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Additional value of inflammatory biomarkers and carotid artery disease in prediction of significant coronary artery disease as assessed by coronary computed tomography angiography

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Aims To evaluate the relationship between an incremental model including cardiovascular risk factors, carotid disease, and inflammatory biomarkers to predict the presence of obstructive coronary artery disease (CAD).

Methods and results A total of 134 consecutive and asymptomatic intermediate-risk patients (mean age 61 ± 9 years, 52% men) were enrolled. Each subject underwent circulating levels assessment of interleukin (IL)-2r, IL-6, IL-8, IL-10, high-sensitivity C-reactive protein (hs-CRP) and carotid and coronary artery evaluation using carotid ultrasound and coronary computed tomography angiography (CCTA), respectively. Carotid disease was diagnosed in 71 (53%) patients. Obstructive and multi-vessel CAD were found in 50 (37%) and 18 (14%) patients, respectively. Patients in whom CCTA showed multi-vessel CAD had a higher rate of carotid disease (89 vs. 46%, $P = 0.001$) and increased values of all interleukins when compared with patients without multi-vessel obstructive CAD. The univariate and multivariate analysis showed that male gender, diabetes, carotid disease, and IL-6 were independently associated with obstructive CAD. At receiver operating characteristic curve analysis, the multivariate model (including male gender, carotid disease, IL-6 > 5.9 pg/mL, and diabetes) showed the highest area under the curve for prediction of obstructive CAD, multi-vessel CAD, and high-risk plaque defined as mixed and/or remodelled plaque when compared with all other models ($P < 0.001$).

Conclusion Among asymptomatic intermediate-risk patients, the presence of increased IL6 levels in addition to traditional risk factors (male gender with diabetes) and carotid artery disease predicts higher rates of obstructive CAD and it could be of help to identify which subset of asymptomatic patients could be referred to CCTA for screening.

Keywords inflammatory biomarkers • cytokines • carotid disease • coronary artery disease • computed tomography • risk stratification

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Introduction

Myocardial infarction represents the first clinical manifestation of coronary artery disease (CAD) in up to 65% of previously asymptomatic patients.[1] Regrettably, the efficacy of 'primary prevention' of cardiovascular disease (CVD) is compromised by the modest predictive accuracy of clinical scores currently utilized for the evaluation of pre-test probability of CAD.[2] Moreover, the total direct medical costs of CVD are projected to rise three-fold in the next 25 years.[3] Therefore, there is a major need for more effective strategies aimed at the prevention of unheralded cardiovascular events in intermediate-risk subjects.

Carotid artery disease has been identified as an independent predictor of myocardial infarction and stroke.[4] Moreover, in view of the inflammatory mechanisms involved in the atherosclerotic process and acute coronary syndrome, the assessment of inflammatory activation may be also useful for risk stratification of subjects with CAD.[5] However, both approaches alone seem weakly correlated with the extent of CAD. On the other side, coronary computed tomography angiography (CCTA) is a well-established non-invasive imaging technique able to detect atherosclerotic plaques and to evaluate the degree of coronary artery stenosis.[6,7] Despite the proven excellent prognostic value in symptomatic intermediate-risk patients,[8,9] the extensive use of CCTA in asymptomatic patients as screening strategy is still debated owing to its cost and radiation exposure.[10] Therefore, non-invasive and non-expensive prognostic predictors without the use of ionizing radiation to identify which asymptomatic intermediate-risk patients could be referred to CCTA for screening could be of interest.

The aim of our study was to evaluate the integrated value of inflammatory markers and carotid artery disease for the identification of higher-risk patients for cardiac events among asymptomatic intermediate-risk category to be addressed to CCTA for screening.

Methods

Study population

A total of 224 consecutive asymptomatic patients were screened for this study (*Figure 1*). The exclusion criteria were patients with prior history of known cardiac disease, contraindications to CCTA and the presence of autoimmune disease or current neoplastic disease or recurrent infections, and use of anti-inflammatory drugs within 1 month were excluded from the study. Accordingly, 134 patients were included in the study and underwent structured clinical interview, cardiovascular risk factors collection (according to the National Cholesterol Education Program guidelines[11]), biomarkers assessment, carotid ultrasound (CUS), and CCTA within 2 weeks from the enrolment. Written informed consent was obtained from all patients, and the institutional ethics committee approved the study protocol.

Inflammatory markers dosage

Circulating levels of inflammatory markers were assessed on venous blood samples drawn from the antecubital vein, using an ethylenediamine-tetra-acetic acid (EDTA) test tube. Blood samples for cytokines analysis were immediately centrifuged and serum was separated after coagulum retraction by centrifugation at 2000 rpm for 10 min. They were then frozen at -20 C° until laboratory assay. The IL circulating concentrations were assessed by an immunoassay technique. All cytokines measurements were standardized by the inclusion of a titration of the appropriate purified recombinant cytokines of known concentration: IL-8 (normal value: <62 pg/mL), IL-2R (normal value: 223–710 IU/mL), IL-6 (normal value: <5.9 pg/mL), and IL-10 (normal value: <9.1 pg/mL). Brain natriuretic peptide (BNP) circulating concentrations (normal value: <100 pg/mL) were assessed by a sandwich immunoassay technique (Triade BNP assay, Biotite Diagnostic INC, San Diego, CA, USA). High-sensitivity C-reactive protein (hs-CRP) had been quantified by a high-sensitive immunoassay technique (normal value: <7.44 mg/dL). CA 125 circulating concentrations (normal value: <35 IU/mL) were assessed by a chemiluminescence immunoassay technique (Tumor Markers CA 125 AxSYM System, Abbott, Lake Bluff, IL, USA). α1-Antitrypsin and α1-glycoprotein circulating concentrations (normal values: 95–175 g/dL and 50–120 mg/dL, respectively) were assessed by an immunoenzyme technique.

Carotid ultrasound

All patients underwent CUS evaluation using Hawk 2002 grey-scale ultrasound with a special multi-frequency linear probe (V-K Medical Company, Denmark). Two experienced operators (D.M. and M.D.L.) blinded to the clinical, laboratory, and radiological findings performed the measurements. Briefly, the artery wall layers were examined with the patient sitting upright and looking straight ahead, and the transducer was applied in front of the sternocleidomastoid muscle. Longitudinal and transverse views of both carotid arteries (common carotid, carotid bifurcation, internal and external carotid arteries) were obtained. Carotid artery disease was defined as the presence of carotid plaque on both the near and far walls of the carotid arteries. Carotid plaque was defined as a focal structure encroaching on the arterial lumen of at least 0.5 mm or 50% of the surrounding carotid intima-media thickness (CIMT) value or demonstrating a thickness of $\geq$1.5 mm, as measured from the media–adventitia interface to the intima–lumen interface, according to the Mannheim consensus.[12,13]

CCTA protocol and image analysis

We performed CCTA with a 64-slice multi-detector computed tomography (Toshiba 64 Aquilion, Japan). In order to achieve high-quality images, metoprolol was intravenously administered in case of a resting heart rate >65 bpm before CCTA.[14] Prior to CCTA, we performed quantification of coronary artery calcium score (CACS) with unenhanced CT scan. Then, an enhanced CT scan was performed administering 90 mL of contrast agent (Iomeron 400 mg/mL, Bracco, Milan, Italy) at a rate infusion of 5 mL/s using the bolus tracking technique with a retrospective ECG triggering, as previously described.[15] The dose length product (DLP) was recorded. An approximation of the effective radiation dose (ED) was obtained with the equation $ED = k \times DLP$ ($k = 0.017 \times mGy^{-1} \times cm^{-1}$ for the chest).[16]

The data were analysed using an off-line dedicated workstation (Vitrea, Vital Images, Plymouth, MN, USA) for post-processing and evaluated by two experienced operators (A.I.G. and G.P., both with $\geq$8 years of clinical experience in CCTA performance and analysis) blinded to all patient information. For any disagreement in data analysis between the two readers, consensus agreement was achieved by a third reader. The American Heart Association 16-segment model was used to segment coronary arteries.[17] Evaluation of CACS was performed according to the Agatston method.[18] All segments with a diameter of at least 1.5 mm were included. The coronary segments classified as not evaluable were excluded from the analysis. For each coronary segment included in the analysis, the presence of coronary atherosclerotic

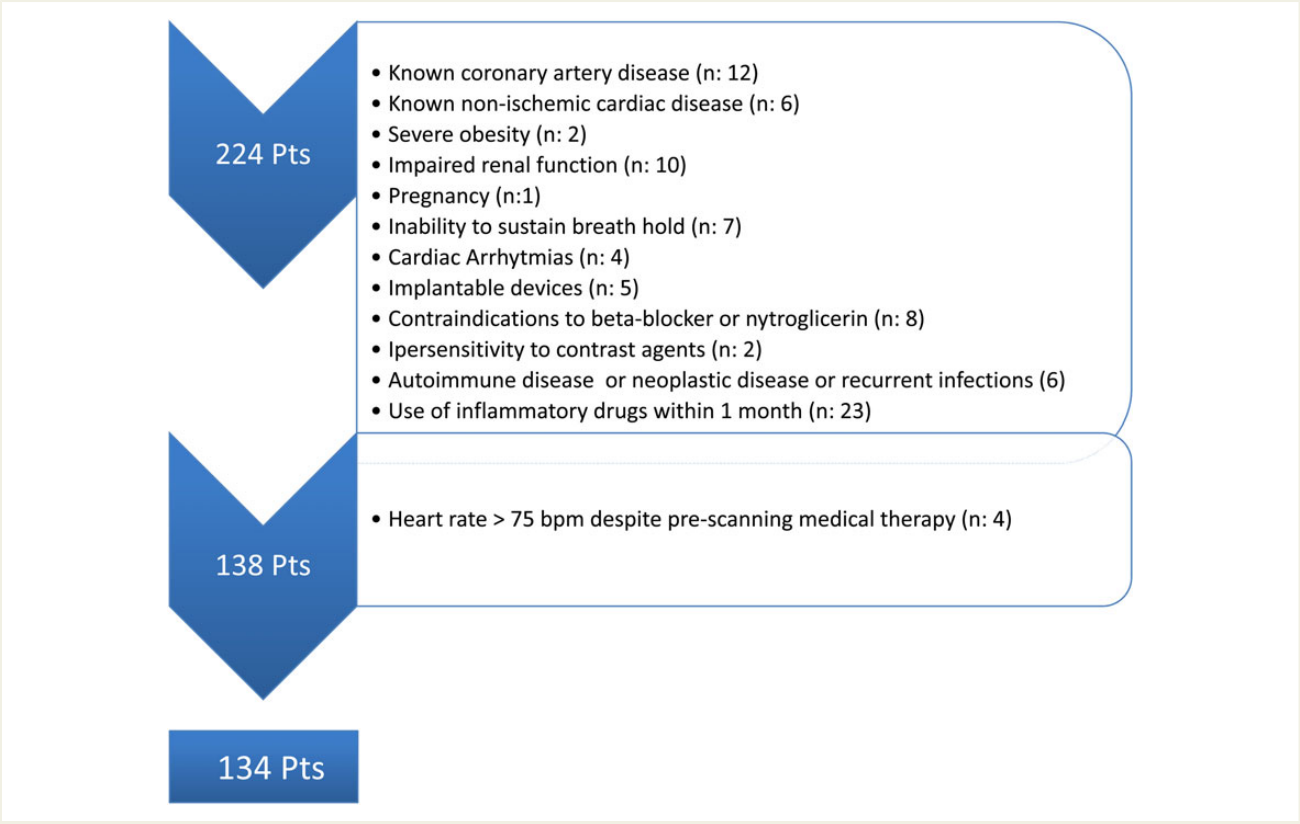

Figure 1 Study population. Pts: patients.

plaque was evaluated and defined as previously described.[7] The degree of stenosis was measured by identifying the luminal diameter of the segment exhibiting obstruction and the luminal diameter of the most normal appearing site immediately proximal to the plaque in multi-planar curved reformatted images. The percentage of stenosis was derived according to the following formula: $(D_{ref} - D_{min})/D_{ref} \times 100$, where D_{ref} is the reference diameter and D_{min} is the minimum diameter.[7] A stenosis $\geq$50% was considered as obstructive CAD. Obstructive CAD was classified into single-vessel or multi-vessel disease (two- and three-vessel disease).

Statistical analysis

Statistical analysis was performed with the SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean $\pm$ standard deviation and discrete variables were expressed as absolute number and percentages. Continuous variables were tested for normality with Kolmogorov–Smirnov test. Unpaired Student's *t*-test or Mann–Whitney *U* test, as required, and χ^2 test or Fisher's exact test were used to compare continuous and categorical variables, respectively. Baseline characteristics, risk factors, and inflammatory markers were tested in a univariate logistic regression analysis in order to determine the predictors of CAD. Significant variables in univariate analysis were included in a multivariate logistic regression analysis. Receiver operating characteristic (ROC) curves were subsequently generated using CAD as the event. Areas under the curve (AUC) were compared for each single factor and the multivariate model using the method of DeLong *et al.*[19] A *P*-value of <0.05 was considered statistically significant.

Results

In total, 134 patients (mean age 61 $\pm$ 9 years, 52% men) were enrolled in the study. The baseline characteristics of study population are listed in *Table 1*. CCTA was successfully performed in all patients. Mean value of radiation dose to patients was 16.2 $\pm$ 4.0 mSv. Overall evaluability of coronary segments imaged by CCTA was 98% (1772 out of 1809 coronary segments). Mean CACS quantification was 146 $\pm$ 285 HU. No CAD, non-obstructive CAD, obstructive CAD, and multi-vessel CAD were found with CCTA in 40 (30%), 44 (33%), 50 (37%), and 18 (13%) patients, respectively. Carotid disease was diagnosed by CUS in 71 (53%) patients (*Table 1*). The κ for inter- and intra-observer variability was 0.87 and 0.91, respectively.

Patients with no or non-obstructive CAD at CCTA showed a lower rate of carotid disease (43 vs. 70%, $P = 0.002$) but no significant differences in terms of IL values when compared with those with obstructive CAD (*Table 2*) with the exception of IL-6 (no CAD or CAD: $<$50%, 2.1 $\pm$ 2.9 pg/mL; CAD: $\geq$50%, 3.5 $\pm$ 4.0 pg/mL; $P = 0.028$). Patients in whom CCTA showed multi-vessel CAD had a higher rate of carotid disease (89 vs. 46%, $P = 0.001$) and increased values of all interleukins when compared with patients without multi-vessel obstructive CAD (*Table 2*). Moreover, TNF-α values were significantly higher in patients with significant and multi-vessel CAD (no CAD or CAD: $<$50%, 14.0 $\pm$ 15.2 pg/mL; CAD: $\geq$50%, 13.9 $\pm$ 5.9 pg/mL; $P = 0.022$; no multi-vessel CAD: 13.7 $\pm$ 13.3 pg/mL; multi-vessel CAD: 16.0 $\pm$ 7.2 pg/mL; $P = 0.019$).

Table 1 Characteristics of the study population

	All (n = 134)
Baseline characteristics	
Age, years	61 ± 9
Male, n (%)	70 (52%)
Risk factors	
Hypertension, n (%)	108 (81%)
Smoker, n (%)	27 (20%)
Hyperlipidaemia, n (%)	92 (69%)
Diabetes, n (%)	34 (25%)
Family history of CAD, n (%)	42 (31%)
BMI, kg/mm^2	28 ± 4
LVEF, %	56 ± 6
Medical therapy	
β-Blockers, n (%)	33 (25%)
ACE inhibitors, n (%)	65 (49%)
Calcium antagonists, n (%)	28 (21%)
Aspirin, n (%)	62 (46%)
Statins, n (%)	54 (40%)
Inflammatory markers	
IL-2R, IU/mL	638 ± 287
IL-6, pg/mL	2.6 ± 3.4
IL-8, pg/mL	95 ± 191
IL-10, pg/mL	0.13 ± 0.89
hs-CRP, mg/mL	4.74 ± 7.74
BNP, pg/mL	35 ± 36
TNF-α, pg/mL	13.9 ± 12.5
CA-125, IU/mL	12.6 ± 9.3
α1-Glycoprotein, mg/dL	77.3 ± 19.5
α1-Antitrypsin, g/dL	157 ± 23
Inflammatory markers	
IL-2R+ (NV: 223–710 IU/mL)	40 (30%)
IL-6+ (NV: <5.9 pg/mL)	14 (10%)
IL-8+ (NV: <62 pg/mL)	35 (26%)
IL-10+ (NV: <9.1 pg/mL)	0 (0%)
hs-CRP+ (NV: <7.44 mg/dL)	20 (15%)
BNP+ (NV: <100 pg/mL)	8 (6%)
TNF-α+ (NV: <8.1 pg/mL)	110 (82%)
CA-125+ (NV: <35 IU/mL)	0 (0%)
α1-Glycoprotein+ (NV: 50–120 mg/dL)	3 (2%)
α1-Antitrypsin+ (NV: 95–175 g/dL)	29 (22%)
Carotid disease, n (%)	71 (53%)
CCTA characteristics	
CACS	146 ± 285
Evaluability, n of segments (%)	1772 (98%)
No CAD, n (%)	40 (30%)
CAD <50%, n (%)	44 (33%)
CAD ≥50%, n (%)	50 (37%)
Number of coronary vessels with CAD ≥50%, n (%)	65 (49%)
Multi-vessel CAD ≥50%, n (%)	18 (13%)

CAD, coronary artery disease; BMI, body mass index; LVEF, left ventricle ejection fraction; ACE, angiotensin-converting enzyme; IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; BNP, brain natriuretic protein; TNF-α, tumoral necrosis factor; CCTA, coronary computed tomography angiography; CACS, coronary artery calcium score; SD, standard deviation; NV, normal value.

Table 2 Distribution of inflammatory markers, carotid disease detected by CUS, and CAD as detected by CCTA

	No CAD or CAD <50% (n = 84)	CAD ≥50% (n = 50)	P-value
Carotid disease	36 (43%)	35 (70%)	0.002
IL-2R, IU/mL	613 ± 237	680 ± 355	0.376
IL-6, pg/mL	2.1 ± 2.9	3.5 ± 4.0	0.028
IL-8, pg/mL	82 ± 151	118 ± 244	0.976
IL-10, pg/mL	0.07 ± 0.67	0.24 ± 1.17	0.290
hs-CRP, mg/mL	4.00 ± 4.96	5.98 ± 10.88	0.191
BNP, pg/mL	37 ± 41	31 ± 26	0.813
TNF-α, pg/mL	14.0 ± 15.2	13.9 ± 5.9	0.022
CA-125, IU/mL	12.6 ± 10.3	12.6 ± 7.5	0.302
α1-Glycoprotein, mg/dL	75.4 ± 17.4	80.6 ± 22.3	0.180
α1-Antitrypsin, g/dL	157 ± 23	158 ± 23	0.506
	No multi-vessel CAD (n = 116)	**Multi-vessel CAD (n = 18)**	
Carotid disease	52 (45%)	16 (89%)	0.001
IL-2R, IU/mL	610 ± 235	790 ± 487	0.220
IL-6, pg/mL	2.3 ± 3.1	4.7 ± 4.8	0.013
IL-8, pg/mL	77 ± 157	214 ± 324	0.047
IL-10, pg/mL	0.05 ± 0.57	0.66 ± 1.91	0.007
hs-CRP, mg/mL	4.10 ± 4.65	9.00 ± 17.41	0.346
BNP, pg/mL	36 ± 38	32 ± 25	0.792
TNF-α, pg/mL	13.7 ± 13.3	16.0 ± 7.2	0.019
CA-125, IU/mL	12.3 ± 9.6	15.3 ± 7.6	0.011
α1-Glycoprotein, mg/dL	76.1 ± 18.2	84.3 ± 26.6	0.184
α1-Antitrypsin, g/dL	157 ± 23	162 ± 23	0.167

CUS, carotid ultrasound; CAD, coronary artery disease; CCTA, coronary computed tomography angiography; IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; BNP, brain natriuretic protein; TNF-α, tumoral necrosis factor.

The univariate (*Table 3*) and multivariate analyses (*Table 4*) showed that male gender, diabetes, carotid disease, and IL6 were independently associated with obstructive CAD. *Figure 2* showed the sensitivity, specificity, negative predictive value, and positive predictive value of all models in prediction of obstructive CAD. The multivariate models showed the highest accuracy [80% (95% CI: 73–87)] when compared with male gender [68% (95% CI: 60–70), $P = 0.03$], IL-6 [58% (95% CI: 49–67), $P = 0.0002$], diabetes [64% (95% CI: 56–72), $P = 0.005$], and carotid disease [62% (95% CI: 54–70), $P = 0.0003$], respectively. Of note, the multivariate model is associated with a higher accuracy in prediction of obstructive CAD even when compared with the combination of male gender plus diabetes [68% (95% CI: 60–76), $P < 0.01$]. At ROC curve analysis (*Figure 3*), the multivariate model showed the highest AUC for prediction of obstructive CAD, multi-vessel CAD, and high-risk plaque defined as mixed and/or remodelled plaque when compared with all other models ($P < 0.001$).

Table 3 Univariate analysis for prediction of obstructive and multi-vessel CAD

	Presence of obstructive CAD		Presence of multi-vessel CAD	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Baseline characteristics				
Age	1.032 (0.992–1.074)	0.113	1.062 (1.000–1.128)	0.049
Male	5.146 (2.349–11.274)	<0.001	3.824 (1.186–12.332)	0.025
Risk factors				
Hypertension	0.915 (0.363–2.305)	0.851	1.044 (0.275–3.965)	0.950
Smoker	1.449 (0.614–3.419)	0.398	2.250 (0.754–6.714)	0.146
Hyperlipidaemia	1.791 (0.796–4.033)	0.159	2.333 (0.634–8.587)	0.202
Diabetes	2.395 (1.079–5.316)	0.032	2.867 (1.019–8.062)	0.046
Family history of CAD	0.771 (0.357–1.666)	0.509	1.071 (0.372–3.089)	0.898
BMI	1.034 (0.950–1.126)	0.434	0.991 (0.879–1.119)	0.887
LVEF	0.993 (0.940–1.050)	0.813	1.002 (0.926–1.085)	0.957
Medical therapy				
β-Blockers	1.124 (0.502–2.520)	0.776	2.360 (0.826–6.740)	0.109
ACE inhibitors	1.250 (0.620–2.520)	0.533	1.843 (0.667–5.098)	0.239
Calcium antagonists	1.944 (0.837–4.517)	0.122	1.685 (0.542–5.243)	0.368
Aspirin	0.983 (0.487–1.984)	0.983	1.260 (0.466–3.410)	0.649
Statins	2.839 (1.375–5.863)	0.005	2.760 (0.993–7.670)	0.052
Inflammatory markers				
IL-2R	1.001 (1.000–1.002)	0.201	1.002 (1.000–1.003)	0.024
IL-6	1.126 (1.010–1.255)	0.033	1.167 (1.035–1.315)	0.012
IL-8	1.001 (0.999–1.003)	0.304	1.002 (1.000–1.004)	0.020
IL-10	1.223 (0.817–1.833)	0.328	1.546 (1.025–2.330)	0.038
hs-CRP	1.037 (0.980–1.098)	0.208	1.057 (0.993–1.125)	0.084
BNP	0.995 (0.984–1.005)	0.322	0.997 (0.982–1.012)	0.687
TNF-α	0.999 (0.971–1.028)	0.999	1.011 (0.980–1.044)	0.485
CA-125	0.999 (0.962–1.038)	0.973	1.028 (0.984–1.075)	0.215
α1-Glycoprotein	1.014 (0.996–1.032)	0.137	1.020 (0.996–1.045)	0.104
α1-Antitrypsin	1.003 (0.987–1.018)	0.734	1.011 (0.988–1.034)	0.362
Carotid disease	3.111 (1.479–6.543)	0.003	9.385 (2.061–42.729)	0.004

CAD, coronary artery disease; OR, odds ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricle ejection fraction; ACE, angiotensin-converting enzyme; IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; BNP, brain natriuretic protein; TNF-α, tumoral necrosis factor.

Table 4 Multivariate analysis for prediction of obstructive CAD

	Presence of obstructive CAD	
	OR (95% CI)	*P*-value
Male	11.564 (3.985–33.563)	<0.001
IL-6	1.213 (1.059–1.389)	0.005
Diabetes	5.153 (1.736–15.299)	0.003
Carotid disease	2.961 (1.236–7.090)	0.015

CAD, coronary artery disease; OR, odds ratio; CI, confidence interval; IL, interleukin.

Discussion

To the best of our knowledge, this is the first study showing that asymptomatic male and diabetic subjects with high IL-6 values in conjunction with carotid artery disease have a very high probability to manifest obstructive CAD or multi-vessel disease or coronary atherosclerotic high-risk plaque. Carotid artery disease has been employed as a marker of generalized atherosclerosis, and the predictive value for CAD has been evaluated for both CIMT and carotid artery plaque.[13] We previously showed the value of CIMT in predicting obstructive and 'potentially vulnerable' CAD diagnosed by CCTA in intermediate-risk patients.[20] Although its widespread use as a surrogate marker of CAD, the ability of CIMT to adequately predict cardiovascular events is still controversial. A recent

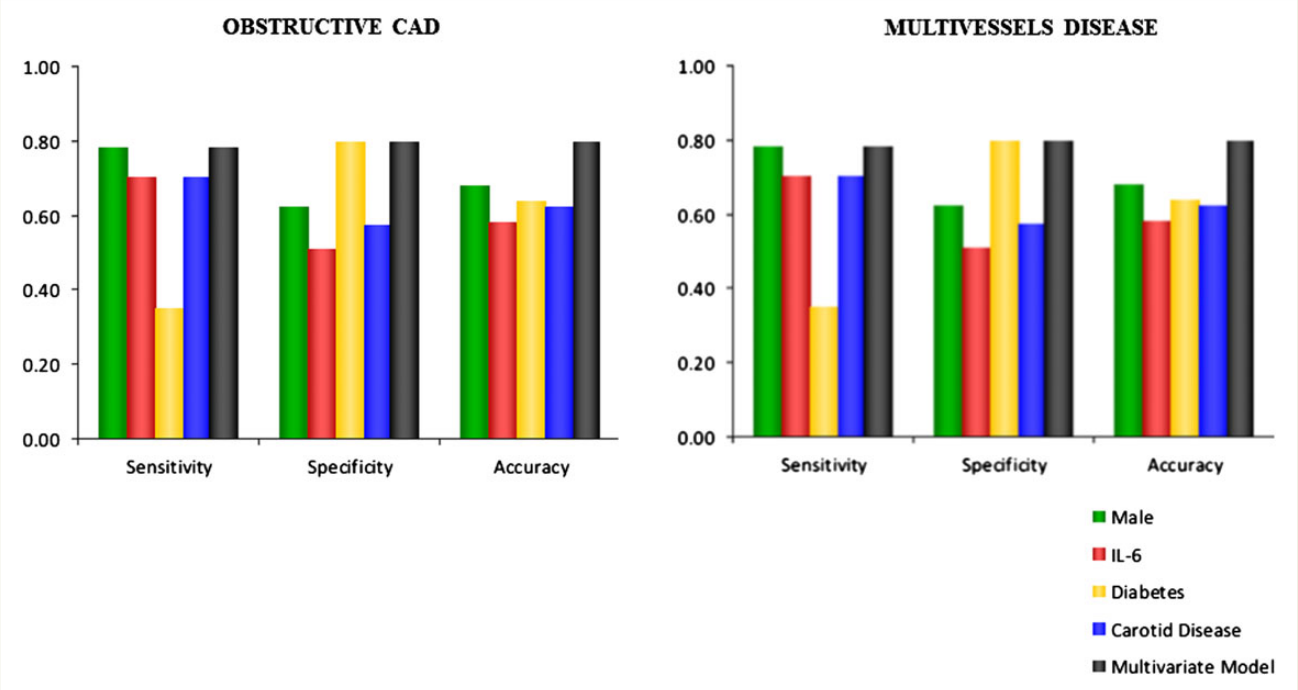

Figure 2 Diagnostic accuracy of four different models to predict obstructive CAD (left panel) and multi-vessel coronary artery disease (right panel). CAD, coronary artery disease; IL, interleukins.

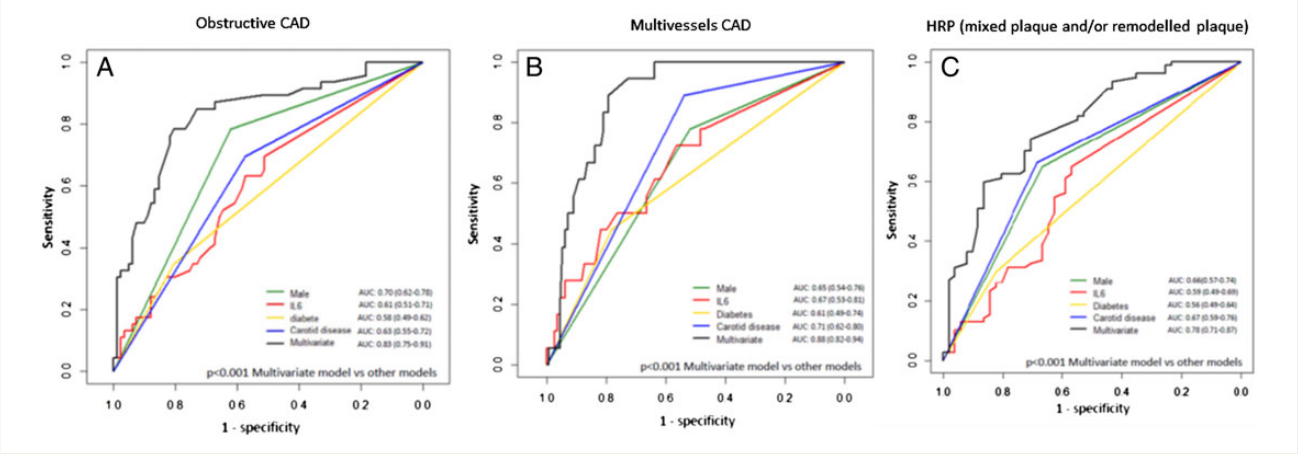

Figure 3 ROC curve analysis generated using CAD (*A*), multi-vessel coronary artery disease (*B*), and presence of HRP (*C*) as endpoints. AUC, area under the curve; CAD, coronary artery disease; HRP, high-risk plaque; IL, interleukin; ROC, receiver operating curve.

meta-analysis including a large cohort of patients[21] demonstrated that carotid plaque detection carries a higher diagnostic accuracy for predicting future CAD events when compared with CIMT assessment. However, Steinvil *et al.*[22] found that the severity of carotid stenosis and CAD extent were poorly correlated. Indeed, significant carotid disease was found in 5.9%, 6.6%, 13%, 17.8%, and 31.3% of patients with normal or non-obstructive CAD, one-vessel, two-vessel, three-vessel, and left main CAD, respectively. For this reason, the use of carotid artery disease detected by ultrasound technique as robust predictor of CAD has not been accepted yet.

To this regard, CCTA has emerged as a robust diagnostic test with incremental prognostic value in the evaluation of suspected CAD.[9,23] However, this technique has a limited use for a screening strategy in asymptomatic patients due to its cost and radiation exposure associated. Therefore, the identification of non-invasive multiparametric approach without the use of ionizing radiation to

identify which patients among asymptomatic population really need to be referred to CCTA is of interest.

Despite the evidence that inflammation plays a pivotal role in the early phases of atherosclerosis,[4,24] many studies showed that the association between inflammatory markers and the extent of CAD is weak.[25] However, in all these studies, the invasive coronary angiography has been used as a reference technique for CAD detection. This could be responsible for some bias. Indeed, the patients referred to invasive coronary angiograophy are usually symptomatic for chest pain at a higher risk for CAD when compared with our study population. Moreover, its well-known limited ability to detect non-obstructive CAD could likely underestimate the association between inflammatory marker and CAD.[26] In our study, inflammatory markers and carotid artery disease showed a significant correlation with the presence of obstructive CAD and multi-vessel disease when detected by CCTA. Such findings may support the hypothesis that an integrated approach combining inflammatory markers and carotid artery evaluation by ultrasound techniques could identify the 'vulnerable patient' to refer to CCTA despite the absence of symptoms suspected for CAD.

To this regard, Freitas *et al.*[27] found a positive association between CACS and maximum CIMT and systemic inflammatory activity only in those above the 75th percentile for CRP or for TNF-α. However, these results are limited by the assumption that CACS may represent coronary atherosclerosis burden. Some large studies demonstrated that CCTA has incremental prognostic value over coronary calcification detection.[28,29] Thus, the study of Freitas *et al.* probably underestimates the association between inflammatory mediators and coronary atherosclerosis.

When compared with previous efforts to identify patients at risk of coronary events by the detection of single 'vulnerable' atherosclerotic plaques (the 'high-risk plaque' concept), our findings move towards the consideration of patient as a whole, highlighting the 'high-risk patient' concept. Our study could have a potential interesting clinical implication, thanks to the widespread availability of systemic inflammatory biomarkers and non-invasive evaluation of carotid artery disease imaging, to identify asymptomatic patients to refer to CCTA for screening. This could be of interest because specifically in the setting of asymptomatic patients, the early detection of subclinical atherosclerosis could to be more important when compared with the detection of perfusion defect with the aim to provide a more aggressive and targeted primary prevention therapy and more personalized follow-up. Indeed, theoretically, aggressive therapy with statins, ACE inhibitors, and Cox-2 inhibitors reduce inflammatory marker levels and improve outcome of patients.[30] This benefit occurs by a direct effect of coronary artery plaque composition, and distribution is still under investigation.

Limitations

Some limitations of this study should be acknowledged. First, the study population was highly selected, and this was mainly due to the current limitations of CCTA. Second, a small number of patients were enrolled, and this may have caused a statistically underpowered analysis. As a result, although a tertile analysis clustering the study population as having no CAD, non-obstructive CAD, and obstructive CAD or one-, two-, and three-vessel disease should be of great interest, the sample size does not allow this sub-analysis. Nevertheless, it is important to remark that the endpoints used in our study (obstructive CAD and multi-vessel disease) are the most robust independent predictors for patient prognosis.[31] Third, we performed manual assessment of coronary stenoses. Although this is a clear limitation, it is also highly consistent with real-world practice. Finally, the radiation exposure of CCTA of our study is too much high to be considered acceptable for screening. However, the latest generation scanner is reliable to provide coronary artery imaging with <3 mSv and this makes CCTA reasonable for this aim.

Conclusions

Obstructive CAD as assessed by CCTA is highly predicted in asymptomatic male and diabetic subjects with increased circulating levels of inflammatory markers and carotid artery disease. Therefore, the combination of carotid disease evaluation and circulating levels of inflammatory markers could be of help to select the subset of asymptomatic patients that needs to be referred to CCTA for screening.

Conflict of interest: G.P. declared to be part of speaker's bureau for GE Healthcare, Bracco and Medtronic; D.A. declared to be part of speaker's bureau for GE healthcare; A.I.G., L.F., M.D.L., F.P.C., M.G., A.B., V.B., G.M., S.M., E.C., G.G, N.D.B., M.D.B., A.B. and M.P. have no disclosures.

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