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RESEARCH LETTER

Evidence of SARS-CoV-2 mRNA in endomyocardial biopsies of patients with clinically suspected myocarditis tested negative for COVID-19 in nasopharyngeal swab

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Infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause coronavirus disease 2019 (COVID-19).[1] Myocardial injury—observed in up to 7–17% of patients with COVID-19[2]—is associated with increased morbidity and mortality due to COVID-19, which is highest among patients with known cardiovascular disease (CVD), but also includes patients without known CVD.[3] Recent reports described cases of acute myocarditis in COVID-19, sometimes with a fulminant course, that occur between 4 and 9 days after initial symptoms.[2,4,5]

We here report cases of two patients admitted to our tertiary medical centre, the University Medical Center Mainz, Germany. Both patients were male, 39 and 36 years old, had shortness of breath, T-wave inversions in the anterolateral leads on ECG, elevated serum levels of natriuretic peptides and cardiac troponin I, as well as echocardiographic signs of left ventricular (LV) dysfunction (decreased global and regional longitudinal strain or reduced LV ejection fraction and increased LV end-diastolic diameter). Both patients were obese and had a history of upper airway infection with headache, fever, and cough up to 4 weeks before admission. Patient B had more pronounced cardiovascular risk factors and co-existing coronary heart disease (Supplementary material online, *Table S1* and *Figure S1*). Cardiac magnetic resonance imaging and mapping analysis were compatible with clinically suspected myocarditis (*Figure 1A*). Nasopharyngeal swab was repeatedly tested negative for SARS-CoV-2 mRNA by reverse transcription–polymerase chain reaction (RT–PCR; Altona Diagnostics; Hamburg, Germany), and negative for influenza A and B, respiratory syncytial virus (RSV), metapneumovirus, and parainfluenza virus when patients presented with clinically suspected myocarditis 4 weeks after possible COVID-19 disease. Endomyocardial biopsies were taken based on the recommendation of current guidelines. Immunohistochemistry and histology revealed myocardial inflammation in the absence of cardiomyocyte necrosis (Dallas criteria of 'borderline myocarditis'[6]), with increased lymphocytes (CD3, LFA-1, and CD45R0) and macrophages (Mac-1), in part with highly abundant perforin-positive cytotoxic T cells. The inflammatory process seemed to be paralleled by increased thickness of small arteries (*Figure 1B*).

Expression analysis of SARS-CoV-2-specific nucleic acid was performed by an RT–PCR assay (TIB MOLBIOL, Roche, Germany). We detected a positive result for the SARS-CoV-2 genome by PCR (*Figure 1C*) and a lower or negative viral load for erythroparvovirus B19, cytomegalovirus, Epstein–Barr virus, adenovirus, Coxsackie virus, and HHV6.

Both patients were monitored during their clinical course on the telemetry ward. They were constantly cardiopulmonary stable with regular blood pressure and heart rate; referral to the intensive care unit was not indicated. They were treated symptomatically, in part with optimization of guideline-directed medication for heart failure and coronary artery disease, respectively (patient B). During hospitalization, in both patients, the levels of cardiac troponin decreased; laboratory values on day 15 were within the reference range, and seroconversion was confirmed by enzyme-linked immunosorbent assay (ELISA) for IgG specific for SARS-CoV-2 (Epitope Diagnostics Inc., San Diego, CA, USA). Patients are currently followed-up in our outpatient clinic to avert or improve development of heart failure (Supplementary material online, *Tables S2* and *S3*; *Figure S2*).

Recently, electron microscopy-based diagnosis of COVID-19 myocarditis was reported.[4] Autopsy studies revealed that 5 out of 12 COVID-19 victims had SARS-CoV-2 mRNA in the myocardium.[7] Our Research

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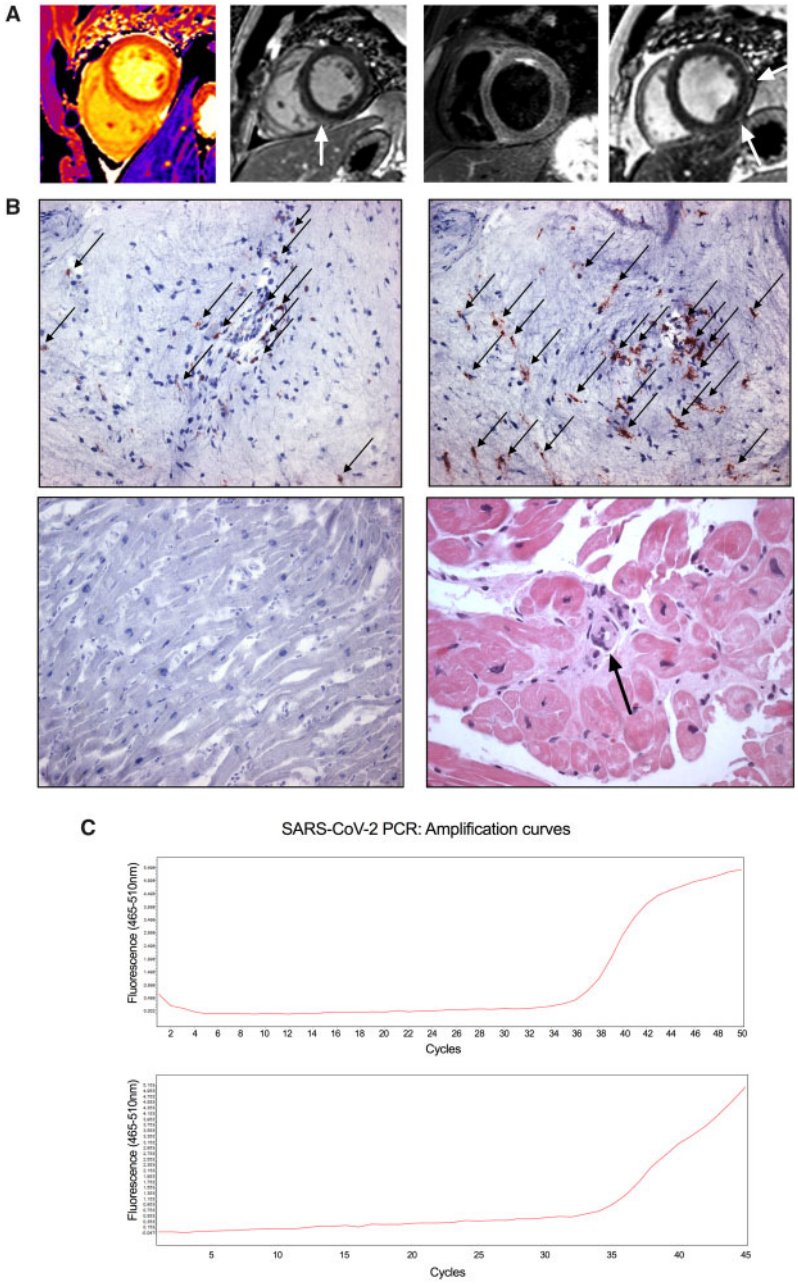

Figure 1 Biopsy-proven SARS-CoV-2 mRNA in clinically suspected myocarditis. (*A*) Representative cardiac magnetic resonance imaging (cMRI) scans. The two left panels: patient A—native T1 map showing prolonged T1 relaxation times in the posterior interventricular septum and corresponding late gadolinium enhancement image (LGE) with enhancement in the posterior septum (arrowhead), consistent with acute myocarditis. Transthoracic echocardiography showed a preserved left ventricular (LV) systolic function (EF 60%) without any wall motion abnormalities, but focal echo-bright appearance of the interventricular septum (not shown) and slightly impaired global longitudinal strain. The two right panels: patient B—representative cMRI scans of the patient who had a history of coronary artery disease treated by percutaneous coronary intervention (everolimus-eluting stent in the right coronary artery). T2-short TI inversion recovery image, showing diffuse myocardial oedema and LGE image with subtle subepicardial enhancement of the lateral wall (arrowheads). Transthoracic echocardiography showed LV dysfunction (EF 30%), decreased global and regional longitudinal strain, as well as increased LV end-diastolic diameter. (*B*) Representative immunohistochemical staining for assessment of inflammation in SARS-CoV-2-positive EMB (patient B). Top left panel: increased CD3+ T lymphocytes. Infiltrates of inflammatory cells (arrowheads) mostly in the neighbourhood of small blood vessels. Top right panel: increased CD45R0+ T memory (arrowheads) cells mostly in the neighbourhood of small blood vessels. Bottom left panel: negative control of CD3 immunostaining. Magnification ×200. Bottom right panel: histological evaluations were performed on paraffin sections with haematoxylin and eosin (HE; patient A). The arrow indicates increased thickness of the small arterial vessel. No active myocarditis according to Dallas criteria ('borderline myocarditis'). Magnification ×400. (*C*) Expression analysis of SARS-CoV-2-specific nucleic acid was performed by an RT–PCR assay (TIB MOLBIOL, Roche, Germany) in cardiac tissue obtained by EMB. Original amplification curves of patient A (top) and patient B (bottom). See also Supplementary material online *Table S1* and *Figure S1*.

Letter is the first report of patients with a history of COVID-19 in whom clinically suspected myocarditis was supported by endomyocardial biopsy (EMB) with evidence of persisting cardiac SARS-CoV-2 mRNA. The pathologist's diagnosis of borderline myocarditis was based on the fact that no cardiomyocyte necrosis was visible in the EMB samples, which is compatible with the clinical picture of a subacute clinical process in both patients.[6]

Since nasopharyngeal swab tested negative for SARS-CoV-2, our data suggest that myocardial inflammation may also evolve as a delayed sequela of aborted or healed COVID-19, in contrast to acute and often life-threatening myocarditis in active COVID-19-infection.[2,4,5] The time course of subacute SARS-CoV-2 myocarditis reported here is very similar to other viral forms of clinically suspected myocarditis[6] (Supplementary material online, *Figure S3*). It can have modest implications for cardiac function (as in patient A) or evolve into heart failure with reduced ejection fraction (as in patient B) which may deteriorate after COVID-19 has healed. Our findings highlight the risk of SARS-CoV-2-infected patients developing heart disease even in young and physically active individuals (patient A, being a cyclist and football player). Co-existing CVD such as in our patient B can foster an unfavourable course of SARS-CoV-2 myocarditis. In COVID-19, mortality has been shown to be approximately twice as high in patients with CVD and myocardial injury as in those with myocardial injury alone, who still had a 50-day mortality rate of 37%.[3] Our results also illustrate that a negative nasopharyngeal swab cannot rule out persistence of SARS-CoV-2,[7] such as presence of the virus in the myocardium in the case of subacute myocarditis. In conclusion, our report underscores the need for more clinical research to understand the usefulness of routine EMB in patients with COVID-19 and myocardial injury as well as disease progression, management strategies, therapeutic options, and long-term prognosis of SARS-CoV-2 myocarditis.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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Data availability statement: The data underlying this article are available in the article and in its online supplementary material.

Conflict of interest: done declared.

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