Immune Profile of Children With Post-COVID-19 Cardiac Contractility Alterations: a Prospective Study

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BACKGROUND

Clinical and subclinical myocardial injury has been reported following COVID-19, irrespective of infection severity. While systemic inflammation and immune dysregulation are recognized contributors to post-COVID cardiac alterations in adults, their role in children remains unexplored.

AIM

This study evaluated the immune phenotype in children with or without subclinical cardiac contractility alterations after mild COVID-19.

METHODS

- **Prospective, observational**, cohort study
- **Children aged <18** years with (C-ACC) or without subclinical (C-NACC) alterations in cardiac contractility **post-microbiologically confirmed asymptomatic/mild** pre-Omicron **COVID-19** between May 2020 and September 2021
- Evaluated at the Pediatric department of the University hospital of Padova, Italy
- **Three months post-infection**, prior to any reinfection or vaccination, children underwent:
  - **cardiac function assessment** via speckle-tracking analysis of transthoracic echocardiography
  - sera and peripheral blood mononuclear cells samples collection for **SARS-CoV-2 antibody and immune cell profiling**
- Cardiac contractility alterations were defined as a regional peak systolic strain <-16% in at least two segments
- **Flow cytometry** assessed immune activated, senescent, exhausted, and regulatory (Tregs) T cells; relative telomere length was measured via **multiplex real-time PCR**
- SARS-CoV-2-specific neutralizing antibodies (NAbs) were quantified via **plaque reduction neutralization test**, defining $PRNT_{50}$ as the highest dilution reducing plaque count by >50%
- Geometric mean titers (GMTs) with 95% confidence intervals (95% CI), medians and interquartile ranges (IQRs) of immune cells and NAb titers, were compared between C-ACC and C-NACC using the Kruskal-Wallis test. Associations between the immune profile and NAbs were assessed through Spearman correlation analysis.

RESULTS

- A total of 67 children were studied (mean age: 7±4.8 years), including 16 C-ACC and 51 C-NACC
- C-ACC were older (p=0.022) and had a higher BMI (p=0.006), but no significant differences were observed in comorbidities (25% vs. 19.6%, p=0.727), sex (female: 37.5% vs. 43.1%, p=0.776), and presence of acute COVID-19 systemic symptoms (68.8% vs. 60.8%, p=0.768) between the two groups
- All children had no history of long COVID symptoms
- All C-ACC children exhibited subclinical cardiac contractility alterations despite preserved global left ventricular ejection fraction.

- **C-ACC exhibited lower frequencies of Tregs** (p=0.003) and **shorter telomeres** (p=0.055) compared to C-NACC (Fig.1)
- At similar frequencies of activated, senescent, and exhausted CD4 and CD8 T cells, **Tregs** were **inversely associated** with **activated CD8** cells in **C-NACC**, suggesting a protective role in dampening T-cell activation (Fig.1)

- **NAb titers** tended to be **higher** in **C-NACC** compared to C-ACC (4.61 [95% CI: 4.17-5.10] vs. 3.85 [95% CI: 3.10-4.77], p=0.124) (Fig.2)
- **NAb** titers were **positively correlated** with **Tregs** in C-NACC
- NAb titers were **inversely correlated** with **activated CD8** cells in C-ACC (Fig.2)

Regulatory T cells dysfunction was observed in children with cardiac contractility alterations post-mild COVID-19

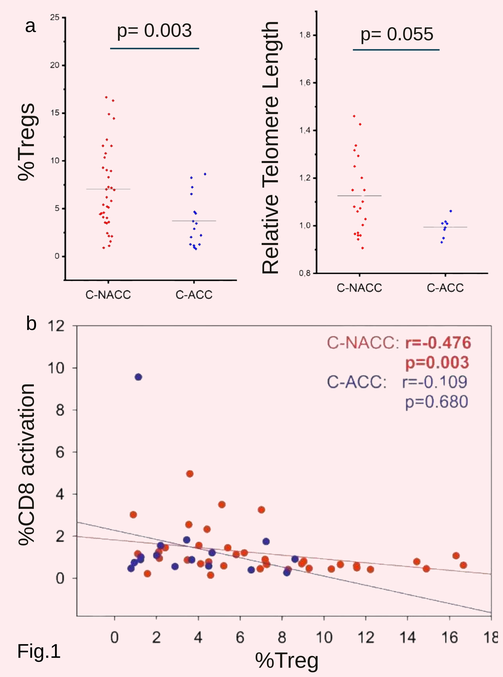
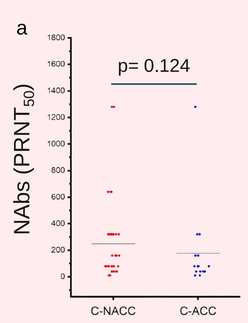


Fig.1

Fig.1 shows T cell phenotypes in children with and without cardiac contractility alterations. a) Frequencies of regulatory T cells (%Tregs), and difference of relative telomere length between C-ACC and C-NACC; b) single correlation graphs among frequency of activated CD8 cells (%CD8 activation) and Tregs (%Treg).

Fig.2



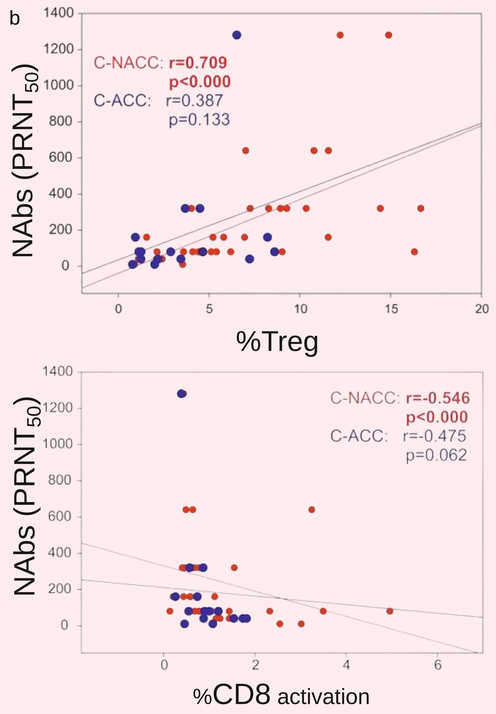


Fig.2 shows humoral immunity and cellular immune profiles in children with and without cardiac contractility alterations. a) Difference of SARS-CoV-2 neutralizing antibody (NAb) ($PRNT_{50}$) titers between C-ACC and C-NACC; b) single correlation graphs among frequency of activated cell (%CD8 activation), regulatory T cells (%Tregs), and NAb ($PRNT_{50}$) titers.

CONCLUSIONS

Impaired Treg function may contribute to defective immune control, as suggested by shorter telomeres, potentially **hindering the development of specific NAbs in C-ACC**. This may delay viral clearance and **contribute to post-COVID cardiac contractility alterations**. The inverse correlation between NAbs and activated CD8 cells in C-ACC further reinforces this hypothesis.

Future studies are warranted to elucidate the mechanisms driving Treg dysfunction in long COVID, as these insights may pave the way for targeted immunomodulatory strategies. Moreover, our findings underscore the need for long-term follow-up studies in larger cohorts to assess cardiac contractility recovery and its clinical implications.

ADDITIONAL KEY INFORMATION

Acknowledgements: This work is partially supported by VERDI (grant agreement No 101045989).

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