophenolate mofetil or cyclophosphamide. Endpoints included were primary efficacy renal response (PERR), complete renal response (CRR), eGFR recovery ≥10%, ≥20%, ≥30%, and

≥50%, and time to renal-related events or death. Kaplan-Meier and log-rank tests were used for time-to-event analysis. All analysis was performed on the intention-to-treat population and stratified by three kidney-biopsy LN classes: Class III or IV, Class III+V or IV+V, and Class V.

RESULTS

In addition to corticosteroids, 41 patients received Belimumab (29 received mycophenolate and 12 cyclophosphamide) and 33 patients placebo (21 received mycophenolate and 12 cyclophosphamide). At time of kidney biopsy, 42 patients (26 on Belimumab; 16 on Placebo) had class III or IV, 9 (4 on Belimumab; 5 on Placebo) had class V, and 23 (11 on Belimumab; 12 on Placebo) had mixed forms. At 52 weeks, PERR was achieved in 19.5% (belimumab) versus 33.3% (placebo), while CRR was 12.2% vs 9.0%, respectively (Table). At 104 weeks, PERR was achieved in 29.3% (belimumab) versus 33.3% (placebo), while CRR was 14.6% vs 15.2%, respectively (Table). No significant differences emerged between the two groups. Notably, eGFR recovery at all thresholds was significantly higher in the Belimumab group compared to placebo one.

CONCLUSIONS

In LN patients with moderate renal impairment, belimumab showed no significant improvement in primary or complete renal response rates compared to placebo. However, it was associated with significantly better recovery of eGFR. These results suggest a potential renal benefit of belimumab in this high-risk population, particularly in preserving or improving kidney function.

Table. Distribution (no., %) of achievement of Primary efficacy renal response rate (PERR), Complete renal response (CRR), and recovery eGFR at weeks 52 and 104 in patients treated with Belimumab or Placebo.

Overall sample	52 week		104 week	
	Belimumab (N=41)	Placebo (N=33)	Belimumab (N=41)	Placebo (N=33)
PEER	8 (19.5)	11 (33.3)	12 (29.3)	11 (33.3)
CRR	5 (12.2)	3 (9.0)	6 (14.6)	5 (15.2)
Recovery eGFR				
≥ 10%	24 (80.0)	21 (72.4)	23 (85.2)	13 (54.2)
≥ 20%	23 (76.7)	18 (62.1)	22 (81.5)	10 (41.7)
≥ 30%	23 (76.7)	14 (48.3)	22 (81.5)	8 (33.3)
≥ 50%	16 (53.3)	10 (34.5)	16 (59.3)	4 (16.7)
Class III and Class IV	(N=26)	(N=16)	(N=26)	(N=16)
PEER	7 (26.9)	7 (43.8)	8 (30.8)	7 (43.8)
CRR	4 (15.4)	1 (6.2)	4 (15.4)	2 (12.5)
Recovery eGFR				
≥ 10%	13 (81.2)	11 (78.6)	13 (86.7)	8 (66.7)
≥ 20%	13 (81.2)	10 (71.4)	13 (86.7)	7 (58.3)
≥ 30%	13 (81.2)	9 (64.3)	13 (86.7)	6 (50.0)
≥ 50%	11 (68.8)	7 (50.0)	9 (60.0)	3 (25.0)
Class III+V, IV+V	(N=11)	(N=12)	(N=11)	(N=12)
PEER	1 (9.1)	3 (25.0)	2 (18.2)	3 (25.0)
CRR	1 (9.1)	1 (8.3)	1 (9.1)	2 (16.7)
Recovery eGFR				
≥ 10%	10 (90.9)	8 (72.7)	9 (90.0)	4 (44.4)
≥ 20%	9 (81.8)	7 (63.6)	8 (80.0)	2 (22.2)
≥ 30%	9 (81.8)	5 (45.5)	8 (80.0)	2 (22.2)
≥ 50%	4 (36.4)	3 (27.3)	6 (60.0)	1 (11.1)
Class V	(N=4)	(N=5)	(N=4)	(N=5)
PEER	0	1 (20.0)	2 (50.0)	1 (20.0)
CRR	0	1 (20.0)	1 (25.0)	1 (20.0)
Recovery eGFR				
≥ 10%	1 (33.3)	2 (50.0)	1 (50.0)	1 (33.3)
≥ 20%	1 (33.3)	1 (25.0)	1 (50.0)	1 (33.3)
≥ 30%	1 (33.3)	0	1 (50.0)	0
≥ 50%	1 (33.3)	0	1 (50.0)	0

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A Non-invasive Diagnostic Tool to Rule Out Left Main Stem Stenosis: the MASTER Study

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INTRODUCTION

In patients with stable coronary artery disease (CAD), medical therapy alone does not increase the risk of ischemic cardiovascular events or deaths, as compared to an initial invasive strategy by percutaneous coronary intervention [1]. However, patients with left main coronary artery disease (LMCAD) have poorer prognosis, and current guidelines recommend revascularization [2]. Therefore, a non-invasive diagnostic method, less expensive than coronary angiography (CAG), which could reliably identify LMCAD, would allow a safe and more sustainable treatment of the vast majority of stable CAD patients.

OBJECTIVES

The MAin stem Stenosis prediction Through Exercise Response (MASTER) multicenter case-control study was designed to develop a diagnostic model for excluding LMCAD among subjects referred to coronary angiogram (CAG) for documented or suspected myocardial ischemia.

METHODS

Eligible subjects were suspected CAD patients with an interpretable exercise stress test (EST) performed before CAG. The training set included patients with a CAG performed between 2010 and 2021 in 5 Italian hospitals; the validation set included patients with a CAG performed between 2022 and 2024 in 3 of the centers used for model training and in two additional hospitals (one in Italy and one in the USA). Cases were patients with either $\geq 50\%$ left main (LM) stenosis or $\geq 70\%$ stenoses of both proximal left anterior descending and proximal circumflex arteries identified through CAG.

In all patients, we collected demographic, clinical, laboratory and EST variables. To deal with missing values, we performed a single imputation using predictive mean matching for numerical variables, logistic regression for binary variables and polytomous regression for categorical variables with more than two levels [3]. The diagnostic model was identified by applying logistic regression with Akaike Information Criterion (AIC)-based backward stepwise selection.

The performance of the selected model in terms of discrimination was quantified by the Area Under the Curve (AUC) with 95% confidence intervals (95% CI). The optimal threshold for the linear predictor corresponded to the point on the ROC curve closest to the top-left corner, assuming a ratio of the cost of misclassifying a case versus a control equal to 100 and a 5% prevalence of LMCAD among patients undergoing CAG for suspected CAD [4, 5]. Based on the optimal threshold, we estimated sensitivity, specificity, negative and positive predictive values (NPV and PPV).

We performed an internal validation estimating the optimism-adjusted AUC based on 500 samples [6].

We performed external validation in the complete validation set, and, as a sensitivity analysis, in the subset of patients from the centers not included in the training set.

RESULTS

The training set included 219 cases and 554 controls. The selected model showed an AUC of 0.80 (95% CI, 0.76-0.83), which after adjusting for optimism became 0.77 (see Figure 1).

The model had a sensitivity of 86.3% and a specificity of 56.2%, with a NPV of 98.7% and a PPV of 9.4%.

The validation set included 137 cases and 274 controls, of whom 53 and 91 in the two additional centers, respectively. The accuracy of the model on the complete validation set

decreased, with an AUC of 0.70 (95% CI, 0.66-0.74). At the best threshold identified from the training set, sensitivity was 81.0% and specificity 45.3%. The NPV and PPV were 97.8% and 7.2%, respectively.

When we limited the external validation to the two centers not included in the training set, we obtained similar results (AUC 0.72, 95% CI 0.63-0.81, sensitivity 79.2%, specificity 47.2%, NPV 97.7% and PPV 7.3%).

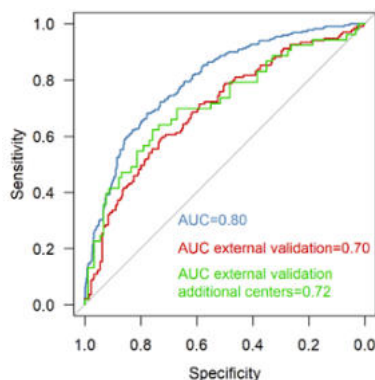


Figure 1. ROC curves in the training set (blue), complete validation set (red) and validation set including only the two additional centers not used for model training

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CONCLUSIONS

This large and multicentric study showed that, based on demographic, clinical and EST variables, it is possible to rule out the presence of LMCAD in patients able to perform a maximal EST, with a negative predictive value of about 98%, with a small difference between internal and external validation. Such results might influence the clinical management of stable CAD patients, by sparing many CAGs to non LMCAD patients.

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Incidence and Modifiable Risk Factors of Dementia: Evidence from Healthcare Utilization Databases of the PREV-ITA-DEM Study

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INTRODUCTION

In Italy, it was estimated that approximately 1,200,000 people were living with dementia in 2018 (1). International reports from the Lancet Commission indicate that intensive interventions targeting modifiable risk factors, such as diabetes mellitus, hypertension, obesity, physical inactivity, depression, smoking, and low educational attainment, could prevent or delay up to 35% of dementia cases worldwide. In Italy, a 20% reduction in these seven risk factors could lead to a 6.4% decrease in Alzheimer's dementia cases (2,3,4,5).

OBJECTIVES

The PREV-ITA-DEM project, a large-scale study funded in 2022 by the Italian Ministry of Health under the National Recovery and Resilience Plan (PNRR-MAD-2022-12375822), aimed to estimate the incidence of dementia in relation to risk factors such as diabetes, hypertension, and depression. The study adopted a population-based approach using data from Healthcare Utilization Databases (including Pharmaceuticals, Exemptions, Hospitalizations, Population Registry, and Mortality) with the goal of supporting prevention efforts.

MATERIALS E METHODS

This retrospective cohort study involved three distinct cohorts, each exposed to one of the three risk factors of interest: hypertension, diabetes, and depression. The reference population included the adult population (aged ≥ 50 years) living in and receiving healthcare in the metropolitan area of Turin, in Bologna Local Health Authority (AUSL), and in Lazio region. Exposure cohorts were defined between January 1, 2011, and December 31, 2020, using a 4-years look-back period (2007–2010) to identify only incident cases. Follow-up, with a maximum duration of 12 years, started on January 1, 2011, and ended at the earliest occurrence of one of the following events: dementia diagnosis, migration, death, or the end of the study period (December 31, 2022). A methodological approach based on a Common Data Model (CDM) was adopted, with a shared operational protocol, harmonized database structures, and a common script applied locally by each participating center, in compliance with data privacy regulations established by the Italian Data Protection Authority. A record linkage procedure of regional administrative health data flows was executed to generate the three studied cohorts.

Quantitative variables were synthesized through means and standard deviations, while categorical variables were reported as absolute and relative frequencies. Incidence rates for dementia and the exposures (hypertension, diabetes, depression) were calculated based on the number of incident cases per person-time, also accounting for censored individuals (due to death or migration). The risk of dementia associated with each exposure was estimated using Cox proportional hazards models, both univariate and multivariate (adjusted for key confounders), with time-dependent exposures. Results were expressed as Hazard ratios (HRs) and 95% confidence intervals (95% CIs). Data were analysed using Stata and SAS software, with a p -value <0.05 for statistical significance.

RESULTS

During the follow-up period, dementia incident cases after a diagnosis of depression added up to 4,161 (12.3%) in Bologna, 15,412 (9.4%) in Lazio, and 6,775 (8.5%) in Turin. Regarding diabetes, the number of individuals who developed dementia was 1,163 (4.1%) in Bologna, 6,854 (3.6%) in Lazio, and 1,917 (2.1%) in Turin. Finally, for hypertension, dementia incident cases during follow-up amounted to 6,729 (3.8%) in Bologna, 13,018 (1.0%) in Lazio, and 3,321 (1.5%) in Turin.

Univariate analysis showed a positive association between depression and the risk of dementia in Bologna (HR: 6.0; 95% CI: 5.6–6.4), Lazio (HR: 5.06; 95% CI: 4.88–5.25), and Turin (HR: 7.56; 95% CI: 7.21–7.94). Diabetes was also significantly associated with dementia across all three studied areas: Bologna (HR: 2.2; 95% CI: 1.9–2.4), Lazio (HR: 1.8; 95% CI: 1.7–1.9), and Turin (HR: 2.4; 95% CI: 2.2–2.7). Hypertension showed a positive association in Bologna (HR: 1.5; 95% CI: 1.4–1.6) and Lazio (HR: 1.06; 95% CI: 1.0–1.1), while an inverse association was observed in Turin (HR: 0.94; 95% CI: 0.89–0.98).

After adjusting for sex, age, and Charlson Comorbidity Index, depression was strongly associated with an increased risk of dementia across all areas: Turin (HR: 6.0; 95% CI: 5.7–6.2), Bologna (HR: 2.8; 95% CI: 2.6–2.9), and Lazio (HR: 2.58; 95% CI: 2.49–2.68). Diabetes showed a significant association only in Turin (HR: 1.7; 95% CI: 1.5–1.8) and Bologna (HR: 1.1; 95% CI: 1.02–1.3), whereas the association was not confirmed in Lazio (HR: 0.98; 95% CI: 0.93–1.03). Hypertension was inversely associated with dementia in all areas: Turin (HR: 0.9; 95% CI: 0.9–1.0), Bologna (HR: 0.63; 95% CI: 0.60–0.66), and Lazio (HR: 0.80; 95% CI: 0.78–0.82).

CONCLUSIONS

These preliminary findings underscore the importance of prevention strategies focused on the early management of psychiatric and metabolic risk factors in the adult population, suggesting potential benefits in reducing the incidence of dementia.

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Validation of the RoRex Questionnaire: The Needed Questionnaire for Gastroesophageal Reflux Disease?

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INTRODUCTION

Gastro-esophageal reflux disease (GERD) is increasingly common worldwide, with a prevalence in Western countries between 10 and 30% of the population. Prevalence could be even underestimated as only typical symptoms (heartburn and regurgitation) are considered in epidemiological studies. At present no questionnaire is universally accepted as the benchmark. Herein we propose a new 15-item questionnaire, named RoRex (Rovereto Reflux), which is intended to be both complete as it considers both typical, atypical and extra-esophageal symptoms, easy to understand, and as objective as possible as it assesses symptom frequency on a numeric scale rather than perceived severity.

AIM

To validate the RoRex questionnaire.

METHODS

The RoRex questionnaire was administered via web from April 2021 to October 2023 to volunteers and patients attending specialized gastrointestinal surgical units in two Italian centres, Rovereto (Trento) and Verona. Each individual answered a RoRex version in scientific jargon and in plain language, and two already validated questionnaires, RSI and GERD-HQRL. Internal consistency was assessed by

Cronbach's alpha, questionnaire dimensions were evaluated by exploratory and confirmatory factor analysis, and item responses were related to subjects' characteristics.

RESULTS

213 individuals (149 patients and 64 volunteers) answered the questionnaire. Of these, 60 patients and 29 volunteers were recruited in Rovereto, while 89 patients and 35 volunteers were recruited in Verona. In the exploratory factor analysis (EFA), two factors (esophageal and extra-esophageal symptoms) were identified which accounted for 97.7% and 94.1% of the total variance of the scientific or plain language versions, respectively (Kaiser–Meyer–Olkin [KMO] statistic = 0.9015 for normal item and 0.8803 for easy item). For the plain language version, overall Cronbach's α of the questionnaire was 0.89, indicating good internal consistency; in detail, Cronbach's α was equal to 0.87 for factor 1 and 0.85 for factor 2. We evaluated the factor correlation matrix of the final exploratory factor analysis to be verified discriminant validity. The correlation between factor 1 and factor 2 was 0.546. The confirmatory factor analysis (CFA) yield similar results.

CONCLUSION

The RoRex questionnaire seems to be an easy clinical tool, useful to diagnose GERD and evaluate therapy effectiveness, whether medical or surgical.

Treatment Strategies of Acute Myeloid Leukemia Relapses after Allogeneic Stem Cell Transplantation: Evidence from the GITMO Nationwide Italian Registry

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INTRODUCTION

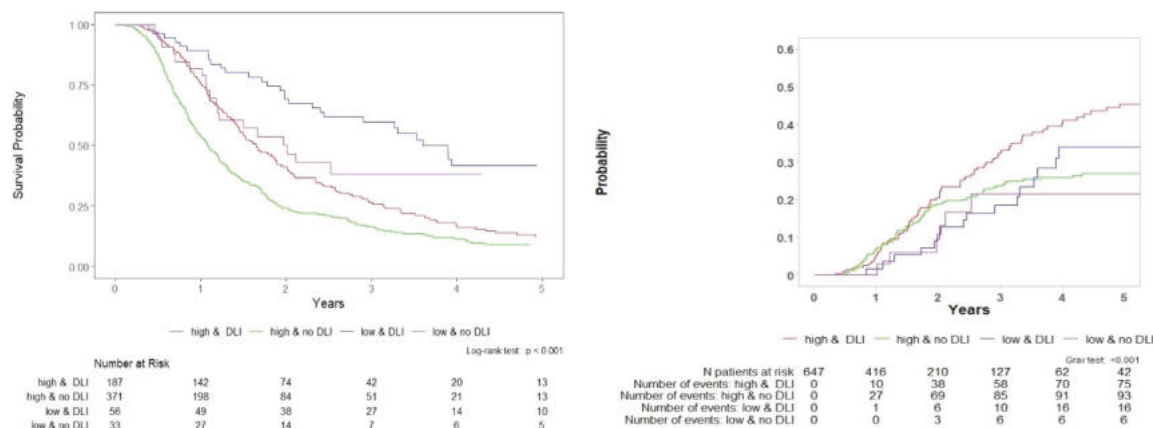
Acute myeloid leukemia (AML) is a clonal bone marrow disorder marked by impaired hematopoiesis, leading to cytopenia and transfusion need. Predominantly affecting older adults, its incidence is expected to rise with population aging. Using data from the latest 2021 Global Burden of Disease (GBD) study, the global incidence of AML was 144,645 in 2021 (+82% with respect to 1990) [1]. The age-standardized incidence rate (ASIR) was increasing in Western Europe (from 2.25 (2.17–2.32) in 1990 to 2.79 (2.57–2.93) in 2021) and particularly in Italy (from 2.0 (1.9–2.1) in 1990 to 2.9 (2.7–3.1) in 2021) [2]. Allogeneic stem cell transplantation (Allo-SCT) is considered the only curative treatment for up to 80% of the cases. Unfortunately, 30–40% of the transplanted patients eventually relapse within the first 2 years after allo-SCT, and accordingly, the identification of the most effective and safest therapeutic alternatives after relapse is of paramount importance, especially in high income countries.

The Gruppo Italiano Trapianto di Midollo Osseo (ClinicalTrials.gov NCT06790680) study was designed with the aim to describe the real-life management of AML/MDS post-transplant relapses in Italy and draw some conclusions on the role of the different treatment strategies in the different settings of relapse. It included data of 859 AML/MDS cases from 33 Italian transplant Centers who relapsed after allo-SCT between 2015 and 2021, which were extracted from the European PROMISE database (n = 3336).

STATISTICAL METHODS

Descriptive statistics were expressed as Mean (SD), median (range) for continuous variables, and as count and percentages for categorical variables. The probability of the OS was calculated by the Kaplan–Meier method. Cox proportional hazards regression model and the log-rank test were used to compare OS across groups. The Grambsch and Therneau score test (1994) for the time-varying coefficient=0 was used to test the proportionality of the PH Cox regression model. For both Treatment-related Mortality (TRM) and Relapse Mortality (RM), the sub-distribution hazard models were estimated, and the Gray's test was used for comparison across groups.

Figure 1. OS and Cumulative incidence of TRM according to disease burden at relapse and DLI-therapy



The association with the independent variables was assessed by the sub-hazard ratios (SHR) with corresponding 95%CI. Factors that were associated with a two-sided P-value of 0.05 in the univariable analysis were included in a multivariable analysis. In the presence of non-proportional risks in the OS, the interaction of individual covariates with time was modelled as an unrestricted smooth cubic spline function [3]. Conversely, when estimating the Fine and Gray competing risk regression model, we did not test nor correct for non-proportional risks, as suggested in [4-5]. Statistical analysis was performed in the R environment (<https://cran.r-project.org/>).

RESULTS

Of 859 AML/MDS relapse cases after allo-SCT, 647(75%) received post-relapse treatment, with 86% classified as HIGH disease burden (hematological relapse) and 14% as LOW disease burden (minimal residual disease positivity and/or molecular mixed chimerism). Hypomethylating agents (HMAs) +/- venetoclax were the most frequently used treatment (308/647 (47.6%)), in combination with DLI in 20.4% (132/647) of the cases.

In this series, OS was 55% and 28% at 1 and 2 years, respectively, RM was 39% and 56% at 1 and 2 years, respectively, and TRM was 6% and 15% at 1 and 2 years, respectively.

Among the 647 treated patients, overall survival (OS) was significantly longer in patients with LOW disease burden receiving DLI-based therapy ($p < 0.001$), and the treatment-related mortality (TRM) was influenced by disease burden at relapse ($p < 0.001$) (Figure 1). By multivariate analysis LOW disease burden at relapse (HR 0.4 95%CI=(0.29-0.55)), complete remission status at transplant (HR 0.6 95%CI=(0.49-0.72)) and DLI-based treatment (HR 0.65 95%CI=(0.52-0.8)) were independently associated with improved OS. The use of DLI was independently associated with reduced relapse mortality (HR 0.56 95%CI= (0.43-0.74)) and with increased TRM (HR 1.45 95%CI= (1.06-1.97)). In the subset of 308 patients treated with HMAs +/- venetoclax group, the OS remained significantly longer in patients with LOW disease burden and DLI-based therapy($p < 0.001$), but TRM was not influenced by disease burden at relapse($p = 0.762$) (data not in Table).

DISCUSSION

This study confirmed the poor outcome for AML/MDS relapsed patients after allo-SCT, consistently with [6-7]. Interestingly, there was a dramatic unbalance between patients submitted to a post-relapse treatment at the time of hematological relapse (86%) and patients treated in the early phase (14%) that suggests a quite widespread clinical practice of delaying treatment to the most advanced stages of post-transplant relapse. However, our study demonstrates that initiating therapy at an early stage significantly improves survival outcomes, particularly in patients receiving immunotherapy with donor lymphocyte infusion. Notably, also in the subset of patients submitted to hypomethylating agents (HMAs), with or without venetoclax, therapy with DLI seems to improve the survival, especially in patients presenting with a low disease burden at relapse. Furthermore, there is evidence of the relatively safety profile of HMAs +/- venetoclax therapy treatment, as the TRM was not significantly influenced by the disease burden at relapse.

CONCLUSIONS

In relapsed AML/MDS following allo-SCT, disease burden at the time of relapse emerges as the key determinant of long-term treatment outcomes. The early use of molecularly targeted agents and donor lymphocyte infusion (DLI) should be considered in clinical practice as soon as a low disease burden is detected.

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Age-Related Patterns of Fine Motor Performance across the Adult Lifespan in a Large Population-Based Study

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INTRODUCTION

Fine motor function, which integrates motor control and cognitive processing, has emerged as a promising non-invasive marker for neurodegenerative diseases and cognitive impairment.[1] However population-based reference data on fine motor patterns across adulthood are limited, making it difficult to distinguish physiological aging from pathological decline. The population-based Cooperative Health Research in South Tyrol (CHRIS) study, includes detailed assessments of fine motor function using digital spiral analysis (DSA).[2,3] DSA is a scalable and valid approach that captures the dynamics of hand drawing movements. It enables the quantification of multiple dimensions of fine motor control by extracting a range of spatiotemporal and frequency-based features from the drawing. These features provide a rich, multidimensional profile of neuromotor performance and hold promises for capturing early age-related deviations or subtle motor impairments associated with neurodegenerative processes.

OBJECTIVES

The objectives of this investigation were to characterize normative, age-dependent patterns of fine motor function across adulthood using spiral-derived kinematic metrics. Specifically, we aimed to estimate age-, sex-, and hand-specific centile curves for multiple motor parameters using flexible distributional modelling that captures central tendency, variability, and skewness. In addition, we sought to identify age of peak performance, inflection points in motor trajectories, and the age of steepest decline through derivative-based analysis.

METHODS

All participants from the CHRIS study who completed three digital spiral drawings per hand (six in total) by alternating hands at each repetition were included in this investigation. Five spiral-derived metrics were analyzed, representing distinct domains of fine motor function: spatial properties (trace length), temporal measures (drawing time), kinematic features (drawing speed and acceleration), and markers of movement irregularity (tremor amplitude estimated from pen displacement and deviations from the ideal spiral path). Separate models were fitted for dominant and non-dominant hands, and all models incorporated sex-specific smooth age trends to account for biological differences in motor aging.

To characterize age-normative patterns, the mean and degree of dispersion of each metrics were modeled as functions of age using the Box-Cox Cole and Green (BCCG) distribution within the Generalized Additive Models for Location, Scale, and Shape (GAMLSS) framework.[4] This approach enabled estimation of age-dependent centile curves for each metric while simultaneously modeling changes in dispersion and skewness. The BCCG distribution estimates three parameters: the median (μ), the coefficient of variation (σ), and the skewness (ν). To allow for greater distributional flexibility, we also fit models using the Box-Cox Power Exponential (BCPE) distribution, which includes a fourth parameter to capture kurtosis (τ). Improvements in model fit were evaluated using Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and visual diagnostics.

To further characterize the shape and timing of age-related changes, we conducted inflection point analysis based on the fitted μ (median) curves. First and second derivatives were

computed to identify key ages of interest, including the age of peak performance, the inflection point (change in curvature), and the age of steepest change (maximum slope). This analysis complements centile modeling by highlighting potential transition points or acceleration peaks in fine motor decline.

As a sensitivity analysis, we repeated the modeling using only the second spiral drawing from each participant to minimize potential practice or fatigue effects.

RESULTS

A total of 8,707 participants (53.6% female) aged 18 to 94 years (mean = 44.8, SD = 16.7) returned 6 valid spirals. After exclusion of a minority of spirals with inconsistent metrics, there were 50,299 valid spirals for the analysis. Across all spiral-derived metrics, the BCCG distribution provided evidence of better fit than the normal distribution, effectively capturing age-related skewness in motor performance. The BCPE distribution further improved fit for selected metrics, namely spiral length and tremor amplitude, by modelling excess kurtosis. Model comparisons using AIC and BIC consistently favored flexible models. Derivative-based inflection point analysis identified distinct phases of motor change, with peak performance typically occurring in the late 30s to early 40s and accelerated decline between ages 60 and 70. For example, centile modeling of tremor amplitude (mm) at peak performance age 40, mean dominant hand values were 0.15 for males and 0.14 for females (5th–95th percentile: 0.08–0.33 and 0.07–0.30, respectively); by age 65, medians rose to 0.25 and 0.24, with broader ranges of 0.12–0.72 and 0.11–0.70, reflecting increased tremor and variability with age (Figure). These patterns remained consistent across sensitivity analyses supporting the robustness of the estimated age trends.

CONCLUSIONS

Digital spiral analysis revealed age-normative patterns of fine motor function across adulthood, with peak performance during advanced young adulthood and accelerated decline after age 60. Flexible GAMLSS modelling effectively captured age-related changes in central tendency, variability, and skewness across metrics, by sex and dominance of the drawing hand. These sex-age-specific centile curves offer a robust normative reference for evaluating fine motor performance and may support future applications in the early detection of motor dysfunction and neurodegenerative disease.

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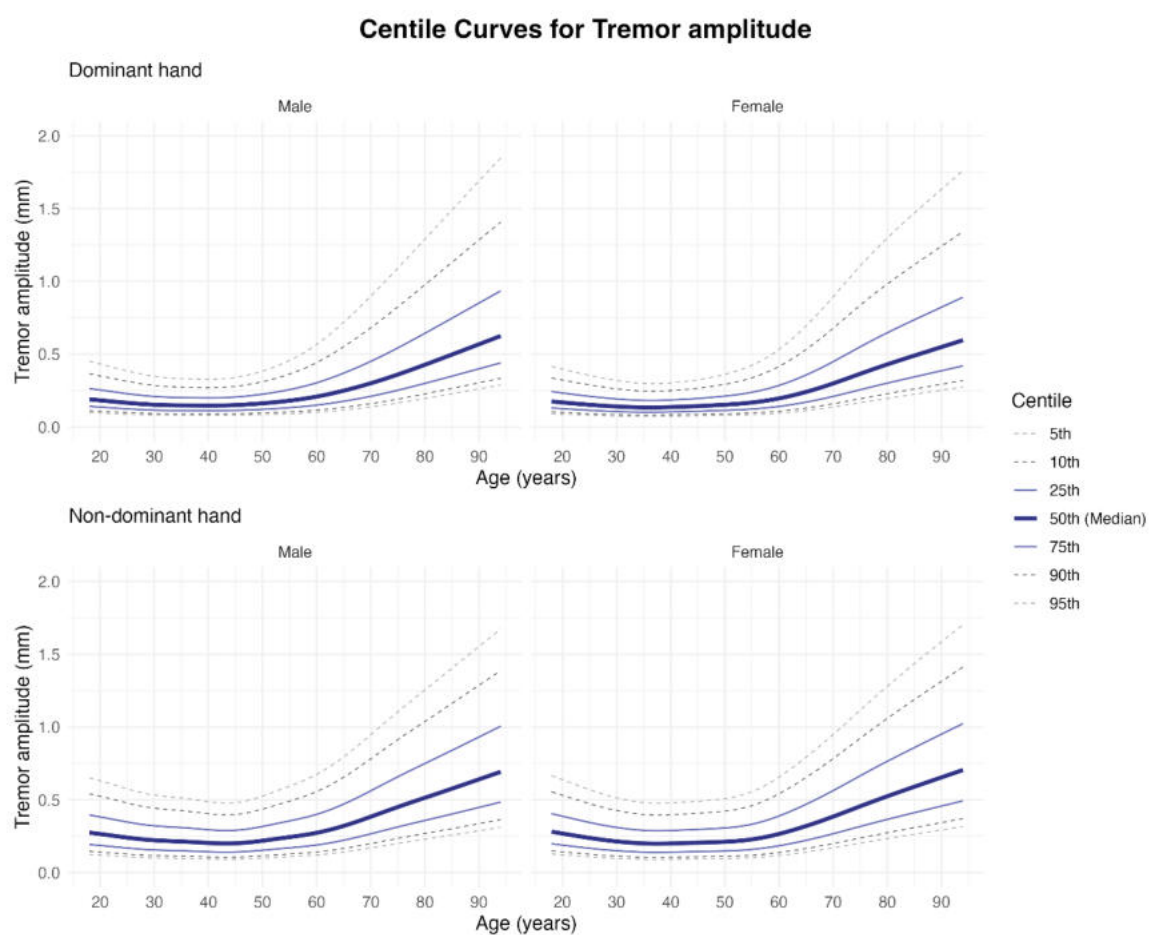


Figure. Continuous age centile curves for tremor amplitude, by sex and drawing hand

Informative Censoring in CDK4/6 Inhibitor Adjuvant Therapy for Early Breast Cancer: A Sensitivity Analysis of Invasive Disease-Free Survival in the monarchE Trial

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INTRODUCTION

The estimation of survival curves and hazard functions relies on the assumption of non-informative censoring—that is, the censoring time for an individual provides no further information about that person's likelihood of survival at a future time, had the individual continued the study [1]. This assumption is inherently untestable [2] and, if violated, may introduce bias in the estimation of treatment effects, such as the hazard ratio (HR). This issue is particularly relevant in oncology trials evaluating disease recurrence or progression, where follow-up may be affected by treatment-related toxicity. Patients with early toxicity or clinical deterioration may be more likely to discontinue treatment and withdraw from follow-up, introducing potentially informative censoring.

This topic has gained attention in the interpretation of the monarchE trial—a large phase III study evaluating adjuvant CDK4/6 inhibitor in early breast cancer patients with hormone receptor-positive, HER2-negative, high-risk of recurrence—where the magnitude of benefit in invasive disease-free survival (IDFS) (HR = 0.68; 95% CI: 0.60-0.77) was not paralleled by an overall survival (OS) advantage (HR = 0.90; 95% CI: 0.75-1.09). Informative censoring due to toxicity-related dropouts has been suggested as a possible explanation for this discordance, potentially resulting in an overestimation of the IDFS benefit [3,4].

A previous reanalysis of the monarchE data applied a sensitivity analysis by balancing reverse Kaplan–Meier curves over the entire 72-month follow-up period, treating all excess

censored patients in the experimental arm as if they had experienced the event [3]. This approach yielded an attenuated IDFS HR of 0.82 (95%CI: 0.72-0.94), casting doubt on the magnitude of the reported benefit. However, this strategy implicitly assumes that all censoring is informative, including late censoring events that are more plausibly administrative that may overcorrect the estimate.

AIM

We reanalysed the monarchE trial data by describing and comparing censoring patterns between treatment arms, then estimating the potential bias on the IDFS HR through clinically motivated sensitivity analyses.

METHODS

In the absence of individual patient data, pseudo-individual data were reconstructed from published Kaplan–Meier curves using Guyot's algorithm [6].

To describe censoring patterns and compare arms, reverse Kaplan–Meier curves were used [7], which invert the roles of events and censoring and estimate the probability of remaining under observation over time, assuming no events occur.

Two sensitivity analyses were conducted using clinically motivated time intervals: i) the first sensitivity analysis (24 months) considered 0–24 months interval, corresponding to treatment duration, during which toxicity could cause drop-

outs; ii) the second sensitivity analysis (48 months) considered 0–48 months interval, representing the minimum time before administrative censoring could occur. Before 48 months, all censoring must be attributed to loss to follow-up.

For each analysis, the difference in censored patients between treatment arms—defined as the ‘excess’ censoring—was calculated. Assuming that this excess censoring could be attributed to treatment-related toxicity, an equal number of censored patients matching this excess were then randomly selected from the experimental arm and reclassified as having experienced the event at their censoring time. A new HR was subsequently estimated.

RESULTS

In the first 24 months, there were 27 excess censored patients in the experimental group (214 vs. 187), with 186 and 268 events in the experimental and control arms, respectively. Over 48 months, the difference in censoring decreased to 23 (351 vs. 328), with 362 and 523 events, respectively.

Reverse Kaplan–Meier analysis showed a marked increase in censoring rates from 48 months onwards, consistent with the expected start of administrative censoring. The censoring rates were similar between arms during the entire study period.

When reclassifying the excess censored patients in the 24-month window in the experimental arm as events, the IDFS HR changed from 0.68 (95% CI: 0.60–0.77) to 0.72 (95% CI: 0.64–0.82). Similar results were obtained in the 48-month analysis, with an HR of 0.72 (95% CI: 0.63–0.81).

To observe a less relevant effect (defined as an IDFS HR higher than 0.80), it was necessary to reclassify 80 censored patients in the experimental arm as events within the first 24 months—the duration of treatment, whose toxicity is suspected to cause the bias. This is a very large number, especially considering that just 186 events were actually observed in the same time interval.

CONCLUSIONS

Informative censoring is a potentially important source of bias in oncology trials. Reverse Kaplan–Meier curves help visualize temporal patterns in censoring, and sensitivity analyses based on clinically justified time windows provide realistic estimates of the possible bias. In the monarchE trial, the observed IDFS benefit appears robust even under conservative scenarios. Therefore, the discrepancy between IDFS and OS does not seem to be attributable to informative censoring and may be better explained by prolonged post-recurrence survival, as the discontinuation of CDK4/6 inhibitors at the time of progression limits their impact beyond recurrence. Extended survival after recurrence can make it inherently difficult to detect an OS benefit, even when a true delay in recurrence—as reflected by improved IDFS—is present [8]. Even where OS remains unchanged between treatment arms, improvements in IDFS can still provide meaningful value, extending the time patients remain free from chronic disease and its psychological, social, and financial burdens.

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Investigation of the SMAD3 Haplotype Structure and Allelic Distribution of two Candidate SNPs

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BACKGROUND

The present study is an extension of two previous gene-environment interaction analyses on asthma that identified two single nucleotide polymorphisms (SNPs), rs2118610 and rs9302242, located on chromosome 15 within the SMAD3 gene. These SNPs were found to modify the association between outdoor air pollutants and two asthma-related outcomes, fractionated exhaled nitric oxide (FeNO) (rs2118610) and the Symptom frequency and anti-asthmatic Treatment intensity Score (STS score) [1] [2] (rs9302242), in adult patients with asthma from the general Italian population (Gene Environment Interaction in Respiratory Diseases - GEIRD [3]).

AIM

This analysis aims at investigating the haplotype structure of SMAD3 gene, in order to assess the distribution of the alleles of the two SNPs across the most common haplotypes of SMAD3 gene.

METHODS

GEIRD is an Italian multicentre (multi)case-control study investigating the role of genetic and modifiable factors in asthma, COPD, chronic bronchitis, and allergic rhinitis, with cases and controls identified from pre-existing cohorts and new population samples through a two-stage process involving a screening

questionnaire followed by clinical examination. Participants' addresses were geocoded and linked to daily outdoor air pollution estimates using BIGEPI [4] exposure models, including three-year (2013–2015) averages of PM_{2.5}, NO₂, and O₃, as well as summer (April–September) O₃ levels. Respiratory symptoms and use of anti-asthmatics treatment were combined into the STS score [1] [2], which is a valid and replicable continuous measure of asthma severity in adults.

We performed quality check steps on 997 subjects and 384 SNPs from the GEIRD [3] study using PLINK [5]. Individuals with more than 10% missing genotype data and SNPs having more than 5% missing data were filtered out. We removed SNPs deviating from Hardy Weinberg equilibrium (p -value $< 1 \times 10^{-6}$), subjects with excessive heterozygosity level, and closely related individuals. In addition, we performed a Principal Component Analysis (PCA) to exclude population outliers based on genetic ancestry, using the 1000 Genomes Project (GRCh37) [6] as reference panel. Genotype phasing was conducted using Eagle v2.4.1 [7] [8] on a subset of 321 patients with asthma who passed the quality checks and on 15 genotyped SNPs located on chromosome 15. Genotype imputation was carried out using Minimac4 [9], based on the 1000 Genomes Project [6] reference panel. Haplotype frequency was estimated using imputed data.

RESULTS

The two polymorphisms (rs2118610 and rs9302242) are located within the intronic region of SMAD3 gene. SNP

rs2118610 lays within an active regulatory region characterized by 11-zinc finger protein (CTCF) and RAD21 Cohesin Complex Component (RAD21) binding [10]. Similarly, rs9302242 overlaps a strong regulatory element marked by transcription factor binding peaks for Homeobox Containing 1 (HMBOX1), and RE1-Silencing Transcription factor (REST) [10]. Both SNPs are in very low linkage disequilibrium (LD; $R^2 = 0.016$) and are located on different haplotype blocks. Both alleles in the two SNPs are uniformly distributed among the common haplotypes.

CONCLUSION

This haplotype analysis suggests that the two SNPs may influence asthma-related outcomes independently in response to environmental exposures, in adult patients with asthma from the general Italian population.

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Clinical Outcomes and Survival Differences in People with Cystic Fibrosis Living in Europe

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INTRODUCTION

Cystic fibrosis (CF) is the one of the most common severe genetic disease in the world. Although advances in care have positively affected CF outcomes, both in terms of lung function and survival in higher-income countries (HIC), the situation remains alarming in lower-income countries (LIC) [1,2].

AIMS

This study aims in comparing characteristics of pwCF and their clinical outcomes in European countries with different income and to compare the situation before and after the introduction of CFTR modulators, a new class of drugs that completely transformed the landscape of CF.

METHODS

PwCF carrying the F508del mutation on at least 1 allele enrolled in the European Cystic Fibrosis Society Patient Registry (ECFSPR) were evaluated in 2017 and in 2022 according to 3 groups created by terciles of the gross national income (GNI) per capita of European countries included in the study (Low Income Countries -LIC-, Middle Income Countries -MIC-, High Income Countries -HIC-) for predicted percent forced expiratory volume (ppFEV1), underweight, and chronic *Pseudomonas aeruginosa* (Pa) infection. Survival was evaluated in 2013-2017 and 2018-2022. Generalised linear models

and Cox regression models were fitted. Prediction of the median survival age were obtained from the regression model, according to the values of the covariates.

RESULTS

From the 31,723 pwCF reported in ECFSPR in 2022, 13.5% lived in LIC, 19.9% in MIC, and 66.6% in HIC. pwCF living in LIC had a significantly lower median survival age, reduced ppFEV1, higher prevalence of Pa infection and underweight compared to pwCF from MIC and HIC. Although some improvements have been observed between 2017 and 2022 in all country groups, the gap between lower and higher income countries became even larger in recent years, after the introduction of CFTR modulator.

Data modeling indicated that the effect of the country group almost "disappear" in the adjusted regression model, indicating that in an ideal situation where underweight status, chronic *Pseudomonas Aeruginosa* infection, use of CFTR modulator is the same among different countries, also their median survival age will be the same. Model prediction showed that avoiding underweight and Pa infection would increase survival by 42 years for pwCF living in LIC. Access to CFTR modulators would further increase their survival by 15 to 29 years depending on their nutrition and infection status, resulting in a survival up to 82 years in the best case scenario.

CONCLUSIONS

Big differences of survival in pwCF are observed according to the country where they live. The reduced survival of PwCF living in LICs can be improved with improvement in standard of care, such as optimal nutrition, anti-infectious care, access to CFTR modulators.

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Beyond Medical Care: Exploring Socio-Psychological Distress, Violence, and Food Insecurity among Gynecological Patients

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BACKGROUND

Gynecological conditions significantly impact emotional well-being, relationships, and overall quality of life, alongside presenting medical challenges [1]. Anxiety, depression, and stress due to concerns about diagnosis, treatment, and body image, are now widely recognized as critical determinants of health [2-5]. Additionally, patients often face social challenges such as financial instability, limited healthcare access, and social isolation, which exacerbate distress [6]. Some studies have examined the social needs experienced by patients with gynecological disorders, but primarily in gynecologic oncology patients [2,7-9].

OBJECTIVES

This study aims to assess the prevalence of distress among patients attending at an Italian tertiary gynecological clinic while also exploring socio-demographic, psychological, clinical, and lifestyle factors influencing distress, violence, and food insecurity.

METHODS

This prospective cross-sectional study received local ethical approval (Prot. N. 10524/23) to enroll women attend-

ing the Gynaecological Outpatient Clinic of Fondazione Policlinico A. Gemelli IRCCS in Rome, Italy. From March to November 2023, an ad-hoc questionnaire, was administered by trained volunteers to all eligible women (≥ 18 years old) seeking gynecological care, excluding those unable to provide consent due to mental disorders. All volunteers were women members of the Associazioni Cristiane Lavoratori Italiani (Acli Roma APS), a national society for social promotion and had at least four years of experience in social projects. The questionnaire was validated through a Delphi procedure involving eight multidisciplinary experts and comprised 42 questions covering socio-demographics, family situation, clinical history, socio-psychological distress, and lifestyle.

Responses were analysed focusing on three key outcomes among women: socio-psychological distress, violence experienced and food insecurity. Inferential analysis was performed including multivariable logistic regression models incorporating statistically significant parameters from univariable analysis. Estimates were reported as Odds Ratios (OR) with 95% Confidence Intervals (CI). The analyses were conducted using STATA software, with a significance level of $p = 0.05$, adjusted using Bonferroni's correction where needed.

RESULTS

408 women were included in the study. 45.6% of patients attended the gynecological outpatient clinic for benign conditions, 38.5% for oncological issues, and 18.6% for preventive check-ups. Over 64.0% underwent medical or surgical treatment, and nearly half (47.1%) had a chronic disease. Sociodemographic findings showed that 97.1% of patients were Italian, primarily from central Italy. Almost half were married or in stable relationships, with 19.4% holding postgraduate degrees and 53.9% employed full-time.

152 (37.2%) reported socio-psychological distress, 136 (33.3%) violence, and 60 (14.7%) food insecurity. About 50% of women reported that the disease had changed their lives, both in terms of self-perception and relationships with others. Additionally, 34.6% reported that people's attitudes toward them had changed because of the disease.

Multivariable analysis shown in Table 1 identified oncological disease, chronic conditions, economic difficulties, and experiencing violence as independent risk factors for socio-psychological distress. Experiencing violence was associated with benign gynecological conditions, alcohol use, economic struggle, and experiencing food insecurity. Economic difficulties were the strongest independent predictor of food insecurity.

CONCLUSION

Socio-psychological distress and experiences of violence were found to be prevalent in over one-third of the studied population, highlighting the urgent need for integrated social support systems within gynecologic healthcare - especially for individuals facing economic hardship and food insecurity. A promising intervention could be trained volunteers specializing in social care, addressing both psychological and social health determinants to enhance patient well-being and overall outcomes through holistic interventions.

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Table 1. Multivariable logistic regression analysis for prediction of presence of socio-psychological distress, of experience of violence and of presence of food insecurity

Characteristics	Socio-psychological distress		Violence		Food insecurity	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Type of disease for gynecological examination					ni	
None	Ref.		Ref.			
Gynaecological benign	2.07 (0.95-4.54)	0.069	1.95 (1.02-3.74)	0.043		
Oncological	3.76 (1.55-9.11)	0.003	1.98 (1-3.93)	0.050		
Treatments for disease	0.92 (0.5-1.69)	0.793	ni		ni	
Chronic diseases (no vs yes)	2.22 (1.38-3.57)	0.001	1.52 (0.98-2.35)	0.062	ni	
Use of alcohol (no vs yes)	ni		1.88 (1.16-3.04)	0.010	ni	
Relationship ^a	ni		1.46 (0.93-2.27)	0.098	1.61 (0.83-3.12)	0.156
Education ^b	ni		ni	ni	0.67 (0.35-1.29)	0.236
Employment ^c	0.68 (0.4-1.15)	0.148	ni	ni	0.58 (0.3-1.12)	0.107
Economic difficulties ^c	3.91 (2.2-6.93)	<0.0001	1.72 (1.02-2.9)	0.040	6.01 (3.06-11.81)	<0.0001
Housing conditions ^d	ni		ni		0.71 (0.37-1.36)	0.297
Food insecurity ^e	0.93 (0.45-1.94)	0.847	1.92 (1.03-3.59)	0.041	-	
Socio-psychological distress ^e	-		ni	-	0.84 (0.41-1.73)	0.640
Violence ^f	4.65 (2.83-7.65)	<0.0001	-	-	1.85 (0.94-3.63)	0.074

Bold font highlights statistically significant values. ni: characteristic not included in the multivariable analysis as not statistically significant at univariable analysis. ^a Married and stable relationship vs other. ^b Elementary, middle and high school degree vs bachelor's and post-graduate degree). ^c None vs other. ^d House owned and parent's house vs other. ^e Absent vs present. ^f Not experienced vs experienced.

Introducing the Four Dimensions 4D Migraine Scale: A Composite Score Proposal Evaluating Migraine Severity and Treatment Efficacy

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INTRODUCTION

Different measures are currently used to evaluate migraine frequency and disability, but a single measure may only partially represent the burden of migraine. A composite measure that includes the most relevant parameters, obtained through a statistically weighted approach, would better assess migraine severity and treatment efficacy.

OBJECTIVES

This study aimed to develop a composite “four dimensions (4D) migraine scale” by selecting four commonly used endpoints: monthly migraine days (MMDs), number of monthly acute medications (MAMs), attack pain intensity (Numerical Rating Score, NRS), and Migraine Disability Assessment (MIDAS) Score.

METHODS

For each parameter, specific levels were chosen to cover the entire empirical range of each scale.

To estimate utilities and develop the 4D migraine scale, a series of pairwise choice tasks were generated using Lighthouse Studio, a Conjoint Analysis platform. In these tasks, respondents (patients and clinicians) were presented with two hypothetical migraine patient profiles, described by scale parameters, and asked to identify the patient in better health.

The design of the Discrete Choice Experiment (DCE) aimed for orthogonality and balance, meaning attribute levels should vary independently and occur equally often. However, a perfectly orthogonal and balanced design was not feasible due to the generation of unrealistic combinations, such as a patient with one monthly migraine day (MMD=1) taking more than 14 symptomatic medications or having a MIDAS score ≥ 16 points. To address this, expert clinicians identified and prohibited certain implausible combinations. Although prohibitions hinder a perfectly orthogonal and balanced design, statistical procedures were applied to adjust for these discrepancies.

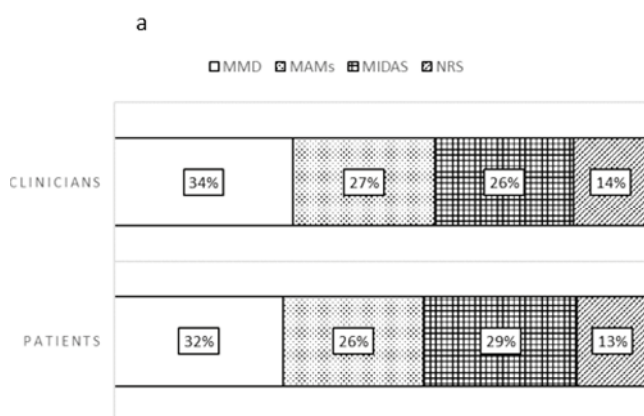
The sample size was determined using Orme's formula, $n \geq 500b/(ac)$. With two alternatives ($a=2$), the largest attribute having seven levels (MAMs, $b=7$), and 12 choice tasks ($c=12$), a minimum sample size of 146 was calculated. Furthermore, simulations using Lighthouse Studio software estimated that the standard errors of the effects for each level, with 300 respondents, were consistently below the recommended threshold of 0.05.

For statistical analysis, discrete choices from patients and clinicians were used to estimate the utilities attributed to each attribute level. This estimation was performed using the Hierarchical Bayes algorithm within Lighthouse Studio. Positive utility values were indicated by values above a reference line of 0, while negative utility (disutility) was indicated by values less than 0. These utility measures were then regressed against the levels of each scale to identify polynomial models that best interpolated the relationship between scores and utility. A log-transformation was applied to MIDAS utilities to account for its lognormal distribution and mitigate outlier bias. Polynomial models were ap-

plied within a General Estimating Equations framework, treating each respondent as a “cluster” with an “unstructured” working correlation matrix. Finally, an overall composite score, weighted by utility, was computed and standardized to a 0-100 scale, representing a range from a patient without migraine to a patient with the most severe migraine condition.

RESULTS

The relative weight of each level per parameter was rated by 197 migraine patients and 118 headache experts using



Polynomial models were developed to estimate the utilities for each scale:

- Utility_MMD = 68.48 - 11.52 MMD + 0.582 MMD² - 0.011 MMD³
- Utility_MAMs = 43.57 - 1.96 MAMs
- Utility_MIDAS = 51.43 - 4.836 (log_e(MIDAS + 1))
- Utility_NRS = 28.85 - 11.33 NRS + 1.916 NRS² - 0.144 NRS³

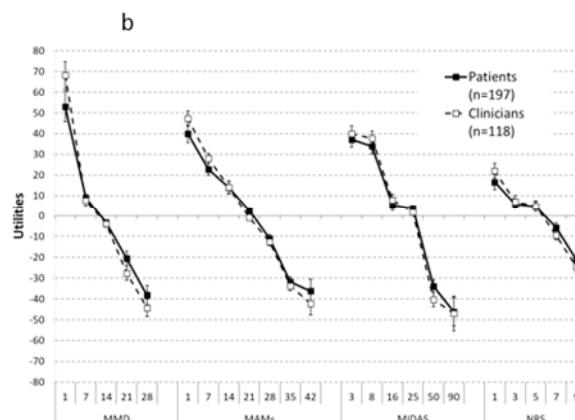
A composite raw 4D score was calculated as: 4D score raw = -(Utility_MMD + Utility_MAMs + Utility_MIDAS + Utility_NRS) / 4 and finally a more intuitive and clinically relevant score ranging from 0 to 100 (0 = no migraine, 100 = most severe migraine) was derived using: 4D score = 100 (4D score raw + 48.1) / 110.7

The 4D score was applied to a sample of 205 migraine patients treated with galcanezumab. It demonstrated sensitivity to changes, effectively summarizing the treatment's effect with a larger effect size compared to single parameters (eta-squared = 0.685). The 4D score also showed concurrent validity with the Head Impact Test HIT-6, an external patient-reported outcome measure.

CONCLUSION

This composite score, based on the preference weights of both clinicians and patients, can serve as a valuable Patient-Reported Outcome to comprehensively quantify migraine burden and treatment efficacy. The study highlights the importance of a multi-dimensional approach to migraine assessment, as single measures often fail to capture the full complexity of the condition and treatment response.

Conjoint Analysis. A substantial agreement was found between clinicians and patients regarding the relative importance (RI) of each parameter. MMDs was identified as the most important attribute for both categories (RI: 34% for clinicians, 32% for patients), while PAIN-NRS was the least important (RI: 14% for clinicians, 13% for patients). MIDAS was marginally more important than MAMs for patients (29% vs. 26%), while for clinicians, their relevance was almost equal (26% and 27%). The strong agreement between clinicians and patients was also confirmed in terms of the utility assigned to each level.



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Integrating Polygenic Risk and Digital Interventions for Cardiovascular Prevention: Design and Preliminary Results of the INNOPREV Randomized Clinical Trial

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading global cause of morbidity and mortality. While conventional preventive strategies have contributed to reductions in population-level risk, they often overlook inter-individual variability in predisposition and behavioral response. Advances in genomics and digital health offer novel opportunities for personalized prevention. Polygenic risk scores (PRS) [1], which estimate inherited susceptibility to CVD by aggregating common genetic variants, and mobile health (mHealth) tools, such as wearable devices and smartphone applications, are two promising interventions. However, evidence on their clinical impact—individually or in combination—remains limited.

OBJECTIVES

The INNOPREV randomized controlled trial evaluates the impact of PRS disclosure and digital health interventions on lifestyle behavior and cardiovascular risk modification among high-risk individuals without established CVD [2].

METHODS

A total of 1,020 participants aged 40–69 years with an estimated 10-year CVD risk of 2.5–10% (SCORE2) were recruited across three Italian centers (Rome, Catania, Palermo) and randomized into four arms: (A) traditional risk assessment (control); (B) risk assessment + PRS; (C) risk assessment + digital tools; and (D) risk assessment + both PRS and digital tools.

Participants in arms B and D provided saliva samples for genotyping and PRS calculation. Genetic risk was stratified into low, intermediate, or high categories and disclosed through structured counseling sessions. Participants in arms C and D received a smart band and mobile app, enabling real-time tracking of physical activity, heart rate, sleep, and caloric expenditure. The app delivered personalized behavioral prompts and feedback.

All participants underwent assessments at baseline (T0), 6 months (T1), and 12 months (T2), including CVD risk reassessment and lifestyle profiling using the American Heart Association's Life's Essential 8 (LE8) framework. Biomarker analysis was performed at T0 and T2 only. The primary endpoints are changes in LE8 score and SCORE2-estimated CVD risk. Secondary endpoints include changes in lipid profile, BMI, psychological response to PRS disclosure, and adherence to digital tools.

Comparative analyses will evaluate the marginal effects of each intervention (Arms B, C, and D) versus standard care (Arm A). Adjusted mixed-effects models for repeated measures will be used to assess changes in lifestyle patterns, lipid levels, and CVD risk over time. Subgroup analyses will explore potential effect modifiers, including age, sex, educational level, and baseline lifestyle profile.

RESULTS

Recruitment of 1,022 participants concluded in March 2025. As of now, 382 individuals have completed the T1 assessment, and 60 have reached T2. The baseline characteristics of the study sample were as follows: 52% male, mean age 56 ± 6.95 years, BMI 25.72 ± 4.0 kg/m², total cholesterol 201.23 ± 35.15 mg/dL, Score2 4.69 ± 2.14 , and LE8 score 63.98 ± 10.93 . Randomization across the four intervention arms was successful, with no significant differences observed in the key baseline variables. Among genotyped participants, 76% had a low PRS, 16% intermediate, and 8% high.

Across the full sample, a statistically significant improvement in LE8 score was observed, increasing from 64.0 ± 10.9 to 65.6 ± 10.9 ($p = 0.003$). BMI showed a modest but non-significant reduction (25.7 ± 4.0 to 25.5 ± 4.0 kg/m², $p = 0.46$).

The most marked improvement in LE8 was seen in the combined intervention group (Arm D), with an increase from 61.93 ± 11.75 to 64.11 ± 10.83 ($p = 0.012$). In the usual care group (Arm A), LE8 improved from 62.05 ± 12.25 to 63.98 ± 11.62 ($p = 0.09$); in the genetic intervention group (Arm B) from 64.10 ± 10.77 to 65.48 ± 10.72 ($p = 0.16$); and in the digital intervention group (Arm C) from 67.47 ± 9.87 to 67.56 ± 10.42 ($p = 0.90$). Although all arms showed trends toward improvement, only the combined intervention reached statistical significance, likely due to the limited sample size at this stage.

CONCLUSIONS

INNOPREV is the first large-scale randomized trial in Italy to evaluate the marginal and combined effects of genomic risk stratification and digital health tools in primary CVD prevention. Interim findings suggest meaningful lifestyle improvements, particularly with combined intervention. Final 12-month results will determine the long-term clinical impact and scalability of precision prevention strategies.

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Preoperative CT Radiomics for Prognosis Prediction in Resected Early-Stage Non-Small Cell Lung Cancer

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BACKGROUND

Approximately 20% of non-small cell lung cancer (NSCLC) cases are diagnosed at an early stage (ES), allowing for potentially curative surgical resection. However, a significant proportion of these patients still experience disease recurrence. Although the TNM staging system remains the cornerstone for prognostic assessment and clinical decision-making, it does not fully account for outcome variability among patients within the same stage [1]. This highlights the need for novel biomarkers to complement TNM staging and support more personalized treatment strategies. Despite extensive efforts to identify such biomarkers, stage remains the sole factor currently guiding treatment and follow-up in ES-NSCLC. In this context, radiomics has recently gained attention as a promising, non-invasive tool to enhance prognostic evaluation [2].

OBJECTIVE

This study aims to develop and preliminarily validate models that use preoperative CT radiomic features—alone and in combination with clinically relevant factors—to predict post-surgical outcomes for ES-NSCLC.

METHODS

Imaging and clinical data were obtained from the MIRACLE study—a multicenter, retrospective and prospective inves-

tigation aimed at developing a prognostic algorithm by integrating biological, radiological, and clinical information. This project was supported by Italian Ministry of Health, under the frame of ERA PerMed (project code: ERP-2021-23680708). The current analysis focuses exclusively on retrospective data and preoperative CT images from patients enrolled at IRST-IRCCS between 2018 and 2021. The primary endpoint was disease-free survival (DFS), defined as the time from surgery to disease recurrence or death from any cause, whichever occurred first. The last follow-up update was in January 2024.

Tumors were manually segmented by two independent expert radiologists. Radiomic features were extracted from preoperative CT scans, acquired with or without contrast medium, using the open-source package PyRadiomics [3]. In some cases, both contrast-enhanced and non-contrast scans were available for the same patient.

Two analytical approaches were employed: one based on an extension of the Cox model, and the other using random survival forests (RSF). For the Cox-based models, radiomic feature selection involved bootstrap resampling, feature inclusion frequency analysis, and consensus clustering. In each bootstrap replicate, an elastic net Cox model was fitted, accounting for within-patient scan correlations. Features most frequently selected were then clustered via consensus clustering using Kendall's tau distance and complete linkage, and one representative feature per cluster was chosen. For RSF, two modeling strategies were considered: one using all radiomic features, and one incorporating feature selection via hierarchical clustering on Kendall's tau distances, with one representative feature retained per cluster.

All models underwent hyperparameter tuning using stratified 3-fold cross-validation (CV), and final models were trained with the optimal parameter set. Evaluation was performed using the same 5 repeats of 5-fold CV across models, with concordance index, integrated Brier score, and 3-year time-dependent AUC as performance metrics. Results are reported as mean $\pm$ standard deviation across the repeated CV runs.

RESULTS

A total of 78 patients were included, accounting for 115 CT scans. The majority were male (60.3 %), with a median age at surgery of 71 years [IQR: 65–75]. Adenocarcinoma was the most common histotype, observed in 83 % of cases. Most patients (87.2 %) underwent lobectomy, and 68.0 % presented with a stage I tumor. The median follow-up time was 42.5 months (95 % CI: 37.9–45.43) and the median DFS was not reached. Overall, 25 failures were observed.

From the Cox-based pipeline, two radiomic features—GLCM Cluster Shade and Shape Maximum 2D Diameter Column—were ultimately selected and included in a standard Cox model. This model achieved a C-index of 0.767 ± 0.103 , IBS of 0.153 ± 0.032 , and 3-year AUC of 0.804 ± 0.136 . Adding pathological stage improved performance to a C-index of 0.777 ± 0.098 , IBS of 0.152 ± 0.034 , and AUC of 0.815 ± 0.134 . The stage-only model performed worse across all metrics (C-index: 0.729 ± 0.119 ; IBS: 0.155 ± 0.042 ; AUC: 0.739 ± 0.155).

Similar patterns were observed with RSF models. The stage-only RSF model yielded a C-index of 0.720 ± 0.120 , IBS of 0.163 ± 0.041 , and AUC of 0.739 ± 0.160 . Incorporating all radiomic features improved performance (C-index: 0.776 ± 0.095 ; IBS: 0.145 ± 0.041 ; AUC: 0.828 ± 0.135), but the best results were obtained using selected radiomic features (C-index: 0.788 ± 0.096 ; IBS: 0.147 ± 0.032 ; AUC: 0.837 ± 0.115). These included morphology-based (Shape Elongation and Shape Least Axis Length), intensity-based (Firstorder 10th Percentile, Firstorder Entropy, and Firstorder Interquartile Range), and texture-based (GLCM Difference Variance and GLCM ID) features. Adding stage to the selected radiomics model did not yield further improvement.

Additional analyses incorporating patient characteristics (e.g., age and sex) did not improve predictive performance and are not reported.

CONCLUSIONS

Our study shows that CT-derived radiomic features improve prognostic performance compared to stage alone. Although these results are promising, external validation on an independent dataset is essential to confirm their generalizability. Future work will also focus on investigating the explainability of the models to better understand the biological relevance of selected radiomic features.

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A High-Dimensional Mediation Analysis Integrating Genomics and Epigenomics to Understand Adaptive Advantages and Health Risks of Chronic Hypoxia in andean Highlanders

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This study is part of the HIGHCARE LAPS project (HIGH Altitude Cardiovascular Research

– Latin American Population Study), which investigates the biological impact of chronic hypoxia in high-altitude populations, focusing on Andean communities in Peru. These highlanders have developed distinctive genetic, physiological and lifestyle adaptations that support survival in low-oxygen environments and may reduce the prevalence of certain diseases. At the same time, some health conditions might be overlooked, such as hypertension, which could be underestimated due to altered blood pressure regulation at high altitude [1,2]. In this cohort, genomic analyses revealed only minor differences between individuals living at sea level in Lima and those residing above 4000 meters in Cerro de Pasco, while analyses of DNA methylation, a well-established marker of epigenetic regulation, identified several differences, particularly at CpG sites involved in adrenergic signaling in cardiomyocytes, suggesting a potential epigenetic contribution to high-altitude adaptation. However, it remains unclear whether DNA methylation actively shapes phenotypic peculiarities associated with high-altitude adaptation, such as enhanced oxygen transport, or contributes to health risks related to chronic hypoxia.

AIM

The study investigates whether epigenetic regulation, assessed through genome-wide DNA methylation, mediates phenotypic effects of chronic hypoxia in a Peruvian cohort of

96 highlanders (above 4000 meters) and 96 lowlanders (at sea level). The focus is on traits including hemoglobin, hematocrit, oxygen saturation, 24-hour systolic and diastolic blood pressure, 24-hour heart rate, respiratory rate, and hypertension risk, accounting for genetic background and various clinical and environmental confounders.

METHODS

DNA methylation and genotyping data were obtained from whole blood using the Illumina MethylationEPIC v1.0 array (866000 CpGs) and the Illumina Global Screening Array (650000 SNPs); raw data were subsequently processed for quality control and normalization using R (minfi and ChAMP packages) and PLINK [3,4]. Genetic background related to hypoxia adaptation was captured using principal components from SNPs in 25 hypoxia-related genes. Factorial analysis of mixed data (FAMD) was used to summarize a wide set of variables including lifestyle, diet, psychological, anthropological, exposure-related, and immune cell components. Selected FAMD and genetic principal components were screened for collinearity and included as covariates. High-dimensional mediation analysis (HDMA) was performed to test whether DNA methylation mediates phenotypic traits related to chronic high-altitude hypoxia. The approach combined Sure Independence Screening (SIS) for CpG preselection with de-biased Lasso regression to estimate the exposure–mediator (α) and mediator–outcome (β) paths. Mediation was considered significant based on the maximum p-value from both paths,

with global indirect effects calculated by summing all significant $\alpha \times \beta$ products, and direct effects obtained by subtracting the total indirect effect from the total effect [5].

RESULTS

DNA Methylation significantly mediated the effect of high altitude on several traits that were themselves significantly associated with altitude. For 24-hour heart rate, the total effect was +1.79 bpm, with 50% mediated by two CpG sites in B3GNT2 and KIAA0368. Hemoglobin increased by 3.87 g/dL, with 2% mediated by two CpG sites in SEMA4F; hematocrit increasing by 11.41 percent, with 1.7% mediated through two CpG sites in SEMA4F. Respiration rate increased by 2.22, with an inverse mediation effect of -0.21 (9%) involving three CpG sites associated with: MRPS34, EME2, and ARHGEF4. Oxygen saturation dropped by 2.2 points, 2.8% of which was mediated by an unannotated CpG. 24 hour systolic and diastolic blood pressure decreased by 8.25 and 2.21 mmHg, with 8.1% and 24% mediation through ST3GAL1 and CSGALNACT2, respectively. Finally, high-altitude exposure was associated with an estimated 80% reduction in hypertension risk (OR =0.20, 95% CI [0.078, 0.515]), with 3% of this protective effect mediated by DNA methylation at three CpG sites in ABCG1 and ARHGEF4.

CONCLUSION

These findings suggest that DNA methylation may contribute to high-altitude adaptation by modulating physiological functions, particularly those related to oxygen transport and cardiovascular regulation. In addition to quantifying mediated effects, the analysis also provided insights into the genomic context in which these effects occur, by identifying specific CpG sites within genes that may hold biological relevance. For example, ABCG1 included one of the mediating CpG sites; although this gene has been previously linked to hypertension, its role in chronic hypoxia remains to be clarified [6]. This may offer a novel entry point for future research into the molecular mechanisms of high-altitude adaptation. Other, less-characterized genes also emerged and require further investigation to understand their potential contribution.

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Antibiotic Prophylaxis and Ventilator-Associated Pneumonia in Traumatic Brain Injury Patients: Insights from The CREACTIVE Study

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INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide [1, 2]. It is now recognized as a condition involving multiorgan dysfunction, characterized by non-neurological complications, particularly respiratory ones such as ventilator-associated pneumonia (VAP), being common and associated with worse outcomes. VAP occurs frequently in intensive care unit (ICU) patients, and the incidence among those with TBI ranges from 21% to 60% and an average of 36% [3]. Prevention strategies for VAP include daily sedation interruption, spontaneous breathing trials, oral decontamination, continuous monitoring of endotracheal tube cuff pressure, the use of an endotracheal tube with subglottic drainage ports,

and, most importantly, antibiotic prophylaxis (AP) [4, 5]. However, the role of AP in preventing VAP remains unclear. While some studies suggested that AP has a protective effect, particularly against early-onset VAP [6-9], others found no association between AP and VAP occurrence, length of hospital stay, or mortality [9-12]. Moreover, prolonged AP use has been associated with an increased incidence of antibiotic-resistant Gram-negative pathogens and other complications [13].

OBJECTIVES

To investigate the effect of AP on the incidence of VAP in patients with TBI admitted to ICU. We also assessed the role of AP on secondary outcomes, including the duration of mechanical

ventilation, ICU and hospital length of stay, ICU and hospital mortality, and the six-month Glasgow Outcome Scale-Extended (GOS-E), using data from the large, multicenter, prospective CREACTION cohort [14].

METHODS

We included adult TBI patients requiring mechanical ventilation for more than 48 hours. AP was defined as administration of antibiotics in the absence of documented infection within the first 7 days of ICU stay. The primary outcome was the incidence of VAP, defined according to international criteria.

To create well-balanced AP and no-AP groups for all relevant confounding factors, we used a propensity score-matched design, a robust methodology for estimating causal effects in observational studies [15]. Propensity scores were estimated for each patient using a logistic regression model based on 22 covariates, including variables that were identified to impact both the decision to administer AP and the patient outcome (i.e., demographics, TBI severity, extracranial injuries, and ICU characteristics). We used the full matching algorithm [16], which requires weighted post-matching analyses, in which the weights depend on the size and composition of the matched sets [17]. Differences between no AP and AP groups for the primary and secondary outcomes were investigated using appropriate weighted tests. The probability of experiencing VAP was assessed using the weighted Kaplan-Meier analysis, and a time-to-event comparison was conducted using the log-rank test.

RESULTS

A total of 2,518 patients from 70 European ICUs were included, of whom 1,392 (54%) received AP, while 1,183 (46%) did not. Compared to patients in the no-AP group, those with AP at ICU admission were younger, had fewer comorbidities, presented lower Glasgow Coma Scale scores, higher Marshall scores, more injuries in body areas other than TBI, and were more frequently involved in high-impact or traffic-related trauma. After weighting, the groups were well balanced, with weighted standardized mean differences below 10% for all variables used in model to estimate the propensity score, except for country (11.8%) and penetrating trauma (10.4%).

After weighting, patients in the no-AP group had higher probability of experiencing early VAP than those in the AP group (18.9% vs. 14.7%, p -value<0.01), although there was no significant difference in the overall occurrence of VAP (Table 1). Time-to-event analysis confirmed a reduced risk of early VAP in the AP group, particularly during the first days of mechanical ventilation (Log-rank p -value<0.05). Compared to AP patients, those without AP had higher ICU mortality (35.0% vs. 27.1%, p -value<0.01) and higher hospital mortality (43.5% vs. 37.1%, p -value<0.01). ICU and hospital stays were significantly longer for AP patients, while no difference was detected in the duration of mechanical ventilation. There were no differences between groups in the 6-month GOS-E.

Among patients who developed VAP and had available microbiological data, those in the AP group reported a lower proportion of Gram-positive bacteria compared to the

no-AP group (29.3% vs. 47.2%), and a higher proportion of Gram-negative bacteria (80.9% vs. 71.4%). Moreover, AP patients showed higher rates of MDR bacteria, both Gram-positive (17.4% vs. 11.9%) and Gram-negative (32.3% vs. 15.8%).

CONCLUSIONS

Our findings suggest that AP is effective in reducing early-onset VAP among TBI patients, consistent with previous studies [6, 8, 12, 18, 19]. The benefit is pronounced during the early phase of mechanical ventilation, when patients are especially vulnerable. Patients who received AP had more Gram-negative infections and fewer Gram-positive ones but also showed higher rates of MDR in both types. The higher MDR rates in the AP group may be attributable to longer antibiotic courses, which was also evident in our results. This finding aligns with existing literature, which indicates that greater antibiotic exposure may promote the selection of resistant strains, complicating future treatment [20-23].

These results underscore the need to balance the benefits of VAP prevention with the risks of antimicrobial resistance. In conclusion, AP appears effective in reducing the incidence of VAP in TBI patients, but its use should be carefully considered. Clinicians are encouraged to apply AP selectively in high-risk cases, aiming to prevent infection while preserving antibiotic efficacy.

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Table 1. Outcomes of the of 2,575 patients included in the analysis, according to the AP at the ICU admission before and after propensity score matching

	No AP		AP	p-value ^b
	N (%)	Weighted distribution, n% ^a	N (%)	
N of patients	1,183	1,392	1,392	
Mechanical ventilation length, days				
Mean (SD)	8.15 (9.91)	10.33 (12.63)	11.34 (11.37)	0.1271
Median (Q1-Q3)	5 (1-12)	7 (2-15)	8 (3-16)	
ICU stay, days				
Mean (SD)	10.67 (12.13)	13.40 (15.85)	15.18 (14.68)	0.0328
Median (Q1-Q3)	7 (2-15)	9 (3-18)	11 (5-21)	
Hospital stay, days				
Mean (SD)	18.34 (22.29)	22.10 (26.73)	26.79 (31.18)	0.0010
Median (Q1-Q3)	11 (4-25)	14 (6-30)	18 (7-35)	
VAP	236 (19.95)	26.57	349 (25.07)	0.3677
Early VAP ^c	168 (14.20)	18.93	204 (14.66)	0.0025
ICU mortality				
Alive	738 (62.38)	64.96	1,012 (72.91)	<.0001
Dead	444 (37.53)	35.04	376 (27.09)	
Last hospital mortality				
Alive	623 (53.07)	56.52	872 (62.91)	0.0006
Dead	551 (46.93)	43.48	514 (37.09)	
GOS-E (6 months)				
Upper good recovery	91 (7.69)	7.09	108 (7.76)	0.1567
Lower good recovery	82 (6.93)	7.61	91 (6.54)	
Upper moderate disability	78 (6.59)	7.13	93 (6.68)	
Lower moderate disability	45 (3.80)	4.43	78 (5.60)	
Upper severe disability	66 (5.58)	7.84	117 (8.41)	
Lower severe disability	186 (15.72)	15.54	232 (16.67)	
Vegetative state	40 (3.38)	3.94	78 (5.60)	
Dead	595 (50.30)	46.41	595 (42.74)	

SD, standard deviation. ^a Data for patients in the no AP group are weighted to make them comparable with those in the AP group with respect to pretreatment covariates. Weights are defined by the matched design. ^b P-value of the weighted tests comparing the no AP and AP groups. ^c Defined as occurred <7 days from start mechanical ventilation

Effectiveness of Surveillance in Reducing Reoperations in Spinal Surgery

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INTRODUCTION

Surgical site infections (SSIs) are among the most frequent complications in spinal surgery, ranking as the third most common complication in this field [1-3]. The reported incidence in the literature varies significantly, ranging from 0.7% to 16.1% [4-7]. This broad variability reflects both methodological differences among studies, which are predominantly retrospective, and the heterogeneous characteristics of the analyzed patient populations.

AIM

The aim of this study is to analyze and quantify the outcomes associated with the implementation of targeted interventions to prevent SSIs. Specifically, the investigation focuses on evaluating the effectiveness of these measures in reducing the incidence of SSIs and improving clinical outcomes.

MATERIALS AND METHODS

A prospective surveillance study on SSIs in spinal surgery was conducted starting January 1, 2023, at a hospital in Genoa, Italy. All patients undergoing elective spinal arthrodesis were enrolled, and key infection risk factors were assessed: age, sex, body mass index (BMI), ASA score, diabetes status, type of surgery, modality and timing of antibiotic prophylaxis, hair removal practices, surgery duration, number of operating

room personnel, and peri- and postoperative glucose levels. A 30-day follow-up was performed. For each SSI case, the time of onset relative to surgery, infection type, and the need for surgical revision were recorded. Following the observation of an SSI outbreak, specific prevention interventions were developed and implemented.

RESULTS

A total of 309 patients were enrolled, including 203 during the period preceding the outbreak and 106 in the period following the implementation of the interventions. After the interventions were introduced, a statistically significant reduction in SSI incidence was observed ($p < 0.05$), decreasing from 10.89% in the pre-implementation period to 3.77% in the post-implementation period. To assess the effectiveness of the intervention, the expected number of infections in the absence of the implemented measures was estimated at 11. Based on this comparison, a 63.64% reduction in observed infections compared to expected values (11) was calculated, along with a 57.14% reduction in revision surgeries relative to expectations.

CONCLUSIONS

The study underlines the critical importance of active surveillance for the timely identification of rising incidence and the rapid implementation of tailored corrective strategies. The adoption of these measures significantly reduced both surgi-

cal site infections and related revision surgeries. These findings highlight the necessity of effective surveillance systems, integrated with structured data communication processes, to support the development of targeted interventions and foster a system of continuous quality improvement in healthcare.

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Discontinuity of Sacubitril/Valsartan in a Cohort of Hospitalised Individuals for Heart Failure

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INTRODUCTION

Heart failure (HF) is a complex, multifactorial clinical syndrome that originates from an alteration in the pump function of the heart, either systolic and/or diastolic. This condition typically manifests itself with symptoms such as dyspnoea, easy fatigability and water retention, which are often responsible for a reduced quality of life. The prevalence of HF increases exponentially with increasing age [1] and is one of the most main cause of hospitalisation of geriatric population. This is associated with a high mortality risk and a significant burden of comorbidities [2-3]. The management of these patients is particularly complex and relevant in public health terms. Over the years, guidelines for the management of heart failure have undergone some modifications following the introduction of new drugs. Specifically, from 2021 the guidelines ESC [4] suggest, as first-line for HF patients, the use of sacubitril/valsartan. The efficacy of sacubitril/valsartan has been investigated in two randomized clinical trials (RCT): PARADIGM-HF (Prospective Comparison of Angiotensin Receptor–Neprilysin Inhibitor with Angiotensin-Converting–Enzyme Inhibitor to Determine Impact on Global Mortality and Morbidity in Heart Failure Trial) [5] and PARAGON-HF (Prospective Comparison of Angiotensin Receptor–Neprilysin Inhibitor with Angiotensin-Receptor Blockers Global Outcomes in HF with Preserved Ejection Fraction) [6].

However, few real world data is available about persistence of this pharmacological treatment.

AIM

To investigate the therapeutic discontinuity of sacubitril/valsartan in a cohort of individuals hospitalised for heart failure using data from the healthcare databases of the Lombardy region.

METHODS

The study was conducted according to a retrospective cohort design. Patients in the Lombardy Region aged between 40 and 80 years with a hospitalisation for heart failure (ICD9-CM codes: 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.91, 404.03, 404.13, 404.93, 427.4x and 428.x) in the period 2021-2022 were identified. For each subject, the first hospitalisation for HF was considered and the discharge date was taken as the index date. Patients with a hospitalisation for heart failure in the 5 years preceding the index date were excluded. The final cohort included all patients with a fill of sacubitril/valsartan in the first 90 days after index date.

All sacubitril/valsartan prescriptions dispensed to cohort members during follow-up were identified. The duration of each prescription was calculated by dividing the total amount of drug prescribed by the DDDs (Defined Daily Dose). From the index prescription onwards a patient was considered to be discontinuing (primary outcome) if the time lapse between the end of the prescription and the start of the next one was greater than or equal to 90 days. The first day of non-coverage of the drug was considered as the event date. A sensitivity analysis was performed considering a reduced length of 60 days for the window used to define discontinuation.

Each cohort member was followed from the index date until 31 December 2023. The proportion of discontinuation was estimated with its 95% confidence interval (95% CI). A log-binomial model was implemented to investigate the determinants of discontinuation including the following variables: gender, age at index date, the use of antihypertensive, antidiabetic, statins and antidepressants as well as Multi-source Comorbidity Score (MC score at 3 classes) evaluated in the 5 years before index date. The relative risk (RR) and its confidence interval (95% CI) was reported.

RESULTS

Final cohort was composed by 1985 patients with a first admission for HF in the period 2021-2022. The mean age was 65 (SD 10) years and 77% were male. Of these, 27% were in treatment with antidiabetics while 45% with statins and 74% with antihypertensive drugs. In addition, 11% were treated with antidepressants. Patients in the lowest MC score class were 1177 (59%) while those in the highest class were 323 (16%). The S/V discontinuation proportion was 20% (95% CI 18% to 22%). We observed that for discontinuers the proportion of alternative pharmacological drug (ACE, ARB, CCB, BB and Diuretics) during the 90 days with no S/V treatment were lower respect to their proportion during the 3 months after index date suggesting either a clinician indication or the onset of collateral effect.

The model showed that increasing age was statistically associated with a lower risk of discontinuation (RR 0.987; 95% CI 0.976 to 0.998, p-value = 0.019) while the use of antidepressants increased the risk (RR 1.430; 95% CI 1.072 to 1.91, p-value = 0.015). When the outcome was defined by considering 60 days, age was no longer significant.

CONCLUSIONS

Preliminary data based on few years after ESC guidelines showed a quite high S/V discontinuation proportion similar to that reported in other studies [7]. Young age and use of antidepressant seems to increase the risk to interrupt an efficacy treatment like S/V. Continuous monitoring of healthcare data, in the next years, will allow efficiently to evaluate the effectiveness and persistence to S/V treatment.

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From Polygenic Scores to Phenotypic Screening: A Multi-Trait Framework for Cost-Free Risk Stratification in Endometriosis

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ABSTRACT

Introduction: Endometriosis is a chronic inflammatory condition affecting approximately 10% of women of reproductive age and is often diagnosed late due to nonspecific symptoms, overlap with common conditions such as primary dysmenorrhea, and the reliance on invasive laparoscopy [1,2]. Early detection could reduce patient burden and long-term complications, but current diagnostic tools remain limited. Genome-wide association studies (GWAS) have identified several genetic risk variants [3], yet their individual effects are modest. Polygenic risk scores (PRSs), which aggregate the effects of multiple variants, show promise but still lack the accuracy required for clinical application due to limited replication, small effect sizes, and population-specific variability [4,5]. Recent findings suggest that endometriosis is linked to a range of genetically influenced traits—such as immune, metabolic, and psychiatric characteristics—pointing toward the potential of multi-trait approaches to improve early, non-invasive risk stratification.

OBJECTIVES

This study aims to develop a non-invasive, cost-effective strategy for endometriosis risk stratification using a genetics-informed, two-phase approach. First, we evaluated whether polygenic scores (PRSs) related to a broad spectrum of complex traits could predict disease risk and reveal genetically defined subgroups among patients. Next, we identified the most informative traits associated with these genetic risk profiles and translated them into a targeted phenotypic questionnaire. We then assessed whether this phenotype-only model could accurately classify endometriosis cases, offering a feasible alternative to genetic testing for early detection in real-world settings.

METHODS

We analyzed 1,996 genotyped women (862 cases, 1,134 controls) and computed 4,490 PRSs across complex traits. After filtering and trait mapping, 645 scores were retained; one per trait was selected via bootstrap logistic regression (218), then reduced to 40 via LASSO. Supervised machine learning models (logistic regression, random forest, XGBoost, neural networks) [6,7] were trained to evaluate the predictive performance of the PRS-based model. Top-ranking PRSs from the best-performing model were used to cluster endometriosis cases, identifying genetically defined subgroups. Traits linked to these PRSs were used to design a targeted phenotypic questionnaire. The questionnaire was tested in an independent cohort ($n = 506$), where curated phenotypic features were used to train classification models. The best model was then used to generate a non-invasive, phenotype-only risk score for endometriosis stratification.

RESULTS

The multi-PRS model significantly outperformed the endometriosis-specific PRS (AUC = 0.636 vs. 0.546, $p < 0.001$), with key contributions from traits related to height, early menarche, schizophrenia, and autoimmune disorders. Clustering based on the most informative PRSs identified two genetically defined subgroups with distinct clinical characteristics, including differences in endometrioma prevalence, gastrointestinal symptoms, and disease stage. A phenotype-only model trained on questionnaire data demonstrated high discriminative ability (AUC = 0.904), with CA125, fatigue, gynecological symptoms, and muscle pain emerging as the most informative features, supporting its potential as a cost-effective and non-invasive tool for early risk stratification.

CONCLUSIONS

Our results demonstrate that leveraging polygenic information to identify trait-level predictors enables the development of accurate, phenotype-based models for endometriosis risk stratification. The use of AI-driven approaches allows robust prediction from a minimal set of non-invasive, low-cost clinical features—reducing reliance on genetic testing and supporting more accessible, early diagnostic strategies within precision gynecology.

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Incidence, Prevalence and Patterns of *Stenotrophomonas Maltophilia* Infection in People with Cystic Fibrosis across Europe

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INTRODUCTION

Progressive airway disease with recurrent infections is a hallmark of cystic fibrosis (CF). While *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common pathogens, emerging bacteria such as *Stenotrophomonas maltophilia* (SM), non-tuberculous mycobacteria, and *Achromobacter* species are increasingly identified. SM, a gram-negative bacterium, has been cultured from the sputum of people with CF (pwCF) with variable prevalence across countries. While its role remains debated, a 2012 review suggests that chronic SM may be a marker of more severe lung disease [1]. The clinical impact of *Stenotrophomonas maltophilia* in European pwCF remains underexplored, highlighting the need for better epidemiological data to guide research and care.

AIMS

Aims of this study are to estimate the prevalence and incidence of SM in Europe, to compare the clinical characteristics of pwCF according to their SM infection status and to characterize the most common patterns of infection during a period of 6 years (2018-2023).

METHODS

This longitudinal study is based on data provided by the European Cystic Fibrosis Society Patient Registry (ECFSPR), which collects demographic and clinical data of pwCF from 42 countries in Europe. Data are collected annually, according to specific inclusion criteria and standardized definitions.

In the ECFSPR, SM status is classified as negative, intermittent, or chronic, based on standardized criteria [2]. De-

mographics and clinical outcomes within SM-negative, intermittent, and chronic groups in 2023 were described and the differences among groups were assessed using Pearson's Chi-squared test for categorical and Kruskal-Wallis test for continuous variables.

SM overall incidence and prevalence from 2008 to 2023 were computed. Prevalence was the proportion of infected (both intermittent and chronic together) in each year, while incidence was the number of newly infected individuals divided by those uninfected in the previous year. To assess whether trends in prevalence and incidence over time were statistically significant, logistic regression models were applied, using infection status as the response variable and calendar year as a continuous explanatory variable. Additional models were then fitted, accounting for time of introduction of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) modulators and COVID-19 pandemic (pre- or post- period).

Chronic and intermittent SM infection were used as response variables in further logistic regression models to assess whether a significant trend existed with age at follow-up, included as a continuous explanatory variable, also accounting for CFTR modulator use.

Infection patterns from 2018–2023 were used to classify patients accordingly.

Analyses were run using R Core Team versions 4.5.0.

RESULTS

In 2023, ECFSPR included 52977 pwCF, of whom 51566 (97.3%) had complete information regarding SM infection status.

The overall prevalence of SM (including both chronic and intermittent cases) in 2023 ranged from 0.26% in Romania to 19.3% in Serbia, accounting for 5.0% (95% CI: 4.8 to 5.2)

across Europe. Four small countries did not report any SM detection.

Age, gender, BMI in adults, BMI z-score in children, and the country's income category (all $p < .05$) differed across the three infection groups: SM-negative (49010, 95%), intermittent (2000, 3.9%), and chronic (556, 1.1%). SM-chronic had the highest median age (28.7y) and lowest BMI (22.1 kg/m²), while SM-intermittent were the youngest (16.5y), and SM-negative had the highest BMI (22.5 kg/m²).

In contrast, genotype ($p = 0.7$) and use of pancreatic enzymes ($p = 0.2$) showed no significant differences. All concomitant infections—chronic *P. aeruginosa*, chronic *S. aureus*, MRSA, *H. influenzae*, *Achromobacter* spp., and nontuberculous mycobacteria—differed significantly across groups (all $p < .001$), with highest prevalence in those chronically infected with SM; only chronic *Burkholderia cepacia* complex showed a borderline difference ($p = .041$).

The prevalence of SM increases significantly with age ($p < .001$) in both the unadjusted model and the model adjusted with CFTR modulator use. The unadjusted model showed no significant trend in SM prevalence over time ($p = 0.609$); however, after adjusting for CFTR modulator/COVID period (≤ 2019 vs > 2019), a significant trend was observed ($p < .001$); furthermore, the stratified analysis revealed a significant rise in SM prevalence from 2008 to 2019 ($p < .001$), followed by a significant decline from 2020 to 2023 ($p < .001$).

Among 32,724 pwCF, 64 distinct infection patterns were identified. Most (24,594, 75.2%) showed no infection, while 24.8% (8,130) showed infection in at least one year and 11.1% (3640) for at least 2 years. Specifically, one year (13.7%), two years (5.3%), three years (2.8%), four years (1.5%), five years (0.8%), and six years (0.6%). Only 1.6% (524) of pwCF showed a continuous infection, remaining positive through 2023.

CONCLUSIONS

This large multinational analysis offers a detailed view of SM infection in the European CF population, revealing that while most individuals remained uninfected, nearly one-quarter experienced infection between 2018 and 2023. Chronic infection was strongly age-associated and consistently linked to a higher burden of co-infections. The shifting temporal trends—marked by a rise in prevalence up to 2019 and a subsequent decline during the CFTR modulator and post-COVID era—suggest that both medical advancements and broader changes in healthcare practices may significantly shape SM epidemiology. These findings highlight the need for continued surveillance to guide care and inform future research.

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Comparative Analysis of Antithrombotic Strategies in Atrial Fibrillation Patients with Ischemic Stroke: A Propensity Score and Inverse Probability Weighting Approach with Three Groups

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INTRODUCTION

Acute ischemic stroke (AIS) is a severe complication in patients with atrial fibrillation (AF), even in those already treated with direct oral anticoagulants (DOACs). Growing evidence indicates that systemic inflammation plays a key role in promoting the prothrombotic state and increasing the risk of thromboembolic events in AF. In observational studies, treatment allocation is not assigned randomly but rather reflects real-world clinical decision-making. DOACs treatment is prescribed based on the patient's estimated thromboembolic risk, typically assessed using established scoring systems such as CHA2DS2-VASc score. However, despite appropriate anticoagulation, a residual thromboembolic risk persists in a subset of patients, as documented in real-world studies. The characterisation of this subgroup of patients is essential to elucidate pathophysiological mechanisms and guide potential therapeutic modifications. Accordingly, within a cohort of AIS patients, different antithrombotic strategies can be encountered among patients with AF. This variability, combined with the lack of randomization, can introduce confounding and selection bias, making it difficult to accurately compare clinical outcomes [1]. In this context, methods such as propensity score analysis are essential to adjust for these imbalances and improve the validity of comparisons. In this retrospective cohort of patients aged ≥ 70 with acute ischemic stroke (AIS) due to large vessel occlusion (LVO), three groups were identified: (1) AF patients treated with direct oral anticoagulants (AFwDOACs), (2) AF patients without anticoagulant therapy (AFwDOACs), and (3) patients with large-artery atherosclerosis (LAA). The primary aim of the study was to compare the inflammatory profiles between the two AF groups -those

receiving DOACs and those not- within the context of cardioembolic AIS related to LVO. The LAA group served as a reference cohort, allowing for contextualization of inflammatory markers in a non-cardioembolic stroke etiology.

OBJECTIVES

To compare the impact of different statistical methods -Propensity Score Matching (PSM) and Inverse Probability Weighting (IPW)- in identifying clinical and laboratory differences between treatment groups. Specifically, we aimed to: (1) evaluate the ability of these methods to balance covariates across groups; (2) compare findings across methods in both pairwise and three-group analyses; (3) assess how statistical approach choice impacts the identification of clinically meaningful biomarkers.

METHODS

In this study, we retrospectively analyzed patients admitted between 2015 and 2024 with acute ischemic stroke due to large vessel occlusion (LVO). Patients were categorized into three groups based on prior clinical characteristics: (1) AF patients treated with direct oral anticoagulants (AFwDOAC), (2) AF patients not receiving oral anticoagulants (AFwDOAC), and (3) patients with large-artery atherosclerosis (LAA). The third group served as a reference cohort, functioning as a control group to contextualize inflammatory profiles in a non-cardioembolic stroke population.

A major challenge of this observational design was achieving covariate balance across three distinct groups. While

propensity score (PS) methods such as matching and inverse probability weighting (IPW) are widely used, they are most commonly applied in two-group comparisons [2]. To address this, we implemented two complementary strategies: (1) pairwise balancing (comparing Group 1 vs Group 2 and Group 1 vs Group 3 separately), and (2) simultaneous balancing across all three groups using a multinomial PS model.

Covariates included in all PS models were: age, sex, baseline NIHSS score, pre-stroke statin use, and vascular territory. To improve covariate balance and ensure model stability, the analysis was restricted to patients aged 70 years or older, as the inclusion of younger patients introduced instability in PS and IPW estimation.

PAIRWISE COMPARISONS:

For each pairwise comparison, propensity score matching was performed using nearest-neighbour matching with a 1:2 matching ratio and a caliper of 0.2 standard deviations. The caliper ensured that only treated and control individuals with sufficiently similar propensity scores were matched, reducing potential bias from poor-quality matches [3]. The standardized mean difference was used to evaluate balancing between covariates.

In parallel, IPW was applied using stabilized weights to reduce the influence of extreme weights [4]. The Average Treatment Effect (ATE) was used as the estimand. To limit the impact of outliers, weights were truncated at the 1st and 99th percentiles.

THREE-GROUP COMPARISON:

To balance covariates across all three groups simultaneously, propensity scores were estimated using a multinomial logistic regression model using stabilized and truncated weights, again with the ATE as the estimand. This model extends binary logistic regression to multiple categories, estimating the conditional probability of belonging to each group given the covariates. These probabilities were used to generate stabilized inverse probability weights for multi-group IPW, allowing for covariate adjustment across all groups within a single model. This approach allowed for the inclusion of all patients in a single weighted model, improving comparability across the three clinical profiles.

Covariate balance was assessed graphically using Love plots (see Figure 1), which display standardized mean differences before and after weighting or matching. An STD value ≤ 0.1 was considered a good balancing. For statistical testing, weighted t-tests were conducted for both pairwise and three-group comparisons.

A broad panel of biochemical and haematological markers was analyzed across groups, including lipid profiles, inflammatory indices (e.g., neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio), and coagulation parameters (e.g., D-dimer). When global differences were identified in the three-group analysis using weighted ANOVA, post hoc pairwise comparisons were performed using model-based t-tests.

For additional comparison, an unweighted ANOVA was also conducted on the raw data, to evaluate differences in the absence of statistical adjustment.

Residual inflammatory risk (RIR) -defined as hsCRP > 2 mg/dL- and residual cholesterol risk (RCR) -defined as LDL >

70 mg/dL-were analyzed using chi-square tests.

All analyses were performed using RStudio (v2025.05.496).

RESULTS

Both PSM and IPW effectively improved covariate balance (see Figure 1), although the number of units used varied between methods:

- Three-group IPW: 48/59 in group 1, 287/294 in group 2 and 125/151 in group 3;
- IPW pairwise (Group 1 vs 2) 52/59 in group 1 and 293/294 in group 2;
- IPW pairwise (Group 1 vs 3): 43/59 in group 1 and 138/151 in group 3;
- PSM (Group 1 vs 2): 56/59 in group 1 and 111/294 in group 2;
- PSM (Group 1 vs 3): 54/59 in group 1 and 89/151 in group 3.

In pairwise comparisons:

- PSM identified significantly higher MLR and lower triglycerides in Group 3 vs Group 1 ($p = 0.041$ and $p = 0.003$ respectively).
- IPW identified significantly lower triglycerides ($p = 0.008$) and lower D-dimer ($p = 0.012$) in Group 3 vs Group 1 and detected significantly higher D-dimer in Group 1 vs Group 2 ($p = 0.024$).
- In the three-group IPW-weighted ANOVA, MLR, triglycerides, and D-dimer were all significantly different across groups. Interestingly, Total-Cholesterol also reached significance under IPW but not in unadjusted models, potentially due to improved covariate balance revealing subtler metabolic effects.
- In the unweighted (raw data) ANOVA only triglycerides were statistically significant.

The post-hoc analyses confirmed significant differences in D-dimer (Group 1 vs 2 and Group 1 vs 3) and triglycerides (Group 1 vs 3). Other significant results from the weighted ANOVA were primarily attributable to differences between Group 2 and Group 3.

These findings demonstrate that the choice of adjustment method can shift which biomarkers appear relevant, reinforcing the importance of using multiple statistical approaches to capture meaningful clinical signals.

CONCLUSIONS

The results showed suggest that Propensity Score Matching (PSM) identified a significant difference in MLR between groups; however, this result was not confirmed by post hoc analyses following the three-group IPW-weighted ANOVA. This discrepancy may be attributed to the matching ratio (1:2), which necessarily excludes a substantial portion of observations from Groups 2 and 3, potentially limiting representativeness and power. In fact, MLR was only significantly different between Groups 2 and 3 in post hoc tests, but this comparison is outside the primary scope of this study. Moreover, PSM failed to detect significant differences in D-dimer between Groups 1 and 2, which were instead clearly identified

through IPW, both in pairwise and three-group comparisons.

The limitations of PSM in studies involving more than two groups are underscored by the need to select separate matched datasets for each pairwise comparison. This results in inconsistent representation of Group 1 across comparisons and makes integration of findings more difficult. In contrast, IPW applied across all three groups, in combination with post hoc weighted analyses, successfully identified clinically relevant differences in D-dimer and lipid markers. IPW appears to be a robust and flexible method for covariate adjustment in in three-group observational.

Unweighted analyses were not able to detect differences beyond triglycerides, this highlights the importance of using covariate balancing techniques, particularly when dealing with groups of highly unequal size. These results emphasize the critical role of appropriate statistical methods in observational research and support the application of multinomial IPW as a valuable strategy when multiple treatment groups are involved.

The variation in findings across PSM, IPW, and unadjusted models underscores the importance of triangulating results from multiple analytical strategies, especially in unbalanced observational datasets.

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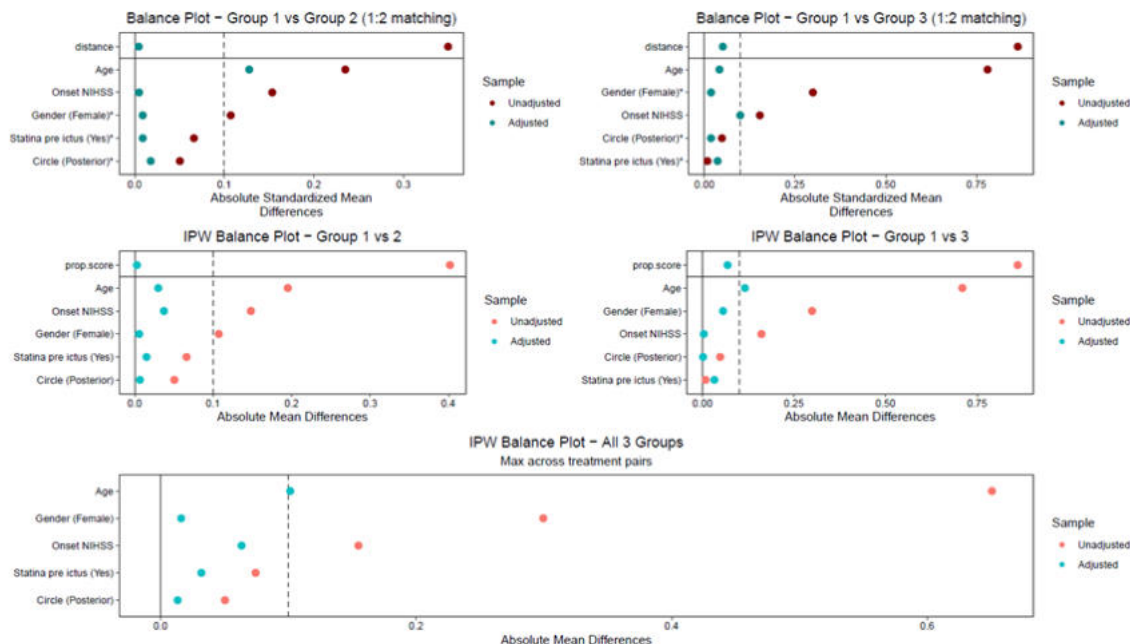


Figure 1. Covariate balance across groups before and after adjustment using PSM and IPW. Plots show standardized mean differences across age, sex, NIHSS, statin use, and vascular territory. Adjustment substantially reduced imbalance in all two-by-two comparisons and the overall three-group analysis

Ventricular Volume as the Most Informative Biomarker of TSPO-PET Binding Status in Multiple Sclerosis Patients

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INTRODUCTION

Ability to identify multiple sclerosis (MS) patients burdened by smouldering inflammation is of great importance for therapeutic and clinical trial purposes [1]. PET imaging using 18 kDa translocator protein (TSPO)-binding radioligands can be used as an imaging biomarker for quantitation of glial activation in vivo [2]. However, large scale use of PET is challenging. Proxy biomarkers for TSPO-PET outcomes would therefore be helpful.

OBJECTIVES

The objective of the study was to identify key predictors of high TSPO-binding status in MS brain (HOT-PET phenotype) to provide widely usable tools for identification of patients with significant smouldering inflammation. Moreover, establishing a valid link between TSPO-PET and MRI measures would support the use of MRI as a proxy for microglial activation, given the limited accessibility of TSPO-PET.

METHODS

The sample included 128 MS patients (92 RRMS, 36 progressive). 3T magnetic resonance imaging (MRI) was performed and the images were processed in MATLAB and segmented using FreeSurfer. PET imaging using the TSPO-binding radioligand [¹¹C]PK11195 was performed. Patients were classified according to their TSPO binding status: threshold for HOT-PET was set at 9.6% of active voxels in the white matter (based on TSPO-binding in an SPMS cohort). Partial least squares discriminant analysis (PLS-DA) extracted independent subspaces of variables best explaining outcome variability

(high or low TSPO-PET binding status). Based on variable importance in projection (VIP) scores, significant predictors of high TSPO binding were combined into a decision tree model to quantify misclassification error and refine the predictive framework [3,4].

RESULTS

Based on the highest VIP scores, we constructed a decision tree using MRI-derived features alone. Ventricle parenchymal fraction (%PF) consistently emerged as a key predictor across all models, with a stable threshold around 2.12%. When combined with thalamus %PF, brain %PF, and age, and after tuning model parameters, we achieved an improved test accuracy of 0.84, outperforming the ventricle-only model (accuracy = 0.74). Key decision nodes included ventricle %PF ≥ 2.12 and age ≥ 38.4 , which were strongly associated with the HOT-PET phenotype. Even among patients with smaller ventricles, low thalamic and brain %PFs contributed to identifying those with high glial activation.

To further characterize these patterns, we plan to stratify patients by MS subtype (RRMS vs PMS) and ventricle size, assessing whether enlarged ventricles in RRMS suggest a progressive-like inflammatory profile or whether some PMS patients with preserved brain volumes show lower microglial activation.

CONCLUSION

MRI-based volumetrics offer a practical strategy to identify MS patients with high glial activation when PET is unavailable or difficult to perform. While ventricle PF% alone was a strong proxy biomarker, classification improved by including

thalamus and brain volume PF%, as well as age. This multi-variable approach can support better patient stratification for phase 3 trials targeting microglial activation and help clinicians screen those most likely to benefit from microglia-targeted therapies.

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Informative Censoring and Outcome Definition in a Target Trial Emulation Framework using Real-World Data

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INTRODUCTION

Oral anticoagulants are commonly prescribed for patients with atrial fibrillation (AF) to prevent stroke and systemic thromboembolism. However, anticoagulant treatment, regardless of whether it involves a vitamin K antagonist (VKA) or a direct oral anticoagulant (DOAC), can be associated with the development of kidney events and/or progression of kidney damage [1-2]. When using Electronic Health Records (EHR) in a Target Trial Emulation (TTE) framework [3] to evaluate the impact of a treatment on an outcome defined by a dynamical decline process, some issues arise not only due to the baseline confounding factors, but also for the potential informative censoring caused by treatment discontinuation or switch and related to the definition of the outcome itself. If these aspects are not taken into account, a biased estimate of the treatment effect is highly probable.

OBJECTIVES

To investigate accelerated renal function decline in patient with AF using anticoagulation therapy, exploring differences among VKA vs DOAC users using EHR of the Observatory of CardioVascular Diseases (OCVD) in the Friuli-Venezia Giulia region (FVG, Italy) in a TTE framework.

METHODS

To define renal outcomes as kidney failure (KF) and AKI (Acute Kidney Injury), we considered hospital admissions for renal causes identified by ICD9-CM codes but also the worsening in the eGFR biomarker defined in two ways: the date of the first eGFR value that showed a decline $\geq 30\%$ with respect to the baseline value or was below 15 ml/min/1.73 m². In the alternative approach, for each subject a linear regression

line was fitted through all his/her eGFR measurements. To be considered a sustained decline the linear regression slope needed to be negative, the time to the event was defined as the moment the regression line crossed the 30% decline threshold (or was below the 15 ml/min/1.73 m²) [4]. Two approaches were compared to define the follow up: Intention to Treat (ITT), where the censoring date was the first date among the administrative censoring date or death, whatever come first, and Per-Protocol (PP) where the follow-up terminated at the first date among administrative censoring date, drug group switch (from VKA to DOAC or viceversa) or therapy discontinuation date or death. Therapy discontinuation was defined as the date of the last anticoagulant medication purchase, extended by the number of days the purchase was expected to cover based on the quantity dispensed, plus an additional 90 days. In all the analyses, death as a first event was treated as a competing risk for the renal outcomes. To take into account baseline confounders, Inverse Probability of Treatment Weights (IPTW) were estimated using logistic regression to predict the treatment group assignment, according to characteristics of subjects at the index date. Results of the weighting procedure were considered appropriate if the standardized mean difference (SMD) between weighted treatment groups was < 0.1 . Since a differential rate of loss to follow-up between treatment groups (due to switch and discontinuation) was highly expected, in order to take into account simultaneously differences at baseline and informative censoring, adjustments were also performed using “combined” weights. These weights were the product of IPTW and time-dependent Inverse-Probability-of-Censoring Weights (IPCW) [5] taking into account monthly or 6-months’ time intervals and different set of baseline covariates for sensitivity analyses. Weighted incidence rates of events and weighted cumulative incidence curves were estimated in the overall population and in treatment groups [6]. Hazard ratios (HRs) for renal outcomes were estimated using cause-specific Cox regression models.

RESULTS

The study cohort was composed by 6873 subjects, 49% treated with DOAC and 51% with VKA. Significant differences at baseline were present between treatment groups, in particular as expected in the year of enrolment. After IPTW estimation, all the differences were below the 10%SMD (21 subjects were excluded due to the positivity violation). Under the ITT approach, no differences in any of the renal outcomes were observed, on the weighted cohort. Conversely adopting the PP, an increased risk of sustained eGFR decline (with the regression method definition) and KF was detected (respectively HR=1.18, [1.03 – 1.35], HR=1.69, [1.10 – 2.60]) in the IPTW-weighted cohort. No differences in AKI were found. When the renal event was defined using the first eGFR measurement below the decline threshold, a significant impact was observed only for KF but not for eGFR decline and AKI (KF HR: 1.43 [1.09-1.87], eGFR Decline HR: 0.99 [0.90-1.10], AKI HR: 1.16 [0.90-1.50]). When IPTWIPCW weights were adopted, the results were substantially confirmed, with less precision of the estimates (wider 95% CI) across the different time-intervals and set of covariates used.

CONCLUSIONS

When using observational data in the context of a TTE framework, it is crucial to take into account both the confounders issue due to the non-randomized study design but also the informative censoring induced by therapy discontinuation or switch. Moreover, the methods used to define the outcome when a longitudinal biomarker is involved are also relevant.

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Cangrelor in Patients with Percutaneous Coronary Intervention (PCI) after out-of-Hospital Cardiac Arrest (OHCA): A Propensity Score Matchig Analysis

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INTRODUCTION

Propensity Score Matching (PSM) is used in observational studies to measure the effect of a treatment by removing the bias of confounders, as randomisation is not possible. A multivariable logistic regression is performed to estimate the association of previously selected variables with the treatment administration, whilst the coefficients estimated from this regression are used to calculate a predicted probability of each patient receiving the treatment. Each patient in the treatment group is then matched to one or more patients in the untreated group based on the PS [1]. There are several ways to check whether PSM is successful or not: the two groups can be compared to confirm that there are no significant differences in covariate characteristics or the distribution graph of PS in both groups can be compared to ensure that they are similar. Once PSM is performed, unmatched patients are removed and analysis can be performed to test the treatment effect [1,2]. Out of Hospital Cardiac Arrest (OHCA) is defined as a sudden cessation of cardiac function with loss of consciousness and circulation occurred in out of hospital setting and acute coronary syndrome is the most common cause of OHCA. Emergent invasive coronary angiography (ICA) and percutaneous coronary intervention (PCI) has been shown to improve outcome in patients with ACS. Survivors of OHCA undergoing PCI are at higher risk of thrombotic and bleeding complications and cangrelor use has been shown to induce a faster, higher and more sustained inhibition of platelet aggregation function compared to all three P2Y12-inhibitors [3]. However, few data are available regarding OHCA victims.

AIM

The aim of this work is to apply PSM based analysis to investigate survival at hospital discharge of cangrelor use in OHCA survivors undergoing PCI.

METHODS

This is a multicentric, prospective, observational study involving all OHCA patients enrolled in the LombardiaCARE Registry from January 1, 2015, to December 31, 2022, who underwent PCI in seven centers in Lombardy region, Italy. Categorical variables were described as number and percentage and compared with the chi-squared test or Fisher exact test depending on the expected frequencies. Continuous variables were described as mean $\pm$ standard deviation and compared with the t-test or described as median and interquartile range (IQR) and compared with the Mann-Whitney test and according to their normal distribution tested with Shapiro Wilk test. All the variables that differed significantly between patients treated with cangrelor and patients in whom cangrelor was not administered were included in a multivariable logistic model for cangrelor administration. Model goodness of fit was assessed with Pearson test. The Area under the ROC Curve (AUC) was also computed. From the resulting coefficients PS was calculated. Patients were randomly matched according to the PS to generate random samples. The number of needed samples was established according to the convergence of the median chi-squared. The goodness of PSM was evaluated

in term of balance of the baseline characteristics comparing the propensity distribution graph in the unmatched population and matched population and Kolmogorov-Smirnov test [2]. For each sample, considering only matched patients, chi-squared test and logistic regression were performed to test the association between cangrelor administration and patient in-hospital survival. The median chi-squared test and the overall odds ratio (OR) derived from each sample were taken into account to confirm the association between cangrelor administration and survival at discharge. Statistical analyses were performed with STATA 17. A two-sided p-value of < 0.05 was considered statistically significant.

RESULTS

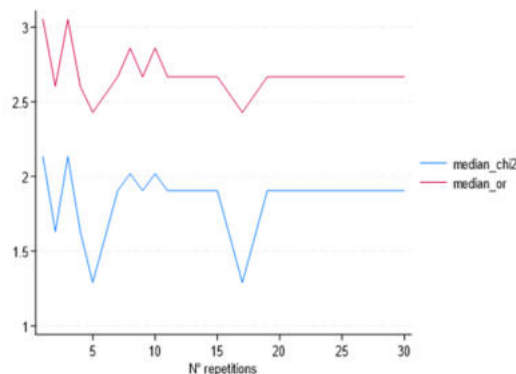
A total of 612 patients were admitted to the seven centres after OHCA and 414 (67.4%) underwent PCI. Among those patients 34 (8.2%) were treated with cangrelor. In the cangrelor group, 82.4% of patients were alive at discharge, compared to 65.3% in the no-cangrelor group (chi-square: 4.1; p-value: 0.04). A multivariable logistic regression model for the probability of receiving cangrelor was performed with all the significantly different variables between cangrelor and no-cangrelor group. (p-value: 0.001; PseudoR2: 0.2; AUC: 0.8). The model showed a good goodness of fit (Pearson chi2: 128.7; p-value: 0.8). Patients were randomly matched 25 times according to the PS to generate 25 random samples with 20 patients per each group, as indicated by the convergence of the median chi-squared (fig.1.a). Figure 1a demonstrates how the median Chi2s and ORs resulted from the Chi2 test and logistic regression for survival at discharge converge after 25 repetitions of PSM. The propensity distribution graph and Kolmogorov-Smirnov test showed a good PSM (p-value > 0.05) in all 25 samples. The resulting ORs from the 25 samples with their 95% confidence interval are plotted in Figure 1b which shows that in all samples OR was $OR > 1$ and an overall OR of 2.1 (95% CI 1.2-3.0) of survival at discharge, confirming the association between cangrelor administration and survival at discharge.

CONCLUSION

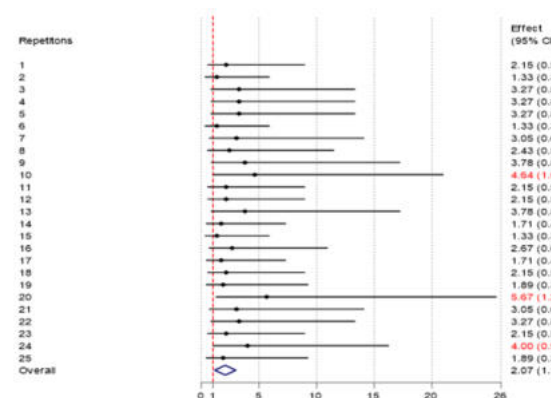
The multivariable logistic model for the association with cangrelor administration showed a good AUC and a good goodness of fit. The low number of patients treated with cangrelor prompted us to perform a random PSM to generate 25 random samples. The PSM was able to balance the baseline characteristics, making the two groups comparable. Moreover, repeating the PSM could help to achieve significant results in case of low numbers of patients and to overcome the limitation of PSM that leads to a reduction of the number of patients that can be included in the analysis due to the matching itself.

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1A



1B

Fig.1: A: Median chi2 convergence graph B: Forest plot displaying the effect of cangrelor administration on the probability of survival at hospital discharge in all 25 random samples.

Risk Scores Validation: An Example in Cardiology

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INTRODUCTION

Clinicians often make important decisions about patient care by estimating the likelihood of a particular disease, condition or event occurring. Prediction models are useful in this context. Development studies aim to develop a prediction model by selecting clinically relevant predictors and statistically combining them in a multivariable logistic or cox model [1]. Once a model has been developed, its performance must be assessed in the same cohort (internal validation) and in a new cohort (external validation). The performance of a model can be assessed in terms of calibration (comparison between the observed and predicted proportions of events) and discrimination (ability to predict patients who will or will not have the event of interest) [2]. There are several calibration methods: calibration in large, calibration curve associated with the Hosmer-Lemeshow test and calibration slope. Discrimination can be assessed by the area under the ROC curve (AUC) or the Harrel-c index, depending on the regression model used for development [3]. The development and validation of predictive scores are useful in cardiology: a study was conducted to investigate whether the ECG acquired after return of spontaneous circulation (ROSC) could play a prognostic role for 30-days mortality in patients surviving from out-of-hospital cardiac arrest (OHCA), defined as sudden cessation of cardiac function with loss of consciousness and circulatory signs occurring in an out-of-hospital setting. The study was conducted considering post-ROSC ECGs related to OHCA patients from 2015 to 2018 in the populations of Lugano, Vienna and Pavia. Multivariable cox regression was performed and age ≥ 62 years, female, ECG acquisition time ≥ 8 min, presence of >1 segment with ST elevation, a QRS ≥ 120 msec and the diag-

nostic pattern for Brugada syndrome resulted associated with higher 30-days mortality. The coefficient of each variable was multiplied by 10 and rounded to the nearest whole number and, by summing the rounded coefficients, a score between 0 and 26 was created. The study showed that the risk of death increased as the score increased. Furthermore, by dividing the population according to score tertiles, 30-days mortality risk classes were identified: low (score 0-4), intermediate (score 5-7) and high (score 8-26) [4].

AIM

The aim of this study is to validate a post-ROSC ECG score in predicting mortality risk and stratifying 30-days mortality risk after OHCA in a new cohort.

METHODS

This is a multicenter, prospective, score validation study to predict 30-days mortality in OHCA survivors. Post-ROSC ECGs of patients enrolled in the LombardiaCARE registry from 01/01/2015 to 31/12/2023 and ECGs of OHCA patients admitted to Saint-Pierre Hospital, Brussels, from 01/01/2017 to 31/12/2023 were collected. The same outcome and the same predictors of the previous work were considered. Categorical variables were described as numbers and percentages and compared using the chi-squared test or the Fisher exact test, depending on the expected frequencies. Continuous variables were described as mean $\pm$ standard deviation and compared with the t-test or described as median and interquartile range (IQR) and compared with the Mann-Whitney

test and according to their normal distribution, tested with the Shapiro-Wilk test. The risk score and mortality risk groups were identified according to previous work [4]. Univariable cox regression was performed with 30-days mortality risk category as the independent variable. The assumption of hazard proportionality was tested using the Shoenfeld test. Calibration was assessed by plotting the observed proportions of events against the predicted probabilities, while the c-index was assessed for discrimination. Moreover, the prognostic index (PI) was calculated from the cox regression model with the same predictors of the previous work [4] as covariates and Kaplan-Meier (KM) curves of PI tertiles were plotted. Long-rank test was used to test the difference between the three curves. All values $p < 0.05$ were considered statistically significant. Statistical analyses were performed using Stata 17.

RESULTS

A total of 1167 ECGs were collected in the two centres and score calculation was possible for 1075 of them. Of these patients, 431 (40.1%) were alive at 30 days. The median score was 10.0 (6.0-12.0) and 175 (16.35%) patients were classified as low risk, 300 (27.9%) as intermediate risk and 600 (55.8%) as high risk. Cox regression showed that patients in the intermediate risk group had a higher risk of death compared with those in the low risk group (HR: 1.3 [95% CI: 1.1-1.9]; p -value: 0.049), as did patients in the high risk group (HR: 1.9 [95% CI: 1.4-2.5]; p -value<0.001). The harrel-C of the model is C:0.56 [95% CI: 0.54-0.59].

CONCLUSION

The discrimination is lower compared to the original model (Harrel-c: 0.66 [95% CI, 0.57-0.76]), though acceptable. Indeed, the model retains the ability to discriminate patients at low, intermediate and high risk of 30-days mortality. Figure 1A shows the KM curves of the PI tertiles (p -value<0.001) and the graph suggests that the model discriminates better patients at high risk rather than low and intermediate risk, as expected considering the harrel-c. Figure 1B shows similar predicted and observed survival probabilities in all groups, confirming good calibration of the model. A limitation of the study is the non-homogeneous distribution of patients in the 3 risk groups. Our results suggest that the post-ROSC ECG score can predict the risk of 30-days mortality after OHCA. This provides a possibility for risk stratification in post-cardiac arrest care, assisting clinicians in clinical decision making and underlining the prognostic role of ECG.

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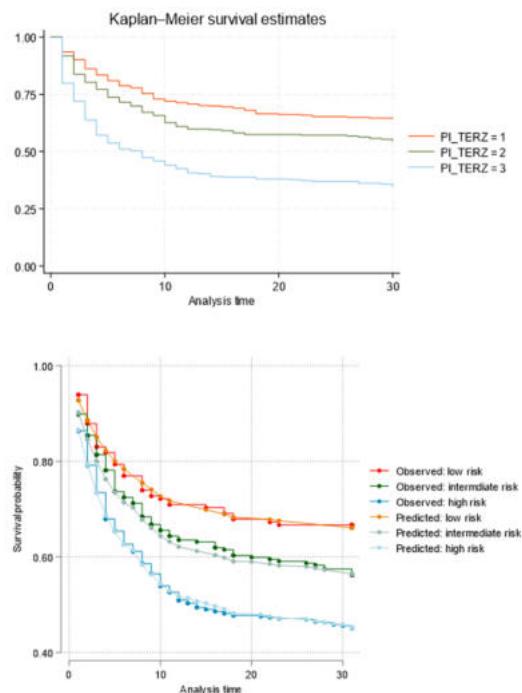


Fig.1. A: Kaplan-Meier curves of PI tertiles. B: Calibration graph.

Integrated PET/MRI in Pediatric CNS Tumors: Diagnostic Complexities

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INTRODUCTION

Brain tumors are the most common solid tumors in children, with an annual incidence ranging from 1.12 to 5.14 cases per 100,000. Advances in treatment have led to a 70% five-year survival rate. Follow-up imaging—particularly in differentiating tumor recurrence from therapy-related toxicity—remains a critical clinical need. Advanced quantitative imaging techniques, such as Perfusion-Weighted Magnetic Resonance Imaging (PW-MRI) and Positron Emission Tomography (PET) with ¹¹C-Methionine [1], may play a crucial role in therapy-related decision making in these patients. The use of simultaneous hybrid PET/MRI is highly beneficial in children as it allows for a one-stop-shop examination that limits the scanning procedures. Since both techniques generate a large number of imaging features, identifying and integrating those with the greatest impact on clinical outcomes could represent an effective strategy to improve and accelerate diagnostic accuracy. Machine Learning algorithms have significantly enhanced the analysis of multi data. In the specific context of PET imaging, missing data are often categorized as missing not at random [2] (MNAR), where the absence of data is systematically related to unobserved variables or the underlying condition being studied. This introduces potential biases and challenges for integrating and extracting meaningful features from the data.

AIM

The present study is aimed to achieve main objectives in the managing patients of care:

- i) to investigate how features extracted from PW-MRI and PET can be integrated to develop a combined diagnostic score

- ii) to manage missing data in the context of PET imaging

METHOD

PET/MRI features associated with tumor progression were analyzed both individually and in combination with the reference, introducing new variables into the model. Given the presence of missing not at random (MNAR) data in the PET set, missing values were imputed using a Bayesian approach [3] with the “stan” function using Monte Carlo sampling (MCMC), based on a truncated normal distribution. The algorithm DIABLO [4] has proven effective in integrating datasets from different sources and in identifying features across data from PET and from MRI. The DIABLO was used to integrate PET data (18 features, 42 patients) and MRI data (52 features, 40 patients). More specifically a sparse multiblock partial least square-discriminant analysis (sPLS-DA) was employed to integrate them, implemented via the `block.plsda()` function from the `mixOmics` R package [5]. sPLS-DA integrates an intrinsic variable selection procedure into the model fitting by applying a LASSO penalization to the loading vectors of the X data, thereby identifying the most discriminative features through latent components (`ncomp=2`) that maximize the shared covariance between data blocks. Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC) as a measure of classification accuracy. 95% Confidence Interval [CI] was computed by resorting to a bootstrap method.

RESULTS

A total of 42 patients were enrolled in the study, including

18 females. The median age at enrolment was 9 years, with an interquartile range (IQR) of 5 to 14 years. Tumor histologies were classified into the following categories: embryonal tumors ($n=16$), high-grade gliomas ($n=14$), germ cell tumors ($n=5$), ependymal tumors ($n=5$), and low-grade gliomas ($n=2$). For the following analysis, 40 out of the 42 patients were included, since two patients did not undergo the MRI. The overall correlation between the MRI and PET datasets, calculated using Pearson's method on the first latent component of each block, was 0.41 (95% CI: 0.12–0.60). Regarding diagnostic performance, assessed via AUC, the second components of both MRI and PET data showed stronger discriminative ability. For MRI, the first component yielded an AUC of 0.722 (95% CI: 0.453–0.932), while the second component achieved 0.795 (95% CI: 0.515–0.951). Similarly, for PET, the first component demonstrated an AUC of 0.725 (95% CI: 0.517–0.906), and the second component obtained 0.753 (95% CI: 0.547–0.923).

CONCLUSIONS

Our findings reveal a moderate overall Pearson correlation of 0.41 between the MRI and PET datasets. This correlation is a positive indicator for a multi-modal approach; it suggests the modalities capture complementary, rather than entirely redundant, information. To enhance the generalizability and robustness of these findings, further validation of these results is essential.

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AI-Based Tool for Early Diagnosis and Progression Prediction in Alzheimer's Disease: A Multicenter Validation Study

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of neurodegenerative dementia and poses a major healthcare challenge worldwide. Despite the availability of biological biomarkers, their application in routine clinical settings remains limited. Recent recommendations from eleven European scientific societies and Alzheimer Europe propose a patient-centered diagnostic workflow for memory clinics [1]. Within this context, artificial intelligence (AI) may offer valuable support for clinical staging and diagnosis based on widely available neuropsychological and MRI data [2-3].

OBJECTIVES

This study aimed to evaluate the clinical performance of TRACE4AD™, a CE-marked AI-based medical device, in supporting memory clinics during key diagnostic steps, specifically by assessing its ability to correctly stage cognitive decline, to classify clinical syndromes and formulate causal hypotheses (distinguishing AD from non-AD profiles), and to predict conversion to AD dementia within 24 months.

METHODS

A total of 797 subjects were enrolled from 66 centers (Italy, US, Canada). All underwent 3D T1-weighted MRI and a detailed neuropsychological battery assessing multiple

cognitive domains [4]. In 482 cases, CSF biomarkers (A β 42, t-tau, p-tau) and/or [¹⁸F]FDG PET imaging were available [5]. TRACE4AD™ automatically analyzed imaging and cognitive data using an ensemble of Support Vector Machines (SVMs), with feature selection via Principal Component Analysis (PCA) and Fisher Discriminant Ratio (FDR) [6-7]. Clinical performance was assessed in terms of agreement with expert clinical staging (Cohen's kappa), diagnostic accuracy against biomarker-based classification for syndrome identification, and predictive accuracy of conversion to AD dementia at 24 months using clinical follow-up as reference.

RESULTS

TRACE4AD™ showed substantial to almost perfect agreement with clinical staging ($\kappa=0.81$ for HS/SCI/WW, $\kappa=0.70$ for MCI/MD, $\kappa=0.90$ for moderate/severe dementia). In the subset of subjects with biomarker data ($n=130$), the tool correctly classified AD-related syndromes with 91% accuracy, achieving a positive predictive value of 91% and a negative predictive value of 100%. For prediction of conversion to AD-dementia at 24 months ($n=341$), TRACE4AD™ reached 89% sensitivity, 82% specificity, 85% overall accuracy, and an AUC of 83%. Furthermore, AI-derived brain volumetric features significantly correlated with CSF biomarkers, particularly in medial temporal regions, and cognitive performance, supporting the tool's biological validity and interpretability.

CONCLUSIONS

TRACE4AD™ demonstrated high performance in staging, syndrome classification, and prediction of AD conversion, supporting its utility as a statistical and clinical decision-support tool. Its ability to integrate multimodal data in a reproducible, interpretable manner aligns with current intersocietal recommendations [1], providing an innovative and practical solution to enhance early diagnosis and personalized care in memory clinics.

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Evaluation of Artificial Intelligence-Generated Synthetic Data for Clinical Research in Secondary Cardiovascular Prevention of Patients with Dyslipidemia

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INTRODUCTION

Data is crucial in modern healthcare, bearing the potential to improve patient care by powering clinical research and enhancing public health. However, the promise of real-world data to enable personalized medicine, guide policymaking, and respond to rapidly evolving healthcare needs is often constrained by challenges in accessing high-quality datasets. Synthetic data offers a compelling alternative, addressing privacy concerns, simplifying ethics reviews, reducing costs, and ensuring access to sufficiently large and reliable patient cohorts [1]. While synthetic data has already been successfully validated in domains outside healthcare, its application in the medical field remains limited.

OBJECTIVES

This study aims to compare synthetic and real-world data in healthcare by applying various statistical methodologies to both types of datasets.

METHODS

Data synthesis techniques will be used to create cohorts of patients with specific attributes that are statistically similar to real patients by using AI tools released by Aindo SpA. The real datasets originate from the clinical platform of Centro Cardiologico Monzino and include structured data system-

atically collected by the Atherosclerosis Prevention Unit from 2002 to 2024 during outpatient visits of 1000 patients in secondary cardiovascular prevention and with a personal history of dyslipidemia. A comparison was performed between real and synthetic datasets. For categorical variables ($n = 49$), Jensen–Shannon Divergence (JSD) was used. For continuous variables ($n = 25$), differences in means were evaluated using 95% confidence intervals (CIs). A logistic regression model with stepwise selection and cross-validation was developed using both real and synthetic data.

RESULTS

Only 1 out of 49 categorical variables showed a statistically significant difference (~2%), which is below the expected 5% due to type I error. For continuous variables, 4 out of 25 (16%) showed CIs that did not include zero. Logistic regression with cross-validation identified the same predictors in both datasets: CPK (UI/l) and Therapy modification. Odds Ratio (OR) for CPK was 1.01 (real) and 1.01 (synthetic), with a 70% overlap of CIs. OR for Therapy modification was 0.10 (real) and 0.28 (synthetic). The model developed on the synthetic database was used for training and then validated on the real database. All variables selected by the stepwise model on synthetic data were validated on real data, confirming the model's transferability.

CONCLUSIONS

The synthetic dataset demonstrated reliability and comparability with real-world data. While having a slightly higher margin of error, cross-validation of SAMS (statin-associated muscle symptoms) predictors indicates that models trained on synthetic data can be transferred effectively to real-world data applications, supporting wider use of synthetic datasets in clinical and epidemiological studies.

This study has been realized thanks to the support of NOVARTIS.

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Translation and Content Validity of a Tool for Measuring Perception of Nuclear Issues among Students Visiting Pavia Research Reactor

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INTRODUCTION

There is a lack of national data describing students' perceptions of nuclear safety. Moreover, no validated instruments currently exist for investigating this topic.

OBJECTIVES

The objectives of this study were to translate and back-translate from French to Italian and from Italian to French the questionnaire used in the study "Special Eurobarometer 271 / Wave 66.2 – Europeans and Nuclear Safety"1 conducted by the European Commission; validate the questionnaire using the Content Validity Ratio (CVR); assess the content and face validity of the tool through expert panel reviews; and collect preliminary data (pilot study) to support the planning of future research strategies.

METHODS

This pilot observational study involved the administration of a questionnaire as an additional procedure during routine educational reactor activities. Phase 1 consisted of the translation and back-translation of the questionnaire. Phase 2 involved the evaluation of content and face validity by a panel of nine experts with diverse backgrounds in nuclear science. In Phase 3, pilot data were collected using the survey instrument and subsequently analyzed through descriptive statistics. Content validity was assessed using the Item Content Validity Index (I-CVI), with a threshold of 0.78 considered acceptable, and the Scale Content Validity Index/Average (S-CVI/Ave), with a threshold of 0.80. Face validity was assessed through expert panel reviews. Items scoring below 0.60 were individually reviewed by the expert board.

RESULTS

Content and face validation revealed the removability of seven items, which were individually reviewed by the expert board. As a result, two of these items were retained. The final version of the questionnaire included twelve items, with a final S-CVI/Ave of 0.89. The pilot study (n = 50) showed a divided perception among adolescents regarding nuclear energy: some participants perceived it as highly risky, while others held more favorable views. Data collection is ongoing. Sixty percent of respondents believe that the benefits of nuclear energy outweigh its risks. However, 98% reported feeling not at all or only slightly informed about nuclear energy. Sixty-seven percent believe that nuclear power plants are not very risky or not risky at all for the country, and 88% are completely or somewhat in agreement that such plants can be operated safely. Finally, 67% believe that the use of nuclear energy should be increased.

CONCLUSION

The results of this pilot study support the successful translation, back-translation, and initial validation of the questionnaire in terms of content and face validity. These findings suggest that the instrument is a promising tool for assessing perceptions of nuclear safety and can be reliably used in future research within educational and healthcare contexts.

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Association Analysis of Rare Variants in Endometriosis

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INTRODUCTION

Endometriosis is a chronic, inflammatory gynecological condition characterized by the presence of endometrial-like tissue outside the uterus. It appears in various forms and can be classified based on location into several subtypes: superficial peritoneal, ovarian, deep infiltrating, extra-abdominal, and iatrogenic [1]. In recent years, advances in genetics have underscored the complex, multifactorial nature of endometriosis. Genome-wide association studies (GWAS) have identified common genetic variants linked to disease susceptibility. However, Whole Exome Sequencing (WES) has emerged as a powerful tool for uncovering rare, high-impact mutations that may explain severe or familial cases. WES not only complements GWAS findings but also opens new ways for understanding disease mechanisms, identifying biomarkers, and developing personalized therapeutic strategies.

AIM

The aim of this study is to identify and compare genetic variants present in a cohort of healthy women and women with endometriosis by using WES. The goal is to discover potential genetic biomarkers and gain a better understanding of the molecular mechanisms underlying the disease, to improve early diagnosis, prognosis, and the development of targeted treatments for women with endometriosis.

METHODS

This case-control study included 400 Italian women, 200 with laparoscopically confirmed endometriosis and 200 healthy controls. A key focus of the study was an in-depth qual-

ity control analysis: particular attention was devoted to evaluating the distribution and consistency of sequencing quality metrics—such as average coverage, read depth, genotype quality, and mapping quality—across the target regions. This thorough assessment was crucial to ensure homogeneous and comparable data across all samples. Only variants meeting stringent criteria—read depth >10, genotype quality ≥ 30 , and mapping quality ≥ 40 —and located in regions shared by at least 95% of the samples were retained, strictly following GATK best practices. This rigorous filtering was essential to achieve consistent variant calling across the cohort. To support this process, a comprehensive bioinformatic pipeline was developed for the processing, filtering, and statistical analysis of genetic data, ensuring high reliability and reproducibility across the entire workflow. Following quality control, we focused on rare (MAF <1%), exonic and non-synonymous variants. To assess the association between these variants and endometriosis, we applied the Sequence Kernel Association Test (SKAT) using RVTES [2, 3]. SKAT is a powerful, regression-based method designed to evaluate the combined effect of multiple rare variants within a gene, accommodating variants that may have effects in different directions. This approach allowed us to evaluate the cumulative effect of rare variants within each gene, increasing the power to detect associations in a genetically complex disease like endometriosis. Genes with a p-value < 0.01 from the SKAT test were considered significant and were subsequently analyzed using DAVID for functional annotation and GTEx for evaluation of their tissue-specific expression, in order to explore their potential involvement in the pathophysiology of endometriosis [4].

RESULTS

After quality control, we obtained 451195 variants, of which 134113 were rare, exonic, and non-synonymous. These 134113 variants were analyzed, and the SKAT test identified 98 genes with significant association ($p < 0.01$). Functional annotation revealed enrichment in glycoprotein-related genes and those involved in immune response, cell adhesion, and metabolism. Twenty-seven candidate genes showed a higher mutation burden in cases than controls, with a case-to-control burden ratio ranging from 1.1 to 5.3. Among them, ENG, PTEN, and HLA-DPB1 were implicated in known pathogenic pathways, while novel candidates like CDHR3, CSMD3, and PLA2G3 were linked to cell adhesion and inflammation. Gene expression analysis revealed relevant tissue-specific expression in reproductive organs, supporting their potential involvement in disease pathogenesis.

CONCLUSIONS

We identified 27 genes potentially implicated in the disease's development, focusing on those involved in immune response, inflammation, and tissue remodeling. Notably, genes such as ENG, PTEN, and HLA-DPB1 play key roles in cellular processes that could contribute to endometrial tissue proliferation, immune dysregulation, and fibrosis, all of which are central to endometriosis pathogenesis. Moreover, the discovery of genes like CSMD3, CDHR3, and PLA2G3 highlighted the importance of immune regulation and cellular adhesion in the progression of endometriosis. Additionally, genes such as FMO2, FMO4, SLC2A4, and TET2 suggested that metabolic dysfunctions and epigenetic alterations may also play crucial roles in disease development. These findings support the notion that endometriosis is driven by a combination of disrupted signaling pathways, immune dysfunction, altered metabolism, and epigenetic modifications. Ultimately, further research and validation of these genetic variants could lead to the identification of novel biomarkers and treatment options for endometriosis, offering hope for improved patient outcomes.

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Weight Changes during Early Breast Cancer Treatment and their Association with Survival: An Exploratory Functional Data Analysis of GIM-2 Trial

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INTRODUCTION

Limited evidence is available on the impact of early breast cancer (EBC) treatments on body weight. Notably, robust data quantifying the magnitude and timing of weight changes during treatment are lacking, and existing findings on the association between weight changes and long-term survival outcomes are conflicting [1–3]. Since weight changes may reflect metabolic shifts [4], influence treatment tolerability [5], affect dosing [6], or serve as prognostic markers, our findings could help optimizing both therapeutic and supportive care strategies.

AIMS

We have two objectives. First, to describe how patients' weight changes during the administration of EBC treatments. Second, to explore whether these short-term weight changes are associated with long-term survival outcomes.

METHODS

We used data from the GIM-2 trial, a randomized phase III trial in patients with EBC, which compared the addition of fluorouracil (FEC-P) to standard anthracycline-taxane chemotherapy (EC-P), administered either dose-dense (q14) or at standard intervals (q21). Body weight was recorded at randomization and at each treatment visits. We used Functional

Data Analysis (FDA) to model individual weight trajectories [7], compare them across subgroups [8], and identify groups of patients with similar trajectories [9]. Two grid search approaches were used: (1) to find the parameters that minimized the generalised cross-validation criterion for curve fitting, and (2) to identify the optimal clustering strategy based on the largest average silhouette score. Long-term outcomes included overall survival (OS), disease-free survival (DFS), and breast cancer-free interval (BCFI), defined according to the STEEP Criteria [10]. Associations between weight changes and outcomes were evaluated using landmark analyses, applying either the standard Cox Proportional Hazard Model (CPH) or its extension [11] to handle functional covariates. In both approaches, models were adjusted for relevant baseline characteristics.

RESULTS

A total of 17,361 weight measurements were analyzed from the 1,978 patients with complete data on treatment administration, stage, and grade. By the end of the treatment, the average weight change from baseline was +0.5%, with 13% of patients experiencing weight changes of $\pm 5\%$ or more. Older and obese patients tended to lose more weight, while tumor characteristics were not associated with weight change. Patients on q21 had a modest but significant weight gain compared to those on q14 (+0.8%, 95% CI: 0.5–1.2), with divergence in mean groups trajectories emerging early during treatment (L2N Test statistic = 1.38, p-value < 0.001). No statistically significant differences were observed between FEC-P and EC-P (L2N Test statistic = 0.01, p-value = 0.76). Conventional CPH models suggested a modest OS benefit associated with weight gain during treatment (HR per 1% increase=0.97; 95%CI=0.94–1.00; p-value=0.039). However, this association was not consistent across all outcomes and appeared to conflict with the observed beneficial treatment effect of the q14 versus q21. FDA revealed a more nuanced picture: the impact of weight gain varied according to its time, with mid-treatment gains appearing less favorable. Notably, a subgroup of patients—more frequently in the q21 treatment group—who experienced weight gain during mid-treatment had significantly increased risk of death compared to those with stable weight (adjusted HR: 1.58, 95% CI: 1.10–2.27). Similarly, patients with mid-treatment weight loss—a pattern more common among older and obese patients—had a higher risk of adverse outcomes across all endpoints (adjusted HR: 1.46, 1.38, and 1.47 for OS, DFS, and BCFI, respectively), and also received reduced dose intensity across all drugs.

CONCLUSIONS

By overcoming the limitations of traditional analyses based on weight changes between two arbitrary fixed time-points, FDA provided a comprehensive, time-based characterization of weight trajectories. This approach identified distinct patient subgroups with similar longitudinal patterns of weight change, which were also associated with differences in dose intensity and clinical outcomes. These findings support the potential of FDA-informed profiling to guide personalized management strategies in early breast cancer care.

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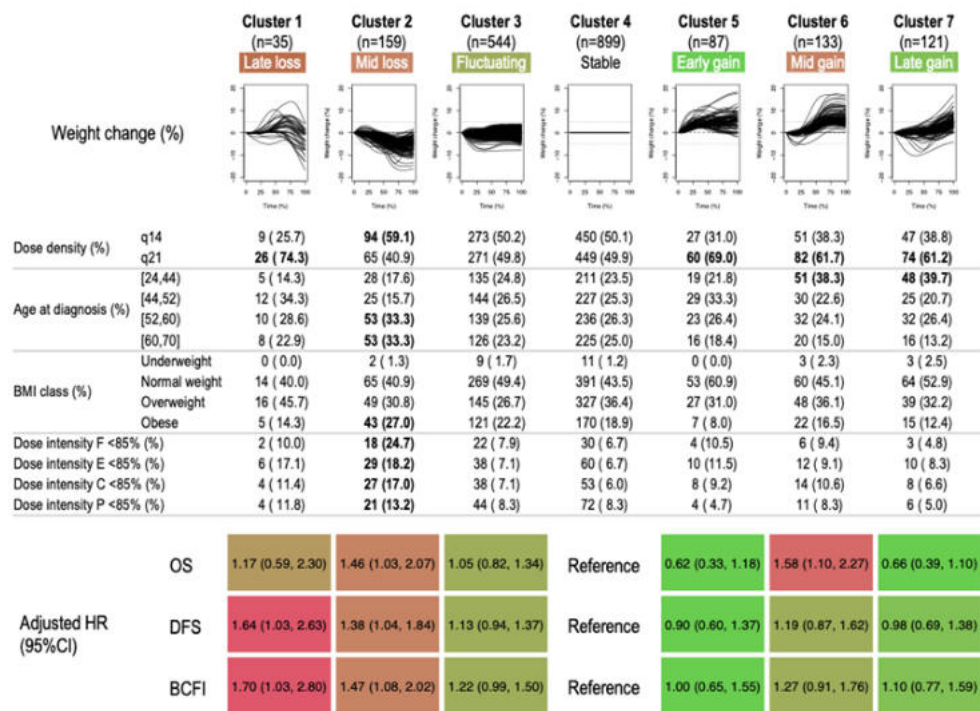


Figure 1. Characteristics associated with patients clusters based on weight changes

Unveiling the Dynamics of Physical Activity and Mood in Schizophrenia Spectrum Disorders: A Bayesian Approach from the Multicentric DiAPAsen Project

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INTRODUCTION

Schizophrenia spectrum disorders (SSD) affect approximately 1% of the global population [1], leading to significant quality-of-life impairments and functional disabilities. Physical inactivity is a critical modifiable risk factor in SSD, with meta-analytic evidence showing markedly reduced physical activity (PA) levels compared to the general population. While PA is a promising intervention to improve both physical and mental health outcomes, its real-time interplay with mood in SSD remains poorly understood. Despite the potential of Ecological Momentary Assessment (EMA) and accelerometry for monitoring mood and activity in natural settings, few studies have examined these variables together in SSD populations. Most traditional approaches study PA and mood separately, overlooking their bidirectional relationship. In this study, we address this gap by integrating EMA and accelerometry within a Bayesian framework to examine the dynamic interactions between PA and mood in SSD patients, capturing their complex temporal dependencies.

AIMS

This study aims to examine the bidirectional relationship between PA and mood in individuals with SSD through the use of EMA and accelerometry. By employing a Bayesian framework, we aim to model the dynamic interactions between these variables and investigate potential differences between SSD patients and healthy controls. The primary goal

is to provide empirical evidence supporting the integration of PA interventions into the clinical management of SSD.

METHODS

As part of the Italian DiAPAsen project, we conducted a multicenter cohort study involving 120 patients diagnosed with SSD and 113 healthy controls (HC), matched for sex and age. Over seven consecutive days, participants underwent ecological monitoring that combined smartphone-based EMA of emotional states with continuous 24-hour PA tracking using the ActiGraph GT9X Link device. The EMA protocol included seven daily prompts to assess current mood, calculated as the difference between self-reported happiness and sadness scores.

Our analytical strategy followed a two-step approach. First, we employed a generalized linear mixed-effects model (GLMM) to examine the association between daily mood and aggregated daily PA, adjusting for demographic variables and group membership. This provided insight into between-subject differences in mood-PA associations at the day level.

Moreover, we investigated the dynamic, within-day bidirectional relationship between mood and PA using a Bayesian Network approach. For each EMA evaluation, we computed the average PA level in the 30 minutes before and after the mood rating, allowing us to jointly estimate the prospective influence of PA on mood and the reciprocal effect of mood on subsequent PA. The model accounted for first-order

autoregressive effects in both mood and PA time series, and controlled for circadian patterns using time-slot indicators. The analytical framework was formalized through a DAG (Fig. 1) and estimated via Monte Carlo Markov Chain (MCMC) sampling, enabling robust multilevel inference adapted to our high-frequency, hierarchical data structure.

RESULTS

The GLMM confirmed that SSD patients exhibited significantly lower mood levels compared to healthy controls, but revealed no significant association between daily physical activity and mood in the overall sample. However, temporal factors may play a crucial role, as both PA and mood show marked diurnal variations which daily-level analyses may fail to capture. Further analysis using a Bayesian multilevel model provided deeper insights into the bidirectional relationship between PA and mood. This model jointly estimated the prospective influence of PA on mood and the reciprocal effect of mood on subsequent PA, accounting for time-lag effects, autocorrelations, and time slots throughout the day. The posterior distribution for HC predominantly indicated a positive relationship, with most values above zero, suggesting consistent evidence for PA leading to mood improvements. For SSD patients, the posterior distribution also remained mostly positive, but with greater variability and uncertainty, indicating more individual differences in the effect of PA on mood. These findings highlight the more variable and individualized nature of the relationship between PA and mood in SSD patients, compared to the clearer pattern observed in HC individuals.

CONCLUSIONS

Our findings highlight the potential of PA as a valuable addition to psychiatric care for improving outcomes in patients with SSD. Although the impact of PA on mood was more variable in SSD compared to healthy controls, the overall trend supports the integration of structured PA programs to address functional and emotional challenges, improve adherence, and enhance long-term benefits. Personalizing PA interventions to individual needs and timing is crucial for maximizing therapeutic outcomes. Methodologically, our innovative use of Bayesian multilevel modeling represents a methodological breakthrough in understanding complex bidirectional relationships between lifestyle factors and psychological states. The framework we present offers a robust statistical foundation for investigating complex temporal dynamics across diverse biobehavioral domains, with broad implications for advancing personalized medicine beyond psychiatric contexts.

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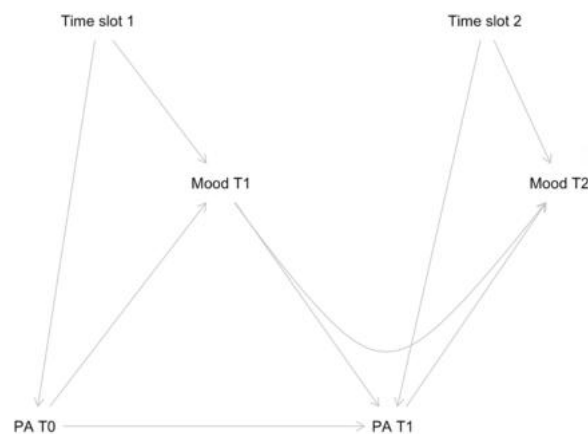


Fig. 1: DAG shows the joint effect of PA on each mood evaluation and of mood levels on following PA patterns. For each EMA evaluation, the average PA level in the previous 30 minutes was estimated

Real or Synthetic? Dermatologist Agreement on Synthetic vs. Real Melanoma and Pattern Recognition

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BACKGROUND

The validation of synthetic dermatological images generated by Generative Adversarial Networks (GANs) [1] is crucial for their integration into clinical and research workflows. Despite rapid progress in image synthesis, a standardized framework for evaluating the realism and diagnostic utility of synthetic skin lesions through expert review is still lacking [2]. Existing automated evaluation metrics, while informative, do not always align with human perception and diagnostic expectations. Particularly in medical domains, subtle visual cues and contextual interpretation often elude algorithmic assessment [3]. Human evaluations remain the most direct means of determining whether synthetic images capture the nuanced features necessary for clinical utility. Without structured expert-based validation, synthetic images may introduce bias or mislead models and clinicians, hampering their responsible deployment in diagnostic support systems, training datasets, or educational tools.

OBJECTIVES

This study aims to conduct an expert-based qualitative evaluation of synthetic melanoma images. Specifically, it investigates the subjective perception of image realism, diagnostic quality, and the recognizability of key dermoscopic features. By engaging dermatologists in a blinded assessment of synthetic and real images, we seek to establish a foundation for systematically validating synthetic dermatological

data for use in AI development, medical education, and clinical decision support. This work emphasizes the importance of subjective expert validation as a complement to technical performance metrics in assessing the fidelity of GAN-generated skin lesion images.

MATERIALS AND METHODS

StyleGAN3-T [4] was trained on a dataset of dermoscopic images of melanoma [5–7] with adaptive discriminator augmentation and transfer learning. A total of 25 synthetic melanoma images were generated and randomly mixed with 25 real melanoma images, resulting in a 50-image dataset. Seventeen board-certified dermatologists with varying levels of experience (low <4 years, medium 5–8 years, high >8 years) participated in the evaluation. Participants were blinded to image origin and asked to classify each image as real or synthetic. They also assessed the presence of 16 defined dermoscopic patterns according to standardized definitions and rated four dimensions—image quality, skin texture, visual realism, and color realism—on a 7-point Likert scale. Additionally, participants reported their confidence in each classification decision. Statistical analyses included Chi-square tests for categorical comparisons, and Fleiss' Kappa and Krippendorff's Alpha were used to measure inter-rater agreement.

RESULTS

Real images were consistently rated higher than synthetic images across all qualitative dimensions: image quality (high: 15.8% real vs. 11.3% synthetic), skin texture (high: 22.4% vs. 13.4%), and visual realism (high: 22.6% vs. 13.2%), all with $p < 0.001$. Confidence in evaluations was also significantly greater for real images, with high confidence reported in 17.4% of real cases compared to 8.7% for synthetic ones ($p < 0.001$). Regarding the recognition of image origin, the overall classification accuracy was 64%. Real images were correctly identified in 73% of cases, while only 56% of synthetic images were correctly classified as synthetic. Accuracy increased with expertise: from 59% in the low-experience group to 71% among high-experience dermatologists. Similarly, higher self-reported confidence was associated with improved performance (accuracy 74% at high confidence level).

Recognition of specific dermoscopic features showed differences between real and synthetic images. The blue-white veil was detected in 29.1% of real images compared to 13.8% of synthetic ones ($p < 0.001$), and shiny white streaks in 22.6% vs. 7.9% ($p < 0.001$). Conversely, synthetic images were more frequently associated with irregular pigmented blotches (45.0% vs. 30.9%, $p < 0.001$). The multicomponent pattern, typically indicative of melanoma complexity, was identified in 40.6% of real images versus only 23.2% of synthetic ones ($p < 0.001$), suggesting a gap in the synthetic images' structural fidelity (Table 1).

Inter-rater agreement for the classification of real versus synthetic images was low, with a Fleiss' kappa of 0.183. Pattern recognition agreement also remained weak (e.g., kappa < 0.3 for most features), underscoring variability in expert interpretations. Further subgroup analyses showed that images rated as highly realistic or evaluated with high confidence were more likely to be classified correctly, with accuracy rising to 74% in the highest-confidence subgroup.

CONCLUSIONS

Synthetic melanoma lesions generated using StyleGAN3-T demonstrate visually convincing features and were frequently perceived as real, yet consistently underperformed compared to real images in diagnostic quality and structural detail. Participants often struggled to distinguish synthetic from real lesions, particularly when realism ratings were medium to high. Critical diagnostic patterns, such as the blue-white veil and shiny white streaks, were significantly underrepresented in synthetic images. These limitations were reflected in the lower classification confidence and weaker inter-rater agreement.

Despite these challenges, the study highlights the potential of synthetic data to approach realism levels sufficient for research and educational use. Qualitative validation by dermatologists is essential to benchmark the readiness of synthetic images for real-world medical applications. As generative models continue to evolve, expert evaluation should remain a key component of validation pipelines to ensure clinical and pedagogical reliability.

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Table 1 - Distribution of dermatological patterns identified by dermatologists during the assessment between real and synthetic. P-values reflect the statistical significance of differences between presence of patterns in the two classes. Results are grouped by level of expertise. The level of agreement between raters for the two classes is also reported (Fleiss' Kappa)

Pattern	Real	Synthetic	p-value	Fleiss' Kappa (real)	Fleiss' Kappa (synthetic)
	N=340	N=340			
Atypical network	241 (70.9)	245 (72.1)	0.799	0.175	0.151
Hypopigmented areas	97 (28.5)	65 (19.1)	0.005	0.205	0.149
Irregular dots and globules	126 (37.1)	99 (29.1)	0.034	0.198	0.204
Irregular streaks	59 (17.4)	48 (14.1)	0.292	0.149	0.124
Irregular pigmented blotches	105 (30.9)	153 (45.0)	<0.001	0.261	0.34
Blue with veil	99 (29.1)	47 (13.8)	<0.001	0.336	0.34
Blue-grey globules	29 (8.5)	8 (2.4)	0.001	0.053	0.008
Blue-grey peppering	20 (5.9)	4 (1.2)	0.002	0.077	0.020
White scar-like areas	71 (20.9)	39 (11.5)	0.001	0.268	0.236
Shiny white streaks	77 (22.6)	27 (7.9)	<0.001	0.381	0.381
Atypical vascular pattern	20 (5.9)	11 (3.2)	0.141	0.223	0.213
Pink areas	85 (25.0)	58 (17.1)	0.014	0.245	0.189
Reticular pattern	59 (17.4)	63 (18.5)	0.764	0.067	0.014
Globular pattern	16 (4.7)	4 (1.2)	0.013	0.197	0.083
Homogenous pattern	30 (8.8)	29 (8.5)	1.000	0.086	0.039
Multicomponent pattern	138 (40.6)	79 (23.2)	<0.001	0.114	0.087

Counterfactual Estimates of Pneumococcal Disease Incidence in England after Vaccine Introduction

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INTRODUCTION

Streptococcus pneumoniae is a leading cause of serious bacterial infections worldwide, including pneumonia, meningitis, and sepsis, especially in young children. The World Health Organization estimates that it is responsible for approximately 5% of global infant deaths [1]. Pneumococcal conjugate vaccines (PCVs) have been developed to protect against the most clinically relevant serotypes and introduced into infant immunization programs across multiple countries. PCV7, targeting seven serotypes, was followed by PCV13, extending protection to thirteen. These vaccines have substantially reduced vaccine-type invasive pneumococcal disease (VT-IPD). Nevertheless, over 80 additional serotypes remain uncovered [2; 3]. In recent years, several settings have reported increases in non-vaccine-type (NVT) IPD, suggesting possible serotype replacement. In England, this phenomenon has been particularly marked, raising concerns about whether the population-level benefits of PCVs might be offset. However, interpreting post-vaccination trends in IPD is challenging. Observed changes in disease incidence may reflect not only biological responses to vaccination but also coincident changes in surveillance systems, healthcare-seeking behaviour, diagnostic practices, or case definitions. Traditional before–after comparisons may misattribute such secular trends to vaccine effects, especially in ecological designs where randomized controls are absent.

OBJECTIVES

We aim to estimate the causal impact of PCV7 and PCV13 introduction on IPD incidence in England, focusing on both direct reductions in VT-IPD and potential increases in NVT-IPD. A key goal is to disentangle true serotype replacement from surveillance-driven artifacts by constructing a data-driven counterfactual using unaffected pathogens as controls.

METHODS

We analysed monthly national IPD surveillance data in England from 2000 to 2018, covering the introduction of PCV7 in 2006 and PCV13 in 2010. To estimate the impact of vaccination, we employed a Bayesian structural time series (BSTS) model [4], a causal inference framework designed for population-level interventions without randomized control groups. The model accounts for seasonality, underlying trends, and time-varying confounders. To adjust for secular changes unrelated to PCVs, we used time series of other bacterial infections (*H. influenzae*, *E. coli*, *S. aureus*, *P. aeruginosa*, and others) as control outcomes. These pathogens share similar diagnostic pathways and reporting mechanisms but are unaffected by pneumococcal vaccination. The model included a spike-and-slab prior for Bayesian variable selection, allowing only those control series with high predictive value in the pre-intervention period to inform post-intervention counterfactuals. This synthetic control design improves robustness over simple before–after approaches and helps isolate vaccine effects from unrelated system-level changes.

RESULTS

We estimate a 60% overall reduction in IPD incidence following the introduction of PCV7 and PCV13, comparing the pre-vaccine (2000–2006) and post-PCV13 (2011–2018) periods. The greatest reductions occurred among children under five (–73%). Specifically, PCV7-type IPD fell by 92% across age groups, and PCV13-type IPD declined by 42% following its introduction in 2010. These effects were consistent across subpopulations and robust to alternative model specifications. In contrast, NVT-IPD incidence increased by 36.5% after PCV7 and by 31.8% after PCV13 in raw surveillance data. However, when adjusted for confounding trends using control pathogens, the estimated increase in NVT-IPD was attenuated to +16% overall, with wide credible intervals

and non-significant effects in most age groups. This suggests that previous unadjusted analyses may have overestimated the magnitude of serotype replacement by not accounting for coincident improvements in detection and reporting.

CONCLUSIONS

Our findings demonstrate that PCVs have had a substantial and sustained public health impact, dramatically reducing IPD caused by vaccine-covered serotypes. While serotype replacement is evident, much of the apparent increase in NVT-IPD can be explained by concurrent changes in surveillance and diagnostic practices rather than biological displacement alone. By leveraging control pathogens and a Bayesian synthetic control approach, we provide more credible causal estimates than conventional time series analyses. These results are important for public health planning and support continued investment in pneumococcal immunization, particularly as higher-valency PCVs are developed. Future evaluations of vaccine impact should incorporate similar causal modelling strategies to avoid misinterpretation of surveillance-based trends.

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Microbiota Data: A Statistical Analysis Workflow

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INTRODUCTION

The microbiota includes the collection of microorganisms that colonize our body, playing a pivotal role in maintaining homeostasis and modulating immune responses. An imbalance in the composition of these microbes is associated with various diseases, including oncological conditions [1]. From a methodological perspective, several aspects should be considered during microbiome analysis, such as the compositional nature of the data, its high dimensionality, overdispersion, and the prevalence of zero-inflated count data [2]. The institutional microbiome project currently active at the Fondazione IRCCS Istituto Nazionale dei Tumori was designed to assess the feasibility of investigating the microbiota in different tumors settings and biological matrices, with the final aim of identifying microbial communities able of distinguishing patients characterized by worse prognosis from those with less aggressive disease. The cancer settings included in the projects are lung, breast and prostate cancer, as well as pseudomyxoma peritonei.

OBJECTIVES

Given the unique characteristics of microbiome data, we propose a unified workflow designed for analysing data from various cancer settings to ensure consistency and comparability across the different cancer types.

METHODS

Biological samples analysed in this study were disease-specific and include, tumor tissues and matched normal counterpart for lung cancer, tumor tissues for breast and prostate cancer, matched tumor tissue, feces and mucin for pseudomyxoma peritonei. Samples were processed using the same standardized analytical pipeline across all pathologies.

This process includes bacterial DNA extraction, enrichment, library preparation and sequencing of the variable regions of the bacterial 16S rRNA gene. Microbial communities were firstly characterized in terms of alpha- and beta-diversity: the first one quantifies the diversity within a given sample in terms of richness or evenness [3], whereas second one assesses diversity differences between sample-groups. Specifically, for alpha-diversity we used a set of different indices: Chao1, Hill and Observed for richness, Gini-Simpson and Shannon for evenness [3]; for beta-diversity the Principal Coordinate Analysis (PCoA) analysis [4,5] was adopted together with the PERmutational Multivariate ANALysis of VARIance (PERMANOVA) [4] and PERmutational Multivariate analysis of DISPersion (PERMDISP) tests [6] by using the Bray-Curtis distance metrics [5]. Then, the workflow incorporates tests and statistical models for both continuous and categorical data to identify bacterial taxa that are differentially expressed or present in distinct biological matrices or associated with clinical-pathological characteristics investigated in each cancer context. Test for paired or unpaired groups comparison were included for both continuous and categorical data analysis.

RESULTS

Using lung cancer as a model setting, matched tumor and normal counterpart of 155 lung cancer patients (stage I-III), were profiled using 16S rRNA gene sequencing for a total of 310 observation and 63 identified bacteria taxa (i.e. genus level). The analysis revealed distinct microbial compositions, in terms of beta-diversity, according to histology. Moreover, by modelling the count data and its presence/absence, a specific subset of bacteria was significantly associated with tumor progression and aggressiveness; specific bacteria were also found differentially expressed between tissue types (tumor or normal counterpart). We are now applying this workflow at the other settings, with the final aim of better understanding the bacteria that characterize tumor aggressiveness.

CONCLUSION

The developed workflow allows: (i) the use of a shared pre-analytical and analytical workflow of analysis among the different tumor settings under investigation, (ii) the characterization of the microbial community within/between samples and (iii) the evaluation of associations between specific taxa bacteria and tumor characteristics.

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Prediction of Risk of Disease in Children at Risk of Facioscapulohumeral Muscular Dystrophy with Machine Learning Approach

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INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) (MIM#158900) is one of the most prevalent forms of muscular dystrophy, characterized by progressive skeletal muscle weakness, primarily affecting the face, shoulders, and upper arms. Its genetic basis is complex and typically involves contractions of the D4Z4 repeat region on the 4q subtelomere, even though it might be still incompletely described [1].

As a hereditary disease, an accurate risk assessment is crucial for improving genetic counselling, especially in the context of pregnancy planning. However, due to the significant variability in clinical manifestation and progression and the age-dependent penetrance of the disease, predicting the probability and severity of FSHD in newborns poses several challenges [2], and no tools are available for clinicians for this purpose.

OBJECTIVES

The aim of this study was to develop a machine learning model aimed at enhancing FSHD disease risk prediction for child of D4Z4 alleles of reduced size (DRA) carriers. In particular, our study focused on designing a predictive tool which can estimate the probability of FSHD and the age of disease onset in newborns, given the information of parents and other family members.

METHODS

This predictive model was estimated on the basis of genetic, clinical and socio-demographic data collected in the

Italian National Registry for FSHD [3]. Clinical data includes presence and severity of FSHD symptoms, measured using the FSHD Score [4], and a standardized description of clinical phenotypes, obtained through the Comprehensive Clinical Evaluation Form (CCEF) [5]. The availability of detailed family trees allowed the model to include the information carried by each family member, weighted by the degree of kinship with respect to subject involved in the genetic counselling.

To be included in this study, each family must be composed by a child, a DRA carrier parent, and may include one or more relatives. Families which did not fit in this structure were excluded. Since the expected FSHD onset age lies between 15 and 30 years of age, subject with age at visit less than 30 were also excluded, to limit misclassification.

For the development of the predictive model, we relied on a stacking approach: 4 base learners (a generalized regression model (GLM), a random forest (RF), a support vector machine (DVM) and a Bayesian network (BN)) provided a first-level individual prediction. Subsequently, these first-level predictions were combined by a random forest meta-learner to obtain the final predictions. Figure 1 outlines the model structure.

Leave-One-Out cross-validation was used to train each base learner (except BN) and the meta-learner. The parameters and hyperparameters of GLM, RF and SVM were estimated via grid search within each cross-validation loop, using the classification error as performance measure.

The BN learning procedure articulated in two steps: in first place, the structure of the network is established by defining the causal connections among all features, relying on expert knowledge. Then, the probability parameters that describe how the variables influence each other are estimated with a

Bayesian approach. Non informative prior distributions were assumed at each non-deterministic node of the network. Since all variables have been previously categorized, each node likelihood was a Multinomial distribution, and a Dirichlet prior was used [6]. The network was embedded also with a set of deterministic nodes, which were introduced to process family trees with different structure and depth, and to reduce the model complexity.

The prediction accuracy of the model for each outcome (occurrence of FSHD and age at onset of first symptoms) was estimated using Leave-One-Out cross validation.

Based on the probabilities estimated from the model, the child of each family was predicted as with FSHD phenotype or as asymptomatic/healthy and assigned to an estimated age at onset class (No Onset, ≤ 22 years, < 22 years). Youden Index was used to estimate the optimal probability cutoffs.

RESULTS

A total of 293 families were included in the study. Of these, 121 families contributed to the estimation of risk of disease base learners, whereas 104 families contributed to the age at onset base learners.

For the evaluation of risk of disease, the model showed an area under the ROC curve (AUC) equal to 0.89. With the selected probability cutoff, the sensibility was equal to 0.90 and the specificity 0.70. The accuracy was 0.75, with 91 out of 121 children correctly assigned to their actual clinical status.

For the estimation of age at disease onset, the model reached a multi-class AUC equal to 0.88, with an accuracy of 0.72 (75 out of 104 children's age at onset correctly predicted).

Overall, 70 children (67.3%) were correctly assigned to both their actual clinical status and their onset age class.

CONCLUSION

The developed predictive model was able to provide accurate estimates of disease probability in children of patients characterised by FSHD symptomatology, even though it was not able to discriminate between finer clinical categories. These findings further support the hypothesis that additional elements, such as other genetic variants and environmental factors, must be considered for predictive purposes.

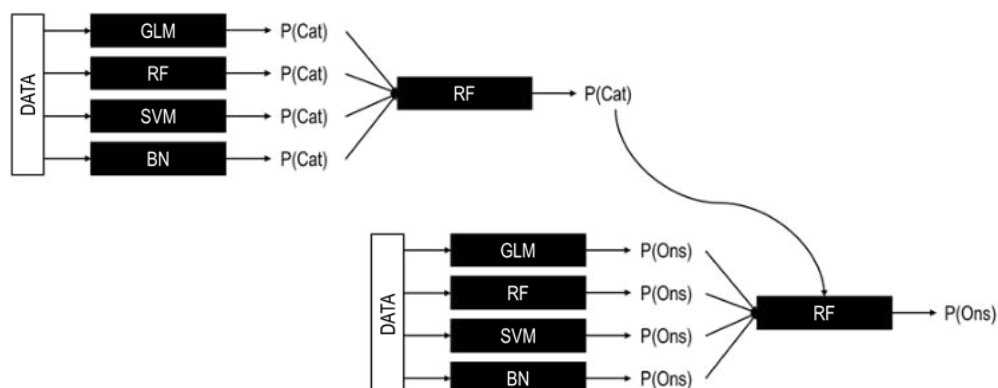
Nevertheless, this model can lead to significant advancements in FSHD genetic counselling and in implementing personalized medicine practices. Notably, our model is based on a very limited number of variables. So, it can be easily applied to provide tailored advice for families at risk of FSHD in real life scenarios.

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Figure 1. Predictive model structure. Black boxes represent machine learning models, with inputs and outputs denoted by incoming and outgoing arrows, respectively. Abbreviations: $P(\text{Cat})$: Probability of occurrence of disease; $P(\text{Ons})$: Probability of age at onset; GLM: Generalized linear regression model; RF: Random Forest; SVM: Support vector machine; BN: Bayesian network



A Study of Tremor Classification in Parkinson's Disease using Unsupervised Learning Methods and Wearable Sensor Signal Processing

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INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by motor-related symptoms as tremor, slowness of movement, rigidity and difficulty with balance [1].

Although symptoms may vary from person to person, resting tremor is usually the most common symptom [2]. At the onset of the disease, it may be mild and unrecognized, and may only be a barely perceptible tremor in a hand, or sometimes in a foot or jaw. It often starts on one side of the body and then affects both sides, but usually one side remains the more affected than the other.

A diagnosis of PD is made based on neurological and physical examinations. The Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [3] is the most common clinical scale used to track the longitudinal progression of PD. The assessment is based on disease severity, as determined through interview and clinical observation. Therefore, the evaluation may be subjective and affected by variability, reflecting the need for more objective measures for tremor classification.

Machine learning algorithms have recently been used to process data collected by wearable sensors [4].

Aims: Explore the use of an unsupervised learning model (k-means) to solve two classification problems: CP1: distinguish patients from controls (i.e. tremor vs. non-tremor); CP2: classify different tremor severities, where $k=n$. We also consider a third problem, CP3, which aims to distinguish between severe and mild tremor (i.e. $k = 2$), with the aim of simplifying CP2.

METHODS

We used a publicly available dataset accessible via the website <https://doi.org/10.21227/g2g8-1503> [5].

This dataset includes activity, gait, and tremor measures from 17 individuals diagnosed with PD and 17 healthy control (HC) subjects who were matched for age. These measures were collected using five adhesive sensors (one on each limb and one on the trunk) which captured triaxial accelerometer data during a clinic visit. Annotation files were also collected during this visit, when subjects underwent an evaluation using the MDS-UPDRS. We only considered data from sensors placed on the upper limbs of people with PD, and we excluded four subjects due to missing clinical data. Thus, our population included 13 PD patients (mean age $\pm$ SD: 66.1 ± 11.8 years; 38.5% female) and 11 HCs (66.0 ± 8.4 years; 90.1% female).

Before to apply k-means we performed a data segmentation process with the aim of extracting time intervals in which subjects were seated in a resting state. We used the start and end timestamps of the resting periods performed during the clinical assessment according to file tasks annotations. Then, the extracted segments were concatenated into a single string, i.e. a recording instance. We analyzed the tremor-prevalent arm recordings for each PD patient, except for one patient for whom we analyzed both arms, for a total of 25 recording instances.

We pre-processed the raw accelerometer data performing the mean-centering and computing modulus. This transformation was necessary because of the effects of sensor orientation and individual bias. We chose the Euclidean distance as the distance metric because of its effectiveness in measuring the similarity of the movement intensities represented by the modulus values [6].

To ensure a balanced distribution of tremor events across subjects and enhance the sensitivity of the clustering algorithm, we only considered signal periods within the 95th percentile

of movement intensity. We then identified the 'dominant cluster' as the cluster label that occurred most frequently in the top 5% of modulus instances.

In order to evaluate the accuracy of the K-means algorithm, we compared its assignments with clinician diagnoses. Specifically, for CP2, we compared cluster assignments with tremor labels assigned by the neurologist according to item 3.17 of the MDS-UPDRS, which evaluates resting tremor amplitude using a scale of 0–4. HCs had an at-rest tremor score of 0. Finally, we used a best matching approach to align the cluster labels with the clinical labels, selecting the mapping that resulted in the highest accuracy percentage among all possible permutations.

RESULTS

CP1: the algorithm achieved an accuracy of 76.0%. Specifically, most PD patients were correctly assigned to the tremor cluster, while the majority of HCs were assigned to the non-tremor cluster. CP2: The algorithm achieved an accuracy of 57.1%, with significant overlap in cluster assignments. CP3: The algorithm achieved an accuracy of 71.4%. Figure 1 shows the k-means performance in all CPs.

CONCLUSIONS

The results emphasize that raw motion data can provide valuable information independently of predefined clinical labels, achieving a high level of accuracy in distinguishing tremor or states from non-tremor states. Although the results of tremor or severity classification, especially in multiclass scenarios, demonstrate the complexity of subtle tremor differentiation, highlighting the importance of improving feature extraction to achieve greater accuracy, the usefulness of unsupervised learning to enable scalable and objective tremor analysis is clear. Integrating such models into wearable systems could improve continuous monitoring, enhance rehabilitation strategies, and support standardized clinical assessments. Future work should focus on developing advanced algorithms, enriched feature sets and larger datasets to enhance the robustness and generalizability of these models.

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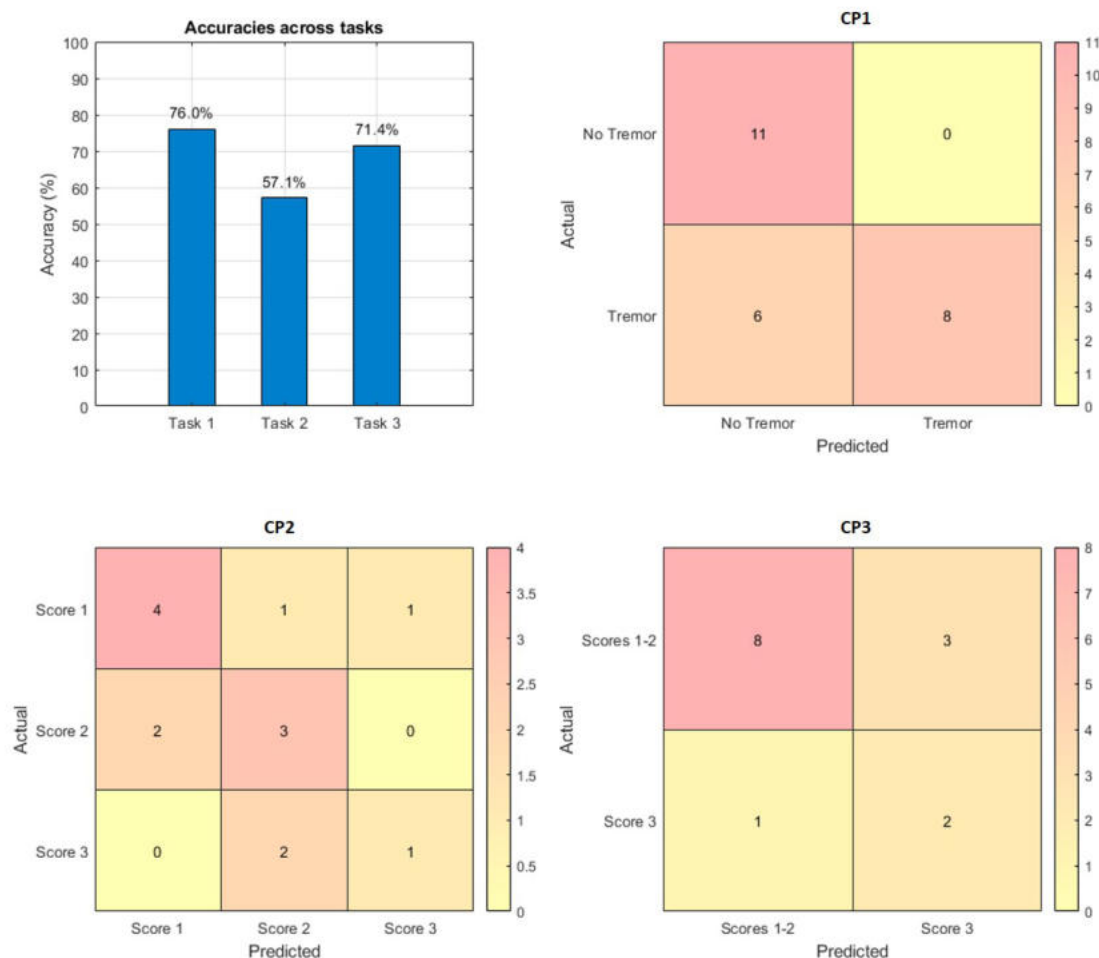


Figure 1. Classification tasks performances. The top left panel displays a bar chart summarizing the accuracy of each classification problem. The remaining panels provide confusion matrices for each CP, in clockwise direction: Tremor vs. Non-Tremor, Multiclass Tremor Severity, and Severe vs. Milder Tremor

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Assessing Methods for Predictive Cut-Point Estimation: A Simulation-Based Comparison

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INTRODUCTION

The identification of an optimal cut-point for continuous biomarkers plays a crucial role in defining patient subgroups likely to benefit from specific treatments. While the literature has extensively covered prognostic biomarkers, those that provide outcome prediction regardless of treatment, the methodological framework for identifying predictive effect, which inform treatment effect heterogeneity, is less developed. This is primarily due to the added complexity of modelling treatment-biomarker interactions, which poses challenges related to statistical power, overfitting, and bias.

OBJECTIVES

This study aimed to compare three statistical methods for the identification of predictive cut-points in time-to-event data. Our goal was to assess their performance in estimating the correct interaction effect and identifying a responder subgroup, under simulation settings that account for variability in treatment efficacy, biomarker predictive effect, and subgroup prevalence.

METHODS

We implemented three approaches: Procedure B of the Biomarker-Adaptive Threshold Design (M1), which combines test statistics across possible cut-points using a permutation

test based on likelihood-ratio statistics; the Differential Hazard Ratio method (M2), which selects the cut-point with the largest difference in HRs across adjacent thresholds; and a Minimum P-value method (M3) adapted for interaction terms in the Cox model [1,2]. We conducted a simulation study with 1000 replications from an exponential distribution with an expected censoring rate of approximately 40%. Eight main scenarios were defined by all possible combinations of two sample sizes ($n = 300$ and $n = 500$), two treatment effect sizes ($HR = 1$ or 0.5), two interaction effect sizes ($HR = 1$ or 0.5), and a biomarker prognostic effect set to $HR = 0.6$. In addition, we included two extra scenarios calibrated to achieve 80% power: one based on the interaction effect test (β for treatment-biomarker interaction) and one on the subgroup effect test (β within responders). In each replication, the true cut-point was randomly drawn from the biomarker distribution between the 20th and 80th percentiles. For each method, we evaluated statistical power, cut-point estimation bias, subgroup and predictive coefficient estimation bias, and type I error. A significance level of 0.05 was used for all three methods. The procedures were also evaluated on a real case on a prostate cancer clinical trial conducted by the Second Veterans Administration Cooperative Urologic Research Group [3].

RESULTS

M1 consistently demonstrated robust performance, with type I error close to the nominal level (**S2**, 5.6%) and minimal bias in cut-point estimation (**S1**, $\hat{c}$: 0.005 ± 0.06). It maintained

good power even when the subgroup size was small. M2 showed unstable cut-point estimates (**S1**, $\hat{c}$: 0.055±0.42) and high variability in interaction estimates (**S1**, β_3 : 0.463±1.46), yielding a very low power (**S1**, 16.2%). While the M3 achieved the highest power in some scenarios (**S1**, 82.1%), it exhibited significant type I error inflation (**S2**, 50.1%) and substantial bias due to multiple testing without correction (**S1**, β_3 : 0.401±1.730). In small subgroups, all methods experienced reduced performance, but M1 remained the most stable. On the prostate cancer dataset, M1 identified a plausible treatment-responsive subgroup, while the other two methods produced conflicting or less reliable results.

CONCLUSIONS

Our results highlight the need for robust methods in predictive cut-point estimation. M1 showed the best balance between error control and accuracy. In contrast, M2 and M3 may lead to overfitting, unstable estimates, and inflated first error rates. Future research should extend these comparisons to more complex models including multivariate biomarkers.

Table 1. Empirical power, type-I error and parameter estimation bias from setting scenarios in using the three compared methods

Scenarios ($N, \exp(\beta_1), \exp(\beta_3)$)	Empirical power / Type-I error α			$\hat{c}^b$ (SD)			β_3^b (SD)			γ_e^b (SD)		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
S1 (300, 1.0, 0.5)	0.636	0.162	0.821	0.005 (0.06)	0.055 (0.42)	0.003 (0.24)		0.463 (1.46)	- 0.401 (1.730)	- 0.167 (0.26)	0.385 (1.30)	- 0.356 (2.33)
S2 (300, 1.0, 1.0)	<u>0.056</u>	<u>0.084</u>	<u>0.501</u>	0.030 (0.15)	0.045 (0.37)	0.010 (0.37)		0.003 (1.19)	- 0.040 (1.77)	- 0.419 (2.98)	- 0.009 (1.12)	- 0.077 (2.55)
S3 (300, 0.5, 0.5)	0.998	0.144	0.737	- 0.001 (0.09)	0.041 (0.41)	0.021 (0.28)		0.632 (2.47)	- 0.955 (3.74)	- 0.825 (1.02)	- 0.161 (2.42)	- 2.065 (5.02)
S4 (300, 0.5, 1.0)	0.918	<u>0.062</u>	<u>0.476</u>	- 0.003 (0.19)	- 0.005 (0.36)	- 0.005 (0.36)		- 0.033 (1.93)	- 0.275 (2.97)	- 0.818 (1.07)	- 0.559 (1.68)	- 1.165 (3.91)
S5 (500, 1.0, 0.5)	0.853	0.331	0.932	- 0.000 (0.03)	0.100 (0.43)	- 0.005 (0.19)		0.392 (0.60)	- 0.226 (0.84)	- 0.072 (0.19)	- 0.259 (0.55)	- 0.159 (1.02)
S6 (500, 1.0, 1.0)	<u>0.057</u>	<u>0.158</u>	<u>0.504</u>	0.006 (0.12)	0.075 (0.44)	0.009 (0.37)		0.023 (0.70)	0.020 (0.93)	0.077 (0.39)	- 0.014 (0.38)	0.022 (0.85)
S7 (500, 0.5, 0.5)	1.000	0.178	0.881	0.000 (0.05)	0.062 (0.43)	0.019 (0.22)		0.486 (1.50)	- 0.461 (2.07)	- 0.707 (0.26)	- 0.378 (1.42)	- 1.272 (3.02)
S8 (500, 0.5, 1.0)	0.998	<u>0.093</u>	<u>0.509</u>	- 0.001 (0.12)	0.020 (0.40)	0.002 (0.37)		0.063 (0.82)	0.015 (1.45)	- 0.705 (0.26)	- 0.533 (0.78)	- 0.790 (1.84)
S9 (264, 1.0, 0.5)	0.616	0.189	0.812	0.006 (0.06)	0.074 (0.36)	0.017 (0.22)		0.630 (1.88)	- 0.394 (2.21)	- 0.228 (0.88)	0.556 (1.67)	- 0.374 (2.85)
S10 (481, 1.0, 0.5)	0.838	0.305	0.920	0.003 (0.03)	0.082 (0.43)	- 0.011 (0.18)		- 0.292 (0.57)	- 0.910 (0.83)	- 0.774 (0.20)	- 0.423 (0.49)	- 0.853 (1.19)

0.8 Power target for test on γ_e

0.8 Power target for test on γ_e

^aDepending on the scenario, the Empirical power/Type-I error column shows the power (when H_0 is true) as non-underlined values and the type-I error (when H_1 is true) as underlined values

^bRepresents the empirical bias, mean and (SD)

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Relationship between Non-Optimal Air Temperature and Mortality Risk in Italy using High-Resolution Data: A Case Time Series Analysis

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INTRODUCTION

Non-optimal air temperature has been associated with an increased mortality [1]. The relationship is usually inverse J-shaped, with higher risk at more extreme temperatures [2]. Nonetheless, estimates of excess mortality due to non-optimal temperature exhibit marked geographical heterogeneity [3]. This variability depends on a range of individual- and area-level determinants, including socioeconomic, demographic, and environmental factors [4]. Characterizing such variability is crucial to trace differences in health impacts and identify hotspot areas [5]. Limitations in this research area include the availability of relevant data and their spatial and temporal resolution as well as study design and statistical methods [6]. Previous studies investigating the association between air temperature and mortality risk have mainly relied on aggregated data at a broad spatial scale [7-9]. The growing availability of environmental high-resolution data (i.e., small-area level) together with advancements in record linkage procedures and computation capability enables the investigation of health risks at a finer spatial level [10].

The case time series (CTS) design, originally developed for spatial analysis, has been recently adapted to handle spatio-temporal data collected longitudinally [11, 12]. The CTS method allows the analysis of high-resolution data to identify small-scale risk patterns across the whole geographical domain [13].

OBJECTIVES

We investigated the association between non-optimal air temperature and all-cause mortality across Italian municipalities, using high-resolution satellite data.

METHODS

We conducted a CTS analysis using an adaptation of the two-stage design to model country-wide small-area data. We collected time series daily data on all-cause mortality and temperature for 7895 Italian municipalities between Jan 1, 2011, and Aug 31, 2024. Deaths were provided by the Italian National Institute of Statistics. Daily mean temperatures on a 1x1 km grid across Italy were extracted from Copernicus Satellite Data. We derived the corresponding municipal-specific daily temperature series by computing the area-weighted average of the temperatures of all grid cells intersecting the municipal boundaries, with weights proportional to the intersection areas. We also collected several municipal-area variables that are potentially linked with differential vulnerability to extreme temperatures. These variables comprised demographic (e.g., proportion of population aged 65+ years and population density), socioeconomic (e.g., income, employment, education, motorization and recycling), landscape (altitude, surface imperviousness degree, normalized difference vegetation index, land cover), and climatological (average annual range of temperature) characteristics. We aggregated municipal-area variables at the province level using population-weighted averages. A principal component (PC) analysis was conducted, and the extracted PCs were used as composite indicators of temperature vulnerability to assess geographical risk differences.

We estimated the association between non-optimal air temperature and all-cause mortality across municipal areas using a two-stage analysis followed by a downscaling procedure. In the first stage, we applied the CTS design, modeling municipal-specific series within each province through a conditional Poisson regression. Four distinct models were fitted for the following age groups: 0-64, 65-75, 75-84, and 85+

years. First-stage models included terms to flexibly control for long-term and seasonal trends at both municipal and province levels, and indicators for day of week. Temperature-mortality associations were modelled through a distributed lag non-linear model (lag window: 0-21 days), a technique to estimate complex non-linear and lagged dependencies [14]. In the second stage, we cumulated the risk over the lag dimension to obtain the overall temperature-mortality association. Then, we pooled the estimated coefficients for each age group and each province using a multivariate repeated-measure meta-regression. The second-stage model included age and the PCs as meta-predictors to explain variations across provinces.

As a final step, we used the meta-analytical model to downscale risks at municipal-area level. We derived the minimum mortality temperature (MMT) and the MMT-related percentile (MMP) from municipal-specific temperature-mortality curves. We summarized the risk for heat and cold by computing relative risks (RR) and related 95% confidence intervals (CI) at, respectively, the 99th and 1st temperature percentile versus the MMP. We derived a measure of effect of heat and cold by estimating the age-specific excess mortality attributable to non-optimal temperature as well as the standardized rate of excess all-cause mortality and related 95% CI using Monte Carlo simulations.

RESULTS

In Italy, 8938346 deaths occurred in the study period, approximately 653900 per year. Non-optimal temperature was associated with an annual mean excess of deaths of 9502 (95% CI: 7791-10881) and 85535 (95% CI: 75602-94814) each year in Italy attributable to heat and cold, respectively (Table 1). The corresponding standardized excess all-cause mortality rates (deaths per 100,000 person-years) were 12.3 (95% CI: 11.1-13.1) for heat and 110.9 (95% CI: 104.1-117.2) for cold. With regards of Italian regions, we observed slightly higher heat-related standardized excess all-cause mortality rates in the Southern.

CONCLUSIONS

We provide a comprehensive assessment of excess mortality related to non-optimal temperature in Italy, accounting for several determinants of temperature vulnerability. This work also provides a detailed risk map that could be useful for designing effective climate and public health policies at both local and national levels.

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Table 1. Annual excess deaths and standardized excess all-cause mortality rate and corresponding 95% confidence interval attributable to non-optimal temperature by Italian regions in the period Jan 1, 2011 – Aug 31, 2024

Region	Annual excess deaths (95% CI)		Standardized excess all-cause mortality rate (95% CI) ^a	
	Cold	Heat	Cold	Heat
Piemonte	6790 (6062-7514)	562 (483-636)	107.8 (98.5-116.9)	8.7 (7.8-9.5)
Valle d'Aosta	194 (142-244)	26 (4-44)	115.1 (82.6-145.3)	18.2 (2.6-30.6)
Lombardia	15321 (13204-17084)	971 (636-1110)	121.3 (105.4-133.3)	7.5 (5.4-8.3)
Trentino-Alto Adige	1438 (1252-1614)	76 (41-104)	112.0 (99.0-123.9)	6.1 (3.7-8.0)
Veneto	6644 (6018-7271)	582 (496-662)	105.3 (98.1-112.6)	9.1 (8.0-10.0)
Friuli-Venezia Giulia	1903 (1645-2138)	210 (162-251)	105.1 (92.1-116.0)	11.6 (9.0-13.6)
Liguria	2455 (1938-2941)	353 (247-445)	91.5 (72.4-109.2)	13.3 (9.4-16.6)
Emilia-Romagna	6350 (5635-7062)	555 (458-646)	99.0 (89.3-108.1)	8.3 (7.1-9.4)
Toscana	5299 (4726-5873)	691 (586-781)	95.0 (86.5-102.4)	12.2 (10.5-13.6)
Umbria	1242 (1121-1366)	141 (122-161)	91.4 (85.0-98.8)	10.1 (9.0-11.1)
Marche	2143 (1928-2352)	264 (222-304)	93.0 (85.4-100.1)	11.2 (9.7-12.6)
Lazio	7183 (6314-7960)	978 (852-1096)	102.6 (93.8-109.7)	13.8 (12.6-15.1)
Abruzzo	1935 (1782-2086)	231 (203-257)	106.5 (101.0-111.8)	12.5 (11.5-13.6)
Molise	493 (447-538)	73 (65-82)	109.6 (101.2-117.0)	16.3 (14.8-17.8)
Campania	7581 (6580-8496)	1096 (890-1287)	134.8 (119.6-149.1)	19.4 (16.2-22.4)
Puglia	5093 (4604-5575)	848 (736-942)	106.7 (98.5-115.1)	17.9 (16.2-19.4)
Basilicata	827 (750-904)	132 (117-146)	110.0 (101.4-117.8)	17.5 (16.0-19.0)
Calabria	2762 (2519-3007)	369 (322-413)	119.2 (111.3-127.3)	16.0 (14.5-17.5)
Sicilia	7545 (6873-8186)	1074 (929-1198)	133.4 (124.4-142.7)	19.1 (16.9-20.7)
Sardegna	2337 (2062-2603)	270 (220-316)	112.5 (101.0-123.4)	13.1 (11.1-14.8)
Italy	85535 (75602-94814)	9502 (7791-10881)	110.9 (104.1-117.2)	12.3 (11.1-13.1)

^aDeaths per 100000 person-years

Conditional Power and Model Selection Based Sample Size Reestimation with Type I Error Recalibration

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INTRODUCTION

The sample size estimation at study design depends on initial assumptions regarding the target power, treatment effect, accrual/follow-up duration and the underlying exponential distribution for time-to-event outcomes. However, observed data often deviate from these assumptions and the study may not progress as planned. Obtaining an updated sample size estimation after study initiation represents a valuable resource for monitoring, statistics and ethical considerations.

OBJECTIVES

We introduce a methodological framework for sample size reestimation at interim stages of clinical trials with time-to-event endpoints using conditional power (CP) and model selection procedures. We developed an R function to compute the updated sample size and to recalibrate the type I error rate, based on the number of events required to achieve the target CP and the number of events observed at interim.

METHODS

The input data include design-stage parameters (type I error rate, hazard ratio, follow-up duration, target number of events and power), subject-level information (identifier, treatment arm, enrollment date, event status, event date, date of last observation), and user-defined updates (extended accrual and/or follow-up). Subjects are categorized by their follow-up status: lost to follow-up, event-free at the interim stage, or having experienced the event of interest. Time-to-event is computed in days for each subject and four parametric models (exponential, Weibull, log-normal and log-logistic) are fitted for each treatment group and compared using the Akaike

Information Criterion (AIC) to identify the optimal arm-specific fits. Following the standardization of the chi-square statistic from the log-rank test, the interim CP is computed with Jennison and Turnbull's equation [1]. The lower boundary of the interim CP acceptance region is derived using the Broberg's methodology [2]. If the observed CP is below this boundary, the function flags potential study futility and no sample size is updated. In the event the interim CP is greater than or equal to the target CP, the function confirms that the study is progressing as planned and the sample size remains unchanged. When the interim CP falls within the region, the required number of events to achieve the target CP is computed using the Newton–Raphson algorithm, the updated sample size is estimated via a generalized Schoenfeld formula based on Lachin and Foulkes' framework and the type I error rate recalibration is performed using the technique proposed by Uemura, Matsuyama and Ohashi [3] [4] [5].

RESULTS

We applied our method at an interim stage of a phase III trial that evaluates the superiority in terms of Progression Free Survival (PFS) of an experimental treatment versus the control in metastatic colorectal cancer subjects. Starting from a hazard ratio of 0.58, a one-sided type I error of 5%, one-year follow-up and a planned enrollment of 140 subjects to observe 106 PFS events with a 80% target power, we updated the sample size and recalibrated the type I error assuming one additional year of accrual. At the interim analysis (three years after trial initiation), 18 PFS events and 44 enrolled subjects corresponded to an interim CP of 50.9%. For the experimental group, the exponential distribution provided the optimal fit for the time-to-event data, whereas the log-normal was identified as the best model for the control group. To achieve the target

CP of 80%, the function increased the sample size to 269 subjects to observe 154 events. Consequently, the recalibrated one-sided type I error rate decreased to 3.1%, consistent with the slow accrual and low event rates observed at interim.

CONCLUSIONS

This method enhances the clinical trials management effectively by providing the updated sample size at interim stages of clinical trials in a timely and methodologically sound manner. This function supports the operational and statistical aspects of clinical trials, contributing to their overall success.

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Comparison of Different Methodological Approaches to Simulate Geo-Referenced Populations to Be Used in a Cluster Analysis of Childhood Leukaemia Cases in Germany

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INTRODUCTION

A key methodological challenge in epidemiological studies using a cluster analysis approach is the choice of an appropriate set of controls. This challenge becomes particularly complex when cases are geo-referenced and the outcome is rare. In fact, in such situations, controls need to be sampled from a comprehensive primary base, where population is defined both geographically and temporally. Furthermore, if cases are geo-referenced, the controls need to be geo-referenced too. However, selecting and geo-referencing such controls can be highly resource-intensive, both in terms of time and cost.

OBJECTIVE

Thus, the main objective of our study is to use publicly available data and established geo-statistical techniques to simulate a geo-referenced population (GRP). This simulated geo-referenced population will be then used as the primary basis for the extraction of controls in a cluster analysis that will focus on childhood leukaemia incident cases in Germany.

METHODS

For the period 2000-2020, we used population counts of persons aged 0 to 14 years from the WorldPop's (WP) project at the University of Southampton and available in 100×100m

grid cells [1]. The WP project employs a top-down modelling approach and uses different types of variables (rural settlements, industrial areas, schools, etc.) to estimate age-specific (0, 1–4, 5–9, 10–14 years) and sex-specific counts of persons in a grid [2,3,4]. The observed population figures (RP) at the municipality level were provided by the German Childhood Cancer Registry and were used as constraint values. To simulate a georeferenced population, the WP was used as a probability distribution function for sampling, with replacement, a number of cells equal to the RP. Afterwards, a uniform distribution was applied to randomly sample inside each picked cell a number of points (coordinates) equal to the times the cell was extracted.

Here are shown results for three years: 2004, 2011 and 2019 and three simulated GRPs. Whereby the three simulations refer to the geographical level used to constrain the simulated population to the real population, i.e. the overall Childhood German population (S1), the childhood population at the state level (Bundesland) (S2), and the population at province level (Landkreis). For evaluation purposes, the percentage differences between the SP and the RP for all German municipalities were computed and summarized as the median and interquartile range (IQR). In addition, the root mean squared error (RMSE) was calculated.

RESULTS

In Germany, the number of children between 0 and 14 years old was 12,045,019 in 2004, 10,832,081 in 2011, and

11,396,196 in 2019. The WP estimations for the same years were 12,178,611, 10,886,770, and 10,457,921, respectively. When using the overall childhood population of Germany as the constrain for the simulation (S1), in 2004 we observe an overestimation of the population in the eastern Germany and an underestimation elsewhere (Figure 1; a) (median percentage difference = -7.1; IQR: -13.3 – 7.8); RMSE of 17.8. Percentage differences decreases in the third simulation (S3: median = -3.5; IQR: -12.2 – 5.1; a RMSE = 6.5). Simulations for 2011 show, in general, better results with S3 as best performance (median = -1.2; IQR: -10.1 – 7.3; RMSE 4.4). Generally, results observed in 2019 are similar to those observed in 2011.

CONCLUSIONS

Despite being computationally the most time-consuming, S3 shows the best performances in terms of narrower inter-quartile ranges and a more centered distribution. Thus, the simulated georeferenced population obtained using the RP at the province level as the constrain can be considered the optimal one. Further investigations are needed to shed light on the geographical differences observed in 2004.

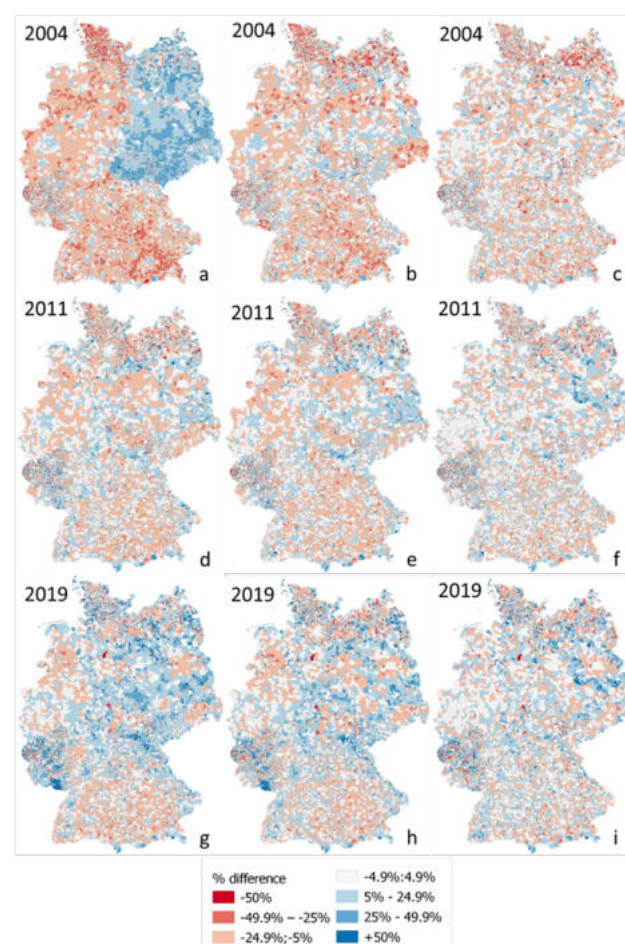
The inconsistencies between the WP and the RP must be considered as a limitation when interpreting our results. However, this is an innovative method which allows the future use of the overall georeferenced population or a selection of it for cluster analyses.

The application of this method to each year of interest and the cluster analysis itself are pending.

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Figure 1. Percentage difference between the Population given by the registry and the GRP S1, GRP S2 and GRP S3 in 2004 (a, b and c), in 2011 (d, e and f) and in 2019 (g, h and i).



Impact of Distance from Healthcare Facilities and Quality of Hospital Care on Patient Healthcare Travel: A Study of Oncological Surgery for Colon Cancer in Italy

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INTRODUCTION

Colon cancer surgery is a complex and essential procedure in the treatment of this disease, requiring advanced medical infrastructure and highly specialized personnel to ensure optimal patient outcomes [1,2]. The geographic distribution of healthcare resources shapes accessibility to critical interventions such as colon cancer surgery, and greater distance from treatment centers has been associated with more advanced stage at diagnosis and higher mortality among patients with this carcinoma [3,4,5]. Furthermore, hospital- and provider-related factors—such as high procedure volumes and greater specialization—also influence patient outcomes [1,2,6,7]. All of these factors can affect patients' decisions to travel for care. In Italy, the uneven distribution and variable quality of centers performing colon cancer surgery may impact equity in service delivery. Analyzing disparities in access to care is crucial for understanding how regional variations in infrastructure and service quality influence patient mobility [8].

OBJECTIVES

To assess the impact of hospital care quality and distance from healthcare facilities (both hospitals and specialist outpatient oncology centers) on patient healthcare travel among those undergoing colon cancer surgery, and to identify any territorial inequalities in access to services.

METHODS

This study examines the interaction between geographic accessibility and hospital quality in shaping patient healthcare travel for colon cancer surgeries across Italy, using maps to visually represent spatial dynamics of access to care and quality [9]. Two primary distance metrics were calculated: the actual travel time from each patient's municipality of residence to the hospital where surgery was performed, and the potential travel time from each municipality to the nearest capable facility. These metrics quantify the geographic impedance patients face when seeking specialized oncological surgery. Geographic coordinates of all Italian hospitals and municipal centers were used, and car travel times were computed via the OpenStreetMap Routing Machine [10] and the R statistical software. To gauge the phenomenon at the health district level, we computed both a "healthcare escape index" (indicating the propensity to travel outside one's area for care) and an "outpatient oncology service supply index" (for chemo- and radiotherapy services) [8,11].

The cohort was identified through the National Repository of Hospital Discharge Records (SDO), linked to the Tax Registry Information System for vital status and follow-up data, and includes all patients aged 15–100 years, resident in Italy, diagnosed with colon cancer and undergoing elective partial or total colectomy in any public or accredited private hospital from 1 January 2015 to 30 November 2023 [12].

Facility-level quality indicators were integrated into the analysis according to the National Outcomes Program (PNE)

classification framework, with particular focus on the Treemap tool's classification for colon cancer surgery quality, which employs the 30-day postoperative mortality indicator under a predefined protocol [13].

RESULTS

To capture geographic disparities at a finer granularity than prior Italian healthcare travel studies (which were limited to regional or ASL levels), we performed detailed mapping at the level of individual ASL health districts. In addition to care quality, we examined the healthcare escape index—measuring the tendency to seek care outside one's area relative to local health needs. We also developed a local outpatient care network indicator, based on the distribution of chemo- and radiotherapy centers and their distance from patient residences, to assess the effectiveness of the territorial oncology outpatient network, given that these treatments form an integral part of the oncological care pathway alongside surgery. Both indicators provided a more granular understanding of how the oncology care network and patient care travel dynamics operate across the territory.

CONCLUSIONS

This study explores the complex relationship between geographic accessibility to healthcare services, the quality of those services, and patient healthcare travel, focusing on colon cancer surgery across Italy. We map the distribution of surgical centers and the broader network of linked outpatient oncology services, offering a detailed visual representation of the national geographic landscape of care provision and its association with patient healthcare travel. The findings can serve as a key tool to identify determinants leading patients to forego local healthcare services.

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Identifying and Characterizing Shared and Ethnic Background Site-Specific Dietary Patterns by Hispanic/Latino Background and Site: The Use of Bayesian Multi-Study Factor Analysis in the Hispanic Community Health Study/Study of Latinos

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INTRODUCTION

Dietary patterns (DPs) are combinations of dietary components intended to summarize key aspects of diet, while taking advantage of synergies between single components. A posteriori DPs are defined from the application of multivariate statistics, including principal component and factor analyses. New statistical methods like multi-study factor analysis have been recently used to distinguish subpopulation-specific DPs (i.e., study/country-specific features within an international consortium or subpopulation-specific features within a single study), as well as those shared among all groups in a population [1].

The Hispanic Community Health Study/Study of Latinos (HCHS/SOL), the most extensive and ongoing community-based cohort of Hispanic/Latino adults from 4 US sites to date, provides a unique opportunity to identify shared and subpopulation-specific a posteriori DPs.

AIMS

The present work aims to: 1. identify shared and ethnic background-site (EBS)-specific (nutrient-based) DPs within

the HCHS/SOL study and 2. characterize the identified DPs in terms of food-group consumption, an overall measure of diet quality, socio-demographic and lifestyle characteristics.

METHODS

THE HCHS/SOL

The HCHS/SOL is a population-based cohort study designed to identify disease prevalence rates and risk factors of Hispanic/Latino populations residing within 4 urban US communities (Bronx, Chicago, Miami, and San Diego) and representing individuals with 7 ethnicity backgrounds (Cuban, Dominican, Mexican, Puerto Rican, Central and South American, and mixed). Participants were selected using a probability sampling design [2].

Dietary habits at baseline (16,415 subjects from 2008 to 2011) were assessed using two 24-hr recalls, the first conducted in person and the second via telephone ≤ 30 days after. The Nutrition Data System for Research software allowed for nutrient intake estimation [3].

SELECTION OF SUBJECTS AND VARIABLES

We excluded Hispanics and Latinos from other/mixed backgrounds, with unreliable dietary recalls, or providing extreme energy intake. We also excluded subpopulations <200 participants after previous exclusions. This gave a final sample size of 15,021 participants.

We selected 42 nutrients that well represent the overall diet for Hispanics/Latinos. For each participant, nutrient intakes were derived from either one available reliable recall or the mean of the two available reliable recalls.

STATISTICAL ANALYSIS

Bayesian multi-study factor analysis (BMSFA) was carried out on the correlation matrices of the log-transformed nutrient intakes. The total number of factors to retain was selected using the spectral decomposition of the factors. After the singular value decomposition method used in the BMSFA for identifiability, the varimax rotation was applied to the shared factor-loading matrix to achieve a better-defined loading structure [4]. Characterization of DPs against selected food groups, a measure of diet quality, selected socio-demographic and lifestyle factors was based on survey-weighted regression models. Calculations were carried out using the R software [5].

RESULTS

The selected model included 4 shared (62.5% total variance explained) and 12 EBS-specific DPs (variance around 10%), one for each of the 12 EBS combinations (Figure 1). Among shared DPs, the first, named **Plant-based foods**, loaded highly on vegetable protein, several minerals, vitamin B1, niacin, natural folate, soluble and insoluble fiber, the second, named **Dairy products**, loaded highly on short- and medium-chain saturated fatty acids and calcium, vitamins B2, B12, D, and retinol; the third shared factor, named **Seafood**, loaded highly on EPA, DPA, and DHA and the fourth, named **Processed foods**, loaded highly on several fats, including long-chain saturated and monounsaturated fatty acids, linoleic and linolenic acids, total trans fatty acids, and natural alpha-tocopherol. Most EBS-specific DPs were further grouped into overarching profiles: *Animal vs. vegetable source*, *Animal source only*, and *Poultry vs. dairy products*, to capture nuances within animal-based DPs. Puerto Rican background participants from Chicago expressed a strikingly different DP.

When interpreted in terms of food groups, the identified DPs confirmed the names based on nutrients. Higher overall diet quality was observed with increasing categories of **Plant-based foods**, **Seafood**, and the "Puerto Rican background-Chicago" EBS-specific DP, whereas increasing categories of **Dairy products**, **Processed foods**, and the remaining EBS-specific DPs were related to lower diet quality. Compared to non-US-born participants, US-born individuals exhibited lower adherence to the **Plant-based foods** and **Dairy products** DPs but higher adherence to **Processed foods**, **Seafood**, and 6 EBS-specific DPs.

CONCLUSIONS

In its first application in nutritional epidemiology, BMSFA succeeded in simultaneously estimating well-interpretable shared and EBS-specific DPs within 12 combinations of background and site.

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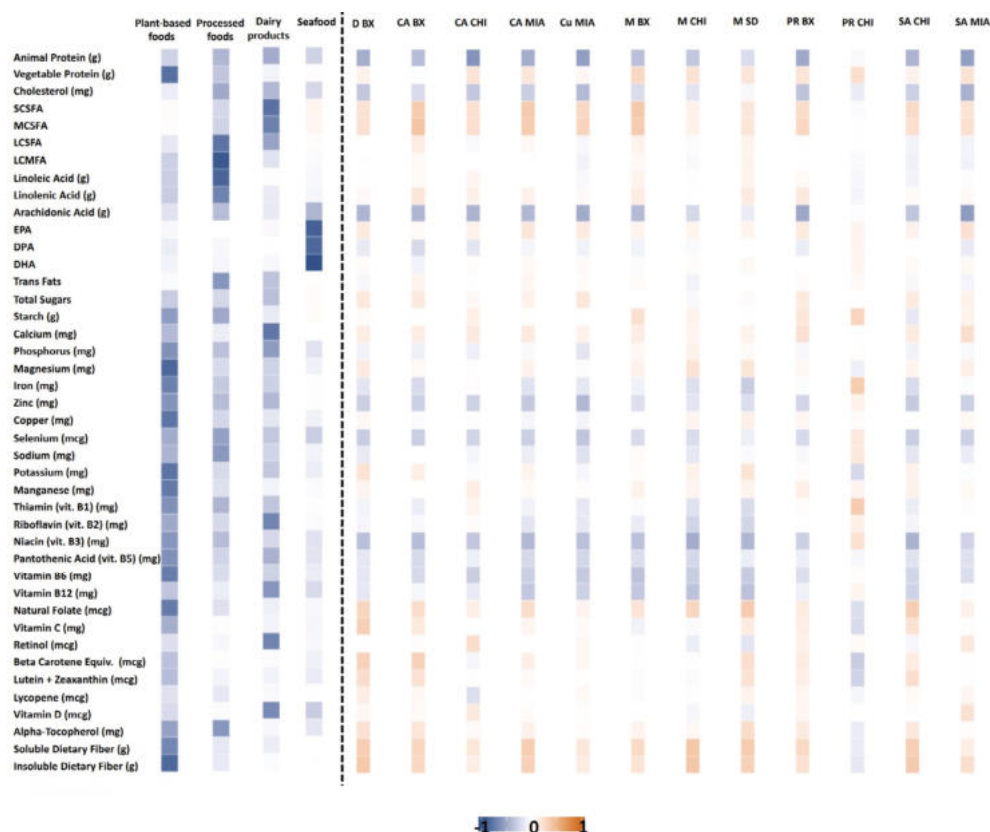


Figure 1. Heatmap of the estimated factor loadings for the shared (left) and background/site-specific (right) dietary patterns. Hispanic Community Health Study/Study of Latinos – baseline examination (2008-2011). Abbreviations: BMSFA: Bayesian multi-study factor analysis; BX: Bronx; CA: Central American; Cu: Cuban; CHI: Chicago; D: Dominican; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; M: Mexican; MCSFA: medium-chain saturated fatty acids; LCMFA: long-chain monounsaturated fatty acids; LCSFA: long-chain saturated fatty acids; MIA: Miami; PR: Puerto Rican; SA: South American; SCSFA: short-chain saturated fatty acids; SD: San Diego

Modelling Nurses' Intention to Leave the Hospital and the Profession Using a Bivariate Additive Ordered Model via Penalized Likelihood with the R Package Pblm

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INTRODUCTION

Addressing the shortage of healthcare workers requires a clear understanding of the factors associated with nurses' intention to leave their current hospital (ITL1), or more critically, to leave the healthcare profession altogether (ITL2). Univariate models, which analyze these outcomes separately, often fail to account for their dependence, resulting in reduced estimation precision and a higher risk of Type II errors. Modelling their joint behavior is essential to improve estimation efficiency and reduce false negatives. Moreover, explicitly capturing the association structure enables the identification of discordant profiles, such as individuals intending to leave the hospital but not the profession, or vice versa.

OBJECTIVES

This paper investigates the determinants of nurses' intention to leave the hospital and the profession using a bivariate additive ordered logit model, emphasizing its ability to model complex dependence structures in ordinal categorical data. The analysis is implemented using the new R package pblm.

METHODS

The data derive from the METEOR [1] cross-sectional survey conducted in 2022 in eight hospitals across Belgium, the Netherlands, Italy, and Poland. The METEOR Turnover Intention questionnaire (MTI), administered to nurses in these hospitals, was based on the Job Demands–Resources (JD-R) model. It included validated instruments measuring job satisfaction, work engagement, burnout, and turnover intentions

(ITL1 and ITL2), both assessed on five-level Likert scales, along with individual and hospital-level covariates.

A previous analysis [2] addressed these outcomes separately. In contrast, this study applies a bivariate additive ordered logit model [3] with an association structure governed by a penalty term [4], which constrains the association intercepts (log-global odds ratios, log-gOR) to follow a data-driven polynomial structure. P-splines are used to model non-linear effects of age in both marginal and association equations. The model is fitted using the R package pblm, soon to be released on CRAN.

RESULTS

The survey collected 1350 complete responses. In the marginal model for ITL1, significant factors included younger age (gOR = 0.95, $p < 0.001$), having experienced bullying (gOR = 1.31, $p = 0.040$), emotional exhaustion (gOR = 2.24, $p < 0.001$), low opportunities for professional development (gOR = 1.80, $p = 0.022$), low support from supervisors (gOR = 2.10, $p < 0.001$), low work prospects (gOR = 2.20, $p < 0.001$), poor physical working conditions (gOR = 1.30, $p = 0.038$), underuse of professional abilities (gOR = 1.64, $p = 0.001$), and low salary (gOR = 1.50, $p < 0.001$). The Netherlands showed the highest country effect (gOR = 1.84, $p < 0.001$), while Italy showed the lowest (gOR = 0.55, $p = 0.005$).

In the marginal model for ITL2, significant factors included younger age (gOR = 0.96, $p < 0.001$), experiences of bullying (gOR = 1.31, $p = 0.043$), emotional exhaustion (gOR = 2.16, $p < 0.001$), depersonalization (gOR = 2.24, $p < 0.001$), work-life conflict (gOR = 1.56, $p < 0.001$), low professional development (gOR = 1.76, $p = 0.026$), low supervisor support

(gOR = 1.56, $p = 0.012$), low work prospects (gOR = 1.86, $p < 0.001$), poor physical working conditions (gOR = 1.48, $p = 0.002$), underuse of professional abilities (gOR = 1.38, $p = 0.030$), low salary (gOR = 1.62, $p < 0.001$), and low overall job satisfaction (gOR = 1.56, $p = 0.020$). Again, the Netherlands exhibited the strongest country effect (gOR = 1.46, $p = 0.001$), while Italy the weakest (gOR = 0.28, $p < 0.001$).

Regarding the association between ITL1 and ITL2, the strength of association increased significantly with age (relative gOR = 1.046, $p < 0.001$) and high working pace (relative gOR = 1.622, $p < 0.001$). Conversely, the association decreased significantly in the presence of health problems (relative gOR = 0.60, $p = 0.021$), low work prospects (relative gOR = 0.35, $p < 0.002$), underuse of professional abilities (relative gOR = 0.52, $p = 0.025$), and when working in the Netherlands (relative gOR = 0.50, $p = 0.004$).

Figure 1 displays the observed and predicted log-gOR structures, along with the partial effects of age (centered at 22 years) estimated using P-splines. These indicate a non-linear increasing effect of age in both marginal and association equations.

CONCLUSIONS

The use of a bivariate additive ordered logit model allowed for the identification of a wider set of significant predictors for nurses' intention to leave the hospital or the profession, compared to previous univariate analyses [2]. Specifically, five additional factors were identified for ITL1 (bullying, low professional development, low supervisor support, poor physical conditions, and low salary) and six for ITL2 (bullying, high working pace, work-life conflict, low supervisor support, poor physical conditions, and salary).

These findings offer more comprehensive insights into the drivers of nurses' turnover intentions and may support the development of more targeted retention strategies. Furthermore, the association model highlighted the presence of discordant profiles—nurses whose characteristics are associated with a weaker connection between ITL1 and ITL2. These insights may guide the design of future surveys or the refinement of questionnaire items. For instance, among more experienced nurses, the intention to leave the hospital and the profession may reflect a single underlying construct, whereas among younger nurses these outcomes appear more distinct.

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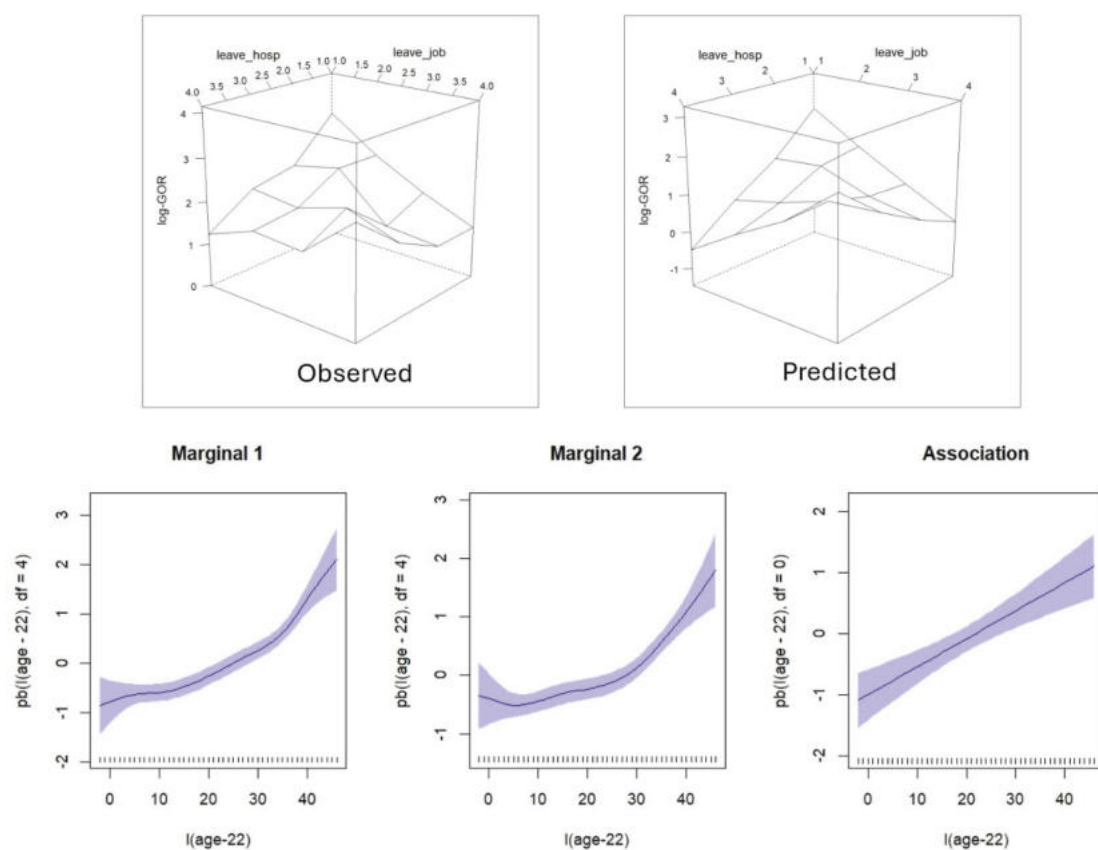


Figure 1. Top panels: observed (left) and predicted (right) association structure of log global odds ratios. Bottom panels, from left to right: partial effects of age (centered at 22 years), modeled using P-splines, for intention to leave the hospital (marginal 1), intention to leave the profession (marginal 2), and their association on the log scale

Clustering of Exposome and Lifestyle Data to Support Differential Expression Analysis of Circulating miRNAs in Multiple Sclerosis

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INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and are influenced by various metabolic and environmental factors[1]. The functional exposome concept can be seen as a new strategy to study the effect of the environment on health and is increasingly studied to understand its role in Multiple Sclerosis (MS) development[2]: it describes the harmful biochemical and metabolic changes (internal exposome) that occur in our body due to the totality of different environmental exposures throughout the life course, ultimately leading to adverse health effects and premature deaths.

OBJECTIVE

Variations in circulating miRNAs, as biomarkers of inflammation and oxidative stress, may identify subgroups of MS patients at risk. Cluster and study the characteristics of those groups and prospect different pattern of miRNAs, that may reflect distinct health status.

METHODS

A cohort of 139 people with Multiple Sclerosis (pwMS) was evaluated with detailed external exposome and lifestyle (air quality, urbanization, nutritional and occupational status) and internal exposome data (microbiome, oxidative stress and inflammation biomarkers).

Differential expression levels of five circulating miRNAs (miR-30, miR-146, miR-330, miR-574, and miR-664) have been measured and normalized with respect to an endogenous control in all blood samples, for each miRNA; they were obtained from a reduced subset of participants (sample sizes ranging from 15 to 25 subjects per miRNA).

Principal Component Analysis (PCA)[3] was used to reduce dimensionality of datasets and identify key patterns among the external exposome and lifestyle data. The first two principal components, which account for the most significant portion of variance, were selected based on parallel analysis of eigenvalues. Elbow Method was used to validate the optimal number of cluster and K-means clustering was then performed using these components.

To assess the miRNA expression differences among cluster membership, we performed Levene's test for homogeneity of variance followed by Kruskal–Wallis non-parametric tests. When appropriate, Dunn's test with Bonferroni correction was used for post hoc comparisons.

RESULTS

PCA identified two meaningful components that captured the primary axes of variation in lifestyle and nutritional profiles (Figure 1A). PC1 was primarily characterized by negative loadings on EDSS while the PC2 by positive; regarding anthropometric variables (e.g., BMI, waist-related measures) both PCs are characterized by negative loadings. Whereas PC2 is correlated negatively with quality of life (MSQOL-29 both physical and mental) in contrast, PC1 showed a positive contribution of those variables.

Based on the Elbow Method, three clusters were identified as optimal. K-means clustering was then applied to the first two PCA-derived components, resulting in the classification illustrated in Figure 1B. Three distinct clusters emerged: Cluster 1 (blue), positioned predominantly in the upper-left quadrant, Cluster 2 (yellow), mainly located on the right-hand side of the plot, and Cluster 3 (grey), concentrated in the lower-left area. These clusters differ along both PC1 and PC2 axes, suggesting heterogeneity in the underlying data structure. The clustering indicates that individuals were grouped based on shared patterns in anthropometric, clinical, and lifestyle variables.

No statistically significant differences in expression were found for any of the five miRNAs across clusters. Levene's tests confirmed the homogeneity of variances in all comparisons ($p > 0.1$) and, since miRNA levels were not normally distributed, the Kruskal–Wallis test was used. Kruskal–Wallis tests for miR-30, miR-146, miR-330, miR-574, and miR-664 all yielded non-significant p-values.

CONCLUSIONS

PCA and clustering analysis should be considered as valuable tools to summarize complex lifestyle variables and explore their interrelationships in MS research. This exploratory analysis using unsupervised clustering of exposome-lifestyle data did not reveal significant associations with expression of selected circulating miRNAs in pwMS, but further comprehensive miRNA profiling on the full sample is warranted to validate the negative results obtained or change the proof of concept.

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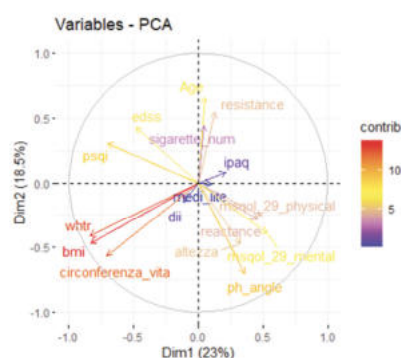


Figure 1A: Variables contributions to Principal Components (PCA) 1 and 2

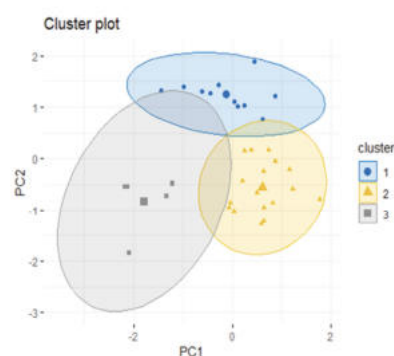


Figure 1B: Cluster assignment based on PCA derived components

A Novel One-Class Classification Framework for Highly Imbalanced Binary Outcomes: the OC-Cat Approach

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INTRODUCTION

Extremely rare events can challenge traditional classification models, which may exhibit reduced power in highly unbalanced datasets (i.e., when two or more target groups are unevenly represented). Moreover, this effect seems to be accentuated by the reduction of the sample size. Some of the easiest and intuitive methods proposed to handle unbalanced datasets, while still using a classical statistical models, are random under- or oversampling or hybrid methods[1]. Alternatively, other approaches have been proposed with different strategies, such as ensemble models (e.g. AdaBoost, XGBoost), or novelty detection models[2].

In medicine, this kind of scenario can occur when analysing catheter related/associated blood stream infections (CRBSI/CABSI), whose incidence usually remains <1/1000 catheter days[3], but could be higher in very frail patients[4]. Catheter insertion has a potential risk of complications and longer hospitalization: the use of decision-making algorithms is of great importance in order to avoid complications for these patients[5].

OBJECTIVES

The main purpose of our study is to adopt a novel anomaly detection model focused on binary/categorical covariates to predict risk of CRBSI/CABSI occurrence at baseline. To reach this result, we use a combined approach: features reduction, novelty detection algorithm and importance grid for model explainability.

METHODS

Data from hospital patients who received a vascular access device (VAD) placements at the University Hospital Luigi Sacco in Milan between January 2021 and January 2025 were analysed. All patients underwent central or peripheral catheterization in a non-ICU department. Parameters were collected at catheter insertion: age, sex, any major comorbidities, active intravenous drug usage, parenteral nutrition, regimen of hospitalization, transfer from the ICU, type of catheter, number of lumens, tunnel, exit site and number of placement attempts. All continuous variables were discretized into categorical format, yielding 29 Boolean and 2 categorical features.

The designed framework (OC-Cat) combines:

1. a graph-search-based feature selection method;
2. a one-class soft classifier designed (based on characterization of patients who didn't incurred in catheter infection);
3. a feature ranking that clarifies the classifier's decisions by ordering features based on their unique role in identifying uninfected patients.

In details:

1. we assess the redundancy of each pair of features using the excess over independence metric[6]. Then, we design a undirected connected graph where each node represents a feature, and the edge weights reflect the excess over independence between feature pairs. From each node, we apply the Bellman-Ford algorithm[7] to find the shortest closed path. Among all paths, we select the one that best represents the original data based on the Bayesian Information Criterion (BIC). The features included in this optimal path constitute the final selected feature set;
2. to design the soft-classifier, we rely on the assumption that a higher occurrence of a specific feature combination in majority class records (uninfected) implies that each new instance with those values is less likely to be infected. The learning phase consists of estimating the probability for a majority-class record occurring, given the distribution of uninfected patients. The prediction phase, instead, consists of estimated the majority-class probability for a new record (based on its i th attribute combination) using a weighted inverse Hamming distance [8]. The weight increases with the record's frequency among uninfected patients;
3. accordingly, the method ranks features based on a tailored definition of importance, stating that a feature - or a features set - is more important if it consistently exhibits the same value in majority-class data. To achieve this, we build a tree where nodes represent subsets of features, and each step measures the contribution of each new feature in reducing the majority-class data entropy. Last, once exploring all feature combinations and identifying the path with minimal entropy, the algorithm reports the features ranking as the order in which features appear along the path: from the root (most important) to the leaf (least important).

To evaluate the framework performance in terms of one-class classification, we compared OC-Cat probability distribution with that obtained from Isolation Forest (iForest) and One-Class Support Vector Machine (OCSVM). For the analysis, dataset was split into training and test set (August 2023 as threshold: ~75% vs 25%).

RESULTS

Data from 2836 hospitalized patients with VADs were retrieved. After keeping only the first VAD placement for each patients, we considered 2275 subjects (1222 women and 1053 men between 18 to 101 years) Among them, 148 become infected: 62 patients developed a CRBSI, 80 a CABS and 3 both. In the first step, our approach retained 16 out of 29 variables, which were then inserted in the novel model in

the second step. Figure 1 displays the risk factor index distributions for the training and test sets of our model, iForest, and OCSVM, along with their respective ROC curves. Lastly, catheter insertion site (upper vs lower limb vs neck), biological sex, hypertension, Charlson Comorbidity index, neurological disease and diabetes resulted the first most characterizing feature.

CONCLUSION

Our model introduces a novel, integrated approach for both characterizing and forecasting outcomes under severe imbalance in the target variable. It outperformed the iForest and OCSVM models applied to categorical and Boolean variables in a specific clinical contest. We are currently conducting further analysis and refinements to optimize performance on both our internal and external datasets, enhancing the model's generalization.

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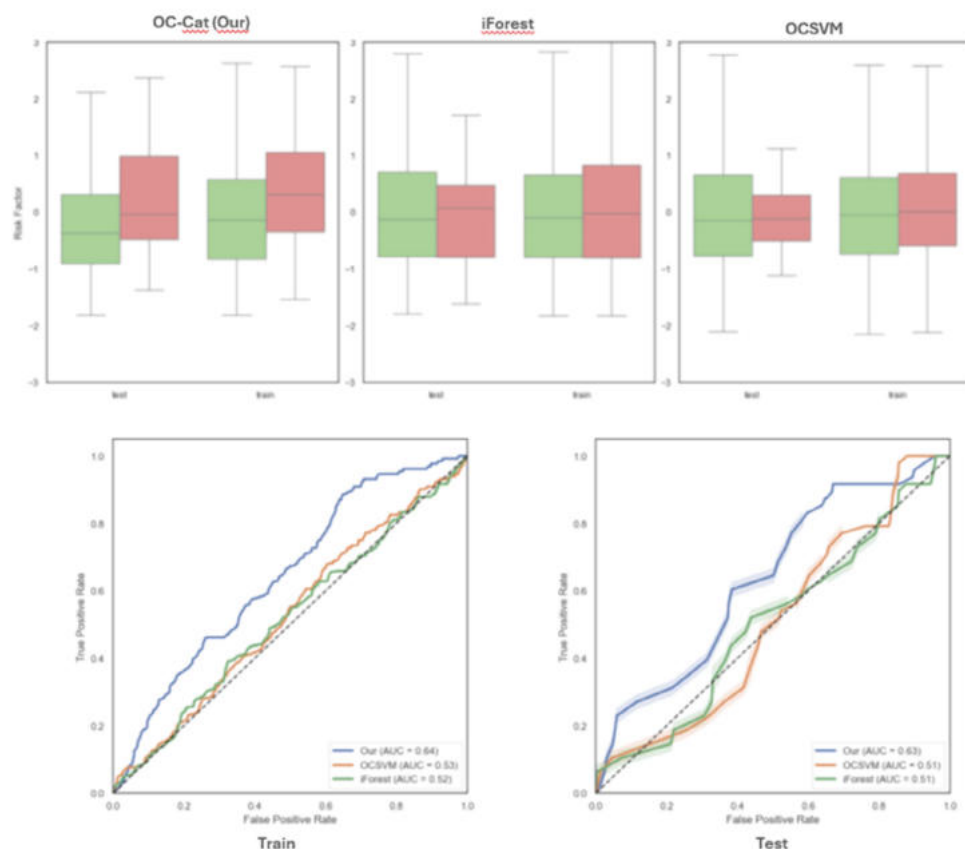


Figure 1. Risk distributions for the two patient classes (top of the figure): patients who didn't experience a CRBSI/CABSI infection during the follow-up period (green boxplot) vs patients who experienced a catheter infection (red boxplot). The distributions were calculated separately for the training set (left) and the test set (right). The same analyses, using the selected covariates, were conducted with two other models: Isolation Forest (iForest) and One-Class Support Vector Machine (OCSVM). In the lower part of the figure, the Receiver Operating Characteristic (ROC) curves for the three models are shown, separately for the training set (left) and the test set (right)

Use of Network Analysis for Identifying Drug Combinations to Prevent ADRs

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INTRODUCTION

The identification of drug combinations is critical to prevent adverse events, such as bleeding, often associated with the concomitant use of different active ingredients. Analysis of interactions using graphs is a useful tool for intuitively and quantitatively representing drug-drug relationships in real-world clinical settings.

OBJECTIVES

This study aimed to estimate the prevalence of potentially clinically important drug-drug interactions (DDIs) and the average causal effect of DDI exposure on hospitalization for bleeding related to adverse drug reactions (ADRs). In addition, identify the type of DDI that could lead to bleeding and the most common co-prescribed therapies responsible for ADRs through a network analysis approach.

METHODS

We performed a retrospective cohort single-center study including all consecutive patients admitted to the Internal Medicine Units of the Niguarda Hospital in Milan for bleeding-related complications from 1 January 2015 to 31 December 2018. Clinical characteristics, comorbidities, and pharmacological treatments were collected for each patient. Medication exposure was defined as the therapy assumed by the patient at the moment of admission. Polytherapy was defined by concomitant chronic use of ≥ 5 drugs. DDIs were identified using the LexiDrug database. Network analysis was applied to go to identify drug-drug interaction. Networks are a widely used tool for describing and analyzing complex systems, such as biological systems, in which relationships between entities-such as molecules, genes or drugs-play a central role [1,2,3]. In the biomedical context, graphs make

it possible to visualize and study molecular interactions, such as those between proteins, genes, or drugs. A graph is a structure composed of a set of nodes (also called vertices) and a set of arcs (or edges) connecting pairs of nodes. Each arc can be characterized by a weight, which represents the strength or frequency of the interaction. This approach allows highlighting which drug combinations are most common in the analyzed dataset. There are several types of graphs, but in this analysis we focus on an undirected, weighted graph. This type of representation is particularly suitable when the relationship between two elements is bidirectional or symmetric, as is the case when two drugs are simply taken together by a patient, without implying a direction of effect [1]. One of the main questions that network analysis seeks to address concerns the identification of the most relevant or central nodes. A frequently used metric for this purpose is the degree of a node, which represents the number of connections (i.e., that the number of arcs) it has with other nodes in the network. Nodes with a high degree can be considered potential hubs, that is, central elements that contribute to the connectivity and robustness of the network. Analyses were conducted in R, using packages for data manipulation, graphs and visualization.

RESULTS

Overall, 604 patients, 242 women, and 363 men, were admitted for bleeding: 215 clinically relevant non-major bleeding, 389 major bleeding. Among major bleeding 209 in >80 elderly, 62 in patients between 75-80, 67 between 65-75, and 51 in under 65 patients. Patients using more than 2 drugs were included and they were 87.15% in case of major bleeding and 84.65 with minor bleeding. The most used drugs are proton pump inhibitors, followed by platelet aggregation inhibitors excl heparin, and beta-blocking agents. The dataset contains 392 ddis associated with the risk of bleeding. These associations represent specific combinations of drugs that could be linked to

bleeding incidents, highlighting the importance of monitoring these combinations in clinical settings. The post frequent ddi associated with bleeding are co-somministrations of cns depressants and agents with antiplatelet properties, but also vitamin k antagonists with omeprazole/ pantoprazole, corticosteroids (systemic) / salicylates, aspirin / selective serotonin reuptake inhibitors, enoxaparin / agents with antiplatelet properties.

A drug x drug adjacency matrix was then constructed, in which each cell represents the absolute frequency with which two drugs were taken in combination by at least one patient. An undirected graph was derived from this matrix, in which the nodes represent the drugs and the arcs represent the observed interactions, with weight proportional to frequency. The degree of each node (number of interactions) was calculated and then the top 10% of the most connected nodes were selected to construct a filtered graph. A highly connected node appears frequently in combination with other active ingredients, while a less connected node is present in only a few associations. The degree of a node corresponds to the number of connections it has with other nodes. In addition, the weight of the arcs is proportional to the number of patients sharing the same drug combination: a thicker arc indicates a recurrent combination, while thinner arcs signal less frequent combinations.

The dataset included 604 patients, with 348 total drugs and 2542 interactions. Limiting the analysis to bleeding-associated interactions, the number of drugs considered drops to 121, with 392 interactions. In this subgraph, warfarin emerges as the most connected node (rank = 46), followed by amiodarone, clopidogrel, enoxaparin sodium, and acetylsalicylic acid. The most frequent interaction is warfarin - omeprazole (17 times). The filtered graph, showing only the 10% most connected drugs in the bleeding risk subgroup, is shown in Figure 1, where the intensity of the arc reflects the frequency of the observed interaction.

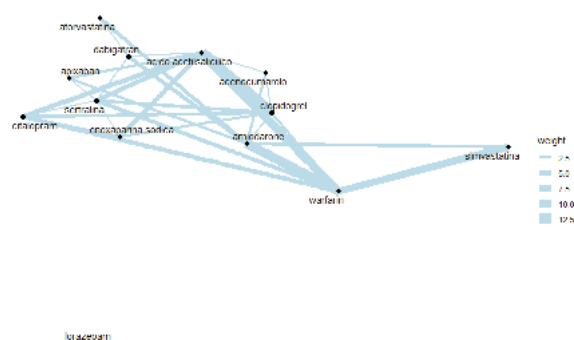


Figure 1. Graph of drug interactions associated with bleeding (10% most connected nodes).

CONCLUSIONS

Potentially clinically important DDIs carry an increased average causal effect on ADR-related admission. Especially by exposure to DDIs that increase bleeding risk, which should be targeted for medicine optimization. The analysis highlighted key drugs in the network of interactions, particularly warfarin, which confirms its clinical relevance as a critical node in high-risk bleeding settings. This approach is a valuable tool for surveillance of drug interactions and can support clinical decisions, especially in polytreatment settings.

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Bayesian Detection of Break-Points and Seasonal Patterns for Spontaneous Preterm Birth in Lombardy

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BACKGROUND

Preterm birth (<37 weeks gestation) is the leading cause of death in children under the age of 5 worldwide, accounting for 900k neonatal deaths a year [1]. Preterm birth rates vary widely within and between countries (4-16%) [2], with environmental causes of yet unclear aetiology suspected to be the main drivers [3, 4, 5]. Emerging evidence points to physical environmental factors—such as extreme weather and exposure to air, water, and soil pollutants—that may influence maternal biology, including the microbiome and inflammatory responses, through pathways like infection and nutrition [6]. However, numerous concurrent environmental factors present a challenge for robust inference analysis resulting into weak causal evidence.

OBJECTIVE

This study presents a general method for identifying break-points in time series data to help isolate the impact of environmental interventions on health outcomes, illustrated with preterm births in Lombardy.

METHODS

Preterm births were derived from a cohort of nearly 1M pregnancies among 750K women in Lombardy (2012–2023) from linked administrative health data. Monthly and weekly rates of spontaneous singleton births (22–37 weeks) were compiled across 12 provinces and stratified by maternal age (≥ 35), education, country of origin, and offspring sex. A Bayesian interrupted time series model was used to detect breakpoints—defined as immediate or sustained changes in baseline or slope. A Cauchy prior was applied to shrink the number of potential breakpoints to those with meaningful ef-

fects. The model incorporated seasonality to improve validity and provided uncertainty estimates for all parameters, enabling trend and seasonal pattern extraction. This approach was validated through a case study examining the impact of smoking bans in Italy on hospital admissions for cardiovascular events, where the breakpoints were already known.

RESULTS

In Lombardy, nearly 42k (6.3%) children were born preterm between 2012-2023 including 32k (4.8%) due to spontaneous labour. Time series analysis shows a 5.3-4.0% declining trend in spontaneous preterm birth rates. The seasonal component shows the expected biannual peaks in summer and winter, corroborating literature results from other countries. The trends and seasonality patterns are mostly consistent across provinces and socio-demographic risk factors. The model identified 5-10 break-points where an environmental policy might have unintended consequences on preterm birth rates (Fig 1).

CONCLUSION

The Bayesian breakpoint method proved effective in identifying significant changes in the time series. Summer and winter have emerged as high-risk seasons for spontaneous preterm birth over the last 12 years in Lombardy. Future work will explore potential causal drivers by incorporating control time series, detailed characterization of relevant policy changes and concurrent events, and triangulating findings with prior evidence to strengthen causal inference.

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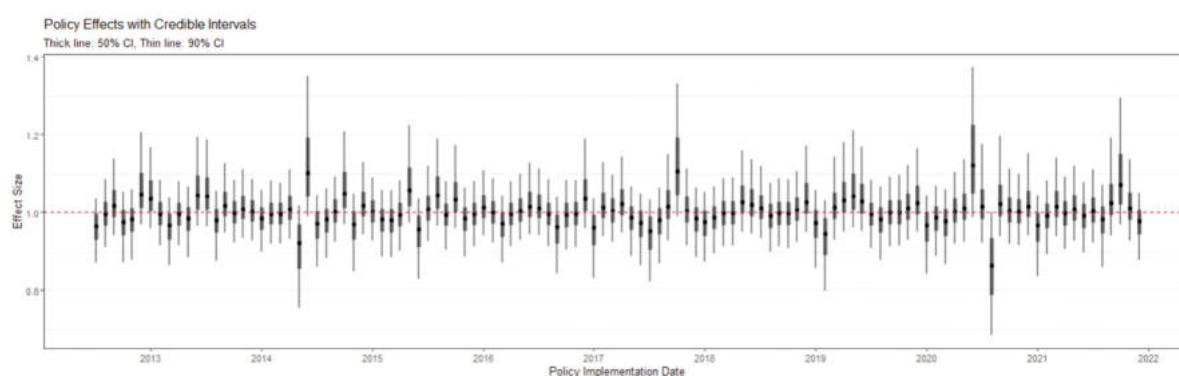


Figure 1. Detection of breakpoints in the time series of preterm birth in Lombardy. Each dot represents the estimated immediate effect of a breakpoint occurring in that month, with corresponding credible intervals

Statistical Strategies for Olink Proteomics Data: A Comparative Approach and Future Directions

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INTRODUCTION

Olink® proteomics platforms offer a powerful tool for high-throughput biomarker discovery through multiplexed protein quantification. Their application in cardiovascular research provides novel opportunities to identify predictive biomarkers, but the complexity and dimensionality of the resulting Omics data require tailored statistical methodologies for robust analysis and interpretation.

OBJECTIVES

This study aimed to compare multiple statistical techniques to analyze Olink data from coronary artery disease patients, with the goal of identifying plasma biomarkers associated with cardiovascular mortality.

METHODS

We analyzed 69 plasma samples from patients with coronary artery disease, of whom 17 (24.6%) experienced cardiovascular mortality. Protein expression was assessed using four Olink Target 96 panels (cardiometabolic, cardiovascular II and III, inflammation), yielding 333 Normalized Protein eXpression (NPX) values. A multi-method analytical pipeline was employed, including univariate t-tests, principal component analysis (PCA), Gene Set Enrichment Analysis (GSEA), heatmap visualization, Boruta feature selection, and multivariate logistic regression with stepwise variable selection. Analyses were conducted using SAS v9.4 and R v4.3.1, including the OlinkAnalyze R package [1].

RESULTS

Initial univariate analyses did not identify statistically significant differences between outcome groups after multiple testing correction. Volcano plots of adjusted p-values confirmed this lack of significance. PCA revealed low explanatory power of the first two components, suggesting limited separation between cases and controls based on the protein profiles. GSEA and heatmap analyses failed to detect any significant enrichment patterns. In contrast, the Boruta algorithm identified several relevant features, which were further evaluated in a multivariate logistic regression model. Stepwise selection based on unadjusted p-values led to the development of a predictive model with good performance (AUC = 0.89, 95% CI: 0.81–0.97). Clinical collaboration played a key role in contextualizing these findings.

CONCLUSIONS

This study highlights the importance of integrating diverse statistical methodologies for the analysis of high-dimensional Olink proteomics data. While no single approach yielded definitive results, the combination of techniques allowed for the identification of promising biomarkers and construction of a performant predictive model. However, the small sample size remains a major limitation, affecting the robustness and reproducibility of the findings. Future research should explore the integration of synthetic data generation techniques to simulate larger datasets. This could enhance the stability of statistical inferences and allow more confident identification of clinically relevant biomarkers in small-scale Omics studies.

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Leveraging Large-Scale ECG Representations for Interpretable Detection of Cardiac Amyloidosis in a Clinical Cohort

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INTRODUCTION

Deep neural networks (DNNs) have shown strong performance in diagnostic classification of raw electrocardiogram (ECG) signals. However, their clinical utility remains limited due to the scarcity of large annotated datasets and the inherent lack of interpretability. Variational autoencoders (VAEs), a class of unsupervised deep learning models based on encoder–decoder convolutional neural networks, can address these challenges by compressing input signal into a lower-dimensional latent space, obtaining latent representations that preserve key information and that can be explored using explainability techniques.

In this study, we investigate the application of this approach to the detection of transthyretin cardiac amyloidosis (CA)—a progressive and often underdiagnosed disease caused by the deposition of misfolded amyloid proteins in the myocardium, leading to substantial morbidity and mortality. Clinical suspicion typically arises from a combination of specialized examinations, often beginning with the ECG, but a definitive diagnosis requires bone tracer scintigraphy. Notably, only a subset of individuals with suspected CA are ultimately confirmed to have the disease.

AIMS

Among cardiology patients with suspected CA, we aimed to develop a diagnostic model using a two-step approach: first, by compressing ECG signals into a reduced set of independent and explanatory generative factors using a VAE; and second, by applying an interpretable classification algorithms to predict the presence of CA.

METHODS

In this retrospective cross-sectional study, we included patients referred to the Trieste Cardiovascular Department with clinical suspicion of CA who subsequently underwent bone tracer scintigraphy to confirm the diagnosis. For each patient, the ECG closest in time to the scintigraphy was selected for analysis. All ECGs were exported from the Mortara system in raw voltage format. From these, 1.2-second median beats were extracted and reformatted into the MUSE ECG format required by the variational autoencoder (VAE) model developed by van de Leur et al. that was trained on 1.1 million ECGs [1].

The VAE compressed each ECG waveform into 21 latent variables (ECG factors), which were initially assessed for univariate associations with CA status. These factors were then used as input features in a multivariable classification model. The dataset was randomly split into training and test sets. A LASSO (least absolute shrinkage and selection operator) logistic regression model was trained using various combinations of input variables. The optimal regularization parameter (λ) was selected via 10-fold cross-validation using the glmnet package in R. Model performance in terms of discrimination and calibration was evaluated in the holdout test set.

RESULTS

A total of 370 individuals were included in the study, of whom 119 (32%) were diagnosed with CA. The median age was 79 (IQR=[72, 83]), and 142 (28%) were females. In univariate analysis, 3 ECG factors showed significant association with CA status: F11 (OR=66 per 1 SD increase, 95% CI [0.51, 0.83]), previously linked to subtle QRS- and T-wave

changes; F25 (OR=1.63, [1.28, 2.09]), linked to longer QRS duration; and F30 (OR=0.71, [0.56, 0.89]), linked to longer QT-interval.

Compared to a simple model based on sex and age that show an ROC-AUC of 0.67 (95% CI [0.56, 0.77]), the model including all ECG factors as predictor led to an ROC-AUC of 0.69 (95% CI [0.59, 0.79]). The full model combining sex, age and ECG factors reached an ROC-AUC of 0.70 (95% CI [0.60, 0.80]). Calibration metrics for models including ECG factors were acceptable, with calibration slopes between 0.77 and 1.07 and intercepts ranging from 0.08 to 0.31.

CONCLUSIONS

These findings highlight the potential of VAE models to leverage representations learned from large-scale datasets and apply them to smaller, specialized diagnostic tasks such as the detection of CA. Although the discriminatory performance observed in this study was modest, the model's interpretability offers valuable insights into ECG patterns associated with CA, which may guide and inform future research into its underlying electrophysiological characteristics.

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Implementation of a Bundle for Gram-Negative Bloodstream Infection: Impact on 30-Day Mortality Analyzed with a Path Model

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INTRODUCTION

Gram-negative bacteria are the first cause of community bacteremia and the second cause of nosocomial bacteremia with increasing incidence, relevant morbidity and mortality. The current lack of international guidelines and/or evidence-based care bundles focused on Gram-negative bloodstream infection (GN-BSI) led to challenging management.

OBJECTIVES

Our aim was to verify whether implementing a bundle in the management of GN-BSI which comprised follow-up blood culture (FUBC), diagnosis imaging (DI) and source control (SC), optimized antibiotic administration (OAA) and shortened treatment duration for uncomplicated BSIs reduced mortality after a GN-BSI event.

METHODS

The study population included patients with monomicrobial GN-BSI aged ≥ 18 years enrolled at IRCCS Azienda Ospedaliero-Universitaria di Bologna. From January 2017 to December 2019 patients were administered standard clinical practice (pre-phase); from January 2022 to December 2023 (post-phase) all patients with monomicrobial GN-BSI were managed according to the predefined bundle designed to provide a structured, standardized approach to GN-BSI management, aimed to reduce mortality and improve patient outcomes. To verify the objective of the study a path model was developed, in which the indicator of the study phase was the exposure of interest, 30-day all-cause mortality was the dependent variable and the indicators of FUBC, DI, OAA and

SC were set as mediators. FUBC, DI and OAA were posited as first-level mediators and SC as a subsequent mediator, because the decision to undertake FUBC, DI and OAA stems from the patients' diagnosis, while SC can be undertaken according to FUBC and DI results. Treatment duration was not included in the model because it could be either cause or effect of mortality. The phase indicator was hypothesized as influencing all mediators and the outcome. Several clinical characteristics along with age and gender were included as exogenous variables. From an initial theoretical model, a final model was obtained by discarding non-significant paths and adding paths suggested by modification indices and deemed clinically relevant. Finally, a comparison with other modelling techniques was carried out to verify whether the path model actually added critical information and fitted best to the data.

RESULTS

Overall 3,355 patients were included, 2,092 were managed in the pre-phase and 1,263 with the bundle. Median age was similar (70.4 ± 16.4 vs. 71.2 ± 16.2 years), as the proportion of male patients (55.4% vs. 58.3%). No significant differences were observed in SOFA score, immunosuppression, septic shock incidence, rates of uncomplicated BSI, while the Charlson Comorbidity Index (CCI) was slightly lower in the post-phase (5.57 ± 2.74 vs. 5.79 ± 3.04 ; $p = 0.011$). In post-phase patients the BSI acquisition site showed fewer nosocomial or healthcare-associated cases (64.9% vs. 68.3%, $p = 0.045$) and more community-acquired infections. *Escherichia coli* was the most common pathogen and was less frequent among post-phase patients (47.8% vs. 53.3%; $p = 0.002$). Among these patients, higher proportions of appropriate empirical therapy (77.0% vs. 69.2%; $p < 0.001$), execution of FUBC within 7 days (55.8% vs. 30.2%; $p < 0.001$), use of DI (90.8% vs. 77.5%; $p < 0.001$) as well as SC (26.3%

vs. 23.6%, $p=0.083$) were found, while OAA was administered in similar proportions. Mortality rate at 30 days was slightly higher in patients of the pre-phase (14.1% vs. 12.4%) but non-significant at χ^2 test ($p=0.150$). The final path analysis model was performed on 3322 patients and used 12 independent and 4 dependent variables. It obtained a very satisfactory fit to the data (RMSEA=0.015, CFI=0.973, TLI=0.953) and explained 32.7% variance of the 30-day mortality variable. The model confirmed that bundle administration was not directly associated with 30-day mortality, however an indirect negative association was found, through imaging: patients of the post-phase were more likely to have DI performed, which in turn was associated to a lower risk of 30-day mortality. The total indirect effect on mortality of being in the post-phase, expressed by standardized coefficient, was -0.050 ($p<0.001$). DI was positively correlated with FUBC ($r=0.285$). Other variables significantly affecting mortality (std. effects) were SOFA score (0.275, $p<0.001$), age (0.234, $p<0.001$), UTI (-0.172, $p<0.001$), CCI (0.167, $p<0.001$), stay in surgery ward at the time of infection (-0.130, $p<0.001$), BSI nosocomial infection (0.034, $p<0.001$), carbapenem resistance (0.063, $p=0.009$) and *Pseudomonas* spp pathogen (0.045, $p<0.001$). The relationship between study phase and mortality was not found by a multiple logistic regression model including the same variables used in the path model (std. coefficient 0.004, $p=0.882$); a treatment-effect lasso logistic model run with 75 variables on 2791 patients of this population obtained an ATE of -0.004 ($p=0.798$) with a PO mean of 0.137 ($p<0.001$).

CONCLUSIONS

Interventions like the bundle described in this study are not inherently affecting mortality directly, because they act by deploying several components that may take place with a predetermined priority and sequence, actually one affecting the other. Rather, they are expected to affect the outcome indirectly by triggering activities that can actually be related to the outcome. We have demonstrated that the bundle activation did decrease the mortality rate of patients with monomicrobial GN-BSI by increasing the use of imaging and of its correlated follow-up blood culture, which in turn were related to lower mortality. Indirect effects cannot be estimated by traditional modelling techniques like multiple regression, therefore we advocate the use of path modeling in clinical settings involving temporally related activities.

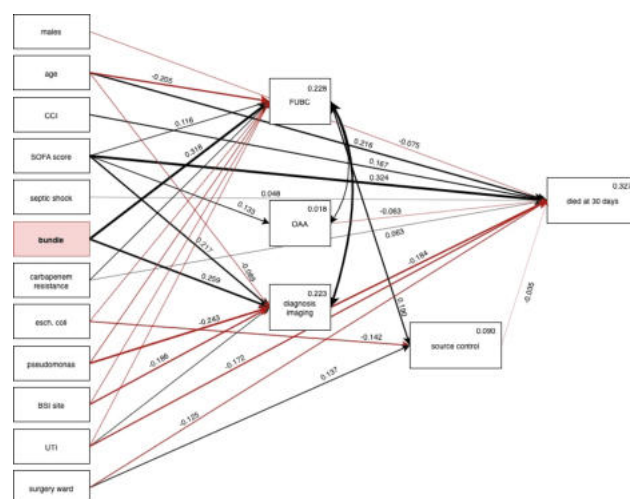


Figure 1. Path model diagram. Arrow lines indicate direct relationships between variables; black arrows represent positive relationships, red arrows represent negative relationships; thickness is proportional to the strength of the relationship, which is written near most of the arrows. Lines with bidirectional arrows indicate correlations between dependent variables. The number inside the box of the dependent variables indicates the R^2 .

Read-ICU: An Ensemble Deep Learning Model for ICU Readmission Prediction

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INTRODUCTION

Intensive Care Unit (ICU) readmissions are linked to increased morbidity, mortality, and healthcare costs [1]. The correct timing of ICU discharge is critical to prevent these adverse outcomes. Traditional predictive models have limited accuracy, while deep learning (DL) models offer potential improvements by extracting richer information from multimodal electronic health record data [2].

OBJECTIVES

To enhance ICU readmission prediction task accuracy by developing READ-ICU, an ensemble DL model trained on harmonized multi-institutional data.

METHODS

We identified standardized, publicly available ICU datasets from different institutions and geographical regions. Existing pipelines to merge and pre-process the datasets were searched. A previously conducted systematic review and meta-analysis informed the selection of DL architectures, outcome definitions, and predictors [3]. Bayesian Model Averaging (BMA) was chosen to ensemble the retrieved DL models whose code was publicly available [4]. The area under the receiving operating curve (AUROC) served as the primary performance metric, which guided the training and fine-tuning process. To improve the explainability of the resulting model, we applied an explainable artificial intelligence (XAI) technique able to identify key predictors influencing model output. Analyses were conducted using Python v. 3.10.12.

RESULTS

We gained access to MIMIC-III, MIMIC-IV, the multi-center eICU US datasets, and the European AmsterdamUMCdb [6-9]. We harmonized the four datasets through the BlendedICU pipeline [10]. The 48-hour readmission interval was selected as the outcome measure due to its stronger association with ICU care quality [11]. Recurrent neural networks (RNNs), long short-term memory (LSTM) networks, and convolutional neural networks (CNNs) were retrieved from the selected articles and were integrated into the ensemble READ-ICU model. Key predictors included static variables (e.g., demographics, length of ICU stay, admission source, comorbidities) and time-dependent variables from the last 48 hours of ICU stay (e.g., vital signs, laboratory test results, diagnosis codes, medications), which improved predictive performance in previous models [12].

CONCLUSIONS

DL enables innovative predictive models that may outperform traditional prognostic tools for ICU readmissions. Our systematic review identified promising DL models and challenges, though further efforts are required to optimize performance through multimodal data integration. This study highlights the potential of using ensemble DL techniques, multi-institutional datasets, and XAI techniques to improve the prediction of important outcomes in critical care.

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Investigating the Role of a Low-inflammatory Diet in Modulating Microbiome Biomarkers of Colorectal Cancer

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INTRODUCTION

Familial Adenomatous Polyposis (FAP) is a hereditary syndrome characterized by chronic colonic inflammation and the development of precancerous adenomas. A low-inflammatory dietary intervention helped reduce inflammation and adenoma development in individuals with FAP. Moreover, this promising strategy also reduced the differences in gene expression between normal and adenomatous tissue, increasing the expression of tumour suppressors and reducing that of potential cancer-driver genes in adenomatous tissue [1].

OBJECTIVES

By comparing the microbiome profile of subjects with FAP that followed a dietary intervention aimed to reduce inflammation in the gut, with that of colorectal cancer (CRC) patients or healthy subjects, we aimed to set up a methodological workflow to be applied in the identification of bacterial species related to the tumor and whose abundance can be modulated with diet. This workflow may contribute to the optimization or development of new strategies for CRC prevention based on diet.

METHODS

Microbiome of stools from 37 patients with stage II-III colorectal cancer who had surgery at INT (Fondazione IRCCS Istituto Nazionale dei Tumori) was quantified by shotgun sequencing. Samples were collected before the intervention (T0) and during the scheduled follow-up visits for two years (1 and 6 months after surgery, then every year) including possible time of relapse. By following the same sequencing pipeline, the fecal microbiome composition of a cohort of 120 healthy

volunteers, equally distributed among vegetarian, vegan, and omnivorous diets [2] and of the FAP cohort of 27 subjects carrying a mutation in the APC gene, who underwent prophylactic total colectomy/ileorectal anastomosis and currently involved in the surveillance program at INT, were determined. FAP stool samples were referred to four different timepoints, before the beginning of the study (T0), after both 15 days (T15d) and three months of active dietary intervention (T1), and after three additional months in which the subjects followed the diet at home (T2) [3]. For all the three cohorts, taxonomic profiling was carried out by using MetaPhlAn 3 [4].

Alpha diversity was quantified using the Simpson index [5,6]. The Alpha diversity between groups was compared using a Wilcoxon rank sum test or a signed rank test for dependent groups. Bray-Curtis dissimilarity [7] was used to measure the beta diversity and Principal Coordinate Analysis (PCoA) to reveal underlying patterns by visualizing differences in beta diversity [8]. Techniques like PERMANOVA (Permutational Multivariate Analysis of Variance) and PERMDISP (Tests of homogeneity of dispersions) were employed to detect significant differences in beta diversity and dispersion between groups [9].

Differential abundance analysis (DAA) of species was performed using Wilcoxon test or signed rank test [10] to compare the groups of interest.

RESULTS

Alpha diversity quantified by the Simpson's index was significantly higher in the CRC cohort compared to the healthy subjects, both in terms of richness and evenness. Wilcoxon rank sum test revealed a statistically significant difference between the two groups ($p < 0.001$). As regards beta diversity, microbial community composition differed significantly between CRC patients and healthy subject, as revealed by

the PERMANOVA test ($p = 0.001$), also due to the dispersion between the two groups ($p = 0.027$). DAA identified 230 differentially abundant bacterial species between CRC patient's cohort and healthy subjects. Subsequently, in order to investigate whether these bacteria were modulated by diet within the FAP population, DAA comparing taxa between samples pre and post diet was performed. This analysis revealed 3 bacterial species that were not only differentially abundant between CRC patients and healthy individuals, but also responsive to dietary intervention. This helped us identify a potentially beneficial change induced by the dietary intervention. As the abundance of all three species was higher in CRC patients compared to healthy individuals, and the diet reduced their abundance in FAP patients.

CONCLUSION

These findings suggest that bacteria may represent potential targets for diet-based modulation strategies in CRC relapsed patients. From a methodological point of view, our findings confirm that by applying a well-structured data analysis workflow to bacterial data could provide valuable insights for the management of CRC patients.

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Prediction of Atrial Fibrillation 10-Year Risk with Optimal Survival Tree Models

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BACKGROUND

Atrial fibrillation (AF) is the most common cardiac rhythm disorder in adults and old subjects with an estimated global prevalence of 35 million cases worldwide and increasing incidence in the next decades [1]. Traditional AF risk scores – Framingham, ARIC, CHARGE AF, CHA2DS2-VASc and SAAFE [2–6] – reach C-statistics around 0.75–0.80. Recently, there has been a growing interest in applying machine learning (ML) techniques to develop predictive models for AF. Many of these models have pushed discrimination performance a little higher, sacrificing interpretability, since the “black-box” nature of the employed algorithms [7].

OBJECTIVE

To build and internally validate an interpretable model that predicts the 10-year probability of AF-free survival, using the recently proposed Optimal Survival Tree (OST) algorithm [8].

METHODS

Data analyzed came from the Catanzaro Atrial Fibrillation project [9], an observational prospective cohort study that included outpatients enrolled from January 1998 to December 2018, referred to the University Hospital of Catanzaro - Italy, for cardiac clinical evaluation.

Patients with end-stage renal disease, active malignancy, thyroid dysfunction, cardiomyopathy, rheumatic and non-rheumatic valvular heart disease, or prosthetic valves, were excluded as well as those with previous acute myocardial infarction or stroke.

Predictors included in the analyses were: i) Demographic and anthropometric measures: age, sex, BMI, waist circum-

ference; ii) Medical history: hypertension, diabetes, heart failure, vascular disease, COPD, previous TIA, CHA2DS2-VASc components; iii) Laboratory measures: fasting glucose, total/HDL/LDL cholesterol, triglycerides, eGFR; Imaging derived variables: Left Atrial Volume index (LAVi), Left Ventricular Mass index, Carotid Intima-Media thickness. Time-to-first AF diagnosis was right-censored at 10 years.

The OST model was benchmarked against three established tree-based algorithms: survival CART, survival conditional-inference trees (cTree) and random survival forests (RF).

The models were trained on a randomly selected subset of patients (70%) and their predictive performances were subsequently evaluated and compared on the remaining 30%. A 5-fold cross-validation based grid search was used to tune the models' hyper-parameters. Discrimination (time-dependent AUC), accuracy (Brier score, integrated Brier score, Index of Prediction Accuracy – IPA) and calibration (Integrated Calibration Index - ICI, E50, E90) were assessed.

RESULTS

A total of 4114 patients were selected (mean age 59.06 ± 11.73 , 48.1% Females). During a mean follow-up of 59 ± 19 months, AF occurred in 533 patients (13%). At baseline, AF patients showed on average a worse clinical profile in terms of anthropometric measures (BMI and Waist circumference), renal function (eGFR), cardiovascular risk factors (Diabetes, Hypertension, Heart failure, previous TIA), CHA2DS2-VASc score and echocardiographic parameters.

The final OST model (Figure 1) relied on only four variables - LAVi, Glucose, Age, and CHA2DS2-VASc - creating six leaves that collapsed into four clinically meaningful risk profiles: i) Very-low risk: Either $LAVi \leq 34$ mL/m², $glucose \leq 97$ mg/dL, $CHA2DS2-VASc \leq 2$, or same LAVi, $Glucose > 97$, and $age \leq 71$ y ($n = 2082$, expected AF-free

survival 115–118 mo); ii) Low risk: Same LAVi/glucose but CHA2DS2-VASc > 2 (n = 213, 106 mo); iii) Moderate risk: Either LAVi ≤ 34 with higher glucose and age > 71 y or LAVi 34–39 (n = 399, 87–89 mo); iv) High risk: LAVi ≥ 40 (n = 186, 56 mo).

On the test cohort OST achieved AUCs of 0.856 and 0.794 and Brier scores of 0.086 and 0.134 at 5 and 10 years, respectively, slightly outperforming CART (5/10-y AUC 0.849/0.764; Brier 0.096/0.137) and cTree (0.846/0.766; 0.096/0.156), and trailing RF in the 5-year (0.894, 0.083), but not the 10-year prediction (0.804, 0.131). Calibration metrics favored OST over RF at both horizons.

CONCLUSIONS

A parsimonious, easily explainable four-variable OST predicted 10-year AF risk almost as accurately as RF yet with superior calibration and bedside transparency. Adding a single echocardiographic measure (LAVi) to routine clinical data may enable personalized AF screening and targeted prevention. External validation in independent, multicentre cohorts is required to confirm the model's generalisability and to support its adoption in routine clinical practice.

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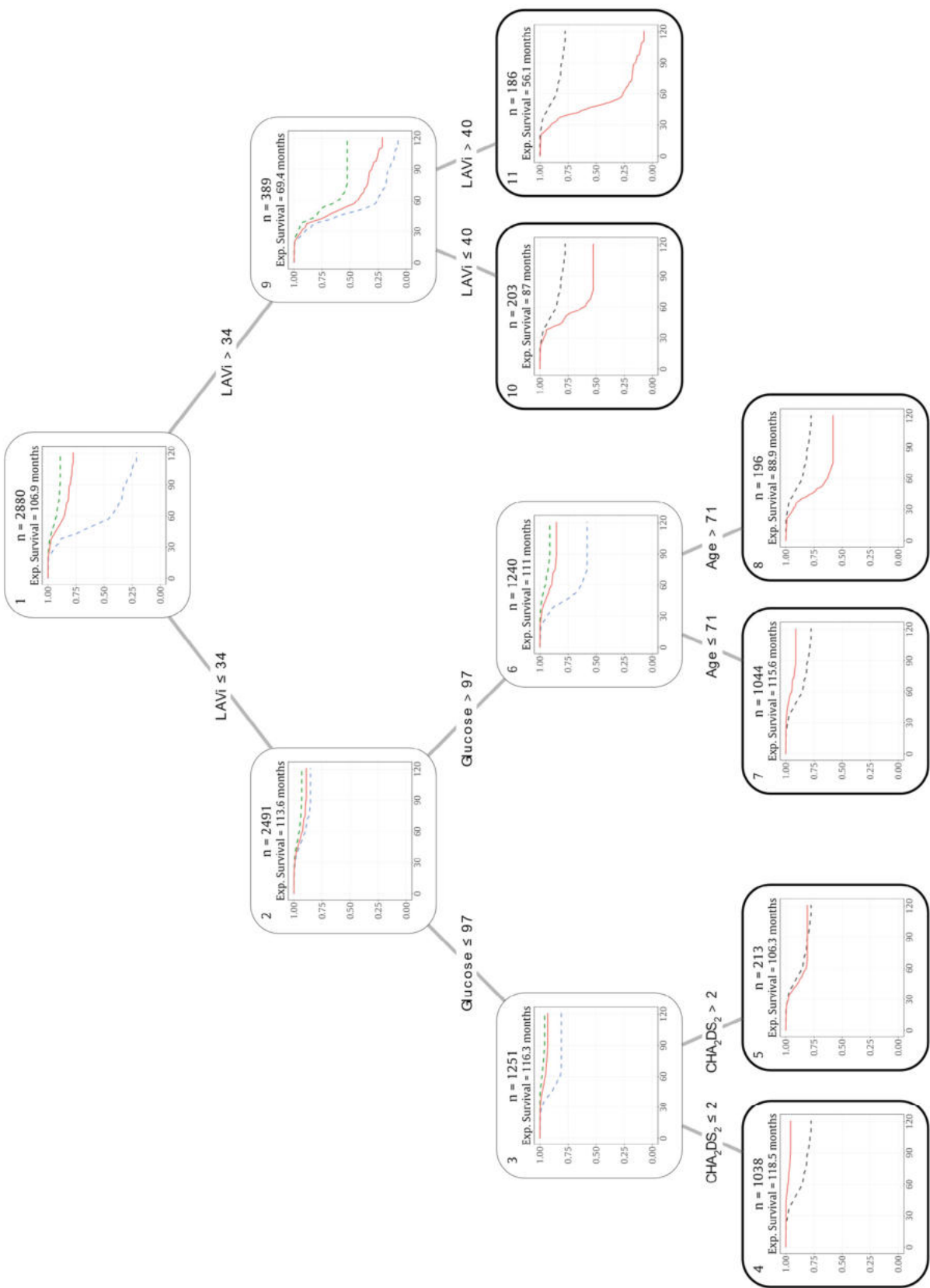


Figure 1. Optimal Survival Tree for 10-years AF risk. The red survival curve shows the survival probability of the subgroup of patients included in the tree node, the dashed green curve shows the survival of the subgroup in the left child, and the dashed blue line shows that of the subgroup in the right child. In leaf nodes (thicker border) the dashed grey curve shows the survival in the entire cohort used to develop the tree.

Applicability of Mendelian Randomization in the Context of Life-Course Epidemiology

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INTRODUCTION

One of the main goals of medical research is to determine whether an observed association between an exposure and an outcome reflects a true causal relationship. Major challenges in this context are the possibility of reverse causation or the presence of confounding variables, which can produce spurious associations. To address this, randomized controlled trials (RCTs) have traditionally been considered as the gold standard for establishing causal associations. However, RCTs face ethical, logistical, and generalizability limitations. They typically have limited follow-up periods, making them less suitable for studying long-term effects or life-course epidemiology.

In recent decades, Mendelian Randomization (MR) has emerged as a powerful tool for assessing causality in observational studies. Based on the instrumental variable approach, MR employs genetic variants that are randomly assigned at conception. This genetic randomization mimics the allocation process in RCTs, enabling researchers to make causal inference using observational data [1].

The growing availability of Genome-Wide Association Studies (GWAS) has further expanded the use of MR, enabling analyses without individual-level data and allowing broader investigation of diverse exposures [2]. Nevertheless, the scientific community continues to debate the applicability, strengths, limitations, and interpretation of MR findings [3].

OBJECTIVES

The aim of our study aligns with the framework of the project “An Integrated Life-Course Approach for Person-Centred Solutions and Care for Ageing with Multi-morbidity in the European Regions – STAGE; Stay Healthy Through Ageing” [4]. In particular, it contributes to one of the project’s tasks, which focuses on investigating the causal nature of associations between biological hallmarks and trajectories of ageing with multi-morbidity.

The specific objective of this study is to explore the feasibility of applying the MR approach to a selected research question. This application, beside obtaining specific MR estimates, should

serve as a basis for critically reflecting on the strengths and limitations of the method, with particular attention to its relevance within the framework of life-course epidemiology.

METHODS

We selected epigenetic age acceleration (EAA) as the exposure and attention deficit hyperactivity disorder (ADHD) as the outcome for our case study. EAA represents an example of a biological hallmark that may be identified within the STAGE project, and it has previously been linked to ADHD, specifically when measured using cord blood.

To conduct a two-sample MR analysis [5], we required summary statistics from GWAS for both the exposure and the outcome. Specifically, this involved identifying a GWAS on EAA to obtain the genetic instruments, and a separate GWAS on ADHD to extract the corresponding associations for those instruments.

For the exposure, we used the most recent and comprehensive GWAS on EAA including more than 40,000 individuals [6], which focused on the Horvath epigenetic clock [7]. For the outcome, we selected two of the latest GWAS on ADHD [8,9]. The first one [8] comprises 38,691 individuals with ADHD and 186,843 controls, while the second one [9], a genome-wide association meta-analysis still unpublished, includes 71,733 unique individuals from 28 population-based cohort.

We performed bidirectional MR analyses, i.e. we investigated the possible causal effect of EAA on ADHD as well as the possible causal effect of ADHD on EAA. The inverse-variance weighted (IVW) method was used as main method, but further sensitivity analyses were performed as well.

RESULTS

Bidirectional MR analyses indicated no evidence of a causal effect of EAA on ADHD (ORIVW = 1.01, 95% CI: 0.98–1.03), nor of ADHD on EAA. This held true when considering both the first ADHD GWAS (β IVW = -0.06, 95% CI: -0.28 to 0.16) and the second, unpublished GWAS focused solely on childhood ADHD (β IVW = 0.23, 95% CI: -0.30 to

0.77).

CONCLUSIONS

Our results did not support a causal effect of EAA on ADHD, nor a causal effect of ADHD on EAA. However, conducting these analyses helped clarify several important methodological considerations.

Firstly, the feasibility of two-sample MR analyses is limited by the availability of appropriate GWAS data for both the exposure and the outcome. In our case, only one GWAS on EAA, based on the Horvath clock, was available. Genetic instruments derived from other epigenetic clocks, such as the Pediatric-Buccal-Epigenetic clock [10], may have been more appropriate for our research question, particularly given prior evidence suggesting that EAA in early life could be causally linked to ADHD onset.

Secondly, the time-varying nature of traits like EAA suggests that GWAS based on repeated measures may be more informative in contexts where exposures are not stable over time [11]. The genetic instruments associated with EAA in adulthood may differ from those influencing EAA at younger ages or over the course of life. The available GWAS on EAA was conducted on individuals with a mean age over 50 years, while the ADHD GWASs included both childhood and adult cases [8] and childhood-only cases [9]. This may suggest the possibility of reverse causation, which was not confirmed by our results.

Finally, within the framework of STAGE, where the outcomes of interest may involve aging with multimorbidity, MR may be less suitable. Constructing genetic instruments for composite phenotypes increases the likelihood of pleiotropy, potentially leading to biased estimates. In this context, more sophisticated methods including MR-Phenome Wide Association Studies (PheWAS) [12] may offer more appropriate frameworks for capturing causal relationships with complex outcomes.

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Structural Equation Modeling and Monte Carlo Simulation in Clinical and Nursing Research: Insights Into Sample Size, Opportunities, and Challenges

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INTRODUCTION

Structural Equation Modeling (SEM) is widely adopted in behavioral, economic, and sociological sciences, and sample size calculations in this regard typically involve Monte Carlo simulation. SEM simultaneously integrates measurement and structural components, enabling both the evaluation of construct validity and the testing of specific hypotheses. Its application is particularly relevant when analyzing self-report data or complex theoretical frameworks, which require testing hypotheses as the primary aim, involving more than one dependent variable in the same model. Clinicians are often unfamiliar with these methods, and studies involving SEM tend to employ convenience sampling without a proper sample size calculation. A critical discussion regarding opportunities and challenges in this regard could facilitate the adoption of best practices when implementing SEM, including appropriate sample size calculations.

OBJECTIVES

To highlight the potential and challenges of adopting SEM in clinical research and nursing science and to discuss the contribution of Monte Carlo simulation for sample size planning in such contexts by using a real-case application as the context of the critical discourse.

METHODS

A real-case application is presented from a randomized controlled trial (RCT) involving Health Easy, a digital ecosystem designed to enhance engagement and health literacy among adolescents with congenital heart disease. The eco-

system integrates medical term simplification (SIMPLE), a patient-centered health search engine (FACILE), and a balanced learning interface (ULearn). The pilot study informed the primary endpoint (self-care behavioral score improvement of 10% after 3 months, on a scale of 0–100, which is Cohen's $d = 0.67$). While classical power-based sample size calculation was applied to the primary outcome, Monte Carlo simulation was used to evaluate the statistical power to test the hypothesized structural paths underpinning the Health Easy model. These included the mediating role of health literacy in the relationship between the intervention (Health Easy) and improvements in patient engagement, where health literacy is hypothesized to mediate and moderate the effects of the digital ecosystem on self-care behaviors and empowerment.

RESULTS

Monte Carlo simulation enabled simulation-based validation of the hypothesized structural paths within the Health Easy conceptual framework, particularly those involving latent variables and indirect effects. The simulation assessed the power and estimation precision for each path, including mediating effects of health literacy and moderated pathways influencing patient engagement and self-care behaviors, by generating multiple synthetic datasets (1000 replications) under specified model parameters. The simulation output supported the stability of parameter estimates and standard errors across replications, reinforcing the robustness of the SEM design.

A traditional power analysis was conducted to detect a 10-point mean difference in behavioral scores between two independent groups (Cohen's $d = 0.67$, $\alpha = 0.05$, power = 0.90), indicating that a total sample of 96 participants (48 per group) would be sufficient for this specific comparison. However, when this same sample size was evaluated within

the Monte Carlo simulation framework, it yielded an empirical power of only 0.49 to detect the hypothesized small-to-moderate indirect and moderated effects typical of SEM (Cohen's $d \approx 0.3$). To achieve adequate power (≥ 0.80) for testing the full model, a substantially larger sample, approximately 344 participants, was required. These findings demonstrate that when the objective is to test a conceptual model rather than a simple group difference, traditional power analysis may be misleading. Simulation-based approaches, such as Monte Carlo methods, are therefore essential for planning the appropriate sample size in SEM-driven clinical research.

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CONCLUSIONS

SEM and Monte Carlo simulation represent valuable yet underutilized tools in clinical research. Their application allows for the rigorous evaluation of complex intervention models, particularly when outcomes are mediated by constructs such as health literacy and patient engagement. Unlike traditional power analysis, these methods accommodate the analytical complexity of real-world frameworks and offer more accurate guidance for study design. Integrating SEM and simulation-based approaches into clinical trial methodology may enhance the interpretability, precision, and validity of intervention research. To support broader adoption, the development of a collaborative research network focused on advancing and disseminating these methods is encouraged.

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Predicting Mortality using Frailty Index and Latent Class Approaches: AUC-Based Evaluation in Simulated Data

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BACKGROUND

The global population is aging rapidly, creating significant challenges for healthcare systems [1]. Traditional disease-centered models often fail to meet the complex needs of older adults, who frequently have multiple chronic conditions. In geriatric medicine, frailty has become a key concept [2-3], representing increased vulnerability due to a decline in physiological reserves and functional capacity. Frailty is a multi-dimensional syndrome that includes physical, cognitive, psychological, and social impairments, making its identification vital for improving patient outcomes and healthcare resource allocation. The Frailty Index (FI) is a widely used tool that quantifies frailty by measuring the ratio of health deficits to the total number of health variables [4]. This approach allows for practical and scalable assessments across various settings.

However, while the FI offers a comprehensive assessment, it remains an observed composite measure that may not fully capture the underlying latent nature of frailty. Frailty can be conceptualized as a latent [5-6] construct. Studying frailty as a latent variable enables a deeper understanding of its structure and heterogeneity. It allows researchers to explore whether distinct frailty phenotypes [7] exist and whether they differ in their relationship to key outcomes such as mortality or functional decline. Moreover, latent variable models can uncover hidden patterns that are not evident from single observed measures like the FI, potentially offering more nuanced tools for risk stratification and clinical decision-making.

OBJECTIVES

To compare the predictive accuracy of mortality models based on a continuous FI and latent class approaches

through simulation, using the area under the curve (AUC) as the evaluation metric.

METHODS

The simulation uses real-world data on 50 binary and ordinal items related to HyperFrail, focusing on marginal probabilities and empirical correlations. Data simulation began by generating multivariate normal data using the empirical correlation matrix for a sample of 1,000 individuals with 50 variables each. These continuous values were transformed into uniform probabilities and mapped to discrete response levels based on specified marginal distributions. A dataset was created with one row per individual and the calculated FI as the sum of the 50-item responses, normalized by the number of items. Five domain-specific subscores were derived: activity (items 1-16), health (items 17-20), psychological (items 21-25), comorbidity (items 26-43), and cognitive (items 44-50). Age was simulated based on the frailty index (FI) levels. Individuals with an FI of less than 0.12 were assigned a random age between 20 and 50 years. For those with an FI of 0.12 or greater, age was sampled between 50 and 80 years, following an exponentially increasing distribution to reflect the greater frailty commonly observed in older adults. Mortality was simulated conditionally based on age: individuals under 50 were assigned a death status of 0 (indicating no death), while those aged 50 and above were assigned a death status with a probability ranging from 30% to 80% (in 5% increments) to represent various levels of risk. Three models were developed to predict binary death outcomes. The first model utilized logistic regression, employing the continuous Frailty Index (FI) on a scale from 0 to 100. The second model applied Latent Class Analysis (LCA) using the polCA [8] package on 50 item variables to identify various frailty classes,

followed by logistic regression where frailty class served as a categorical predictor. The third model used Gaussian Mixture Modeling (GMM) with the Mclust [9] package, analyzing five domain-specific summary scores to identify latent clusters. The Area Under the Curve (AUC) was calculated for each model to evaluate discrimination performance. Finally, a simulation study was conducted to assess model performance across different mortality scenarios.

RESULTS

Latent class analysis using Bayesian Information Criterion (BIC) identified nine distinct frailty classes across five domains. Class 1 represented “low frailty” with minimal deficits, while Class 4 showed “high activity limitation” mainly affecting the activity domain. Class 6 had a “cognitive-predominant” phenotype, marked by significant cognitive impairment. Class 8 displayed a “multi-domain severe” pattern with high frailty scores in activity, health, and comorbidity. Finally, Class 9 exhibited extreme frailty with the highest burden, especially relating to comorbidity and health status. The comparison of the AUC showed that the continuous FI consistently outperformed both latent variable approaches in all mortality probability scenarios. The FI displayed superior discriminative ability, particularly at higher mortality probabilities. It was followed closely by the GMM, while the LCA demonstrated the lowest predictive performance.

CONCLUSION

The continuous FI showed better predictive accuracy for mortality outcomes, while the latent class approach identified significant frailty phenotypes with distinct patterns in specific domains that may have important clinical implications. These findings indicate that, although the comprehensive nature of the FI provides more effective discrimination of mortality risk, understanding frailty as a latent variable offers valuable insights into the diverse characteristics of this syndrome.

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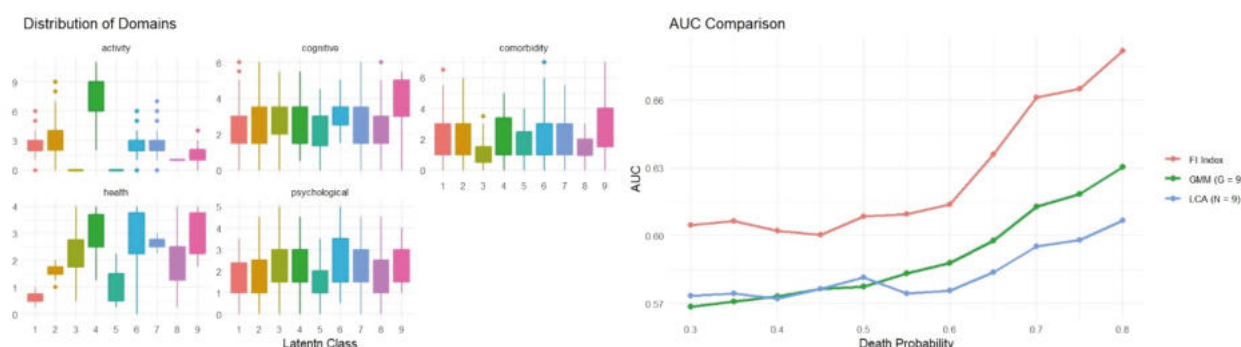


Figure 1. Distribution of Domains across latent classes and comparison of AUC for three models in different death scenarios

Is the Ability to Simulate Chance a Discriminant Property between High Functioning ASD and Typical Subjects?

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INTRODUCTION

The definition of the concept of randomness and the difficulty of humans to simulate it, in the sense of being able to produce unpredictable patterns within reasonably tight margins of error, constitute a profound topic of discussion among neuroscientists, mathematicians and philosophers [1]. The literature has widely demonstrated experimentally the difficulty that humans have in generating random sequences (Chance simulative deficit, CSD) that do not reveal, after an appropriate number of trials, a statistically recognizable response pattern that highlights the dominance of some choice options [2]. Errors in generating random, non-informative and therefore unpredictable sequences are generally explained in terms of prejudices, sometimes misconceptions or in any case with a level of deep structured cognition that prevails in any simulated process [3]. Theories have been proposed as to why there is such difficulty in simulating random sequences by invoking internal languages and, inspired by the artificial intelligence paradigm, recursive information compression algorithms [4]. Although such theories manage to achieve some results in terms of statistical fitting, they certainly require a lot of experimental work to be confirmed. Subjects with Autism Spectrum Disorders (ASD), by virtue of some behavioural peculiarities such as stereotypies, could constitute an important population to test the validity of the algorithmic theory. To date, few studies have investigated the ability to simulate chance in ASD subjects, highlighting a tendency to generate less random, more repetitive sequences and with regular patterns compared to neurotypical subjects [5].

OBJECTIVES

The study tests if the property of simulating chance is discriminating between autistic and typical subjects by introduc-

ing an appropriate metric. Given this difference, it can suggest a first-level strategy on a diagnostic level that takes advantage of the different simulative capacities of the two groups. The hypothesis is that the simulative deficit of randomness typical of human beings can constitute a discriminating property in the case of ASD and that this specificity can be applied for achieving diagnostic suggestions.

METHODS

The study is a pilot cross-sectional study with parallel cohorts aimed at evaluating the Chance Simulative Deficit (CSD). The study took place at the Regional Reference Centre for Autism of the Abruzzo Region in Italy. The sample size calculation assumed a Cohen effect size of namely 0.8, using a power of 80% with a type I error of 5%, requests 25 subjects for each group. The recruitment provided 122 typical subjects with and 45 (27%) ASD. The mean age was 23.2 years for typical and 21.8 years for ASD.

The task administered requests that each player, regardless of being ASD or typical, will play against a computer that simulates random choices, displaying icons with fists, paper, scissors on the monitor, after the human player has selected an icon and regardless of the human player's choice. In this game, the optimal strategy, or the strategy that maximizes the possibility of winning, is the least informative. Consequently, the computer optimally simulates the results using a uniform multinomial distribution with probability equal to 1/3 for each of the three states.

ASD and typical players perform many game trials, in our case 80 (about 20 minutes, assuming 15 s for each trial), so that the empirical frequency distributions of the players can be estimated.

These distributions characterize the specific ability of the ASD groups and typical players to simulate randomness,

namely the distribution used by computer in playing. The performance in the Chance Simulation is defined by the cross-entropy $H(p, q) = \sum_s p_s \ln \frac{1}{q_s}$, between the computer distribution p_s and the empirical distribution of each player q_s . ASD and Typical subjects' groups have been compared without adjustment using the Mann Whitney test with $\alpha=0.05$. This test has been performed to assess the hypothesized difference in chance simulation. A Homogeneous Markov Chain modelled the game. We estimated the transition Matrices of the ASD and Typical subjects. The associated Markov Processes provided different steady states confirming the preliminary non-parametric test. A logistic predictive model adjusted for gender and age has been trained randomly selecting the 80 % of the original sample.

The statistical analysis has been carried using the statistical software R (version 4.5.0).

RESULTS

The mean cross-entropy chance deficit score (CEDS) estimated was 0.49 (SD=0.03; min=0.48; max=0.76) for the typical and 0.48 (SD=0.01; min=0.48; max=0.52) for ASD. The Mann-Whitney test comparison of CEDS in the two groups has been statistically significant ($p<0.01$). The logit score, sets up using CEDS, gender and sex, reached an AUC=83%. Using the Youden cut-off (0.23) the model estimated a sensitivity of 89% and a specificity of 76%. The Kullback-Liebler divergence between the steady states distributions was statistically significant different from zero ($p<0.01$).

CONCLUSIONS

The study checked the hypothesis that ASD high functioning and Typical subjects perform differently in simulating chance. The analysis supports the hypothesis though has to pointed out that the study does not adjust for many potential confounders and the sample is strongly unbalanced between ASD and typical.

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Evaluating Variable Selection Methods in a Classification Framework: A Simulation Study

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INTRODUCTION

Variable selection is a common step in clinical research, where large datasets often include many, potentially highly correlated, variables. The main objective is to identify the most relevant predictors for an outcome, thereby enhancing model interpretability, simplicity, and predictive performance [1]. However, data-driven variable selection also carries several underappreciated risks. These include the potential exclusion of important predictors, inclusion of irrelevant ones, biased coefficient estimates, underestimated standard errors, invalid confidence intervals, and overall model instability [2].

Simulation studies are a valuable approach for evaluating statistical methods, provided they are carefully designed. Yet, many such studies exhibit bias in favor of the newly proposed methods [3]. To address this, we developed a neutral comparison simulation study to fairly evaluate the performance of several variable selection techniques.

OBJECTIVE

To systematically evaluate and compare different variable selection methods across multiple simulated scenarios.

METHODS

To improve the design and reporting of our simulation study, we followed the ADEMP structure [4], this involves specifying the aim (A), the data-generating process (D), the estimand or target of inference (E), the analytical methods (M), and the criteria used to evaluate performance (P).

We designed different simulation scenarios by varying the number of observations, total variables, and number of true predictors. Predictor correlations were modeled to decay exponentially with increasing distance between variables, and

effect sizes for true predictors were varied [5, 6]. Noise was introduced into the correlation structures to better mimic real-world data.

We focused on a binary classification setting, evaluating each method on two key outcomes: model selection accuracy (i.e. whether the true model is selected) and predictive performance. Five methods for selecting variables were compared: stepwise logistic regression, LASSO logistic regression, Elastic net logistic regression, Random Forest Classifier with OOB error based backward elimination [7] and Genetic Algorithm (GA) [8, 9]. Performance metrics included the Area Under the Curve (AUC), number of variables selected, and True Positive Rate (TPR). All the analyses were performed using Python 3.12.

RESULTS

We ran 1,000 Monte Carlo simulations per scenario, varying key factors such as sample size, number of predictors, true signal strength, and correlation strength. Elastic Net consistently achieved the highest mean AUC and TPR, particularly in high-dimensional or strong-signal settings (e.g., Scenarios 5–8), showing robust performance across conditions. Random Forest and Genetic Algorithm performed comparably in some scenarios but incurred substantially higher computational costs. LASSO achieved competitive AUC with significantly lower runtime, though it tended to underselect in weaker signal scenarios. Stepwise selection, while the fastest method, had the lowest overall predictive performance and true positive rates (Table 1).

Table 1. Mean AUC, TRP and number of variable selected (s) over 1000 Monte Carlo simulations by each variable selection methods. The execution time (t) of the 1000 simulations is also reported

Scenario*	Stepwise				LASSO				Elastic Net				RF				GA			
	t	s	TPR	AUC	t	s	TPR	AUC	t	s	TPR	AUC	t	s	TPR	AUC	t	s	TPR	AUC
n=200/m=20/p=3/e=0.15	20s	2	0.259	0.538	4m 4s	1	0.194	0.523	31m 44s	13	0.747	0.558	94m 59s	11	0.683	0.545	122m 14s	6	0.602	0.64
n=200/m=20/p=3/e=0.40	18s	2	0.644	0.753	2m 51s	1	0.391	0.753	32m 20s	5	0.610	0.742	95m 31s	13	0.875	0.679	122m 15s	6	0.686	0.731
n=200/m=50/p=5/e=0.40	1m 1s	4	0.389	0.784	3m 16s	2	0.279	0.794	37m 43d	6	0.371	0.788	188m 49s	12	0.469	0.721	136m 45s	19	0.545	0.767
n=500/m=50/p=5/e=0.45	3m 18s	3	0.103	0.591	9m 2s	6	0.207	0.605	53m 47s	39	0.819	0.616	269m 24s	13	0.258	0.583	164m 12s	19	0.421	0.641
n=500/m=100/p=10/e=0.75	7m 43s	11	0.623	0.852	5m 27s	14	0.626	0.861	77m 16s	26	0.706	0.859	393m 7s	16	0.526	0.821	178m 18s	44	0.643	0.809
n=500/m=50/p=5/e=1.00	1m 13s	4	0.522	0.809	3m 30s	7	0.575	0.809	55m 19s	22	0.752	0.803	252m	16	0.558	0.789	157m 59s	19	0.618	0.760
n=1000/m=100/p=10/e=0.5	8m 36s	11	0.593	0.837	7m 17s	17	0.64	0.838	168m 32s	35	0.760	0.837	553m 16s	14	0.557	0.806	311m 15s	44	0.662	0.776
n=1000/m=50/p=5/e=1.50	2m 7s	5	0.764	0.938	12m 3s	7	0.779	0.938	81m 42s	9	0.783	0.937	321m 21s	13	0.702	0.909	187m 17s	20	0.790	0.865

Parameters of scenarios: n = observations, m = features, p = relevant features, ρ = correlation, e = effect size

CONCLUSION

Among the five evaluated methods, Elastic Net provided the best trade-off between predictive performance and model stability, particularly in realistic, high-dimensional settings. Our results reinforce the importance of carefully considering the variable selection method in the context of the data structure and research goals. This neutral comparison contributes to evidence-based guidance for method selection in clinical research and similar applied settings.

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Decision Rules in Frequentist and Bayesian Hypothesis Testing: P-Value and Bayes Factor

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INTRODUCTION

The P-value is a widely used tool in inferential statistics and represents the probability of obtaining a value equal to or more extreme than the one observed, assuming that the null hypothesis (H_0) is true [1].

One of its main advantages is its intuitive interpretation: a smaller P-value indicates a lower compatibility of the observed results with the null hypothesis [2].

However, the P-value has important limitations that could lead to significant distortions in the interpretation of the results obtained [3].

The most important limitation is its sensitivity to sample size: as the sample size increases, the power of the test also increases. Consequently, even minor and perhaps clinically irrelevant effects can produce statistically significant P-values, while important effects might not be detected in smaller samples [1].

The use of a fixed significance threshold (typically 0.05) can promote a binary interpretation of the results (significant vs. non-significant), oversimplifying the researcher's decision-making process. This approach risks not fully capturing the degree of statistical evidence, thereby increasing the likelihood of assessment errors [4].

Another limitation is that the P-value does not provide information about the evidence in favor of an alternative hypothesis (H_1): a small P-value may suggest that the data do not support the null hypothesis (H_0), but it does not quantify, through a comparative approach, how much more likely the data are under the alternative hypothesis [5].

The excessive use of the P-value encourages researchers to explore alternative approaches, such as the Bayes Factor (BF) [6].

The BF is a Bayesian tool used to compare the evidence in favor of two hypotheses by comparing the likelihood of the data under the null hypothesis with the likelihood of the data under the alternative hypothesis. Therefore, unlike the p-value, the BF directly measures the probability of the data under each hypothesis, providing a quantitative comparison between H_0 and H_1 [7].

Among the advantages of the BF is its ability to provide a continuous measure of evidence, comparing the alternative hypothesis with the null hypothesis while also allowing the incorporation of prior information into the analyses. Its value can be interpreted using specific scales [8].

OBJECTIVES

The objective of this work is to compare the P-value and the BF as statistical tools for hypothesis testing, in order to highlight their behaviors in different scenarios involving (i) sample size and (ii) effect size.

METHODS

A simulation study was conducted with various scenarios constructed by combining sample size and effect size. The proposed simulation uses a t-test on the difference between the means of two independent groups as the endpoint. Nine distinct scenarios were generated, which include: (i) three levels of effect size, defined as the standardized difference between the means of the two groups, equal to 0.1, 0.2, and 0.5; and (ii) three different sample sizes, equal to 50, 100, and 150. A total of 5000 replications were performed, and the results are expressed in terms of medians of the p-value and BF [9].

The Bayesian results were obtained using the R package "Bayes Factor." The default prior was applied, which is identified as a Cauchy distribution centered on 0 and is moderately informative. In the simulation, the default prior of the package was chosen for illustrative purposes, but the process of selecting a prior is not trivial and requires specific considerations related to the research context.

RESULTS

The results of the study show that the Bayes Factor (BF) is less sensitive to sample size compared to the P-value when effect sizes are small (0.1 and 0.2). It can also be observed that the P-value becomes statistically significant for sample sizes of 100 and 150 units with an effect size of 0.5, and its significance increases at a very high rate, compared to the BF where the evidence in favor of H_1 remains moderate. In other words, the P-value becomes extremely low in the presence of an effect size of 0.5 for a sample size of 150 units, whereas the BF remains more cautious, indicating only moderate evidence in favor of the alternative hypothesis.

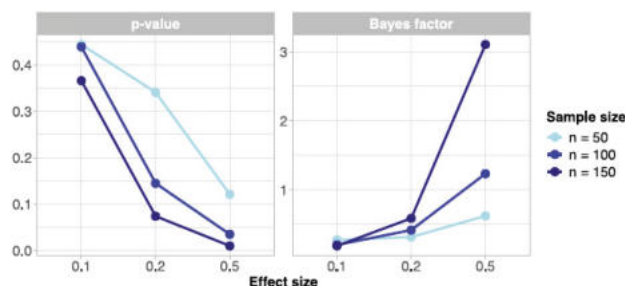


Figure 1. Comparison of Results Between the P-value and the Bayes Factor in the Simulation Study

CONCLUSIONS

The results reveal that the P-value is more sensitive to changes in sample size and effect size compared to the BF. Additionally, the BF provides a more nuanced approach to decision-making, addressing the binary nature of the P-value in rejecting the null hypothesis. The Bayesian alternative can be advantageous for researchers in the healthcare context, as it allows for the incorporation of informative priors that could enhance analysis results and reduce the likelihood of assessment errors. However, a significant challenge of using the BF lies in the choice of the prior distribution, which can significantly impact the final results of the analyses.

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The Importance of Jointly Analyzing Quality of Life and Survival: Insights from a Simulation Study

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INTRODUCTION

Patient-Reported Outcomes (PROs) are a key innovation in clinical research, providing direct insights into patients' perception of symptoms and quality of life (QoL).[1] Beyond their role in measuring well-being, PROs have also shown consistent associations with survival. Nonetheless, they are often analyzed separately from survival, which may lead to biased estimates of treatment effects and loss of clinical information. PROs in fact are only collected from surviving patients and early mortality among those with lower QoL can lead to an overestimation of average QoL, distorting perceived treatment effects. Moreover, survival models that rely only on baseline PRO values may fail to capture crucial changes in QoL that might correlate with prognosis.[2] Joint models (JMs), which combine the analysis of repeated measurements and time-to-event data, were developed to address these issues.[3] However, their use in practice remains limited.[4]

AIM

This simulation study aimed to explore the interplay between QoL and survival under different scenarios and to compare traditional approaches, such as Cox models, with and without time-dependent covariates, with the JM in terms of accuracy and robustness.

METHODS

Six scenarios with 500 samples of 1,500 patients (750 treated, 750 controls) were simulated by combining three different treatment impacts on QoL (worsening, no change, improvement over time) with two survival settings (a halving of mortality vs. no direct survival benefit). The follow-up period was set at 5 years and divided into monthly intervals. In each interval, a linear mixed effect model (LMM) was used to generate QoL score for each patient. The probability

of death within each interval was simulated considering the treatment arm and the current QoL score, assigning the same risk to everyone with the same profile. Informative censoring was introduced by modeling a lower probability of being observed during intervals when subjects had lower QoL scores. Additional scenarios without informative censoring were also simulated to assess how models' performances were affected by the QoL-survival association independently of observation bias. Three statistical approaches were applied: a univariate Cox model with only the treatment variable, an extended Cox model with QoL as a time-dependent covariate, and a JM with a Weibull survival component and a LMM for QoL. Performances were compared through mean estimates of treatment and QoL effects on survival in terms of hazard ratio (HR), bias, standard error, and 95% coverage probability (CP).

RESULTS

Even a modest association between QoL and survival (e.g., HR QoL=0.96) significantly influenced mortality patterns. Mortality increased when treatment had negative impacts on QoL even in the context of a direct survival benefit. Conversely, positive effects on QoL further amplified survival benefits. In the main scenarios with informative censoring, the univariate Cox model, while accurate if treatment had no impact on QoL, tended to overestimate the protective effect of treatment on survival when it was positively associated with QoL, sometimes even indicating a benefit where none existed. Conversely, when treatment negatively affected QoL, the model either underestimated its protective effect on survival or suggested harm where there was none. This pattern highlighted the potential for misinterpretation in clinical settings, where changes in QoL might be mistakenly attributed to treatment effects on survival. The extended Cox model showed mild improvement in certain scenarios but consistently failed to accurately estimate the protective effect of QoL on survival, leading to substantial bias. In contrast, JM consistently produced accurate, unbiased estimates with CPs near 95%, reflect-

ing its robustness in all scenarios, even with a simplified structure that included only a random intercept (Table 1). In the absence of informative censoring, the extended Cox model was able to accurately estimate the effect of QoL on survival when treatment positively influenced QoL and was better than the JM in terms of CP. However, it continued to perform poorly in estimating the treatment effect on survival, showing consistent bias and low CP across most scenarios. The JM remained the most reliable approach, although its performance slightly decreased, likely due to a less clearly defined QoL–survival association.

CONCLUSIONS

JMs offer a more accurate and comprehensive approach for analyzing PROs and survival, capturing both direct and indirect treatment effects. Their capacity to integrate multiple dimensions of patient data make them valuable for analyzing chronic conditions where PROs and survival are linked. Even modest interdependencies can meaningfully influence outcomes and ignoring them may lead to misleading conclusions. Their use should be prioritized in both randomized and observational studies to ensure a valid inference and a deeper understanding of treatment effects.

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Table 1. Estimated treatment and QoL effects on survival (HR trt and HR QoL) across different simulated scenarios.

(HR trt, $\beta_{\text{time trt}}$)	Model	HR trt	95% CP HR trt	HR QoL	95% CP HR QoL
(0.5, -0.333)	Univariate Cox	0.85	0	-	-
	Extended Cox	0.74	0.104	1.03	0
	JM	0.50	0.946	0.96	0.936
(0.5, 0)	Univariate Cox	0.50	0.936	-	-
	Extended Cox	0.74	0.086	1.02	0
	JM	0.50	0.922	0.96	0.964
(0.5, 0.333)+	Univariate Cox	0.33	0.112	-	-
	Extended Cox	1.12	0	1.01	0
	JM	0.54	0.948	0.95	0.924
(1, -0.333)	Univariate Cox	1.65	0	-	-
	Extended Cox	1.04	0.976	1.03	0
	JM	1.02	0.926	0.96	0.946
(1, 0)	Univariate Cox	1.01	0.960	-	-
	Extended Cox	1.00	0.992	1.02	0
	JM	1.00	0.960	0.96	0.950
(1, 0.333)+	Univariate Cox	0.67	0.026	-	-
	Extended Cox	1.39	0.214	1.01	0
	JM	1.08	0.940	0.95	0.904

worsening QoL +improving QoL

Legend: CP, coverage probability; HR, hazard ratio; JM, joint model; QoL, quality of life; trt, treatment.

A Modular Pipeline for the Construction and Validation of Polygenic Risk Scores in Oncology

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INTRODUCTION

Polygenic Risk Scores (PRS) are statistical tools designed to estimate individual predisposition to complex diseases by aggregating the effects of numerous genetic variants (Single Nucleotide Polymorphisms SNPs). In oncology, PRS hold promise for enhancing cancer risk stratification and personalizing screening strategies. However, their effectiveness depends on a well-defined computational framework that guarantees high-quality data processing and consistent predictive performance.

OBJECTIVE

This study aims to describe a modular and reproducible pipeline for the construction and validation of PRS in cancer research, detailing each analytical step from genotype preprocessing to risk score validation. Given that cancer is characterized by a highly polygenic architecture, involving thousands of loci with small effect sizes, such efforts require analytical workflows capable of handling complex and large-scale genomic data in a reliable and scalable manner.

METHODS

The pipeline begins with raw Variant Call Format (VCF) files obtained through genotyping. To ensure statistical power for detecting associations and constructing robust and accurate PRSs, large sample sizes, typically comprising several thousand cases and controls, are essential. Maintaining a balanced case-control ratio of 1:1 is crucial to minimize bias and maximize model stability. When relevant covariates are available, propensity score matching [1] can be applied to further balance cases and controls on clinical or demographic characteristics. Quality control (QC) is implemented using PLINK, a tool for handling SNP data, to remove variants and individuals based on call rate (<98%), minor allele

frequency (MAF<1%), Hardy-Weinberg equilibrium deviations ($p < 1 \times 10^{-4}$), excess heterozygosity or relatedness (PI_HAT>0.2), and sex discrepancies. Population stratification is assessed using Principal Component Analysis (PCA) or Multi-dimensional Scaling (MDS) to control for confounding due to population structure, and outliers are optionally detected via unsupervised clustering methods. The resulting components are included as covariates in downstream models. Imputation is performed via the Michigan [2] or Helmholtz imputation [3] servers using ancestry-matched reference panels to enhance the density of genotype data. Post-imputation filtering excludes SNPs with low imputation quality ($R^2 < 0.3$) and extreme allele frequencies to preserve dataset integrity. To identify genetic variants associated with cancer susceptibility, genome-wide association studies (GWAS) are conducted using logistic regression models, adjusting for age, sex, and leading principal components to mitigate confounding due to population substructure. When multiple cohorts are available, GWAS are initially conducted independently within each dataset. Subsequently, meta-analysis is performed to combine effect size estimates across studies, using either a fixed-effects or random-effects model. The choice of model depends on the extent of between-cohort heterogeneity, which may arise from differences in environmental exposures or other context-specific factors influencing cancer risk. In cases where such heterogeneity is minimal, fixed-effects meta-analysis via inverse-variance weighting is applied; otherwise, a random-effects model is employed to account for variability in genetic effect estimates across cohorts. For PRS construction, we employ a Bayesian regression framework with continuous shrinkage (PRS-CS) as proposed by Ge et al. [4], which integrates GWAS summary statistics with an external linkage disequilibrium (LD) reference panel to infer posterior SNP effect sizes. This approach eliminates the need to specify p-value thresholds or perform LD clumping and produces a single, optimized polygenic model. The PRS is finally calculated by summing allele dosages weighted by GWAS-derived effect sizes. Score performance is internally validated on a held-

out portion of the original dataset and externally tested on independent cohorts. Evaluation metrics include the area under the receiver operating characteristic curve (AUC), R^2 , and calibration plots.

RESULTS

We are currently applying this pipeline to the development and validation of a PRS for gastric cancer (GC) risk in individuals of European ancestry. Despite the growing use of PRS in various malignancies, only few of them have focused on GC, mostly on Asian individuals, and no validated PRS currently exists for GC in European populations. To address this gap, we are leveraging individual-level genotype data from over 8,000 GC cases and more than 350,000 controls across multiple European cohorts, including the Helsinki Biobank, the Rotterdam Study, dataset from Hess et al. [5], and the Spanish sample from the Stomach cancer pooling (StoP) Consortium. These cohorts form the discovery dataset used to conduct GWAS and meta-analysis, followed by PRS construction using a Bayesian framework (PRS-CS). The resulting scores are being externally validated in independent datasets from the UK Biobank and three cohorts from StoP consortium (Rome, Latvia, and Lithuania).

CONCLUSIONS

This pipeline provides a comprehensive and adaptable framework for constructing PRS in oncology, supporting methodological transparency and interoperability. Its modular design ensures flexibility across various datasets and facilitates implementation in clinical research. Future directions include increasing cross-ancestry portability and integrating PRS within clinical decision-making tools.

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Using Two-Sample Mendelian Randomization to Identify Potential Drug Targets: The Case of Desmoplakin

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BACKGROUND

Two-sample Mendelian randomization (2SMR) is a powerful tool for causal hypothesis testing. However, the simplicity of the statistical techniques combined with lack of causal inference reasoning has prompted an explosion of 2SMR studies, flooding scientific literature with findings that are often irrelevant or implausible. Here, we present a molecular biology application where we used 2SMR to advance understanding on the etiological mechanisms of familial arrhythmogenic cardiomyopathy (fACM). fACM is mostly caused by mutations in one of the five genes (DSP, JUP, PKP2, DSG2, and DSC2) that encode proteins constituting the cardiac desmosome, a cell junction responsible for cardiomyocytes mechanical coupling [1].

OBJECTIVES

Because Mendelian traits can be considered as extreme manifestations of common complex traits and share with them genetic architecture [2], the objective of our investigation was to test whether common genetic variants at desmosomal genes would also be associated with the normal variability of cardiac conduction traits in the general population. We show how a 2SMR analysis based on careful design and instrumental variable (IV) selection can help prioritize laboratory experiments, which are ultimately necessary to demonstrate causality.

METHODS

We analysed data of 4342 Cooperative Health Research in South Tyrol (CHRIS) study participants [3], fitting linear mixed models to assess the association between 2742 imputed genotype variants, covering all five genes, and four electrocardiographic traits: the P-wave, PR, QRS, and QT intervals. Replication was assessed in three independent studies from the same (MICROS [4], N=636) or different (SHIP-START and SHIP-TREND [5], N=3779) geographical region. We interrogated the GTEx-v8 database [6] to assess associations with transcriptomic levels in the cardiac left ventricle (N=386). Causality was assessed via Wald-ratio-based 2SMR, followed by in vitro experiments on human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs).

RESULTS

The QRS interval was significantly associated with a genetic variant located in a genomic region shared between DSP and the promoter of DSP-AS1, a long non-coding RNA of unknown function. The association was replicated in the MICROS but not in the SHIP studies. The variant resulted associated with DSP-AS1 but not DSP expression. Supported by evidence of statistical colocalization, 2SMR highlighted a significant causal effect of DSP-AS1 on DSP expression and QRS interval. In vitro experiments in hiPSC-CMs, showed that downregulating DSP-AS1 expression resulted in the upregulation of DSP expression and protein levels.

CONCLUSIONS

The evidence that DSP can be regulated by intervening on DSP-AS1 makes the latter a potential target for pharmacological development for DSP-related diseases such as fACM. Coupling population-based genetic association studies with 2SMR enabled efficient laboratory experiment prioritization to assess the causal molecular connections. Experimental validation also resolved statistical uncertainties related to partial replication and the problem of overlapping samples in 2SMR.

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Integrative Deep Learning of Germline and Somatic Genomics in Glioblastoma: A Translational Approach to Prognosis and Drug Target Discovery

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INTRODUCTION

Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults, characterized by rapid progression, high recurrence rates, and resistance to conventional therapies. The standard-of-care treatment, based on maximal safe surgical resection followed by radiotherapy and concomitant/adjunct temozolomide (Stupp protocol), has modestly improved patient outcomes since 2005. However, the median overall survival (OS) remains limited to approximately 12–16 months, and progression-free survival (PFS) to 6–10 months. A major clinical challenge is the heterogeneity in patient response, which is partially attributable to underlying genetic factors—both germline and somatic. While somatic drivers of GBM have been extensively studied, the role of germline variation and its interaction with somatic alterations in modulating treatment response and survival outcomes remains underexplored.

AIM

The aim is to improve risk stratification in glioblastoma by developing and validating an interpretable machine learning model that integrates germline and somatic genomic alterations with clinical variables, particularly extent of resection (EOR), to predict progression-free and overall survival. The model aims to:

- enhance the early identification of patients at higher risk of rapid progression or death; and
- uncover molecular signatures—across germline var-

iants, somatic mutations, and gene amplifications—that are indicative of a poor prognosis and may inform more personalised therapeutic strategies.

MATERIALS AND METHODS

We retrospectively analyzed a cohort of 119 patients diagnosed with primary GBM who underwent surgical resection at the Department of Neurosurgery, Udine Hospital, between 2014 and 2019. Inclusion criteria were availability of high-quality tumor and blood samples, comprehensive clinical data, and follow-up. All patients received the Stupp protocol, and 44 patients (38%) were additionally treated with carmustine wafer implantation. Targeted next-generation sequencing (NGS) was performed using a multi-gene panel to profile both germline single nucleotide polymorphisms (SNPs) and somatic variants, including point mutations and gene amplifications. After quality filtering, 1,192 high-confidence germline SNPs were retained from an initial 4,680 variants. Somatic alterations in key oncogenes and tumor suppressors (e.g., EGFR, MDM2, TP53, PDGFRA) were encoded as binary features. We employed the GenNet framework, an interpretable deep learning architecture tailored for genotype-phenotype prediction, to model PFS and OS as continuous outcomes. The model incorporated EOR and age as covariates and was trained using mean squared error (MSE) loss. Model performance was benchmarked against traditional machine learning methods, including Random Survival Forests (RSF), to assess prediction accuracy and feature interpretability.

RESULTS

The median overall survival (OS) in the cohort was 16 months (95% CI: 15–19), and the median progression-free survival (PFS) was 10 months (95% CI: 8–12). The median patient age was 60 years (95% CI: 52–69), and the median extent of resection was 98% (95% CI: 95–100).

The GenNet model achieved a test-set MSE of 63.93 for survival prediction (batch size = 32, learning rate = 0.001, epochs = 200, L1 regularization = 0.01). Key predictive features included both germline and somatic variants in genes such as TP53, PAX7, PIK3C2G, CYLD, FGF5, ERG, PIK3R2, and ALK. The inclusion of somatic gene amplifications notably enhanced the model's accuracy and biological interpretability. In comparative analysis, GenNet outperformed traditional RSF models in both predictive power and feature attribution.

CONCLUSIONS

Our study demonstrates the value of integrating germline and somatic genomic information with clinical variables in predicting survival outcomes in glioblastoma. The interpretable deep learning model built using the GenNet framework provides insight into the genomic architecture of disease progression and holds potential for informing personalized therapeutic strategies. These findings underscore the relevance of multi-omic modeling approaches in precision neuro-oncology and support further validation in larger, prospective cohorts.

Explainability in Microbiome-Based Models for CRC Prediction via Partial Dependence Plots

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INTRODUCTION

Gut microbiome profiling through 16S rRNA sequencing has emerged as a promising non-invasive tool for colorectal cancer (CRC) detection. Despite their predictive accuracy, machine learning (ML) models often struggle with interpretability, especially when dealing with high-dimensional and correlated microbial data. Ensemble methods such as random forests provide strong classification performance, but their internal mechanisms are opaque. The fuzzy forest (FF) algorithm extends the random forest approach by improving feature selection under multicollinearity, but still lacks direct interpretability of predictions. To address this limitation, explainability techniques such as Partial Dependence Plots (PDPs) can be used to visualize the marginal contribution of key features, enabling better understanding of the relationships between microbial taxa and disease risk.

OBJECTIVES

This study aims to enhance the interpretability of a microbiome-based classifier applied to Baxter et al.'s 16S rRNA sequencing dataset by using Partial Dependence Plots (PDPs), while also reducing feature importance bias by employing the Functional Forest (FF) method, which effectively addresses the limitations of Random Forests in handling highly correlated features. PDPs allow for the visualization of the marginal effect of each microbial or clinical feature on the predicted probability of CRC. The goal is to offer interpretable insights into the nonlinear and complex relationships captured by the FF model.

METHODS

We analysed faecal samples from CRC patients and healthy controls included in the Baxter et al.'s dataset. After centered log-ratio (clr) transformation of the data, we implemented the fuzzy forest (FF) algorithm for feature selection and classification. FF enhances the standard random forest by incorporating recursive feature elimination and correlation clustering, resulting in an unbiased ranking of features even in the presence of high multicollinearity. We then applied PDPs to the top-ranked microbial and clinical features. These plots allow the visualization of the marginal effect of each feature on the model's predicted probability of CRC, offering a means to interpret the impact of each variable in isolation.

RESULTS

The PDPs highlighted non-linear and threshold effects for both microbial and clinical predictors in the Baxter dataset (Figure 1). Age showed a biphasic relationship with CRC probability: a decreasing effect up to around 65 years, followed by a marked increase in risk thereafter. Among microbial features, *Porphyromonas* (ASV 417) was positively associated with CRC in a monotonic pattern, whereas *Faecalibacterium* (ASV 471) and *Paraprevotella* (ASV 446) showed threshold behaviour, with CRC probability increasing only beyond certain abundance levels. These results support the use of non-linear models for microbiome-based prediction tasks and highlight biologically plausible patterns in feature-response relationships.

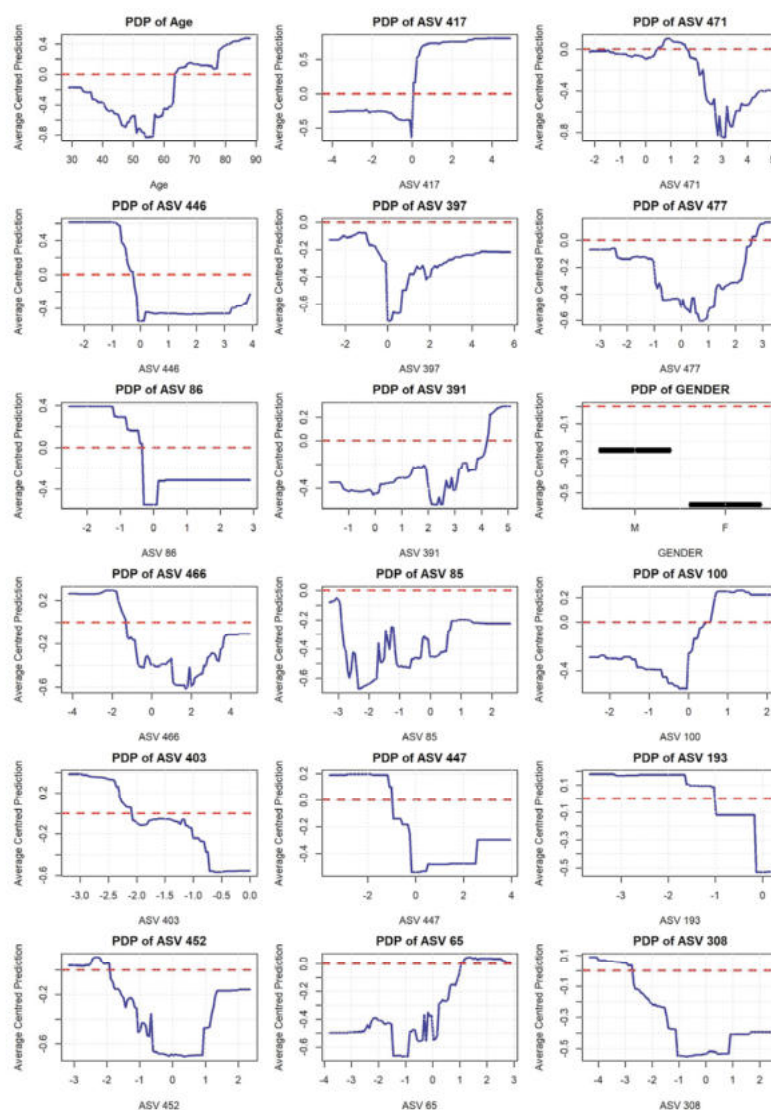


Figure 1. Partial dependence plots (PDPs) from Baxter et al.'s study. They illustrate the non-linear and complex relationships between the key microbial and clinical features and the CRC prediction outcome.

CONCLUSIONS

By combining fuzzy forest feature selection with Partial Dependence Plots, we constructed an interpretable and robust modelling pipeline for microbiome-based CRC prediction. This framework enables a better understanding of the individual contribution of microbial and clinical features to model predictions, enhancing both scientific interpretability and clinical relevance.

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Inclusion of Misanalyzed Stepped Wedge Trials in Meta-Analysis: Findings from a Simulation Study

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INTRODUCTION

The use of the stepped wedge cluster randomized trial (SWT) design to assess the effect of interventions in real-world settings has gained considerable popularity in recent years [1]. In this design, all clusters begin in the control condition, and the intervention is sequentially and randomly rolled out until all clusters transition into the intervention condition [2]. The unique characteristics of this design—for example, the unidirectional crossover to the intervention, variation in the timing and duration of exposure across clusters, and repeated measurements within clusters—pose additional statistical challenges that must be addressed when defining models to evaluate the effects of interventions [3].

In the context of meta-analysis, the inclusion of outcome data from SWTs requires careful methodological consideration. While analytic approaches for incorporating data from parallel cluster randomized trials have been well-documented [4], these methods are frequently misapplied in practice, with many analyses failing to properly account for clustering [5]. Given the added complexity of SWT designs, the potential for analytical errors, such as model misspecification [6], is expected to be higher. Currently, no established methods exist for synthesizing evidence from SWTs within meta-analyses, underscoring an important methodological gap that warrants attention.

OBJECTIVES

This study aims to examine the effects of including misanalyzed SWT in meta-analysis through a series of simulations.

METHODS

RCT and SWT datasets were simulated. RCT datasets were generated with a balanced two-arm design (treatment and control), assuming a normally distributed treatment effect with a mean difference of 0 and a fixed sample size of 1000

participants per study. SWT datasets were generated based on a repeated cross-sectional design with 5 time points, 50 observations per cluster, an average treatment effect of 0, an error variance (σ^2) of 5, and a random cluster effect (τ^2) of 1. Eighteen data-generating scenarios were considered, varying in number of clusters (20, 40 or 60), random treatment effect ($\eta^2 = 1, 2$ or 3), and random time effect (absent ($\gamma^2 = 0$) or present ($\gamma^2 = 1$)). For simplicity, no correlation between random effects was included.

Each SWT dataset was analyzed using three linear mixed-effects models:

1. Unadjusted for time: $y \sim \text{treatment} + (1 \mid \text{cluster})$
2. Hussey and Hughes model: $y \sim \text{treatment} + \text{time} + (1 \mid \text{cluster})$
3. Extended model accounting for random treatment and random time effects, where appropriate:
 $y \sim \text{treatment} + \text{time} + (1 \mid \text{cluster}) + (0 + \text{treatment} \mid \text{cluster}) + (1 \mid \text{clustertime})$

For each model, the estimated treatment effect and standard error were extracted. Meta-analytic datasets were then constructed, each consisting of 3 RCTs and 1 SWT. To enable direct comparison across the three models, each SWT dataset was included in three separate but matched meta-analytic datasets per scenario. For each scenario, random-effects meta-analysis was done using the Sidik-Jonkman estimator of between-study heterogeneity. Model performance was assessed by calculating the mean of the pooled effect sizes, along with bias, model-based standard error, coverage, and the percentage of statistically significant results out of 1000 pooled effect sizes at $p < 0.05$.

RESULTS

The model unadjusted for time consistently yielded the highest percentage of statistically significant results (based on 1000 meta-analyses per scenario), followed by the Hussey and Hughes and the extended model (Figure 1). Out of 18 scenarios, the unadjusted model exceeded the alpha threshold of 5% in 9 scenarios (50%), whereas neither the

Hussey and Hughes model nor the extended model exceeded this threshold in any scenario. Compared to the extended model—considered the correctly specified model in the simulations—the model unadjusted for time yielded, on average, 3.5% more statistically significant results with a maximum difference of 5.8%. The Hussey and Hughes model produced a smaller difference, on average, 1.2% more statistically significant results with a maximum difference of 2.2%.

In terms of coverage, the extended model produced the highest coverage probabilities, while the unadjusted model yielded the lowest. In contrast, the pooled effect size and associated bias averaged over all iterations per scenario were 0 (Monte Carlo standard error ranging from 0 to 0.01), consistent with the treatment effect set during the data generation process.

CONCLUSIONS

The inclusion of SWT data analyzed using misspecified models in meta-analyses can lead to inflated false-positive findings and potentially misleading conclusions about the effect of interventions. Researchers doing meta-analysis that include SWTs should exercise caution in evaluating the appropriateness of the underlying analytic methods to ensure valid and reliable inferences.

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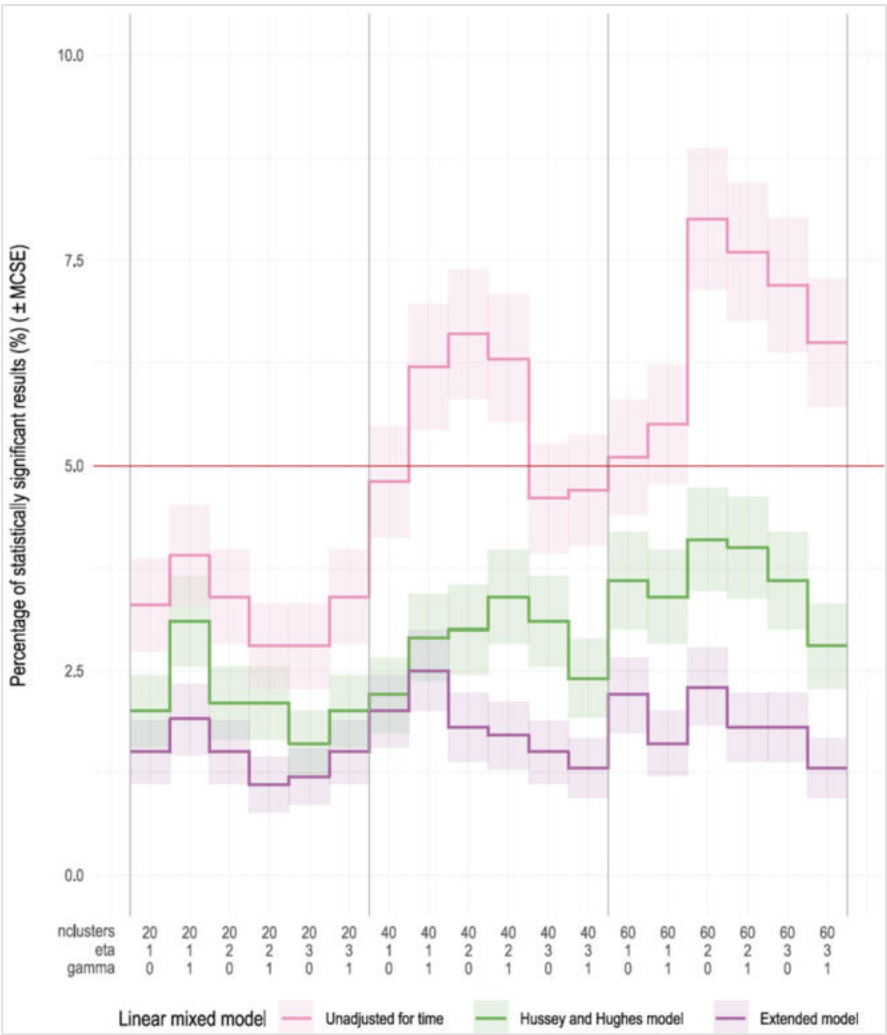


Figure 1. Percentage of statistically significant results across scenarios

Improving the Evaluation of Prognostic Indices for Survival Outcomes

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BACKGROUND

In many clinical settings (especially, in cancer studies), prognostic indices are defined in order to stratify patients with respect to the risk of occurrence of a specific outcome, on the basis of a subset of clinical variables. In the practice, based on the class of risk of the patient, clinicians may decide the patient management or may tailor the treatment. Thus, the prognostic groups must correspond to “clinically relevant” differences with respect to the outcome of interest. In the context of survival outcomes, a good prognostic classification should satisfy at least the following properties: (1) the groups must correspond to “well separated” survival curves, (2) the order of the prognostic levels must be retained in all cohort of the same clinical setting, (3) the groups must be reliable in terms of size, (4) the classification should give a good survival prediction. The assessment of prognostic indices is usually performed by using scores (e.g. the Brier score [1], the c-index [2] or the D-index [3]), which only evaluate one or two of these characteristics.

AIM

In order to have a more comprehensive evaluation of a prognostic index, we defined a new measure of prognostic index evaluation for survival outcomes and its performance has been compared to the one of commonly used scores.

METHODS

The Expected SEparation (ESEP) index is a new score, which represents the expected difference between the survival times of any two patients, given that they belong to “consecutive” risk groups (defined by the prognostic index to evaluate). In the common censored data setting, the estimation of the ESEP index relies on the estimation of the restricted mean survival time, which is performed in practice by using an approach based on pseudo-values [4]. This new score has several advantages: 1) it

evaluates properties (1-3) of a prognostic index, 2) it is based on the restricted mean survival time, which can be used even in case of non-proportional hazards assumption, 3) thanks to its definition, the value of the ESEP index can be judged by physicians with respect to their clinical goals.

The performance of the ESEP index was evaluated and compared with respect to several measures defined in the field through an extensive simulation study. The simulated data were generated mimicking challenging issues characterizing real-world settings. The different scenarios were defined by varying: the sample size, the percentage of censored data, the patient stratification. By using cross-validation, the several measures were also compared on public real data (such as, the Whitehall I Study).

RESULTS

Overall, in the simulation study, the ESEP index outperformed the other scores by enabling to identify wrong prognostic classifications, even in case of small sample size and/or high percentage of censored data. The same behaviour was also observed when comparing different prognostic classifications on public real data, such as the Whitehall I Study. In particular, differently from the other measures, the ESEP index maintained the property of clearly distinguishing the performances of the prognostic indices even when considering subsamples of the entire dataset (and thus in case of reduced sample size).

CONCLUSIONS

The ESEP index was able to well assess and discriminate prognostic models on several simulated scenarios and on real data, even in challenging scenarios. Moreover, since it evaluates three out of the four properties of a prognostic index, it is sufficient to use it together with a measure of survival prediction (such as the Brier score) to achieve a complete assessment.

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A Comparison of Methods to Fit Mortality Curves

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INTRODUCTION

In the field of time series, in particular in the study of mortality or other disease event curves, one of the most broadly used methods is the joinpoint (JP) described by Kim et al. [1]. Despite the present methodology was specifically designed to fit mortality or incidence data, it has some practical limitations such as high computational time, its usage is constrained to the Joinpoint statistical software, meaning that there is less flexibility in adapting the model to different types of generalized linear model (GLM) regressions or repeated measure outcomes.

OBJECTIVES

In this study, we aimed to compare the JP method, the segmented model proposed by Muggeo [2], and the Optimal Knots for Linear Spline (OKLS) regression, a new method introduced in this work, highlighting the strengths and weaknesses of each approach across various scenarios.

METHODS

Given the general formula that represents the regression model of the dependent variable y on a regressor x with k knots:

$$y_i = \beta_0 + \beta_1 x_i + \delta_1 (x_i - \tau_1)^+ + \dots + \delta_k (x_i - \tau_k)^+ + \varepsilon_i^{(k)}$$

where the τ_j 's are the unknown joinpoints, δ_k are the difference in slopes between consecutive segments and $(x_i - \tau_j)^+ = x_i - \tau_j$ for $x_i - \tau_j > 0$ and 0 otherwise. In JP regression a grid search over the $\tau_1, \dots, \tau_k$ is applied where at each step of the grid search the least squares estimates for the other parameters are found by linear model methods, and the set that minimizes the residual sum of squares is chosen. A permutation test is then performed to assess the significance of the new knots included in the model sequentially.

The segmented regression proposes to define the term $(x_i - \tau_j)^+ = (x_i - \tau_j^{(0)})^+ + (\tau_j - \tau_j^{(0)})(-1)I(x_i > \tau_j^{(0)})$ following

the first-order Taylor's expansion around the knot $\tau_j^{(0)}$. In the case of a single breakpoint, we can iterate the optimization of the finding of the optimal knot redefining the formula at each step s as follows:

$$y_i = \beta_0 + \beta_1 x_i + \delta(x_i - \tau^{(s)})^+ + \gamma(-1) \cdot I(x_i > \tau^{(s)}) + \varepsilon_i^{(k)}$$

where $\gamma = \delta(\tau - \tau^{(s)})$. After having fitted the model, the breakpoint can be updated as $\tau^{(s+1)} = \hat{\gamma} / \hat{\delta} + \tau^{(s)}$ until convergence.

In OKLS regression we propose to fit a linear spline starting from a high number of knots (we suggest $k=\sqrt{n}$) and then iteratively remove the knots where a significant change in the slope is found after having defined the place of the breakpoints that maximize the likelihood of the model. To be more parsimonious and to avoid overfitting, a Bonferroni adjustment is applied when testing for the change in slope dividing the level of significance $\alpha=0.05$ by the number of knots. The algorithm stops when all changes in slopes are statistically significant, if present.

Eight different scenarios were simulated to compare the method performances. Four pairs of different numbers of observations and knots were considered and, for each pair, a dependent variable imposing a pseudo R² of 0.3 and 0.7 was generated: 15 observations and $\overline{0}$ knots (scenarios 1 and 2), 10 observations and 1 knot (scenarios 3 and 4), 25 observations and 3 knots (scenarios 5 and 6) and 50 observations and 5 knots (scenarios 7 and 8).

RESULTS

When the number of knots was not prespecified, the three methods showed similar performances in scenarios 1, 2 and 4 while the OKLS model showed to be more accurate in the other scenarios (Table 1). When fixing the correct number of knots, based on the Root Mean Squared Error (RMSE), JP showed the best performances in estimating the regression parameters and the knots 16.7% and 0% of the times, respectively, segmented 20.8% and 0% while OKLS 62.5% and 100% of the

times (Table 1). JP was the most efficient method in scenarios 1,2,3 and 4 where the number of observations and knots was smaller showing the lowest computational times. Segmented and OKLS were faster at increasing number of observations and knots (scenarios 5,6,7 and 8).

CONCLUSIONS

This study allowed us to compare the performance of three methodologies for fitting mortality curves. The method we proposed demonstrated strong performance in estimating regression parameters as well as in identifying the number and placement of knots, outperforming both the JP method, considered the gold standard in this field, and the segmented model, a commonly used approach for fitting piecewise regressions. Future research should focus on evaluating the predictive performance of these methodologies.

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Unsupervised Clustering of Optical Coherence Tomography Data in Patients with Leber Hereditary Optic Neuropathy using Non-Negative Matrix Factorization and K-Means: A Comparison

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INTRODUCTION

Leber Hereditary Optic Neuropathy (LHON) is a rare genetic neurodegenerative disorder of the optic nerve, caused by mitochondrial DNA (mtDNA) pathogenic variants. It leads to sudden and severe central vision loss, mostly bilateral, typically in young adult males (onset age 18–35), though cases from 2 to 87 year of disease onset have been reported [1]. LHON has incomplete penetrance: all family members may carry the causative mtDNA pathogenic variant, but only some develop the disease phenotype. No definite predictors of disease conversion exist. However, subclinical signs can be detected through Optical Coherence Tomography (OCT), which vary between LHON asymptomatic carriers and symptomatic patients [2,3,4]. OCT is a non-invasive imaging technique that measures the thickness of retinal layers and optic nerve fibers. We used the DRI OCT Triton (Topcon), a swept-source multimodal imaging OCT device. LHON asymptomatic carriers may show early retinal alterations, while symptomatic individuals in the acute phase (within 6 months from onset) present distinct OCT phenotypes. Identifying putative OCT parameters predicting clinical conversion is an urgent unmet clinical need.

OBJECTIVES

To apply unsupervised clustering techniques to OCT data to identify latent subgroups of eyes with similar structural patterns, and assess their coherence with known clinical classes.

METHODS

We analyzed 173 eyes from symptomatic LHON patients (acute phase), asymptomatic LHON carriers, and healthy controls, based on 41 OCT parameters related to Ganglion Cell Layer (GCL), Retinal Nerve Fiber Layer (RNFL), and choroidal thickness. Data were normalized and clustered using: (1) Non-negative Matrix Factorization (NMF) via Brunet and Lee methods, running 50 iterations with cluster number (k) optimization based on internal quality indices [5]; (2) K-means clustering with optimal k selected using Elbow and Gap statistics. We also constructed a complete ExpressionSet object including phenotypic and clinical metadata to facilitate integration and visualization [5].

RESULTS

All methods identified an optimal partition into 3 clusters, broadly consistent with the clinical classification. Brunet-based NMF outperformed Lee-NMF in capturing the clinical structure (purity 0.601 vs 0.572; entropy 0.744 vs 0.784), likely due to its ability to model sparse data, such as OCT matrices where a few variables dominate the individual profiles. Then, the extracted metagenes (partitions) showed localized structural patterns in RNFL and GCL sectors. K-means also separated groups meaningfully, although with more overlap, especially among symptomatic eyes.

CONCLUSIONS

Among the clustering methods tested, Brunet-based NMF emerged as the most suitable for unsupervised stratification of LHON patients, carriers, and controls based on OCT data. Its advantage lies in the ability to highlight sparse but informative features — i.e., those OCT parameters that best discriminate between clinical groups — allowing for more distinct phenotypic clustering. These findings support the use of data-driven approaches for structural profiling and future development of predictive tools for LHON conversion.

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Random Forest Regression for Predicting Healthcare Costs using Administrative Databases from a Health Protection Agency in Northern Italy

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INTRODUCTION

Longer life expectancies and increasing prevalence of chronic diseases drive up demand for healthcare services and related costs. In Italy, 32% of people aged 65 and over, and 48% of those over 85, have major chronic conditions and multimorbidity [1]. In 2019, individuals aged 65 and over accounted for 46% of hospital admissions and 60% of pharmaceutical expenditures, highlighting the significant burden of aging on the healthcare system [2]. In terms of costs, population's segments with high prevalence of chronic conditions account for a large portion of healthcare spending [3,4,5]. Accurate predictions of future costs for the whole population and for key segments is crucial for healthcare planning.

AIMS

To predict yearly direct healthcare costs based on data of past National Health Service (NHS) resources utilization for the whole population and for high impacting segments. As a motivating example, we applied our approach to the dialysis patients' segment.

METHODS

Using administrative healthcare databases, we traced NHS resource utilization (i.e., access to inpatient and outpatient services, drug dispensations) and associated costs

for each individual aged ≥ 18 assisted by the Health Protection Agency of Bergamo (Northern Italy) between 2011 and 2023. We analyzed total cost (TC) as the sum of all services and dispensations costs, total scheduled cost (TSC) as the sum of scheduled inpatient visits, all outpatient visits and dispensations costs, and scheduled services cost (SSC) as the sum of scheduled inpatient visits and all outpatient visits costs. In the present abstract we focused on TC prediction.

We used a supervised machine learning approach, namely random forest (RF) algorithm with 500 trees, to address the prediction problem [6,7]. We trained the algorithm on the 70% of individuals' data from 2011 to 2015 ($n=815,553$) with their TC in 2016 as outcome. The 373 input variables included demographic features (such as age and sex) and NHS utilization data over the 4-years period 2011-2014 and in 2015 alone, in order to assess if 2016 cost was more associated with subjects' behavior over the preceding year or with their historic behavior. As test sets, we used the remaining 30% of the dataset (hereafter 2011-16 set) and the subsequent years' datasets (2012-17, 2013-18, 2014-19, 2015-20, 2016-21, 2017-22, and 2018-23 sets). We considered variable importance, measured as the percent increase in mean squared error (MSE) when a given variable is permuted, as a measure of each predictor's impact on the outcome.

For each test set, actual and predicted TCs for the whole population were calculated as the sum of all individuals' actual and predicted TCs, respectively. The ratio of the difference between predicted and actual population TCs to actual population TCs was used as measure of the prediction error (PE). $PE=0\%$ indicates a perfect prediction, $PE > 0\%$ or $< 0\%$ suggests overestimation or underestimation of the actual TC.

Finally, we defined dialysis patients as those who had at least one access to outpatient dialysis services. For this segment, we calculated the mean and sum of predicted and actual TCs, and PE. Also, we derived a variability interval for the mean predicted TC based on the 2.5 and 97.5 quantiles of the distribution of the mean TCs predicted by each tree for subjects included in the segment.

RESULTS

The mean actual annual population TC in the period from 2011 to 2023 was €1,023,636,867 (range: 944,632,707 – 1,111,657,382). High-cost subjects (>€15,000 yearly), accounting for less than 1% of the annual population, absorbed more than 27% of annual TC.

Top 3 most important variables in the RF were the number of outpatient accesses to dialysis over the preceding year, and the frequency of laboratory tests and outpatient services over the 4 preceding years.

Figure 1 shows the PEs calculated across all test sets, overall and in the dialysis patients' segment. Overall, PEs ranged from -3.1 to -1.9 across 2011-16 to 2014-19 sets (for 2014-19 set, actual annual population TC: €1,031,200,509; predicted annual population TC: €1,011,869,922), and widely increased from 2015-20 (range from -6.9 to 8.7; for 2015-20 set, actual annual population TC: €944,632,707; predicted annual population TC: €1,026,878,752)

For the dialysis patients' segment, the lowest PE (-0.7%) was observed in the 2011-16 set (actual mean TC: €38,536; predicted mean TC [variability interval]: €38,259 [35,542 – 41,112]), while the highest was -5.4% in the 2016-21 set (actual mean TC: €38,883; predicted mean TC [variability interval]: €36,785 [33,967 – 39,342]).

CONCLUSIONS

Using a machine learning approach, we predicted health-care TCs based on individual data of past utilization of NHS for the whole population and a high impacting segment. Predictions based on the algorithm trained on data from 2011 to 2015 were consistent until 2019, understandable given the COVID-19 pandemic in 2020. Results highlight the pandemic's impact on the model performance, leading to overestimation of the actual TC in 2020 and underestimations thereafter. Future steps include the identification of key segments and the update of the training algorithm on the subsequent years' datasets. This is a useful tool to assist HPA in resource allocation, e.g. as an integration to the monitoring of chronic diseases in the population.

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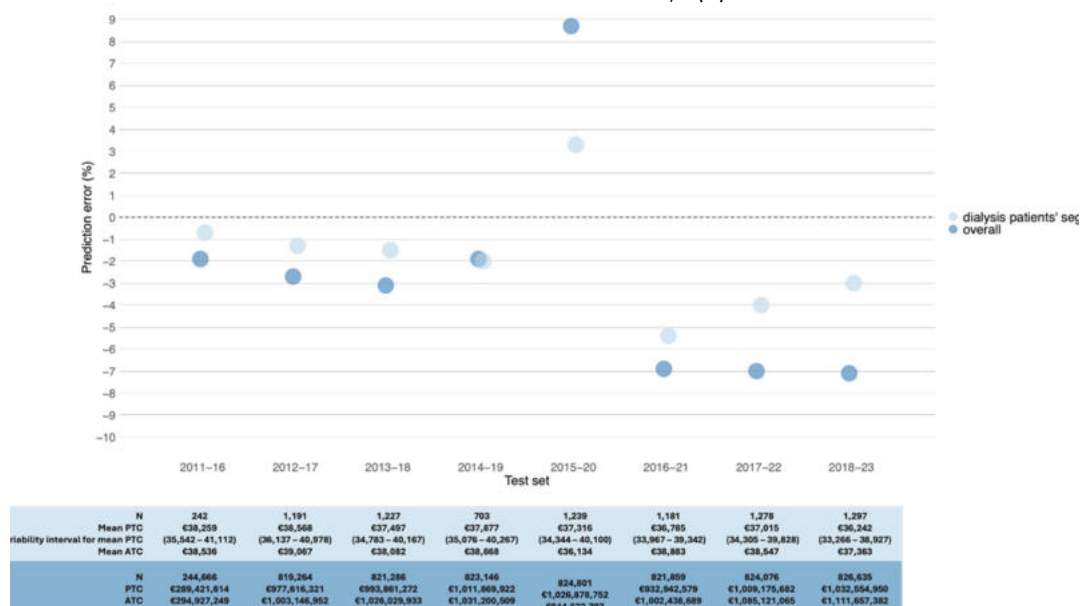


Figure 1. Prediction errors of total cost, overall (blue dots) and for the dialysis patients' segment (light blue dots) across all test sets. Abbreviations: ACT=actual total cost, PTC=predicted total cost.

A Comparison of Baseline and Time-Dependent Approaches in Cox Model

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INTRODUCTION

In clinical and epidemiological research, risk factors vary throughout the observation period. Time-dependent covariates reflect evolving exposures and provide a dynamic view of the individual. In longitudinal settings, repeated observations within a subject are typically correlated and this correlation often decreases as the time interval between measurements increases.

Handling variables that change over time can be challenging in survival analysis framework, especially when their values are influenced by the outcome process itself. This interdependence may limit their application during specific phases of the analysis [1,2]. The extended Cox model provides a robust approach to incorporate such variables under a properly specified time-dependent structure [3,4] and is widely used in epidemiological settings. However, it is common practice, especially in cohort studies, to simplify time-varying covariates by relying on baseline measurements, potentially introducing bias when assessing the instantaneous risk.

OBJECTIVES

This contribution aims to assess the impact of modeling time-varying covariates as fixed at baseline within Cox model. A series of simulations was conducted to quantify the resulting information loss and identify key factors driving the discrepancy in results between baseline and time-dependent specifications.

METHODS

Simulation data were generated for 1.000 individuals, with a single time-dependent covariate drawn from a standard multivariate normal distribution. The covariance matrix was modeled with a first-order autoregressive structure, setting the coefficient to 0.3, 0.6 and 0.9. The repeated measurements, defining the number of change-points, were evenly spaced over a fixed maximum follow-up, with the number of measures M set to 4, 8, or 16. Event times were generated using a Weibull distribution with shape parameter k set to 0.5, 1 and 2, reflecting decreasing, constant and increasing hazard rates. The covariate effect size (log-hazard) was set to 0, 0.2, 0.4 or 1. Survival and censoring times were simulated using the permutational algorithm described in [5]. The expected censoring rate was approximately 50%. Baseline and time-dependent models were implemented and compared across 1000 repetitions for each scenario. For both models, the distributions of the estimated coefficients and their difference (b , bias, and empirical statistical power were assessed over the simulation runs.

RESULTS

The highest discrimination between the models (median was observed in lower correlation and higher measurement frequency scenarios. Baseline models underestimated the true covariate effect, as shown in Table 1. Other scenarios based on a different number of measurements reflected these patterns.

Table 1. Bias of estimated coefficients from the baseline model - 8 measurements per subject

β	ρ	$\hat{\beta}$	$\kappa = 1$			$\kappa = 0.5$				$\kappa = 2$			
			Bias	Rel. Bias	$\widehat{HR}$	$\hat{\beta}$	Bias	Rel. Bias	$\widehat{HR}$	$\hat{\beta}$	Bias	Rel. Bias	$\widehat{HR}$
0.2 (HR=1.22)	0.3	0.06	-0.14	-0.68	1.07	0.13	-0.07	-0.36	1.14	0.01	-0.19	-0.93	1.01
	0.6	0.10	-0.10	-0.51	1.10	0.14	-0.06	-0.29	1.15	0.04	-0.16	-0.79	1.04
	0.9	0.16	-0.04	-0.20	1.17	0.18	-0.02	-0.09	1.20	0.13	-0.07	-0.33	1.14
0.4 (HR=1.49)	0.3	0.12	-0.28	-0.69	1.13	0.26	-0.14	-0.35	1.30	0.03	-0.37	-0.93	1.03
	0.6	0.18	-0.22	-0.55	1.20	0.29	-0.11	-0.28	1.34	0.08	-0.32	-0.81	1.08
	0.9	0.32	-0.08	-0.19	1.38	0.36	-0.04	-0.09	1.44	0.27	-0.13	-0.32	1.31

Note: mean values across the repetition were reported

Across all scenarios, correlation decrease between repeated measurements led to a progressive increase in the relative bias of the estimates. This pattern can be relieved especially in the increasing hazard rate ($k=2$) settings, meanwhile the underestimation was mitigated in decreasing hazard rate scenarios ($k=0.5$).

Moreover, baseline model power improved with higher correlation between measures; conversely, low correlation reduces power, especially when measurement frequency is high. This relationship holds across various effect sizes. Type I error remained controlled in all conditions.

CONCLUSIONS

Our study shows that relying on baseline covariates may lead to underestimation of the true association between variables and outcomes, particularly in scenarios with frequent measurements and low correlation between repeated values. In these settings, the negative bias grows, revealing that the baseline value fails to represent the trajectory of the covariate adequately and in some extreme configurations indicates a near-complete failure to detect the effect. A limitation of our work is using a simulation framework based on controlled assumptions, which may not fully capture the variability and complexity of real-world data. Future research will move towards a formalization of these relationships, while also examining broader settings and additional factors to strengthen these findings. Moreover, future efforts will focus on applying these insights and pursuing further explorations within environmental epidemiology framework, which is characterized by a large amount of data, as well as numerous challenges in individual exposure assessment that may interact in various ways with the time-dependent dynamics of exposure.

ACKNOWLEDGMENTS

This project was carried out with the technical support and funding of the Italian Ministry of Health – PNC PREV-A-2022-12376981 Aria outdoor e salute: un atlante integrato a supporto delle decisioni e della ricerca.

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Comparative Evaluation of Linear, Log-Concentration, and SCHIF Exposure–Response Functions for Estimating Attributable Deaths from Air Pollution: A Simulation Study

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INTRODUCTION

World Health Organization (WHO) has estimated that 4.2 million premature deaths were attributable to ambient (outdoor) air pollution in 2019 [1,2]. The effectiveness of regulatory actions aimed to improve air quality are frequently evaluated by forecasting the specific impacts that these measures will have on public health outcomes, such as reductions in hospital admissions or morbidity and mortality rates, following their implementation. Mainly, the regulatory actions act on long-term exposure to pollution. As consequence, the most common measure in this context is the calculus of attributable deaths (ADs) to levels of air pollution. ADs, can be expressed mathematically using the following equation:

$$\Delta D = M_0 \left(1 - \frac{1}{R(\beta, \Delta z)} \right) \times pop$$

where M_0 is the baseline mortality rate, Δz is the predicted or observed change in ambient concentrations ($z - z_0$), pop is the size of the target population, and $R(\beta, \Delta z)$ is the relative risk function of a vector of unknown parameters β . In this context, $R(\beta, \Delta z)$ denotes the ratio of the probability of an adverse event occurring over a fixed period for a population exposed to z compared to the probability if the same population were instead exposed to z_0 and it is also called exposure-response function. Generally, it is used ERF as linear shape. Nevertheless, in the last years, several studies have

shown that this association is better presented as non-linear form [3,4]. One of the first proposal for non-linear ERF consisted of considering the log of pollutant’s concentration, but in recent year new non-linear ERFs were proposed. In particular, Shape Constrained Health Impact Function (SCHIF) proposed by Nasari et al. in 2016, has the specific aim to be used for health impact assessment [5]. This model is stated on a sigmoidal relative risk shape that appropriately describe the hypothetical bound between air pollution and health’s risk.

OBJECTIVE

The aim of this work is to compare these three ERFs (linear, log-linear and SCHIF) in the calculus of ADs through a simulation study, when the relationship between air pollution and mortality is supposed to have a sigmoidal shape.

METHODS

Firstly, a large cohort study ($n = 100,000$) was generated. For each subject two covariates were considered $PM_{2.5} \sim N(15, 2.5)$ and $age \sim N(52, 5)$, aiming to reproduce a realistic scenario [4,5]. Survival times were generated using a parametric model based on an exponential distribution for both censored and uncensored observations. For the censored observations, a risk of $\lambda_c = 0.00035$ was set. For the uncensored observations, a risk of $\lambda_t = 0.0012$ was used, along with the following coefficients: $\beta = 0.03$ for $PM_{2.5}$ and $\gamma = 0.07$ for age. The coefficient β was weighted by a logistic

weighting function to obtain a sigmoidal shape. Eleven scenarios were evaluated, varying the location parameter (μ) from the 5th to the 50th percentile of the distribution of z and one based on pure logarithm shape. Therefore, ADs were calculated for each scenario, using five different ERFs: linear (L), log-concentration (Log), optimal SCHIF (O), ensemble of best three SCHIF (E3B) and ensemble of all models SCHIF (EA). The European population aged 30+ was set as population; the counterfactual scenario was based on the WHO 2021 Air Quality Guidelines ($PM_{2.5} < 5 \mu g/m^3$ per year) and the estimated β from the five different ERFs for $PM_{2.5}$ was considered. The percentage change of each ERF from the attributable deaths simulated (considered as true) was reported as the median of the 1.000 replications.

RESULTS

We observed that the performance of each ERF varies over every considered setting. O and E3B models appear to be more stable than other ERFs ($\Delta\%$ 0 to 15 and $\Delta\%$ 5 to 17, respectively) in settings based on a sigmoidal shape. If the curvature point in sigmoidal shapes happens at lower concentrations, the L model remains an acceptable approximation of the true shape, $\Delta\%$ -39 to 9 (setting 5 to 20). SCHIF O and E3B in these setting overestimate deaths, but no more than 15%. In the same scenarios the L and Log model reaches 84% and 174% of overestimates in ADs, respectively. On the other hand, the setting based on pure logarithmic shape both O and E3B models have worse performance compared to L and Log models. EA model underestimates ADs in each setting.

CONCLUSIONS

This simulation study highlights the importance of selecting an appropriate ERF when estimating ADs to air pollution. When the true risk relationship is sigmoidal, SCHIF models, O and E3O, provide more accurate estimates than L and Log models, particularly when the curve inflects at higher pollutant concentrations, while it lacks accuracy in detecting correctly ADs in case of pure logarithmic shape. Linear model remain reasonable in context when the shape inflects at low concentrations or in pure logarithmic shape. In conclusion, the adoption of non-linear models represents a significant advancement toward more accurate health impact assessments of air pollution, but more complex scenarios should be evaluated to give robustness to SCHIF results.

ACKNOWLEDGMENTS

This project was carried out with the technical support and funding of the Italian Ministry of Health – PNC PREV-A-2022-12376981 Aria outdoor e salute: un atlante integrato a supporto delle decisioni e della ricerca.

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Table 1. Attributable deaths are reported for each setting, along with percentage change with respect to simulated (true) deaths (first column). Linear (L), logarithmic (Log), optimal SCHIF (O), best three ensemble SCHIF (E3B), all models' ensemble SCHIF (EA)

Set- ting	AD	L	$\Delta(\%)$	Log	$\Delta(\%)$	O	$\Delta(\%)$	E3B	$\Delta(\%)$	EA	$\Delta(\%)$
$\mu=5$	237718	144545	-39%	223818	-6%	238701	0%	249085	5%	137025	-42%
$\mu=10$	226152	186440	-18%	284495	26%	245591	9%	249121	10%	145139	-36%
$\mu=15$	214894	207796	-3%	317079	48%	236554	10%	243606	13%	139837	-35%
$\mu=20$	204116	223069	9%	338523	66%	234393	15%	236927	16%	142806	-30%
$\mu=25$	193489	234385	21%	353349	83%	214574	11%	219462	13%	128863	-33%
$\mu=30$	183023	241854	32%	363895	99%	209881	15%	212982	16%	127838	-30%
$\mu=35$	172879	248262	44%	373684	116%	198342	15%	201901	17%	119199	-31%
$\mu=40$	162825	254148	56%	380610	134%	182839	12%	184422	13%	108112	-34%
$\mu=45$	152727	258234	69%	385618	152%	165088	8%	166401	9%	94293	-38%
$\mu=50$	142272	261652	84%	389836	174%	158699	12%	159890	12%	90244	-37%
log(z)	102441	63474	-38%	97603	-5%	45762	-55%	46169	-55%	18849	-82%

Combined Antiresorptive and Anabolic Drug Approach in Osteogenesis Imperfecta Zebrafish Models: A Geometric Morphometrics and Shape Analysis Perspective

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INTRODUCTION

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder primarily affecting type I collagen biosynthesis, leading to brittle bones and skeletal deformities. Current pharmacological strategies provide only partial therapeutic benefits. Recent preclinical efforts have explored combined antiresorptive and ER-stress modulating therapies. Zebrafish (*Danio rerio*) models of OI, such as chihuahua (*col1a1a* mutant) and *p3h1* knockout lines, mirror key features of human disease and allow high-throughput skeletal phenotyping. Traditional microCT parameters, while useful, fail to capture spatial morphological changes. Geometric morphometrics (GM) enables the quantification of shape variation, offering higher sensitivity in detecting treatment effects and genotype-specific phenotypes [1]. Analyzing biological shape under experimental conditions requires statistical tools that go beyond traditional size or volume measurements.

OBJECTIVES

To develop and test a reproducible statistical pipeline for assessing shape variation and treatment effects in OI zebrafish models using 2D landmark data, with a focus on the methodological robustness of Procrustes ANOVA under small-sample conditions and the use of simulated data for model validation.

METHODS

The study used vertebral landmark data (10 2D landmarks per vertebra, three vertebrae per individual) collected from wild-type and mutant zebrafish (*col1a1a* and *p3h1*), each exposed to four treatments (control, 4PBA, ALN, combined). We performed Generalized Procrustes Analysis (GPA) to align configurations and remove non-shape variation. To assess treatment and genotype effects, we fitted Procrustes linear models (*procD.lm*) with interaction terms and permutation-based ANOVA ($n=3000$) [2,3]. The small sample size was addressed through simulation of shape data using multivariate normal distributions based on the empirical mean and covariance structure for each group [4]. Additionally, Partial Least Squares (PLS) was used to investigate covariation between shape and treatment conditions [5]. PCA was used for visualization of simulated vs observed data in tangent space. Differences between real and simulated data were evaluated visually, verifying that shape variance was adequately captured.

EXPECTED RESULTS

We anticipate that genotype will significantly influence vertebral shape across all types, consistent with known skeletal phenotypes in *col1a1a* and *p3h1* mutants. Treatment effects are expected to be more localized, with the combined antiresorptive and chaperone therapy potentially producing intermediate or synergistic changes in specific vertebral re-

gions. Simulated datasets will allow us to confirm the statistical stability of Procrustes-based models under small-sample conditions, reducing the risk of false negatives and supporting experimental design optimization.

CONCLUSIONS

This work illustrates a flexible and statistically sound framework to analyze shape data in biological models with limited sample sizes. Simulation of landmark configurations allowed us to confirm inference robustness and guide experimental design. Geometric morphometrics, when paired with Procrustes ANOVA and multivariate simulation, can detect subtle effects and validate assumptions, providing a methodological advance for studies in morphometrics, imaging, and developmental biology.

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Improving Calibration Assessment near Clinical Thresholds: The Bayesian Calibration Error

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INTRODUCTION

Calibration of predictive models is essential to ensure the clinical reliability of risk estimates, particularly when decisions are based on well-defined probability thresholds. However, especially in machine learning (ML) applications, calibration is often overlooked, and model performance is typically evaluated using discrimination metrics alone [1,2]. Several calibration metrics have been proposed, including the Brier Score, Expected Calibration Error (ECE), Maximum Calibration Error (MCE), and Integrated Calibration Index (ICI). Each of these has limitations: for example, the Brier Score reflects a global average and may mask local errors; ECE and MCE are highly sensitive to binning strategies and become unstable with limited data; the ICI, while more robust, does not focus specifically on clinically relevant thresholds [3–5]. As a result, these metrics may fail to detect or emphasize calibration errors in the areas most critical for clinical decision-making.

OBJECTIVES

To introduce the Bayesian Calibration Error (BCE), a metric that quantifies both the magnitude and concentration of miscalibration around a clinically relevant threshold, and to evaluate its use alongside the Absolute Calibration Error (ACE).

METHODS

BCE integrates three components: (i) quantile-based adaptive binning, (ii) a Bayesian formulation to estimate local calibration error (LCE), which accounts for the number of events in each bin rather than relying solely on observed

proportions, and (iii) a Gaussian weighting function centered around the decision threshold t . For each bin i , the mean predicted probability p_{pred_i} is compared with the expected value of the observed frequency, modeled using a non-informative $\text{Beta}(1,1)$ prior. The posterior distribution becomes $\text{Beta}(\alpha_{post} = 1 + k, \beta_{post} = 1 + n - k)$, where k is the number of events and n is the number of observations in the bin. The local calibration error (LCE) is then defined as:

$$LCE_i = |p_{pred_i} - E[\text{Beta}(1 + k, 1 + n - k)]|.$$

After defining a decision threshold t (i.e., a predicted probability associated with a clinical “action”), derived through decision curve analysis and/or clinician input, a Gaussian weight is assigned to each bin:

$$w_i = \exp\left(-\frac{(p_{pred_i} - t)^2}{2\sigma^2}\right),$$

where σ (e.g., 0.1) controls the concentration around the threshold. Weights are normalized to have unit mean. BCE is then computed as the weighted average of the LCEs. A high BCE indicates that miscalibration is particularly concentrated around the threshold.

We applied this approach to a dataset of 3,672 pregnant women carrying small-for-gestational-age (SGA) fetuses, enrolled in the TRUFFLE 2 multicenter study. Three predictive models were developed—Logistic Regression (LR), Random Forest (RF), and XGBoost—using 11 routine clinical variables to predict adverse perinatal outcomes. The decision threshold was set at $t = 0.3$ based on prior decision analyses.

RESULTS

The incidence of adverse outcomes was 13%. ACE confirmed the same performance ranking across models (LR: 0.0198, RF: 0.1126, XGBoost: 0.2290). However, BCE imposed a stricter penalty on RF (BCE = 0.1916) and an even higher one on XGBoost (BCE = 0.2633), indicating that miscalibration was concentrated around the decision threshold. Although the RF model showed a more pronounced local peak of miscalibration, XGBoost had a broader spread of error in bins adjacent to the threshold, resulting in a higher overall BCE. Conversely, the LR model maintained a low BCE (0.0216), suggesting good local calibration.

CONCLUSIONS

BCE complements global calibration metrics by quantifying whether miscalibration is concentrated around the clinical decision threshold. While ACE reflects the average accuracy of risk estimates across the entire prediction range, BCE captures local consistency near the threshold, offering a more nuanced evaluation. This distinction is particularly important in clinical contexts, where decisions hinge on specific risk cut-offs. When a clinical “action” threshold is defined, we recommend reporting both ACE and BCE to support informed model assessment. Moreover, BCE enables identification of models that, despite satisfactory global calibration, underperform near the decision threshold—and conversely, models with less favorable global performance that maintain adequate reliability in clinically critical regions.

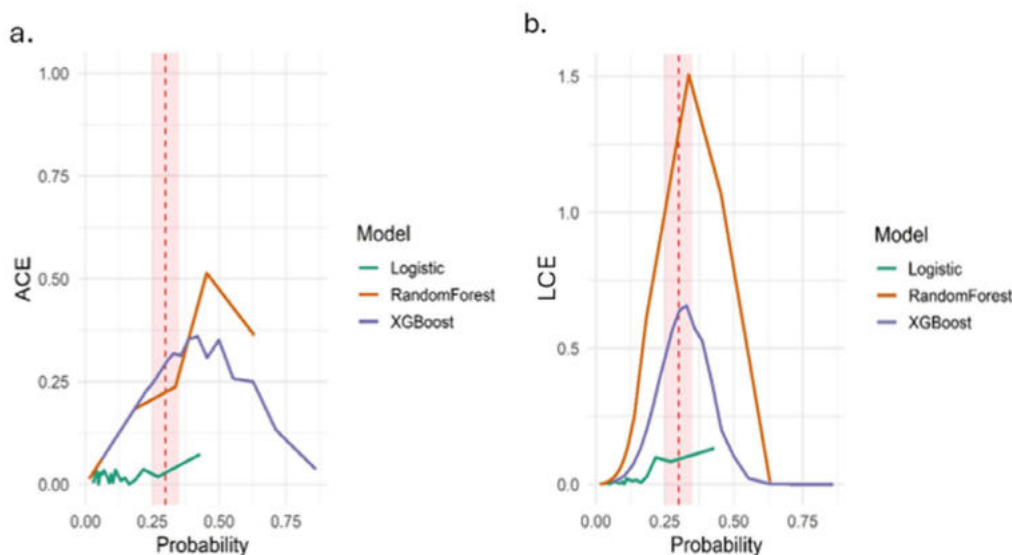
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Figure 1. Absolute Calibration Error (ACE) and (b) Local Calibration Error (LCE), weighted by proximity to the decision threshold. The red dashed line indicates the $t=0.3$ decision threshold, with the shaded red area representing the critical region defined by $\sigma=0.1$



Integrating Calibration into the Evaluation of Clinical Utility: A Proposal for a Weighted Net Benefit

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INTRODUCTION

Decision Curve Analysis (DCA) is a widely used framework for evaluating diagnostic and prognostic strategies, as it explicitly incorporates the clinical consequences associated with decision-making [1]. Within this framework, Net Benefit (NB) is a key indicator of the clinical utility of a predictive model. However, the original NB formulation does not account for model calibration, despite strong evidence that poor calibration systematically reduces clinical utility [2]. In real-world settings, many predictive models, particularly those based on machine learning (ML), often show suboptimal global calibration compared to traditional statistical models, due to their greater complexity and susceptibility to overfitting.

Therefore, when focusing on a specific probability threshold as a decision point, it becomes crucial to evaluate calibration in the vicinity of that threshold. This targeted approach may lead to a different comparative assessment of the clinical potential of various predictive algorithms.

OBJECTIVES

To propose a pragmatic extension of DCA based on the concept of Weighted Net Benefit (WNB), in which a model's utility is penalized more heavily for calibration errors in the decision-making region. This avoids discarding models that, while globally less calibrated, are reliable near the clinical threshold.

METHODS

The framework involves four steps:

- i) the decision threshold is defined a priori through DCA and/or clinical consultation;
- ii) calibration is assessed around the threshold using the Bayesian Calibration Error (BCE);
- iii) the weighted posterior standard deviation (wSD) is computed to quantify the statistical uncertainty associated with local calibration error (LCE) estimates;
- iv) NB at the predetermined threshold is adjusted according to the following formula:

$$WNB = \frac{1}{1+BCE+wSD} NB.$$

Specifically, the BCE is defined as the weighted mean of local calibration errors (LCE), calculated as the absolute difference between the average predicted probability in each bin and the Bayesian estimate of the observed event rate, computed as the posterior mean of a Beta(1 + k, 1 + n - k) distribution, where k is the number of events and n the total number of observations in the bin. Each LCE is weighted using a Gaussian function centered on the decision threshold, thus emphasizing calibration errors in clinically critical regions.

The wSD is calculated as the weighted mean of the standard deviations of the posterior Beta distributions in each bin, using the same weighting function. This penalizes models not only for local miscalibration but also for greater

statistical uncertainty in the estimation of calibration error near the decision threshold [3]. In this way, the WNB provides a more cautious and context-aware estimate of clinical utility, allowing recognition of models that, despite suboptimal global calibration, provide reliable predictions around the decision threshold.

We applied this framework to a clinical dataset of 3,672 pregnancies with small-for-gestational-age fetuses collected in the multicenter TRUFFLE 2 study. Three predictive models—logistic regression (LR), Random Forest (RF), and XGBoost—were developed using 11 clinical variables to predict adverse perinatal outcomes. NB and WNB were calculated at the decision threshold $t = 0.3$.

Additionally, to assess prediction instability, NB and WNB were computed as the mean and standard deviation over 500 bootstrap replicates at the clinical threshold.

RESULTS

The incidence of adverse outcomes was 13%.

RF achieved the best discrimination after bootstrap optimism correction (AUROC = 0.91, 95% CI: 0.85–0.94), while LR showed the poorest performance (AUROC = 0.71, 95% CI: 0.67–0.75). XGBoost had intermediate performance (AUROC = 0.83, 95% CI: 0.74–0.88).

At the threshold $t = 0.3$, LR showed a low NB (0.02 ± 0.001) and an even lower WNB (0.01 ± 0.001), reflecting limited clinical utility at the threshold despite near-optimal global calibration.

RF yielded the highest NB (0.08 ± 0.002), though it received the strongest penalty (WNB = 0.04 ± 0.001), while XGBoost displayed intermediate behavior (NB = 0.05 ± 0.003; WNB = 0.03 ± 0.002).

These results suggest that, when discrimination is high, even a model with suboptimal calibration may retain clinical utility—if the expected decision quality near the critical region compensates for calibration penalties.

Low variability across bootstrap samples ($SD \leq 0.003$) indicates that both NB and WNB are highly stable around the clinical threshold $t = 0.3$ for all models (Figure 1).

CONCLUSIONS

The WNB offers a tailored evaluation of the clinical utility of predictive models by incorporating local calibration information near the decision threshold.

This approach is not intended to replace traditional DCA but rather to serve as a methodologically coherent extension—particularly relevant when comparing predictive algorithms of varying complexity in terms of calibration performance.

In clinical contexts where decisions are based on well-defined thresholds, WNB can support more informed, reliable, and decision-centered evaluations.

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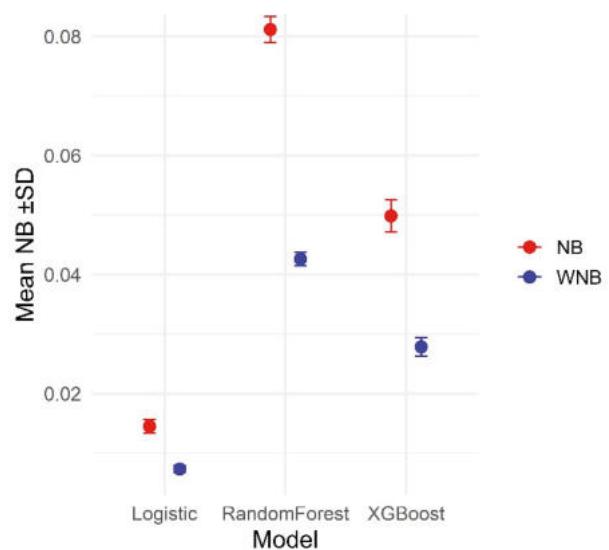


Figura 1. Media e deviazione standard di NB e WNB nei 500 campioni di bootstrap per modello

Index of Abstracts by Author

Anelli Manuela, Ferraro Ottavia Eleonora, Celebrin Mattia, Morandi Anna, Montomoli Cristina	14	Giometto Sabrina, Reilev Mette, Thomsen Ernst Martin, Støvring Henrik, Pottegård Anton	51
Motsa Tengetile, Msibi Velaphi, Sikhondze Mzamo, Nhleko Bongani Alex, Niemann Nicole Rose, Fappani Clara, Gori Maria, Colzani Daniela, Villa Simone, Angelone Paola, Baldi Sante Leandro, Valentini Enrica, Maphalala Gugu, Buthelezi Gcinile, Camilla Torriani, Assefa Mubirhan Alemayohu, Monti Maria Cristina, Amendola Antonella, Tanzi Elisabetta, Raviglione Mario	16	Giusti Emanuele Maria, Ferrario Marco Mario, Veronesi Giovanni	52
Arena Stefania, Simeon Vittorio, Sieri Sabina, Agnoli Claudia, Ricceri Fulvio, Milani Lorenzo, Bendinelli Benedetta, Assedi Melania, Tumino Rosario, Panico Salvatore, Pasanisi Fabrizio, Pollastro Carla, Signoriello Simona, Fordellone Mario, Chiodini Paolo	18	Guaita Arianna, Romano Carolina, Minelli Giada, Binazzi Alessandra, Marinaccio Alessandro, Mensi Carolina, Zona Amerigo, Fazzo Lucia	54
Barbieri Giulia, Naixin Zhang, Marta Trius-Soler, Cristian Pattaro, Marta Guasch-Ferré, Hantikainen Essi Marjatta	21	Iommi Marica, Faragalli Andrea, Fontanarosa Alessandro, Pompili Marco, Carle Flavia, Barbiano di Belgiojoso Elisa, Skrami Edlira, Gesuita Rosaria on behalf of the MIGHTY Project working group	56
Barchitta Martina ^(1,2) , Maugeri Andrea, La Mastra Claudia ⁽¹⁾ , Cortese Noemi ⁽¹⁾ , Enea Marco, Maniscalco Laura, Matranga Domenica, Agodi Antonella ⁽¹⁾	23	A. Ippolito, V. Grieco, A. Romano, MA Aiello, A. Torrisi, A. Di Prima, C. Chillari, G. Giorgianni, R. Ragusa	59
Bartolomeo Nicola, Lorusso Letizia, Trerotoli Paolo	25	Lacalaprice Doriana, Scarsi Nicolò, Gianfagna Francesco, Paladini Andrea, Boccia Stefania, Pastorino Roberta	61
Belli Annalisa, Gobbi Erica, Amatori Stefano, Preti Antonio, Perroni Fabrizio, Rocchi Marco Bruno Luigi, Sisti Davide	27	Lentini Nicolò, Russo Luigi, Giacobini Erika, Osti Tommaso, Pastorino Roberta, Boccia Stefania	64
Bertuccio Paola, Vigezzi Giacomo Pietro, Mosconi Giansanto, Odone Anna	29	Lodico Manuela, Maniscalco Laura, Miceli Silvana, Enea Marco and Matranga Domenica	66
Sartini Marina, Carbone Alessio, Oliva Martina, Pessano Sara, Del Puente Filippo, Buttiron Webber Tania, Schinca Elisa, Ottria Gianluca, Dupont Chiara, Piccinini Carolina, Cristina Maria Luisa	31	Letizia Lorusso, Nicola Bartolomeo, Paolo Trerotoli	68
Centola Carmela, Iommi Marica, Faragalli Andrea, Fontanarosa Alessandro, Pompili Marco, Barbiano di Belgiojoso Elisa, Carle Flavia, Skrami Edlira, Gesuita Rosaria on behalf of the MIGHTY Project working group	33	Giometto S., Convertino I., Pellegrini G., Campelo R., Limoncetta G., Spini A., Massari M., Spila-Alegiani S., Carrasquinha E., Tuccori M., Trifirò G., Lucenteforte E., on behalf of the VALORE Study Group.	71
Cofini Vincenzo, Benvenuti Eugenio, Mancinelli Martina, Di Pangrazio Ginevra, Piccardi Laura, Muselli Mario, Cimino Eleonora, Tobia Loreta, Fabiani Leila, Necozione Stefano	35	Mannocci Alice, Capece Guendalina, David Shaholli, Francesco Belsito, Frantellizzi Ilaria, Gerace Bruno, Guarente Lombardo Floranna, Prataciera Jessica, Quesada Ramos Mren, Sarcoli Claudia, Duraturo Ginevra, Monteduro Antonietta, La Torre Giuseppe	73
Di Maria Gabriele, Pennisi Grazia, Wong Vincent Wai-Sun, de Ledinghen Victor, Sebastiani Giada, Viganò Mauro, Fracanzani Anna Ludovica, Miele Luca, Bugianesi Elisabetta, Ekstedt Mattias, D'Ambrosio Roberta, Ravaoli Federico, Schepis Filippo, Marra Fabio, Aghemo Alessio, Svegliati-Baroni Gianluca, Persico Marcello, Valenti Luca, Berzigotti Annalisa, George Jacob, Armandi Angelo, Nasr Patrik, Kechagias Stergios, Liguori Antonio, Saltini Dario, Mendoza Yuly P., Calvaruso Vincenzo, Lin Huapeng, Infantino Giuseppe, Masarone Mario, Pugliese Nicola, Tulone Adele, Di Marco Vito, Cammà Calogero, Petta Salvatore, Enea Marco	37	Tagliaferro Sofia, Greco Riccardo, Uggetti Lucia, Marchetti Pierpaolo, Locatelli Francesca, Avesani Linda, Marcon Alessandro	75
Dipol Teresa, Zazzara Maria Beatrice, Damiano Cecilia, Costanzo Simona, Palmieri Luigi, Di Castelnuovo Augusto, Donfrancesco Chiara, Lo Noce Cinzia, Magnacca Sara, Marcozzi Benedetta, Casoli Tiziana, Picca Anna, Iacoviello Licia, Vetrano Davide Liborio, Onder Graziano	39	Marinoni Carlo, Caravita Simona Carla Silvia, Zanetti Maria Assunta, Villani Simona, Torriani Camilla	76
Ferraro Ottavia Eleonora, Morandi Anna, Anelli Manuela, Grassi Federico, Ciprian Viviana, Ansaloni Luca, Frassini Simone, Perlini Stefano, Montomoli Cristina	42	Giuseppe Martone, Antonio Bosco, Fabrizio Maturò, Mirella Santaniello, Giuseppe Cringoli, Laura Rinaldi, Alice Mannocci	78
Filosa A., Franchi M., Costantino M.A., Borgatti R., Mensi M.M., Fazzi E., Galli J., Poli V., Leoni O., Limosani I., Reale L.A., Corrao G., Montomoli C.	44	Migliazzo Claudia, Lodico Manuela, Allegra Patrizia, Maniscalco Laura, Tarantino Domenico, Piccoli Tommaso, Vanacore Nicola, Salemi Giuseppe, Matranga Domenica	80
Alessandro Fontanarosa, Flavia Carle, Roberta Papa, Marco Pompili, Giovanni Corrao, Damià Valero-Bover, Josep Roca, Rubén González-Colom, Edlira Skrami	46	Moirano Giovenale	82
Giangrande Patrizia, Fornili Marco, Tramonti Fantozzi Maria Paola, Bardelli Angela, De Vita Erica, Tavoschi Lara, Baglietto Laura on behalf of RISE-Vac consortium.	49	Mazzucco Riccardo, Narcisi Selene, Picasso Erica, Spatarì Gloria, Caltagirone Luca, Carolina Pelazza, Antonio Maconi, Pacileo Guglielmo, Bertolotti Marinella	84
		Valeria Nazzari, Albert Navarro Gallinad, Luisa Zuccolo	86
		Andrea Nova, Teresa Fazio, Giovanni Di Caprio, Davide Gentilini, Luisa Bernardinelli, Roberto Bergamaschi	88
		Das Aditi, Victoria Lee Hampel, Paola Zaninotto, Palla Luigi	90
		Bartoszek Karolina, Palla Luigi	92
		Sartini Marina, Pessano Sara, Alessio Carbone, Martino Oliva, Gianluca Ottria, Cristina Maria Luisa	95

Ponzano Marta, Signori Alessio, Landi Doriana, Bellavia Andrea, Schiavetti Irene, Lavorgna Luigi, Di Sapia Alessia, Fantozzi Roberta, Marinelli Fabiana, Marfia Girolama Alessandra, Sormani Maria Pia	97	Patel Linia, Mignozzi Silvia, Pizzato Margherita, La Vecchia Carlo, Alicandro Gianfranco	143
Ponzo Michela, Di Antonio Federica, Salivetto Marco, Paletta Pasquale, Terzuoli Federica, Battaglia Mario Alberto, Manacorda Tommaso	99	Calvella Alice Sofia, Navarro-Gallinad Albert, Cuccuru Gianmauro, Giambartolomei Claudia, Di Angelantonio Emanuele, Repetto Linda, Zuccolo Luisa	146
Paola Pregel, Benedetta Sensini, Lucia Minelli, Leonardo Leonardi, Elisabetta Manuali, Paola Borrelli	102	Scarmignan Roberta, Iannuzzella Francesco, Solazzo Andrea, Camparini Luca, Donati Gabriele, Gregorini Mariacristina	148
Puliani Natascia, Iommi Marica, Faragalli Andrea, Fontanarosa Alessandro, Pompili Marco, Barbiano di Belgiojoso Elisa, Carle Flavia, Skrami Edlira, Gesuita Rosaria, on behalf of the MIGHTY Project working group	104	Coluccia Sergio, Bianco Rachele, Marinoni Michela, Falcon Alex, Fiori Federica, Serra Giuseppe, Ferraroni Monica, Parpinel Marial, and Edefonti Valeria	150
Rota Matteo, Ceretti Elisabetta, Viola Gaia Claudia Viviana, Marullo Monica, Zani Danilo, Lorenzetti Stefano, Montano Luigi, Calza Stefano, Donato Francesco, FASt study group	107	Adriano De Simone, Mauro Padda, Dario Bruzzese & Daniela Pacella	153
Tomlinson Eve, Holmes Jude, Rutjes Anne W.S., Davenport Clare, Leeftang Mariska M., Yang Bada, Mallett Sue, Whiting Penny	109	Di Gennaro G., Roberti R., Politi C., Russo E., COMPARE Study Group	156
Whiting Penny, Tomlinson Eve, Yang Bada, Davenport Clare, Leeftang Mariska M., Mallett Sue, Rutjes Anne W.S.	111	Faragalli Andrea, Franceschi Roberto, Marigliano Marco, Cherubini Valentino, Gesuita Rosaria, on behalf of the Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology	158
Rutjes Anne W.S., Lachi Alessio, Holmes Jude, Tomlinson Eve, Leeftang Mariska M. G., Whiting Penny, Mallett Sue, Davenport Clare	113	Teresa Fazio, Andrea Nova, Giovanni Di Caprio, Davide Gentilini, Luisa Bernardinelli	161
Rutjes Anne W.S., Lachi Alessio, Holmes Jude, Tomlinson Eve, Leeftang Mariska M. G., Whiting Penny, Mallett Sue, Davenport Clare	115	Franchi Matteo, Gennari Marco, Severgnini Gaia, Biancari Fausto, Bonomi Alice, De Marco Federico, Polvani Gianluca, Agrifoglio Marco	163
Savoia Cosimo, Ciardullo Silvia, Mandolini Donatella, Nardone Paola	117	Giulia Gambini, Eleonora Fresi, Annalisa De Silvestri, Valeria Musella, Valeria Scotti, Virginia Valeria Ferretti, Catherine Klersy	165
Scarale Maria Giovanna, Marino Selenia, Esposti Federico, Di Serio Clelia	119	Gandin Ilaria, Dalena Paolo, d'Adamo Pio, Toffoletti Eleonora, Sinagra Gianfranco, Dal Ferro Matteo	167
Scotti Lorenza, Cena Tiziana, Rossi Maicol, Faggiano Fabrizio	121	Bertolina Carlotta, Erra Stefania, Mazzucco Riccardo, Giacchera Fabio, Biasion Sara, Maconi Antonio, Bertolotti Marinella	169
Skrami Edlira, Iommi Marica, Faragalli Andrea, Fontanarosa Alessandro, Pompili Marco, Barbiano di Belgiojoso Elisa, Carle Flavia, Gesuita Rosaria on behalf of the MIGHTY Project working group	123	Girauda Maria Teresa, Milani Lorenzo, Padroni Lisa, Sieri Sabrina, Agnoli Claudia, Simeon Vittorio, Fordellone Mario, Ricceri Fulvio, Sacerdote Carlotta	171
Speranza Teresa, Simeon Vittorio, Ricceri Fulvio, Gentile Marco, Santucci de Magistris Maria, Pisanisi Fabrizio, Caranci Nicola, Fordellone Mario, Signoriello Simona, Panico Salvatore, Chiodini Paolo	126	Lazzarini Stefano Giuseppe, Martella Donato, Zambon Antonella	174
Torriani C., Scirocco T., Perrone P.M., Angelone P., Maugeri M., Cereda D., Gori A., Monti M.C., Raviglione M.	129	Giorgio Limoncella, Giacomo Emmi, Emanuele Chiara, Marta Calatoni, Gabriella Moroni, Ersilia Lucenteforte	177
Torrighani Ginevra, Baviera Marta, Zambon Antonella	131	Malanima Marco Andrea, De Carlo Marco, Temporelli Luigi, Fornili Marco, Baglietto Laura, De Caterina Raffaele, on behalf of the MASTER Study Group	179
Trerotoli Paolo, Fortunato Francesca, Prato Rosa, Martinelli Domenico, Lorusso Letizia, Bartolomeo Nicola	133	Maniscalco Laura, Zenesini Corrado, Picariello Roberta, Cascini Silvia, Tarantino Domenico, Lodico Manuela, Miglizzo Claudia, Acampora Anna, Fantaci Giovanna, Ribauda Michele, Profili Francesco, Francesconi Paolo, Bargagli Anna Maria, Nonino Francesco, Gnani Roberto, Piccoli Tommaso, Vanacore Nicola, Salemi Giuseppe, Matranga Domenica	181
Veronesi Giovanni, Giusti Emanuele Maria, Loffredo Daniele, Campana Marco, Gianfagna Francesco, Ferrario Marco Mario	135	Zanoni Andrea, Marchetti Pierpaolo, Vesentini Roberta, Guerzoni Giacomo, Sacco Michele, Lauro Enrico, Carrara Alessandro, Giacomuzzi Simone, Giuseppe Verlato, on behalf of SISME (Società Italiana per lo Studio delle Malattie dell'Esofago)	183
Avellone Alessandra, Barbiano di Belgiojoso Elisa, Berta Paolo, Chiodini Paola Maddalena, Pescini Dario, Rimoldi Stefania M.L., Romio Silvana, Solaro Nadia, Zambon Antonella, MIGHTY group	137	Domenica Matranga, Michele Malagola, Luca Castagna, Domenica Russo, Martino Massimo	184
Adamoli Arianna, Orenti Annalisa, Gambazza Simone, Zolin Anna, Ambrogio Federico, De Rose Virginia, on behalf of ECFSPR Steering Group	139	Wang Stella, D'Elia Yuri, Gögele Martin, Blankenburg Hagen, Hopfner Franziska, Kühlenbäumer	
Alemayohu Mulubirhan Assefa, De Giuseppe Rachele, Bertoli Gloria, Ercoli Vittoria, Al-Naqeb Ghanya, Ballante Elena, Bergamaschi Roberto, Camboni Tania, Ceccarani Camilla, Ceriani Chiara, Colombo Elena, Consolandi Clarissa, Costabile Francesca, De Cesari Stefano, Filosa Asia, Gallivanone Francesca, Galuzzi Bruno Giovanni, Gastaldi Matteo, Gervasoni Clarissa, Gugliatti Simona, Itri Teresa, Kalmpourtzidou Alik, Landi Tony, Lanzotti Aurora, Lo Dico Alessia, Loperfido Federica, Maccarini Beatrice, Mazza Antonio, Montomali Cristina, Oddone Enrico, Pellizzer Chiara, Perneti Roberta, Porro Danilo, Scarabotto Anna, Sellaro Francesca, Severgnini Marco, Summa Donato, Tagliaferri Sofia, Tani Flaminia, Torriani Camilla, Vivone Gemine, Cena Hellas, Tavazzi Eleonora and Monti Maria Cristina	141	Gregor, Pramstaller Peter P., Kaat Aaron, Gershon Richard C., Pattaro Cristian, Melotti Roberto	187
		Merlo Federico, Conforti Fabio, Bagnardi Vincenzo	190
		Nicolodi M., Malerba G., Treccani M., Lando V., Casillo L., Margagliotti A., Calciano L., Bombieri C., Locatelli F., Marchetti P., Gariazzo C., Maio S., Stafoggia M., Viegi G., Marcon A., Accordini S.	192
		Orenti Annalisa, Naehrlich Lutz, Bakkeheim Egil, Hatzigeorgou Elpis, Kerem Eitan, Sermet-Gaudelus Isabelle on behalf of ECFSPR Steering Group	194

Tina Pasciuto, Francesca Moro, Drieda Zace, Lidia Borzi, Katuscia Patrizi, Roberta Di Battista, Francesca Ciccarone, Floriana Mascilini, Elena Teodorico, Giulia Zinicola, Maria Luisa Di Pietro, Giovanni Scambia, Antonia Carla Testa	196	Carmisciano Luca, Boschi Tobia, Delmastro Franca, Poggio Francesca, Giuliano Mario, Arpino Grazia, De Laurentiis Michele, Gravina Adriano, Rimanti Anita, Turletti Anna, Nisticò Cecilia, Magnolfi Emanuela, Gasparro Simona, Fabi Alessandra, Garrone Ornella, Alicicco Maria Grazia, Urracci Ylenia, Poletti Paola, Correale Pierpaolo, Puglisi Fabio, Lambertini Matteo, Molinelli Chiara, Vandin Andrea, Chiaromonte Francesca, Boni Luca, Blondeaux Eva, Del Mastro Lucia	240
Patrizio Pasqualetti, Claudia Altamura, Luisa Fofi, Marilena Marcosano, Giulia Bellini, Luigi Francesco Iannone, Marina Romozzi, Sabina Cevoli, Giancamillo Manzoni, Fabrizio Vernieri	199	Martina Carnevale, Elisa Caselani, Cristina Zarbo, Alessandra Martinelli, Donato Martella, Giovanni de Girolamo, DiAPAsen Collaborators and Stefano Calza	243
Pastorino Roberto, Russo Luigi, Proto Luca, Galarducci Riccardo, Mazzucco Walter, Agodi Antonella, De Waure Chiara, Severino Anna, Liuzzo Giovanna, Boccia Stefania	202	Cartocci Alessandra, Luschi Alessio, Tognetti Linda, Iadanza Ernesto, Rubegni Pietro	245
Petracci Elisabetta, von Braun Marie-Sophie, Prochowski Andrea Iamurri, Stella Franco, Aramini Beatrice, Canale Matteo, Urbini Milena, Delmonte Angelo, Christian Martin, Ulivi Paola	204	Chiavenna Chiara, Presanis Anne M., Charlett Andre, Ladhani Shamez, De Angelis Daniela	248
Sacco Davide, De Marco Cesare, Calzari Lucian, Villafuerte Francisco, Zanotti Lucia, Parati Gianfranco, Bilo Grzegorz, Gentilini Davide	206	Ciniselli Chiara Maura, Duroni Valeria, Blanda Adriana, Bernardo Giancarla, Licata Armando, De Cecco Loris, Sfondrini Lucia, Tagliabue Elda, Sozzi Gabriella, Verderio Paolo	250
Claudia Santucci, Aimore Giugni, Greta Carrara, Arturo Chierogato, Akos Csomos, Joanne M. Fleming, Lorenzo Gamberini, Primoz Gradisek, Rafael Kaps, Theodoros Kyprianou, Isaac Lazar, Elisa Marchionni, Malgorzata Mikaszewska-Sokolewicz, Marco Madaghiele, Giulia Paci, Carlotta Rossi, Fabiola Signorini, Nektaria Xirouchaki, Giovanni Nattino, Guido Bertolini, for the CReACTIVE consortium	208	Cuoghi Costantini Riccardo, Nuredini Andi, Pini Sara, Albano Noemi, Spano Maria Carlotta, D'Amico Roberto, Tupler Rossella	252
Sartini Marina, Silvia Boni, Alessio Carbone, Martino Oliva, Sara Pessano, Fedra Guastalli, Mattia Bruzzo, Gianluca Ottria, Cristina Maria Luisa	212	Dattola Serena, Ielo Augusto, Quartarone Angelo, De Cola Maria Cristina	255
Soranna Davide, Malfatto Gabriella, Parati Gianfranco, Zambon Antonella	214	Di Gennaro Piergiacomo, Fordellone Mario, Nicolao Giovanni, Schiattarella Paola, Smimmo Annafrancesca, Speranza Teresa, Simeon Vittorio, Signoriello Simona, Chiodini Paolo	258
Tarantino Barbara, Gentilini Davide	216	Matteo Di Maso, Monica Ferraroni	260
Zanzi Riccardo, Orenti Annalisa, Zolin Anna, Mei-Zahav Meir, Prais Dario, Gambazza Simone on behalf of ECFSPR Steering Group	218	Di Palma Michele Pio, Galli Francesca, Rulli Eliana	263
Ambrogio Arianna, Panetta Valentina, Folcarelli Silvia, Piras Marina, Diomedes Marina, Maestrini Ilaria	220	Di Staso Rossana, Gianicolo Emilio	265
Giulia Nicole Baldrighi(1, 2, 3, 4), Markus Matilainen(1, 2, 3), Maija Saraste(1, 2, 3, 5), Laura Airas(1, 2, 3, 5)	223	Diedenhofen Giacomo, Catania Danilo, Urso Antonino, Brandoni Giordano, Duranti Giorgia, Randazzo Maria Pia, Baglio Giovanni, Vestri Annarita, Di Marco Marco, Cicala Stefano Domenico	267
Federico Turaldo, Giulia Barbatì	225	De Vito Roberto, Stephenson Briana, Sotres-Alvarez Daniela, Siega-Riz Anna-Maria, Mattei Josiemer, Parpinel Maria, Peters Brandilyn, Bainter Sierra A., Daviglus Martha L., Van Horn Linda, Edefonti Valeria	269
Bendotti Sara, Primi Roberto, Raone Luca, Ferlini Marco, Currao Alessia, Bongiorno Andrea, Fava Cristian, Ghiraldin Daniele, Dossena Cinzia, Baldo Andrea, Maffeo Diego, Kajana Vilma, Affinito Silvia, Ulmanova Leila, Baldi Enrico, Savastano Simone	227	Enea Marco	272
Bendotti Sara, Klersy Catherine, Primi Roberto, Pontremoli Silvia Miette, Citterio Bianca, Gianino Elisa, Paggi Margherita, Malinverni Stefano, Currao Alessia, Ulmanova Leila, Baldi Enrico, Savastano Simone	229	Ercoli Vittoria, De Giuseppe Rachele, Bertoli Gloria, Alemayohu Mulubirhan Assefa, Al-Naqeb Ghanya, Ballante Elena, Bergamaschi Roberto, Camboni Tania, Ceccarani Camilla, Ceriani Chiara, Colombo Elena, Consolandi Clarissa, Costabile Francesca, De Cesari Stefano, Filosa Asia, Gallivanone Francesca, Galuzzi Bruno Giovanni, Gastaldi Matteo, Gervasoni Clarissa, Gugliatti Simona, Itri Teresa, Kalpourtzidou Aiki, Landi Tony, Lanzotti Aurora, Lo Dico Alessia, Loperfido Federica, Maccarini Beatrice, Mazza Antonio, Montomoli Cristina, Oddone Enrico, Pellizzer Chiara, Perneti Roberto, Porro Danilo, Scarabotto Anna, Sellarò Francesca, Severgnini Marco, Summa Donato, Tagliaferri Sofia, Tani Flaminia, Torriani Camilla, Vivone Gemine, Cena Hellas(2, 9), Tavazzi Eleonora, Monti Maria Cristina	275
Adriana Blanda, Paolo Verderio, Sara Pizzamiglio, Paola Scifo, Federico Fallanca, Maurizio Barbera, Edoardo Masiello, Giovanna Gattuso, Maura Massimino, Cristina Baldoli, Filippo Spreafico	231	Federico Fassio, Jessica Leoni, Rebecca Fattore, Giovanni Scaglione, Giovanni De Capitani, Fabio Borgonovo, Claudia Conflitti, Daniele Zizzo, Antonio Gidaro, Maria Calloni, Francesco Casella, Chiara Cogliati, Andrea Gori, Antonella Foschi, Marta Colaneri, Valentina Breschi	277
Bonanno Lilla, Maresca Giuseppa, Alibrandi Angela, Marra Angela, Salvatore Christian, Aresta Simona, Zirilli Agata, Cammarato Simona, Biondo Alessia, Quartarone Angelo, Castiglioni Isabella	233	Silvia Folcarelli, Valentina Panetta, Arianna Ambrogio, Agnese Graziosi, Arianna Pani	280
Bonomi Alice, Saccani Sebastiano, Coser Andrea, Valsecchi Carola, Galotta Arianna, Scatigna Marco, Werba Pablo	235	Albert Navarro-Gallinad, Valeria Nazzari, Laura Bondi, Luisa Zuccolo	282
Canziani Letizia, Gambini Giulia, Giorgio Grosso, Gandini Andrea, Salvini Andrea	237	Galotta Arianna ⁽¹⁾ , Mattio Maria Francesco ⁽¹⁾ , Morocutti Chiara ⁽¹⁾ , Brambilla Marta ⁽¹⁾ , Camera Marina ⁽¹⁾ , Bonomi Alice ⁽¹⁾	284
Erika Carbone, Luciano Calzari, Daria Marzanati, Luca Pagliardini, Lisett Solano Narduche, Ludovica Bartiromo, Matteo Schimberni, Errico Zupi, Massimo Candiani, Paola Viganò, Davide Gentilini	238	Gandin Ilaria, Indennitate Carla, Treu Riccardo, Porcari Aldostefano, Merlo Marco	286
		Gibertoni Dino, Grechi Andrea, Bonazzetti Cecilia, Giannella Maddalena	288

Koumantakis Emanuele, Remoundou Konstantina, Xynogalas Stavros, Roussaki Ioanna, Visconti Alessia, Berchialla Paola	290
Lecchi Mara, Pratesi Pietro, Pomposo Oscar Illescas, Gariboldi Manuela, Verderio Paolo	292
Lofaro Danilo, Armentaro Giuseppe, Vizza Patrizia, Veltri Pierangelo, Sciacqua Angela, Conforti Domenico	294
Alessandra Macciotta, Daniela Zugna, Lorenzo Richiardi	297
Arianna Magon, Rosario Caruso, Silvia Belloni, Giulia Maga, Gianluca Conte, Cristina Arrigoni	299
Martella Donato, Zambon Antonella	301
Attanasio Margherita, Di Giminiani Erika, Le Donne Ilenia, Cavone Nicole, Mazza Monica, Valenti Marco, Masedu Francesco	303
Samuele Minari, Dario Pescini, Antonella Zambon, Davide Soranna	305
Nicolao Giovanni, Fordellone Mario, Schiattarella Paola, Smimmo Annafrancesca, Di Gennaro Piergiacomo, Speranza Teresa, Simeon Vittorio, Signoriello Simona, Chiodini Paolo	307
Orieuia Chiara, Specchia Claudia	309
Pascucci Eleonora, Galarducci Riccardo, Boccia Stefania, Pastorino Roberta	311
Foco Luisa, De Bortoli Marzia, Del Greco M Fabiola, Frommelt Laura Sophie, Volani Chiara, Riekschnitz Diana Anna, Motta Benedetta Maria, Fuchsberger Christian, Völker Uwe, Gögele Martin, Dörr Marcus, Teumer Alexander, Pramstaller Peter Paul, Rossini Alessandra, Pattaro Cristian	313
Polano M., Ius T., Di Loreto C., Toffoli G., Dal Bo M., Lombardi G., Miran Skrap	315
Porreca Annamaria, Ibrahimli Eliana, Maturo Fabrizio, Marcos Zambrano Laura Judith, Melo Melisa, Lopes Marta B.	317
Santos Joseph Alvin Ramos, Di Tanna Gian Luca	319
Rancoita Paola M.V.	322
Stefano Renzetti, Matteo Charles Malvezzi	324
Romagnoli Martina, Carbonelli Michele, Amore Giulia, Caporali Leonardo, Fiorini Claudio, Barboni Piero, Vestri Annarita, Luigi Palla, Carelli Valerio, La Morgia Chiara	326
Sala Isabella, Conti Sara, Antonazzo Ippazio Cosimo, Rozza Davide, Losa Lorenzo, Ferrara Pietro, Fornari Carla, Crotti Giacomo, Ciampichini Roberta, Sampietro Giuseppe, Zucchi Alberto, Bagnardi Vincenzo, Mantovani Lorenzo Giovanni	328
Schiattarella Paola, Smimmo Annafrancesca, Speranza Teresa, Di Gennaro Piergiacomo, Nicolao Giovanni, Fordellone Mario, Simeon Vittorio, Signoriello Simona, Massimo Stafoggia, Andrea Ranzi, Laura Bonvicini, Serena Broccoli, Nicola Caranci, Nicolás Zengarini, Paola Angelini, Paolo Giorgi Rossi, Chiodini Paolo	330
Smimmo Annafrancesca, Schiattarella Paola, Speranza Teresa, Di Gennaro Piergiacomo, Nicolao Giovanni, Fordellone Mario, Simeon Vittorio, Stafoggia Massimo, Ranzi Andrea, Bonvicini Laura, Broccoli Serena, Caranci Nicola, Zengarini Nicolás, Signoriello Simona, Chiodini Paolo	332
Torriani C., Tonelli F., Masiero C., Aresi C., Forlino A., Villani S.	335
Zamagni Giulia, Barbatì Giulia	337
Zamagni Giulia, Barbatì Giulia	339