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Impaired Treg Response and Subclinical Cardiac Dysfunction in Children Following SARS-CoV-2 Infection

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Abstract: In a preliminary cohort of children recovering from asymptomatic or mild coronavirus disease 2019, subclinical cardiac contractility alterations were associated with reduced regulatory T cells, shorter telomeres and elevated inflammatory markers 3 months post-infection. These findings suggest that immune dysregulation may contribute to silent post-coronavirus disease 2019 cardiac dysfunction and warrant confirmation in larger cohorts with long-term follow-up.

Key Words: cardiac contractility alterations, immune profile, post-COVID, speckle-tracking echocardiography, children

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Coronavirus disease 2019 (COVID-19) has been associated with post-infectious (sub)clinical cardiac involvement in both adults and children, regardless of acute disease severity.[1,2] In a previous study within a longitudinal cohort of COVID-19 family clusters (University of Padova, Italy), we identified subclinical cardiac contractility alterations in children assessed 3 months after asymptomatic or mild pre-Omicron infection, compared with age- and body surface area–matched healthy peers.[3,4] The clinical significance of these alterations remains uncertain, underscoring the need to investigate underlying mechanisms and validate findings in larger cohorts.

Inflammation and immune dysregulation have been implicated in post-COVID-19 cardiac manifestations in adults, but their role in children—particularly in subclinical myocardial alterations—remains largely unexplored.[5,6] We hypothesized that immune dysregulation may contribute to these subclinical cardiac alterations and aimed to characterize immunological signatures associated with abnormal myocardial contractility.

METHODS

This analysis was conducted within the established longitudinal family-cluster COVID-19 cohort previously described.[3,4] Children 0–<17 years old with laboratory-confirmed asymptomatic or mild severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection underwent standardized transthoracic echocardiography and 2-dimensional speckle-tracking echocardiography 3 months after infection.[3,4] Cardiac contractility alterations were defined as left ventricular regional peak systolic strain <−16% in ≥2 segments. Based on these criteria, participants were classified as having (C-ACC) or not having (C-NACC) alterations in cardiac contractility.

Peripheral blood was collected at the time of cardiac assessment, before vaccination or reinfection, and stored in the University of Padova biobank. Immune, inflammatory and humoral responses were profiled. Flow cytometry on peripheral blood mononuclear cells quantified immune-activated, senescent and exhausted CD4+ and CD8+ T cells, as well as regulatory T cells (Tregs), expressed as percentages of CD4+ T cells, as previously described.[7] Relative telomere length was measured using multiplex real-time PCR, and circulating levels of PAMPs and pro-inflammatory cytokines were quantified by real-time PCR and the Luminex platform. SARS-CoV-2–specific neutralizing antibodies (NAbs) were measured via plaque reduction neutralization test ($PRNT_{50}$).[8]

In a subset of children with available diagnostic nasopharyngeal swabs collected early in the acute phase (median 3 days after symptom onset; interquartile range [IQR]: 1–5), viral RNA was quantified by 1-step reverse transcription digital droplet polymerase chain reaction (ddPCR) and expressed as SARS-CoV-2 copies per 5 μL.

Geometric mean NAb titers and immune cells medians were compared between C-ACC and C-NACC using the Kruskal–Wallis test. Associations between immune cell profiles and NAb titers were examined using Spearman correlation.

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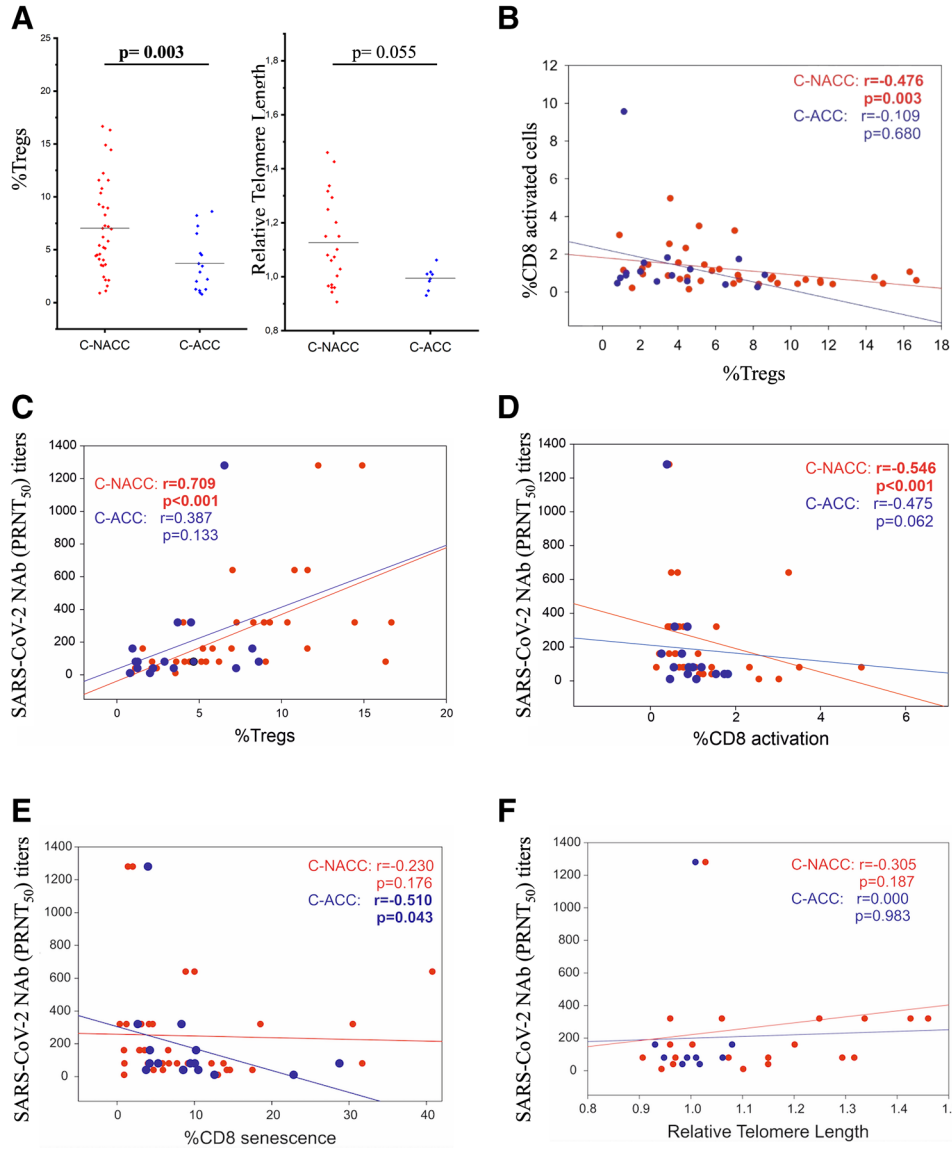

FIGURE 1. Immune profiles and correlations between cellular and humoral responses in children with (C-ACC, N = 16, blue) and without (C-NACC, N = 51, red) alterations in cardiac contractility. A: Frequencies of regulatory T cells (Tregs) and relative telomere length. B: Correlation between activated CD8+ T cells and Treg frequencies. C: Correlations between SARS-CoV-2 neutralizing antibody ($PRNT_{5500}$) titers and Treg frequencies. D: Correlations between SARS-CoV-2 neutralizing antibody ($PRNT_{5500}$) titers and activated CD8+ T cells. E: Correlations between SARS-CoV-2 neutralizing antibody ($PRNT_{5500}$) titers and senescent CD8+ T cells. F: Correlations between SARS-CoV-2 neutralizing antibody ($PRNT_{5500}$) titers and relative telomere length.

The study protocol was approved by the University of Padova's Ethics Committee (Prot. N°0070714). Written informed consent was obtained from parents or legally authorized representatives.

RESULTS

Cohort Description

None of the first 67 consecutive children enrolled between March 1 and December 10, 2020 (mean age 7.0 ± 4.8 years; 56.9% male) reported persistent post-COVID-19 symptoms. At the 3-month post-infection follow-up, subclinical alterations in cardiac contractility were identified in 16 children (C-ACC), despite preserved ejection fraction (see Figure, Supplemental Digital Content 1, https://links.lww.com/INF/G568), while the remaining 51 children C-NACC showed normal strain values. Children in the C-ACC group tended to be older, with a higher proportion of adolescents >12 years of age compared with C-NACC (62.5% vs. 56.9%), and had higher body surface area (see Figure, Supplemental Digital Content 2, https://links.lww.com/INF/G568). Whereas comorbidities—including asthma, allergies, prematurity, and rheumatologic or neurologic conditions—and acute COVID-19 symptoms were comparable between groups.

Immune Profile of Children With and Without Alterations in Cardiac Contractility

Lower frequencies of Tregs ($P = 0.003$) and a trend toward shorter telomeres ($P = 0.055$) were observed in C-ACC compared with C-NACC (Fig. 1A). At similar frequencies of activated, senescent and exhausted CD4+ and CD8+ T cells between C-ACC and C-NACC (see Figure, Supplemental Digital Content 3, https://links.lww.com/INF/G568), Tregs inversely correlated with activated CD8+ T cells in C-NACC ($r = -0.476$, $P = 0.003$) (Fig. 1B).

NAb titers tended to be higher in C-NACC than C-ACC (geometric mean = 4.61 [95% confidence interval: 4.17–5.10] vs. 3.85 [95% confidence interval: 3.10–4.77], $P = 0.124$) (see Figure, Supplemental Digital Content 4, https://links.lww.com/INF/G568). In the NACC group, NAb titers positively correlated with Treg frequencies ($r = 0.709$, $P < 0.001$) (Fig. 1C). Within the C-ACC group, NAb titers inversely correlated with activated CD8+ T cells ($r = -0.546$, $P < 0.001$) (Fig. 1D).

In the subset of children with available nasopharyngeal swabs collected at infection onset, those who later developed cardiac contractility alterations (C-ACC, N = 4) had higher initial viral loads than C-NACC (N = 13) (median = 44,174 [15,737–5,374,164] vs. 5360 [162–15,250] copies/mL). Viral load positively correlated with activated CD8+ T cells in C-ACC ($r = 0.600$). Systemic inflammation also appeared more pronounced in C-ACC compared with C-NACC, with elevated circulating pathogen-associated molecular patterns (PAMPs) (122 [114–131] vs. 32 [10–47] copies/μL, $P = 0.001$) and trend toward higher levels of pro-inflammatory interleukin (IL)-6 and tumor necrosis factor (TNF)-α (IL-6: 1.19 [1.07–1.31] vs.0.34 [0.01–0.89] pg/mL, $P = 0.06$; TNF-α: 4.38 [2.67–6.08] vs. 1.32 [0.01–3.26] pg/mL, $P = 0.08$) (see Figure, Supplemental Digital Content 5, https://links.lww.com/INF/G568).

DISCUSSION

C-ACC displayed a consistent pattern of immune dysregulation characterized by reduced Treg frequencies, diminished humoral responses, ongoing CD8+ T-cell activation and higher inflammatory mediator levels. Together, these findings suggest insufficient immune regulation and prolonged immune activation as potential contributors to subtle myocardial involvement after SARS-CoV-2 infection.

Reduced Treg frequencies may impair control of CD8+ T-cell activation,[5] leading to a suboptimal coordination between cellular and humoral responses in C-ACC. The inverse correlation between NAbs and activated CD8+ T cells in C-ACC supports this hypothesis and aligns with emerging evidence of persistent T-cell activation and altered immune homeostasis in pediatric long COVID.[9] Similar Treg perturbations have been documented in children with chronic post-viral syndromes, reinforcing the possibility of shared pathophysiologic pathways.[9]

The higher initial viral loads in children who later developed cardiac alterations, along with correlations between viral load and immune activation, could point to an insufficient or delayed early antiviral response.[7] This may have sustained viral replication, increased antigenic burden, and promoted prolonged inflammation. Elevated PAMPs and trends toward higher IL-6 and TNF-α further support persistent inflammatory signaling capable of contributing to myocardial strain abnormalities.

Shorter telomeres in affected children may reflect accelerated or more intense immune activation during acute infection, potentially limiting return to baseline immune homeostasis.[9] Although sample size precludes definitive mechanistic conclusions, these observations mirror features of immune senescence described in other pediatric post-viral conditions.

The mechanisms leading to persistent Treg reduction remain unclear and may involve viral, genetic or environmental drivers.[10] Future longitudinal studies should investigate Treg functionality, recovery trajectories and their potential role as biomarkers for risk stratification. If validated, the Treg–CD8–NAb axis identified may help recognize children at risk for post-COVID sequelae and inform early targeted immunomodulatory strategies.

This study is limited by its small sample size, lack of cardiac magnetic resonance imaging to confirm structural myocardial involvement and incomplete availability of viral load data. Larger prospective cohorts, ideally integrating advanced cardiac imaging and immunophenotyping, are needed to clarify long-term implications and define whether the observed abnormalities recover or progress.

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