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Left ventricular longitudinal strain alterations in asymptomatic or mildly symptomatic paediatric patients with SARS-CoV-2 infection

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Aims Compared with adult patients, clinical manifestations of children's coronavirus disease-2019 (COVID-19) are generally perceived as less severe. The objective of this study was to evaluate cardiac involvement in previously healthy children with asymptomatic or mildly symptomatic severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection.

Methods and results We analysed a cohort of 53 paediatric patients (29 males, 55%), mean age 7.5 ± 4.7 years, who had a confirmed diagnosis of SARS-CoV-2 infection and were asymptomatic or only mildly symptomatic for COVID-19. Patients underwent standard transthoracic echocardiogram and speckle tracking echocardiographic study at least 3 months after diagnosis. Thirty-two age, sex, and body surface area comparable healthy subjects were used as control group. Left ventricular ejection fraction was within normal limits but significantly lower in the cases group compared to controls (62.4 ± 4.1% vs. 65.2 ± 5.5%; $P = 0.012$). Tricuspid annular plane systolic excursion (20.1 ± 3 mm vs. 19.8 ± 3.4 mm; $P = 0.822$) and left ventricular (LV) global longitudinal strain (-21.9 ± 2.4% vs. -22.6 ± 2.5%; $P = 0.208$) were comparable between the two groups. Regional LV strain analysis showed a significant reduction of the LV mid-wall segments strain among cases compared to controls. Furthermore, in the cases group, there were 14 subjects (26%) with a regional peak systolic strain below -16% (-2.5 Z score in our healthy cohort) in at least two segments. These subjects did not show any difference regarding symptoms or serological findings.

Conclusion SARS-CoV-2 infection may affect left ventricular deformation in 26% of children despite an asymptomatic or only mildly symptomatic acute illness. A follow-up is needed to verify the reversibility of these alterations and their impact on long-term outcomes.

Keywords paediatric cardiology • SARS-CoV-2 • COVID-19 • speckle tracking echocardiography • longitudinal strain

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), responsible for the coronavirus disease-2019 (COVID-19) pandemic, has rapidly spread worldwide and represents a major concern for healthcare providers.[1] SARS-CoV-2 infects host cells through ACE2 receptors, which are largely expressed in the lungs and heart. Furthermore, cardiac involvement seems to be the result of either direct viral damage or indirect effect, secondary to virus infection's immunological response. COVID-19 has been able to

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induce myocardial injury, myocardial infarction, myocarditis, and Takotsubo syndrome in a relevant number of adult patients.[2–4] While most children with COVID-19 present with mild symptoms and generally have a good prognosis,[5,6] data about the role of cardiovascular involvement in children with COVID-19 is still scarce. Growing evidence shows that some children, following COVID-19 recovery, may develop a severe multisystem inflammatory syndrome (MIS-C) with cardiac involvement in up to 80% of cases,[7] such as reduced left ventricular (LV) systolic function, heart failure, and coronary artery abnormalities.[8–10] However, little is known regarding cardiac involvement in paediatric patients with asymptomatic or mildly symptomatic SARS-CoV-2 infection.

Thus, this study aims to perform a detailed cardiac assessment, including standard echocardiography and speckle tracking echocardiography (STE), in previously healthy children who had an asymptomatic or mildly symptomatic SARS-CoV-2 infection.

Methods

Study design and population

A single-centre, retro-prospective observational study has been conducted on Italian family clusters of SARS-CoV-2 infection evaluated between 1 March and 10 September 2020. The study was carried out by the COVID-19 Family Cluster Follow-up Outpatient Clinic (CovFC), set up at the Department for Women's and Children's Health (W&CHD) of Padua University Hospital, in Veneto region, Italy. The CovFC programme included a clinical assessment provided by either Paediatrician trained on infectious diseases and/or by an Infectious Diseases specialist, an immunological assay for SARS-CoV-2 in both children and parents, and an echocardiographic evaluation only for children younger than 18 years old who had got infected. The study protocol was approved by the Institutional Review Board.

Families were enrolled 1–3 months after COVID-19 infection, through different institutional channels: (i) after hospitalization or after isolation upon diagnosis in the COVID-19 emergency room of the W&CHD; (ii) after receiving a home-based evaluation provided by family paediatricians in the Veneto Region. Inclusion criteria were as follows: (i) having children of paediatric age (0–18 years old); (ii) having a history of at least one confirmed intra-family COVID-19 case; and (iii) providing written informed consent.

The first evaluation included: (i) patient-based data collection; (ii) clinical evaluation of all children; (iii) collection of a blood sample for serological assessment for SARS-CoV-2 for all children/older siblings and their parents; and (iv) standard transthoracic echocardiogram (TTE) for only children with COVID-19 infection confirmed by either a positive SARS-CoV-2 molecular assay at nasal-pharyngeal swab (NPS) or a positive serology.

Data collection and definitions

All information concerning the past and recent history were collected retrospectively at first evaluation through both patients' interviews and clinical file revision. Data were prospectively collected from enrolment according to a case report form and entered into a web-based database using the REDCap platform (Vanderbilt University, Tennessee). Patient-based data collection included demographic information (sex, date of birth), comorbidities and vaccination history, data on COVID-19-related diagnosis (such as clinical features, management infection details, time and result of SARS-CoV-2 molecular assays at NPS), and follow-up (serological assays, cardiac evaluations including echocardiogram). Authorized staff involved in data entry were provided with passwords for secure access to data. All data were collected, maintaining confidentiality, and were anonymized for statistical analysis.

Subjects who had previously tested positive for SARS-CoV-2 by RT-PCR were considered 'confirmed COVID-19' cases, together with patients with no record of virological positivity SARS-CoV-2

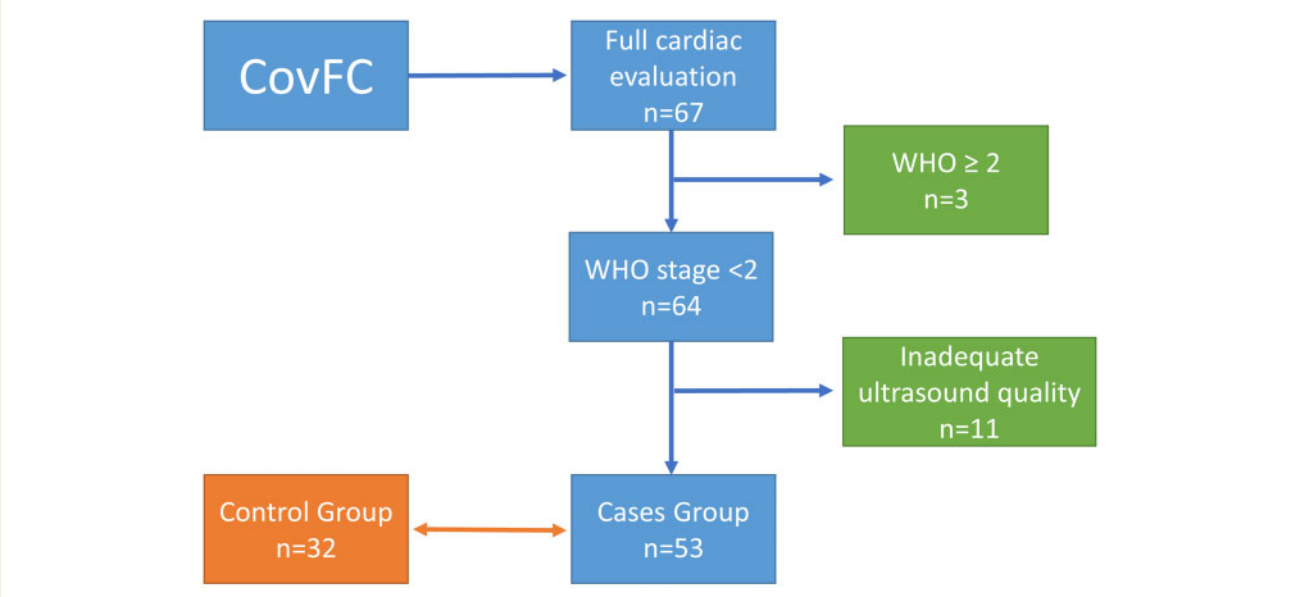

Figure 1 Patient inclusion flowchart. The flowchart shows patients enrolment from the COVID-19 Family Cluster Follow-up Outpatient Clinic (CovFC), with mild or no symptoms and full echocardiogram to perform speckle tracking analysis.

but showed evidence of seroconversion by either of the two serological tests adopted in this study, explained below. Subjects who had no record of infection or seroconversion were considered 'non-COVID-19 cases'; therefore, they were excluded from the analysis. For all COVID-19 cases, a 'baseline time' was defined as the most likely onset of infection, based on either symptoms outset or time of first virological positivity at molecular assay. Furthermore, for subjects with an asymptomatic infection and negative/not done NPS but with a serologically confirmed COVID-19, a baseline time was derived by the family outbreak temporal sequence.

The severity of COVID-19 was scored as mild, moderate, severe, and critical following the WHO classification based on clinical features, laboratory testing, and chest radiograph imaging (when available).[11]

Control group

Thirty-two healthy controls, comparable for age, sex, and weight, were consecutively recruited from the Pediatric Cardiology Outpatient Clinic of the W&CHD. Controls were enrolled among subjects referred for atypical chest pain or innocent murmur, otherwise healthy, not on any medication, and with normal cardiac evaluation, EKG, and echocardiogram. Specifically, we retrospectively selected only the subject with adequate quality and suitable views of 2D TTE in order to perform longitudinal strain (LS) analysis.

Serological assays

Subjects were sampled to collect sera and detect IgG and IgM targeting a recombinant nucleocapsid (N)-spike (S) protein of SARS-CoV-2, with the chemiluminescence immunoassay MAGLUMI™ 2019-nCoV IgM/IgG on the analytical system MAGLUMI™ 2000 Plus (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China). According to the manufacturer's inserts (271 2019-nCoV IgM, V2.0, 2020-03 and 272 2019-nCoV IgG, V1.2, 2020-02), the 2019-nCoV IgM cut-off is 1.0 AU/mL, while the 2019-nCoV IgG cut-off is 1.1 AU/mL. From the same subjects, plasma samples were taken to perform a 50% plaque reduction neutralization test (PRNT50). The neutralization titer was defined as the reciprocal of the highest dilution resulting in a reduction of the control plaque count >50% (PRNT50). Samples recording titers equal to or above 1:10 (or 1 on a $\log_{10}$ scale) were considered positive according to a previous validation conducted on a panel of archive samples collected in 2020 in Italy.[12]

Cardiac evaluation

Children recognized as COVID-19 cases underwent standard cardiac evaluation within 6 months from baseline time, including electrocardiogram (ECG) and TTE. Standard TTE study was performed using the GE Vivid E9 Ultrasound System (GE Healthcare, USA) following the recommendations for cardiovascular imaging during COVID-19 pandemic.[13,14] Left ventricular ejection fraction (LVEF) was calculated by TTE using the modified Simpson method (biplane method of disks), while LS analysis of the left ventricle, through 2D STE analysis, was performed offline using GE EchoPac Software (GE Healthcare, USA). Our methodology for STE study has been previously described.[15,16] Briefly, the best apical four-, two- and three-chamber views to visualize the LV segments were selected. Afterward, three points (two annular and one apical) were positioned, enabling the software to track the myocardium semi-automatically throughout the heart cycle. The region of interest was adjusted with careful inspection of the endocardial border, and manual correction was performed if needed. The automated algorithm allowed global longitudinal strain (GLS) to be calculated. Left ventricular LS by speckle tracking was defined as the average peak negative value on the strain curve during the systole (end of T-wave on the ECG) of all the studied segments.[17] The peak negative systolic strain value for each regional LV segment was also analysed. Analysis of the standard TTE and STE was performed by an experienced echocardiographer blind to the clinical data. Therefore, we compared echocardiographic results with the control group. Coronary arteries diameter was measured on 2D TTE and the respective z-scores were estimated based on previous reported data.[18]

Reproducibility

The data of reproducibility of our Echo Lab for standard TTE parameters as well as for STE has been already published.[19]

Statistical analysis

Categorical variables were presented as percentage (%), and continuous variables as mean ± standard deviation. Shapiro–Wilk test and histogram were used to test normality for each variable. Student's *t*-test was performed for normally distributed continuous variables and Mann–Whitney *U* test for non-parametric continuous variables. Chi-square test was performed for categorical variables to examine if there were significant differences between the groups. In multiple hypothesis testing, the Bonferroni correction test was used

Table 1 Cases and Controls demographical characteristics

	Cases ($n = 53$)	Controls ($n = 32$)	*P*-value
Gender (males)	29 (55%)	18 (56%)	$X^2 = 0.019$; $P = 0.89$
Age (years)	7.5 ± 4.7	8 ± 4.9	0.673
BSA (m^2)	0.98 ± 0.3	0.8 ± 0.4	0.17

BSA, body surface area.

Table 2 Clinical features of children with COVID-19

	N (%)
Asymptomatic patients (WHO = 0)	12 (23)
Mildly symptomatic patients (WHO = 1)	41 (77)
Fever	28 (53)
Congestion or runny nose	8 (15)
Cough	9 (17)
Myalgia	2 (4)
Arthralgia	1 (2)
Sore throat	4 (8)
Smell and taste changes	2 (4)
Abdominal pain	2 (4)
Fatigue	4 (8)
Headache	4 (8)
Nausea or vomiting	3 (6)
Diarrhoea	5 (10)
Loss of appetite	1 (2)
Cutaneous rash	3 (6)
Two or more symptoms	22 (42)

to control the occurrence of false positives. Statistical analysis was performed using STATA 14.0 MP (StataCorp LP, TX, USA).

Results

From 1 March to 10 September 2020, we enrolled 67 children among the COVID-19 cases who had a full cardiac evaluation. Among these, 64 had asymptomatic or only mildly symptomatic COVID-19 infection (WHO stages 0 or 1), while the remaining three patients had an infection with more than mild symptoms (WHO ≥ 2). Therefore, they were excluded from our analysis. All 64 included children were previously healthy, without evidence of previous cardiac disorders. A cardiac evaluation, including ECG and echocardiography, was performed after a mean time of 3.7 ± 1.6 months since COVID-19 disease's onset (baseline time). ECG showed sinus rhythm in all cases. Only five patients (9%) presented an abnormal ECG (four cases presented anomalies in the repolarization phase and one patient sinus bradycardia). Interestingly, there was no significant difference in the prevalence of ECG abnormalities among the two groups of patients with different degree of LV LS impairment. In the offline echocardiogram review, 11 patients were excluded because of inadequate quality of 2D scans (≥2 segments not visualized) to perform STE analysis (*Figure 1*). The remaining 53 patients formed our cases group.

Cases ($n = 53$) and controls ($n = 32$) were comparable for age (7.5 ± 4.7 years vs. 8 ± 4.9 years; $P = 0.673$), gender (29 males—55% vs. 18 males—56%; $X^2 = 0.019$; $P = 0.89$), and body surface area (0.98 ± 0.3 m^2 vs. 0.8 ± 0.4 m^2, $P = 0.17$) (*Table 1*). Among the cases, 12 patients (23%) had asymptomatic COVID-19 infection (WHO = 0), while the remaining 41 (77%) showed only mild symptoms (WHO = 1) (*Table 2*). According to SARS-CoV-2 serological assays performed after a mean time of 96 ± 41 days from baseline, 2019-nCoV IgM mean value was 0.7 ± 0.54 AU/mL, 2019-nCoV IgG mean value was 5.3 ± 6.45 AU/mL, and PRNT Log_{10} mean value was 4.7 ± 1.71.

Standard echocardiographic study

Left ventricular end-diastolic diameter was similar among cases and controls (35.9 ± 7.6 mm vs. 35.8 ± 7.7 mm; $P = 0.964$) (*Table 3*). LVEF was significantly lower in the cases group than controls (62.4 ± 4.1% vs. 65.2 ± 5.5%; $P = 0.012$), although both in the normal range. All cases showed an LVEF ≥ 55%. Among cases, we could not appreciate a correlation between LVEF value and time from infection diagnosis (Ro -0.19, $P = 0.17$). Furthermore, the cases group showed no LV diastolic dysfunction with a mean *E/A* ratio of 1.9 ± 0.55 and mean *E/E′* ratio of 6.4 ± 1.3. We did not appreciate any significant coronary artery dilation (*z* scores all <+2) or pericardial effusion among cases. Finally, right ventricular longitudinal function, measured using tricuspid annular plane systolic excursion parameter, was comparable among the two groups (20.1 ± 3 mm vs. 19.8 ± 3.4 mm; $P = 0.822$).

Table 3 Cases and Controls standard echo characteristics

	Cases ($n = 53$)	Controls ($n = 32$)	*P*-value
LVEDd (mm)	35.9 ± 7.6	35.8 ± 7.7	0.96
LVEDd (z-score)	-0.87 ± 1.45	-0.7 ± 1.4	0.63
LVESd (mm)	22.9 ± 4.0	23.8 ± 6.1	0.68
LVESd (z-score)	-0.20 ± 1.68	-0.22 ± 1.2	0.95
E/A ratio	1.82 ± 0.31	1.7 ± 0.3	0.62
Dec time	140 ± 38.8	139.9 ± 27.5	0.99
E/E′ ratio	5.8 ± 1.6	5.8 ± 1.4	0.93
TAPSE (mm)	20.1 ± 3	19.8 ± 3.4	0.82
LVEF (%)	62.4 ± 4.1	65.2 ± 5.5	**0.01***
GLS (%)	-21.9 ± 2.4	-22.6 ± 2.5	0.21

GLS, global longitudinal strain; LVEDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESd, left ventricular end-systolic diameter; TAPSE, tricuspid annular plane systolic excursion.
**P*-value < 0.05.

Table 4 Regional LV longitudinal strain deformation analysis

LV segmental LS	Cases ($n = 53$)	Controls ($n = 32$)	*P*-value
LV GLS (%)	-21.9 ± 2.4	-22.6 ± 2.5	0.208
LV basal septum	-18.7 ± 2.6	-21.1 ± 6.3	0.021
LV mid septum	-21.6 ± 3.8	-25.5 ± 6.1	**<0.001***
LV apical septum	-26.1 ± 4.8	-20.7 ± 5.1	**<0.001***
LV apical lateral	-24.5 ± 5.3	-21.8 ± 2.9	0.048*
LV mid antero-lateral	-20.7 ± 3.7	-25.4 ± 8.4	**<0.001***
LV basal antero-lateral	-19.6 ± 6.1	-20.3 ± 6.2	<0.617
LV A4C LS (%)	-21.9 ± 2.9	-22.2 ± 2.8	0.582
LV basal inferior	-21.4 ± 3.3	-21.5 ± 4.8	0.846
LV mid inferior	-22.9 ± 2.9	-25.5 ± 5.0	0.005
LV apical inferior	-26.5 ± 4.1	-20.6 ± 11.3	**0.002***
LV apical anterior	-23.4 ± 5.2	-19.4 ± 5.0	0.011
LV mid anterior	-21.3 ± 4.3	-27.4 ± 8.8	**<0.001***
LV basal anterior	-21.2 ± 3.7	-25.2 ± 8.3	0.006
LV A2C LS (%)	-22.8 ± 2.9	-22.9 ± 4.8	0.923
LV basal posterior	-19.1 ± 5.9	-22.2 ± 4.9	0.027
LV mid posterior	-20.5 ± 3.4	-25.0 ± 5.6	**<0.001***
LV apical posterior	-23.3 ± 4.5	-21.5 ± 2.9	0.086
LV apical septal	-24.2 ± 5.8	-20.7 ± 5.5	0.020
LV mid antero-septal	-21.5 ± 4.0	-27.3 ± 8.6	**<0.001***
LV basal antero-septal	-19.1 ± 3.4	-20.3 ± 5.5	0.225
LV A3C LS (%)	-21.2 ± 3.0	-22.8 ± 2.4	0.014

A2C, apical 2 chamber; A3C, apical 3 chamber; A4C, apical 4 chamber; GLS, global longitudinal strain.
**P*-value < 0.002 (Bonferroni correction).

Speckle tracking echocardiographic analysis

LV global longitudinal strain (LV-GLS) did not show any difference between the two groups (-21.9 ± 2.4% vs. -22.6 ± 2.5%; $P = 0.208$).

Similar to LVEF, we did not highlight a correlation between LV-GLS and time from infection diagnosis (Ro 0.21, $P = 0.13$). However, strain segmental analysis of the LV showed significant strain reduction of the LV mid-wall segments among cases, compared to controls. On the other hand, two apical segments displayed higher deformation in cases compared to controls (*Table 4*). Thus, there was a higher base to apex gradient in our patients' cohort than in controls (*Figure 2*).

Furthermore, in the cases group, there were 14 subjects (26.4%) with a strain lower than -16% (corresponding to the mean strain value minus 2.5 SD in our studied healthy cohort) in ≥2 segments. COVID-19 subjects with more compromised LV regional LS (i.e. ≥2 segments below -16%) did not show any difference compared to the remaining cases regarding the presence of symptoms, serological findings (IgM, IgG, and PRNT $\log_{10}$), or age. For the latter, a not statistically significant trend was documented towards older age in the most affected sub-group (9.4 ± 4.9 vs. 6.9 ± 4.5, $P = 0.09$) (*Table 5*).

Discussion

Our study provides new insights on the cardiac impact of COVID-19 among paediatric patients, showing for the first time that 26% of children recovered from an asymptomatic or mildly symptomatic COVID-19 present a mild subclinical cardiac involvement at 3 months after the infection. This finding is of great interest as the cardiac involvement does not correlate to the severity of COVID-19 clinical manifestations.

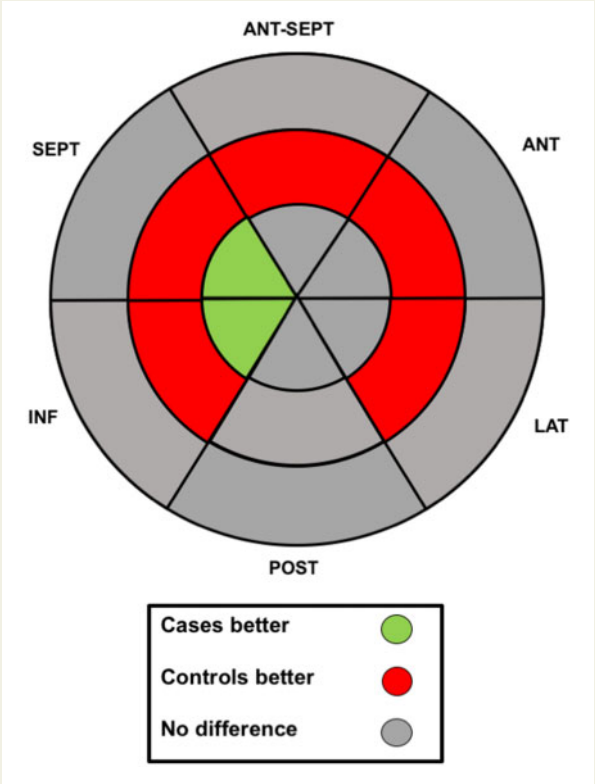

Figure 2 Left ventricular longitudinal strain Bull's Eye plot. Bull's eye representation of segmental LV longitudinal strain differences between cases and controls. In red, the segments with reduced deformation in cases compared to controls. In green, the segments with increased strain values among cases compared to controls. In grey, the segments with no statistical difference between the two groups.

In contrast to adults, SARS-CoV-2 usually leads to a mild illness in children.[20,21] However, it has been described that MIS-C associated with SARS-CoV-2 infection may occur, presenting with significant cardiac involvement in up to 80% of cases[7] and often leading to myocardial abnormalities with a significant reduced LVEF, and abnormal LV regional LS, valve regurgitation and coronary arteries dilation.[10,22–25] On the other side, lack of knowledge still regards the cardiovascular involvement in children with asymptomatic and/or mildly symptomatic COVID-19.

Recent evidence suggests myocardial and pericardial involvement in young athletes after COVID-19 recovery, with a significant proportion of them showing pericardial enhancement on CMR.[26,27] In our population, we did not find signs of pericardial effusion on TTE, however, the shorter timing of imaging in respect to active viral disease and different sensitivity of used imaging modalities might explain this only apparent difference. In our paediatric COVID-19 cohort, standard echocardiography showed preserved LVEF, although significantly lower than controls, and normal LV diastolic function. Despite mean LV GLS in COVID-19 children did not differ significantly from that of the control group, we found differences regarding regional strain analysis of the left ventricle, with the most affected segments in the COVID-19 group being the mid-wall ones and the basal anterior, posterior and septal inferior ones compared with the control group. Conversely, the apical segments showed higher deformation in the COVID-19 group. This finding is in agreement with the distribution of affected areas of the left ventricle in MIS-C patients, which does not follow coronary distribution.[28] Our data are of particular interest since we demonstrated subclinical cardiac involvement even in children who had an asymptomatic or mildly symptomatic COVID-19, persisting at least 3 months after the infection. In accordance with Piccinelli *et al.*[29] we found that apical segments were spared or even showed increased deformation, increasing the base to apex gradient. This pattern has already been described in another cardiac diseases like systemic hypertension.[30]

In COVID-19 adult patients, Croft *et al.*[31] reported a lower mean LV GLS than in healthy populations, even in the presence of preserved LVEF. Moreover, a recent study observed significantly increased mortality alongside with decrease in LV GLS in adult patients with COVID-19.[32] Interestingly, we found a good proportion of our cohort (26.4%) having a regional strain value <-16% in at least two LV segments. Due to the significant inter-vendor variation in normal values, this cut-off value was calculated based on our control group mean LV GLS value minus 2.5 SD. This value was significantly below the normality range proposed in a large meta-analysis in children by Levy *et al.*,[33] and significantly lower than the 5th percentile for LS proposed for healthy children by Cantinotti *et al.*[34] Although abnormal systemic inflammatory response following infection is a described mechanism of indirect myocardial injury, in our subset of patients with reduced LS we could not demonstrate any difference regarding symptoms or levels of SARS-CoV-2 antibodies. These data suggest that COVID-19 clinical features and the degree of the immune system response following the infection may not predict the subclinical myocardial injury's extension. Thereafter, the virus, *per*

Table 5 Clinical and serological characteristics of COVID-19 cases

	≥2 LV segments abnormal (*n* = 14)	<2 LV segments abnormal (*n* = 39)	*P*-value
Age (years)	9.4 ± 4.9	6.9 ± 4.5	0.09
Symptoms (WHO = 1) (%)	11 (79%)	30 (77%)	$X^2 = 0.016$; $P = 0.89$
Time from diagnosis (days)	118 ± 39	104 ± 41	0.27
LVEF (%)	61 ± 3.4	63 ± 4.3	0.14
GLS (%)	-19.6 ± 1.6	-22.7 ± 2.1	<0.001*
Positive NPS (%)	11 (79%)	30 (77%)	$X^2 = 0.016$; $P = 0.89$
IgM (AU/mL)	0.617 ± 0.58	0.76 ± 0.52	0.44
IgG (AU/mL)	4.78 ± 8.56	5.46 ± 5.72	0.75
PRNT log_{10}	4.1 ± 1.8	4.9 ± 1.7	0.17

COVID-19 patients divided in two subgroups according to the number of LV segments with reduced LV longitudinal strain (below -16%).
GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; NPS, nasal-pharyngeal swab; PRNT, plaque reduction neutralization test.
*P-value < 0.05.

se, could directly affect the heart in a significant proportion of patients. Our study may suggest the need to implement a cardiac evaluation for virtually all children affected by COVID-19, irrespectively from their initial clinical presentation. Besides, the clinical value and reversibility of the abnormal longitudinal myocardial deformation properties should be further investigated in order to evaluate the necessity of an extended cardiac follow-up of this cohort of patients.

The single-centre nature of our study may constitute a major limitation. However, we believe that this should be considered a pilot study enhancing further research, including larger cohorts, on this topic. COVID-19 cases were enrolled at least three months after SARS-CoV2 infection. This delay may have influenced the proportion with an abnormal regional strain of the left ventricle. Nevertheless, one-quarter of our cohort presented LV deformation abnormalities late after infection. Unfortunately, we were not able to enroll a higher number of control subject due to the nature of our Institution (tertiary care hospital), the time constrains of COVID-19 and the very limited access to elective cases during the COVID pandemic. Finally, the control group was not matched with cases but resulted in comparable demographic characteristics.

Conclusion

SARS-CoV-2 infection may affect LV cardiac mechanics in a quarter of asymptomatic or mildly symptomatic children, with persistence at least three months after the infection. The cardiac involvement does not seem to be related to the SARS-CoV-2 humoral response nor the clinical presentation of COVID-19.

The clinical significance of these findings is unclear and should be further investigated. Moreover, the development of dedicated paediatric follow-up programmes would be able to verify the reversibility of these alterations.

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Data availability

The data that support the findings of this study are openly available in WHO at https://www.who.int/, reference number [1] and at https://apps.who.int/iris/handle/10665/332196, reference number [11].

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Conflict of interest: none declared.

References

1. WHO | World Health Organization [Internet]. https://www.who.int/ (5 January 2021, date last accessed).
2. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol* 2020;**17**:259–60.
3. Bangalore S, Sharma A, Slotwiner A, Yatskar L, Harari R, Shah B *et al.* ST-segment elevation in patients with Covid-19—a case series. *N Engl J Med* 2020;**382**:2478–80.
4. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A *et al.* COVID-19 and cardiovascular disease. *Circulation* 2020;**141**:1648–55.
5. Parri N, Lenge M, Buonsenso D; Coronavirus Infection in Pediatric Emergency Departments (CONFIDENCE) Research Group. Children with Covid-19 in pediatric emergency departments in Italy. *N Engl J Med* 2020;**383**:187–90.
6. Tagarro A, Epalza C, Santos M, Sanz-Santaeufemia FJ, Otheo E, Moraleda C *et al.* Screening and severity of coronavirus disease 2019 (COVID-19) in children in Madrid, Spain. *JAMA Pediatr* 2021;**175**:316.
7. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF *et al.*; CDC COVID-19 Response Team. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med* 2020;**383**:334–46.
8. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG *et al.* Paediatric Inflammatory Multisystem Syndrome: temporally Associated with SARS-CoV-2 (PIMS-TS): cardiac features, management and short-term outcomes at a UK Tertiary Paediatric Hospital. *Pediatr Cardiol* 2020;**41**:1391–401.
9. Davies P, Evans C, Kanthimathinathan HK, Lillie J, Brierley J, Waters G *et al.* Intensive care admissions of children with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the UK: a multicentre observational study. *Lancet Child Adolesc Health* 2020;**4**:669–77.

10. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P *et al.*; PIMS-TS Study Group and EUCLIDS and PERFORM Consortia. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 2020;**324**:259–69.
11. WHO. Clinical management of COVID-19: interim guidance, 27 May 2020. World Health Organization; 2020. https://apps.who.int/iris/handle/10665/332196. (5 January 2021, date last accessed)
12. Padoan A, Bonfante F, Pagliari M, Bortolami A, Negrini D, Zuin S *et al.* Analytical and clinical performances of five immunoassays for the detection of SARS-CoV-2 antibodies in comparison with neutralization activity. *EBioMedicine* 2020;**62**:103101.
13. Zoghbi WA, DiCarli MF, Blankstein R, Choi AD, Dilsizian V, Flachskampf FA *et al.*; ACC Imaging Council. Multimodality cardiovascular imaging in the midst of the COVID-19 pandemic. *JACC Cardiovasc Imaging* 2020;**13**:1615–26.
14. Sirico D, Castaldi B, Ciliberti P, Sabatino J, Cazzoli I, Secinaro A *et al.*; Working Group on Congenital Heart Disease of the Italian Society of Cardiology. Cardiac imaging in congenital heart disease during the coronavirus disease-2019 pandemic: recommendations from the Working Group on Congenital Heart Disease of the Italian Society of Cardiology. *J Cardiovasc Med (Hagerstown)* 2020;**21**:467–71.
15. Prota C, Di Salvo G, Sabatino J, Josen M, Paredes J, Sirico D *et al.* Prognostic value of echocardiographic parameters in pediatric patients with Ebstein's anomaly. *Int J Cardiol* 2019;**278**:76–83.
16. Di Salvo G, Siblini G, Issa Z, Mohammed H, Abu Hazeem A, Pergola V *et al.* Left ventricular mechanics in patients with abnormal origin of the left main coronary artery from the pulmonary trunk late after successful repair. *Cardiology* 2017; **136**:71–6.
17. Voigt J-U, Pedrizzetti G, Lysyansky P, Marwick TH, Houle H, Baumann R *et al.* Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *J Am Soc Echocardiogr* 2015;**28**:183–93.
18. Dallaire F, Dahdah N. New equations and a critical appraisal of coronary artery Z scores in healthy children. *J Am Soc Echocardiogr* 2011;**24**:60–74.
19. Sabatino J, Di Salvo G, Prota C, Bucciarelli V, Josen M, Paredes J *et al.* Left atrial strain to identify diastolic dysfunction in children with cardiomyopathies. *J Clin Med* 2019;**8**:1243.
20. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z *et al.* Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. *Pediatrics* 2020;**58**:712–13.
21. Cao Q, Chen Y-C, Chen C-L, Chiu C-H. SARS-CoV-2 infection in children: transmission dynamics and clinical characteristics. *J Formos Med Assoc* 2020;**119**: 670–3.
22. Belhadjer Z, Méot M, Bajolle F, Khraiche D, Legendre A, Abakka S *et al.* Acute heart failure in multisystem inflammatory syndrome in children in the context of global SARS-CoV-2 pandemic. *Circulation* 2020;**142**:429–36.
23. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* 2020; **395**:1607–8.
24. Levin M. Childhood multisystem inflammatory syndrome—a new challenge in the pandemic. *N Engl J Med* 2020;**383**:393–5.
25. Abrams JY, Godfred-Cato SE, Oster ME, Chow EJ, Koumans EH, Bryant B *et al.* Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2: a systematic review. *J Pediatr* 2020;**226**: 45–54.e1
26. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP *et al.* Cardiovascular magnetic resonance findings in competitive athletes recovering from COVID-19 infection. *JAMA Cardiol* 2021;**6**:116–8.
27. Brito D, Meester S, Yanamala N, Patel HB, Balcik BJ, Casaclang-Verzosa G *et al.* High prevalence of pericardial involvement in college student athletes recovering from COVID-19. *JACC Cardiovasc Imaging* 2021;**14**:541–55.
28. Piccinelli E, Herberg J, Kang H, Fraisse A, Krupickova S, Bermejo Altamar I *et al.* Abstract 16650: segmental and global longitudinal strain differences between children with paediatric inflammatory multisystem syndrome temporally associated with SARS-COV-2 pandemic and Kawasaki Disease: preliminary data from an ongoing study. *Circulation* 2020;**142**:A16650.
29. Piccinelli E, Herberg J, Kang H, Fraisse A, Krupickova S, Altamar IB *et al.*. Segmental and global longitudinal strain differences between children with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 pandemic and Kawasaki disease. European Heart Journal- Cardiovascular Imaging 2021;**22**:jeaa356.186.
30. Arenas IA, Podesta CA, Issa O, Lin J, Brenes JC. Myocardial longitudinal strain, fitness, and heart failure risk factors in young adults. *Echocardiography* 2020;**37**: 404–11.
31. Croft LB, Krishnamoorthy P, Ro R, Anastasius M, Zhao W, Buckley S *et al.* Abnormal left ventricular global longitudinal strain by speckle tracking echocardiography in COVID-19 patients. *Future Cardiol* 2020;https://www.futuremedicine.com/doi/10.2217/fca-2020-0121 (5 January 2021, date last accessed).
32. Janus SE, Hajjari J, Karnib M, Tashtish N, Al-Kindi SG, Hoit BD. Prognostic value of left ventricular global longitudinal strain in COVID-19. *Am J Cardiol* 2020;**131**: 134–6.
33. Levy PT, Machefsky A, Sanchez AA, Patel MD, Rogal S, Fowler S *et al.* Reference ranges of left ventricular strain measures by two-dimensional speckle-tracking echocardiography in children: a systematic review and meta-analysis. *J Am Soc Echocardiogr* 2016;**29**:209–225.e6.
34. Cantinotti M, Scalese M, Giordano R, Franchi E, Assanta N, Marotta M *et al.* Normative data for left and right ventricular systolic strain in healthy Caucasian Italian children by two-dimensional speckle-tracking echocardiography. *J Am Soc Echocardiogr* 2018;**31**:712–720.e6.