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SARS-CoV-2 infection leads to cardiac pericyte loss, fibrosis, cardiomyocyte hypertrophy, and diastolic dysfunction

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ABSTRACT

Recovered COVID19 patients often display cardiac dysfunction, even after a relatively mild infection. Here, we present the first histological description of cardiac SARS-CoV-2 infection. Within the heart, the ACE2 receptor is mostly expressed by pericytes. Using a COVID19 hamster model, we demonstrate SARS-CoV-2 is replicating in pericytes, and reduced pericyte density is present after infection. In healthy animals, pericytes recover; however, when metabolic comorbidities are present, they fail to recover. These latter animals present with cardiac fibrosis, cardiomyocyte hypertrophy, and early signs of diastolic dysfunction, resembling HFpEF. Biopsies from recovered COVID19 patients showed similar results, with pericyte loss being present.

MAIN

With the number of COVID-19 cases increasing every day, the long-term consequences of SARS-CoV-2 infection, both after clinically mild or more severe manifestations, are not yet understood. SARS-CoV-2 infection has several implications beyond the traditional respiratory syndrome COVID19, including the risk of developing severe cardiac injury, which in turn may exacerbate its severity ¹⁻³. Over 60% of patients hospitalized for COVID19 present with clear signs of myocardial injury⁴, with between 20 and 63% of patients showing increased biomarker levels, depending on the study ^{2,4,5}. Hospitalized patients present with a range of cardiac dysfunctions, including left ventricular wall motion abnormalities (23.7%), ventricular dysfunction (RV 26.3%, LV18.4%), diastolic dysfunction (13.2%), and pericardial effusions (7.2%) ⁴. Additionally, long term consequences on cardiac function are suggested, as evidenced by the presence of cardiac fibrosis ^{6,7}.

The underlying mechanism by which SARS-CoV-2 infection causes cardiac injury is poorly understood. Cardiac damage could occur indirectly, due to thrombotic events or hyperinflammation targeted against the heart, or directly through viral infection of the heart ⁸. In autopsy specimens from patients who died from COVID19, significant amounts of viral RNA were found in the heart ⁹. Similarly, in a SARS-CoV-2-infected hamster model, the heart is among the tissues with the highest viral loads, after the respiratory tract ¹⁰.

Internalization of the virus depends on its binding to the angiotensin converting enzyme 2 (ACE2) receptor. In the human heart, ACE2 is mostly expressed by pericytes, and to a lesser extent by cardiomyocytes ^{11,12}. As pericytes are crucial for the microvascular function and integrity, SARS-CoV-2 infection may induce capillary dysfunction indirectly by attacking pericytes. The combination of microvascular dysfunction, inflammation, and the presence of

post-infective cardiac fibrosis highlights the potential of developing diastolic dysfunction after recovering from COVID19. In fact, most patients with COVID19 that suffer cardiac injury present with preserved ejection fraction, but show either right ventricular dilatation (29%) or left ventricular diastolic dysfunction (16%) ¹³. The former is likely indirectly caused by effects on the lung, whereas the ventricular dysfunction could be a direct effect of the cardiac SARS-CoV-2 infection itself. As such, COVID19 may be an independent risk factor for the development of heart failure with preserved ejection fraction (HFpEF) ¹⁴.

Here, we use a SARS-CoV-2-infected hamster model to investigate the cardiac effects of COVID19. A reduced cardiac pericyte coverage is present very early after infection. Additionally, we show that *in vitro* ACE2 internalization renders pericytes more prone to vasodilatation. Finally, we demonstrate that in the presence of metabolic comorbidities (obesity, hyperlipidemia, and hypertension), SARS-CoV-2-infected hamsters fail to recover their reduced cardiac pericyte coverage and present with fibrosis and cardiomyocyte hypertrophy. Using echocardiography, we demonstrate that in the presence metabolic comorbidities, SARS-CoV-2 infection accelerate the development of diastolic dysfunction. To confirm the relevance for COVID19 patients, we investigated microvascular structure in biopsies from recovered COVID19 patients who had developed cardiac complications. All patients showed reduced pericyte coverage of capillaries, as indicated by $\alpha\text{SMA}^{\text{low}}$ expression.

RESULTS

SARS-CoV-2 infects cardiac pericytes and causes a reduction in cardiac pericyte coverage in hamsters

To study the effect of SARS-CoV-2 infection on cardiac function, we used a previously described COVID19 hamster model ^{15,16}. In this model, hamsters are inoculated intranasally with SARS-CoV-2 virus (2×10^6 TCID₅₀), which leads to a peak in lung viral load at 4 days post infection (dpi). To determine whether SARS-CoV-2 infects the heart tissue, we quantified the viral load in the heart, relative to lung and muscle tissue, by using qPCR at 4 dpi. In the heart, the viral load reached a median of $\sim 4 \log_{10}$ RNA copies per mg tissue, around $3 \log_{10}$ less compared to the lung, while in the muscle only minimal amounts of viral RNA was detected (**Fig. 1a**). Titrations of homogenized cardiac tissue confirmed the presence of infectious particles in the heart of around $2 \log_{10}$ TCID₅₀/mg tissue (**Supplemental Fig. 1**). We next investigated the expression of viral RNA in the heart using *in situ* hybridization at 4 dpi. Our *in situ* hybridization demonstrates that the SARS-CoV-2 virus was present in cells adjacent to capillaries. This indicates that SARS-CoV-2 replication occurs primarily in the pericytes surrounding the capillaries (**Fig. 1b**).

Given the presence of virus in the pericytes, we next investigated cardiac microvascular changes in the infected hamster hearts at 4 dpi. No difference in cardiac arteriolar density was observed, as defined by high α SMA expression (**Supplemental Fig. 2**). In order to identify all cardiac pericyte subsets, we used both PDGFR- β and NG2 as markers. Infected hamsters showed a significantly reduction in the percentage of capillaries with NG2⁺

pericytes (**Fig. 1c-d**), while there was no difference observed in PDGFR- β^+ pericytes (**Fig. 1c-d**). Double-positive NG2/PDGFR- β vessels showed a trend ($p = 0.0542$) towards a reduced pericyte density in the heart (**Fig. 1c-d**). These changes in cardiac pericyte coverage were not associated with any changes in vascular density at 4 dpi (**Fig. 1c-d**).

Pericytes *in vitro* are more susceptible to pro-relaxation cues after ACE2 internalization

As SARS-CoV-2 infection is associated with cardiac pericyte loss in hamsters, we questioned whether it would affect pericyte functioning as well. As such, we simulated SARS-CoV-2 infection by exposing human immortalized pericytes *in vitro* to recombinant SARS-CoV-2 spike protein (**Fig. 2**). The SARS-CoV-2 spike protein causes an internalization of ACE2, resulting in a reduced ACE2 surface expression¹⁷. Exposure of human immortalized pericytes with SARS-CoV-2 spike protein did not induce apoptosis (**Fig. 2a, b**), but significantly affected pericyte function. We analyzed the expression of genes involved in pericyte contraction and relaxation (**Fig. 2c-f**) and their interaction with endothelial cells (**Supplemental Fig. 3**). We found a two-fold and three-fold increase in gene expression of adenosine A1 receptor and adenosine A2A receptor, respectively (encoded by *ADORA1* and *ADORA2*, **Fig. 2c-d**). These genes are involved in adenosine-induced pericyte relaxation¹⁸. Similarly, transcript levels of prostaglandin E2 receptor 4 (*PTGER4*), a receptor involved in prostaglandin-induced pericyte relaxation, were upregulated upon SARS-CoV-2 spike protein exposure (**Fig. 2e**)¹⁹. We observed no change in the Adrenoceptor Alpha 2A receptor, encoded by *ADRA2A*, which induces noradrenaline-induced pericyte contraction (**Fig. 2f**)^{19,20}. In contrast to the strong effects on genes involved in pericyte relaxation, most of the growth factors produced by pericytes sustaining endothelial cell function were only

slightly affected by exposure to the SARS-CoV-2 spike protein, with the exception of Heparin-binding EGF-like growth factor (*HBEGF*), which showed an almost 2-fold increase (**Supplemental Fig. 3**). These results show that ACE2 internalization as a consequence of SARS-CoV-2 spike protein exposure promotes growth factor production by pericytes slightly, but makes them more sensitive to relaxation cues, possibly causing a dysregulation in microvascular flow patterns.

SARS-CoV-2 infected hamsters do not recover their cardiac pericyte coverage when exposed to metabolic comorbidities

Though our results indicate that cardiac pericytes were lost, and possibly dysfunctional, after SARS-CoV-2 infection, it was unclear whether pericytes could recover post-infection. Pericytes are also lost in other pathologies, notably diabetes ²¹. Here, insulin treatment can partly rescue the reduced pericyte density observed ²². Thus, pericytes have at least some regenerative capacities. As such, we investigated whether the heart could also recover after SARS-CoV-2 infection (**Fig. 3**). Since the presence of metabolic comorbidities is associated with both HFpEF and a more severe outcome after COVID19 ^{23,24}, we investigated whether hyperlipidemia and hypertension affects the cardiac phenotype after COVID19 recovery. To this end, infected hamsters and uninfected controls received either high fat diet (HFD) combined with hypertension-inducing N γ -Nitro-L-arginine methyl ester (L-NAME) or a regular chow diet ²⁵. Hamsters were put on HFD/L-NAME one week before infection (**Fig. 3a**).

At 14 dpi, viral RNA was still detected in the lungs, but had decreased substantially compared to levels present at 4 dpi (**Fig. 3b**). In the heart, no viral RNA was detected at 14 dpi. Uninfected hamsters on HFD/L-NAME gained weight after being three weeks on diet, while the weight of SARS-CoV-2 infected animals remained stable (**Fig. 3c-d**).

In uninfected chow-fed hamsters, approximately 80% of cardiac capillaries are covered by pericytes expressing either PDGFR- β and/or NG2 at 14 dpi (**Fig. 3e-i**). Both HFD/L-NAME-treated uninfected hamsters and SARS-CoV-2-infected hamsters on a chow diet showed a slight but insignificant reduction in pericyte coverage, compared to uninfected chow-fed animals (**Fig 3e-f**). Infected animals on HFD/L-NAME, however, presented with a reduced NG2⁺ pericyte density compared to chow-fed hamsters (**Fig. 3e-i**). Approximately 60% of vessels were covered with pericytes, which is similar to the observed level of pericyte coverage of infected hamsters at 4 dpi (**Fig. 1c,d,f**). Overall, our results indicate that cardiac pericyte coverage does recover, but is prevented by metabolic comorbidities. When we investigated α SMA coverage and microvascular density, no differences were observed between the groups (**Supplemental Fig. 4**).

Cardiac damage remaining post-infection could be indicative of future cardiac dysfunction. We therefore investigated cardiomyocyte hypertrophy and cardiac fibrosis in hamster hearts at 14 dpi (**Fig. 4**). Cardiomyocytes from SARS-Cov-2 infected hearts were 20% larger compared to cardiomyocytes from uninfected hamster hearts (**Fig. 4a-b**). This is the a slightly smaller range of hypertrophy reported for animal HFpEF models, where increases between 35% and 45% have been reported^{25,26}. Other viral myocarditis models, such as CVB3 infection in mice, show approximately 15% increases in cardiomyocyte size after five weeks²⁷, further highlighting the unique nature of this cardiac hypertrophy. We found a

significant increase in fibrosis, both due to HFD/L-NAME, as well as infection with SARS-CoV-2 (**Figure 4c-d**). These effects were not cumulative, as infected hamsters on treatment showed the same level of fibrosis as hamsters on treatment or infected alone.

Lastly, we investigated cardiac function at 14 dpi by echocardiography. At this stage, few defects in cardiac function were present (**Fig. 5**). No differences in heart rate (HR) were observed between the groups (**Fig. 5a**). Systolic function was assessed by measuring ejection fraction (EF), fractional shortening (FS), and isovolumic contraction time (IVCT) (**Fig. 5b, Supplemental Table S1**). No difference in FS was observed (**Fig. 5b**). IVCT was increased in chow diet-fed infected hamsters compared to non-infected hamsters (**Supplemental Table S1**). Cardiac hypertrophy was also suggested by the echocardiography, with an increased LV mass present in both infected hamster groups, although statistically insignificant (**Fig. 5c**). Some evidence of the onset of diastolic dysfunction could be observed in the infected HFD/L-NAME hamsters. Although the E-wave increases drastically in severe, late-stage diastolic dysfunction, it decreases early in diastolic dysfunction. Here, a small but statistically significant decrease in the E-wave was present in HFD/L-NAME-treated infected hamsters compared to control uninfected hamsters (**Fig. 5d**). Similarly, the IVRT was slightly increased (**Fig. 5e**). However, the most common indicators of diastolic dysfunction, E/E' , E/A , and mitral valve deceleration time (MV DT), were unaffected in all groups (**Fig. 5f-g, Supplemental Table S1**). Complete echocardiographic analysis is available in **Supplemental Table S1**. Together with the histological data, our echocardiography data suggest that the infected hamster exposed to comorbidities are showing early signs of diastolic dysfunction.

Recovered COVID19 patients present with reduced number of α SMA^{low} pericytes.

Lastly, we investigated whether similar effects were observed in patients that recovered from COVID19 (**Fig. 6**). Biopsies were obtained from patients that developed cardiac complications, warranting catheterization. We limited our analysis to patients who presented with cardiac injury but had a preserved ejection fraction. As control tissue, we used biopsies from patients who had been suspected of developing viral myocarditis but tested negative, as well as patients diagnosed with HFpEF. We found no differences in cardiac microvascular density between control patients, HFpEF patients, and recovered COVID19 patients, based on CD34 endothelial staining (**Fig. 6a-b**). Human cardiac pericytes express several markers, including platelet-derived growth factor receptor beta (PDGFR- β), neuron-glial antigen 2 (NG2), as well as low alpha smooth muscle actin (α SMA) levels ²⁸. However, not all pericytes express all these markers, suggesting that each marker may stain a different subset of pericytes. None of the tested PDGFR- β antibodies worked on our samples, possibly due to the fixation protocol necessary for working with infected cardiac biopsies. We found no statistical difference in the percentage of NG2⁺ capillaries for any of the patient groups, even the HFpEF patients. The data from recovered COVID19 patients was extremely variable (**Supplemental Fig. 5**). However, we found a significant reduction in the percentage of α SMA^{low} capillaries in both HFpEF and COVID19 recovered patients (**Fig. 6a-c**). Together, these data demonstrate that both recovered COVID19 patients and HFpEF patients lose the same amount of α SMA^{low} pericytes, independently of SARS-CoV-2 infection, suggesting that pericytes are indeed targeted by SARS-CoV-2 infection.

Though cardiomyocyte expression of ACE2 is low, several reports show that the expression increases in patients with certain comorbidities ²⁹. We therefore investigated whether

COVID19 affects cardiomyocytes by measuring cardiomyocyte hypertrophy, but could not detect any difference in cardiomyocyte size between either patient group (**Supplemental Fig. 6**). We cannot, however, say with certainty that our control patients lacked hypertrophy since they presented with viral myocarditis-like symptoms.

DISCUSSION

Our results demonstrate that SARS-CoV-2 infection profoundly affects the cardiac microvascular structures by reducing pericyte coverage. This can predispose SARS-CoV-2 infected individuals to developing diastolic heart failure, especially in the presence of metabolic comorbidities. Previous reports stated that ACE2 was largely expressed by cardiac pericytes and to a lesser extent in cardiomyocytes^{11,12}. Our *in situ* hybridization shows that pericytes have the highest levels of viral replication in the heart. This does not mean that the virus is not present in cardiomyocytes, but only that the virus replicates at a much higher level in pericytes.

COVID19 can in some cases lead to viral myocarditis-like syndromes³⁰ and recovered patients show clinical indicators of cardiac injury³¹. Because the infection targets pericytes, our results support the idea that recovered patients are at risk of developing HFpEF. Our group has been investigating HFpEF development in the ZSF1 rodent model (Cuijpers, et al., in preparation). Our unpublished findings show that pericyte loss is the earliest parameter to change histologically and inducing pericyte loss induces HFpEF. Infected hamsters, especially those exposed to metabolic comorbidities, show histological signs of HFpEF. They have increased interstitial stiffness (fibrosis) and cardiac microvascular dysfunction, as

shown by the loss of cardiac pericytes. At the stages investigated, diastolic dysfunction could not yet be detected, as assessed by changes in E/E' and E/A . However, some measures of diastolic dysfunction, specifically IVRT and the E wave velocity, began to indicate dysfunction. Therefore, our results demonstrate that COVID19 recovered patients with metabolic comorbidities should be followed closely to investigate diastolic dysfunction.

The level of pericyte loss observed in our hamster model was much milder compared to the recovered COVID19 patients (around 30% reduction when using $\alpha\text{SMA}^{\text{low}}$ as a marker) and our animal HFpEF models (41% pericyte loss after 14 weeks in ZSF1 rat model, Cuijpers, et al., in preparation). Pericyte loss in patients was also marker specific, possibly indicated that a specific subset of cardiac pericytes is targeted. Only hamsters with pre-existing metabolic comorbidities exhibited a difference in all pathophysiological markers (hypertrophy, fibrosis, and pericyte loss) and showed some level of diastolic dysfunction by echocardiography. Therefore, we suspect that a model with increased cardiac pericyte loss would have an even more pronounced phenotype. Given that patients experience different viral loads, it would be important to investigate different viral loads and the effect on the development of HFpEF after recovery.

Our results highlight that the cardiac pericyte population can recover in the absence of metabolic comorbidities. Pericytes can migrate and make extensions to replace lost pericytes or recover by proliferation^{28,32}. Which of these mechanisms is occurring after SARS-CoV-2 infection is not clear. We can, however, show that pericyte recovery is inhibited by metabolic comorbidities. As such, our results indicate that controlling co-morbidities in COVID19 patients is of primary importance.

Cardiac fibrosis was present in our HFpEF hamster model, as well as in the infected hamsters, regardless of co-treatment with HFD/L-NAME. Cardiac fibrosis has been demonstrated by cardiovascular magnetic resonance techniques after SARS-CoV-2 infection, even months later in young patients with mild or no symptoms during infection ³³. Similarly, a third of COVID19 patients show increased late gadolinium enhancement (LGE) ^{6,7}. LGE increase can indicate myocyte necrosis, myocardial oedema, myocardial scar tissue, and focal areas of fibrosis ³⁴. At the same time, increased LGE predicts mortality in viral myocarditis ³⁴. Our results suggest that the increased LGE observed after SARS-CoV-2 infection is likely due to increased cardiac fibrosis.

Cardiac pericytes were lost, but they may also be hypersensitive to pro-relaxation signals. Pericytes provide a baseline tone to capillaries and loss of pericytes induces capillary dilatation ³². Localized loss of pericyte tone increases blood flow to a region ³⁵, however, massive loss of microvascular tone induces reductions in blood flow ³⁶. Interestingly, loss of vessel perfusion has been noted upon SARS-CoV-2 infection ³⁷. Circulating levels of the pericyte vasodilator adenosine increase rapidly during any viral infection ^{38,39}. Thus, the combination of reduced pericyte numbers and a hypersensitivity to pro-relaxation stimuli may not be beneficial in this case. The receptors for adenosine are also expressed on immune cells, where their activation reduces the intensity of the immune response ⁴⁰. If immune cells also upregulated *ADORA1/2* in response to SARS-CoV-2 spike protein exposure, then adenosine would lead to a more moderate immune response, something of benefit in the response to SARS-CoV-2 infection. Overall, it is unclear whether *ADORA1/2* expression changes represent an overall benefit or detriment. A common variant in adenosine monophosphate deaminase (rs17602729, *AMPD1*) leads to increased circulating

adenosine levels ⁴¹ and is present in 20% of the population ⁴². The possible significance of these *in vitro* results could be confirmed by studying this population and whether they are protected or more sensitive to SARS-CoV-2 infection.

Overall, our results show that SARS-CoV-2 infection primarily targets pericytes in the heart. Microvascular dysfunction and fibrosis are hallmarks of HFpEF. We therefore emphasize the need to manage metabolic comorbidities in COVID19 patients.

MATERIALS

Animal handling

Experiments were performed according to the European Directive (2010/63/EU) and approved by the Animal Care and Use Committee of KU Leuven (P217/2017 and P083/2020). Female Syrian hamsters were inoculated intranasally with 2×10^6 TCID₅₀ SARS-CoV-2, strain BetaCov/Belgium/GHB-03021/2020 (EPI ISL 407976 | 2020-02-03), recovered from a nasopharyngeal swab from a patient who returned from Wuhan in February 2020, and sacrificed after 4 dpi or 14 dpi ^{15,16}. Hamsters received either a regular chow diet or a combination of HFD (60% fat, 20% proteins, 20% carbohydrates; D12492, Research Diet Inc.) with L-NAME (0.5 g/L; N5751, Sigma Aldrich) dissolved in the drinking water, as indicated.

Transthoracic echocardiography

Hamsters were anaesthetized with an intraperitoneal injection of 100 mg/kg ketamine (Nimatek, Eurovet) and 10 mg/kg xylazine (Xyl-M, V.M.D. nv/sa) ⁴³. Complete transthoracic 2D M-mode echocardiography was performed using a MS 250 transducer (13-24 MHz)

connected to a Vevo 2100 echocardiograph (Visual Sonics). Hamsters were placed in a supine position on a heating pad to maintain core body temperature between 37.5-37.7°C, measured using a rectal probe. Electrocardiography recordings were performed to monitor heart and breathing rate. If needed, anesthesia dosage was modified to ensure constant heart rates between all hamsters. HR and cardiac dimensions were assessed on the parasternal short-axis M-mode imaging. CO, EF, LV mass, and FS were calculated based on parasternal short-axis M-mode recordings. Left ventricular filling was assessed by pulsed wave Doppler trans-mitral flow velocity tracings, including peak E and A wave velocities, MV DT, and IVRT. Myocardial E' and A' diastolic peak velocity was measured by tissue Doppler imaging at the lateral mitral annulus. E/A, E/A', E/E', and E'/A' ratios were calculated. At least three stable cardiac cycles were averaged for all measurements. More details are available in the online Supplemental Materials.

Organ collection

Hamsters were sacrificed at 4 dpi, or after echocardiography at 14 dpi. Hamsters were euthanized by excision of the heart. Approximately 5-30 mg of the apex of the heart was placed in RTK buffer for RNA extraction, the remaining was fixed overnight in 4% paraformaldehyde (PFA).

SARS-CoV-2 RT-qPCR and end-point titrations

Hamsters tissues were homogenized with bead disruption (Precellys) in 350 µL RLT buffer (RNeasy Mini kit, 74004 Qiagen) and RNA was extracted according to the manufacturer's instructions. RT-qPCR was performed on a LightCycler96 platform (Roche) with iTaq Universal Probes One-Step RT-qPCR kit (1725140, BioRad) with N2 primers and probes

targeting the nucleocapsid ¹⁶. Standards of SARS-CoV-2 cDNA (IDT) were used to quantitatively express viral load as genome copies per mg tissue. For end-point titrations, Vero E6 cells (African green monkey kidney, ATCC CRL-1586) were cultured in minimal essential medium (15188319, Gibco) supplemented with 10% fetal bovine serum, 1% L-glutamine (25030149, Gibco) and 1% bicarbonate (25080094, Gibco). Homogenized lung tissue in minimal essential medium was titrated on confluent Vero E6 cells in 96-well plates. Viral titers were calculated with the Reed and Muench method and expressed as 50% tissue culture infectious dose (TCID₅₀) per mg tissue.

***In situ* hybridization**

In situ hybridization was performed as described previously⁴⁴ using SARS-CoV-2 anti-sense specific probes (3' ATTAACCCTCACTAAAGGGATTGGTGGACCCTCAGATTC 5') and SARS-CoV-2 sense specific probes (3' TAATACGACTCACTATAGGGGCGCGACATTCCGAAGAA 5') targeting the positive-sense and negative-sense genomic viral RNA, respectively. In brief, after slides were deparaffinized in xylene and rehydrated through a series of graded ethanol, epitope retrieval was performed for 5 min in Proteinase K (3115828001, Sigma) at 37°C, followed by post-fixation in 4% PFA for 20 minutes. Slides were incubated with the antisense and sense probes overnight at 70°C, with a final concentration of 1 µg/ml. Slides were washed through a series of washing buffers and treated with 100 µg/mL RNase A (EN0531, Thermofisher) for 30 min. Slides were blocked with 2% Blocking Reagent (11096176001, Roche) for 1 hour and incubated overnight with anti-digoxigenin-AP Fab fragments antibody (1:2000, 11093274910, Roche) at 4°C. Slides were washed through a series of washing buffers and staining was visualized by BM purple AP substrate (11442074001, Roche) at room temperature, until staining was sufficient.

Patient characterization

After routine non-invasive diagnostic work-up and angiography had failed to elucidate any specific cause of heart failure like-symptoms, all patients underwent endomyocardial biopsy (EMB) and left heart catheterization in a standardized manner, as previously described ⁴⁵. All patients provided written consent for the procedures (local ethic vote number: EA2/140/16 and EA2/066/20). The investigation conforms with the principles outlined in the Declaration of Helsinki ⁴⁶.

Three different groups of patients were investigated and compared:

1. Patients served as controls where a cardiac cause for their chest discomfort were excluded (n=3; age (57±14); male/female (1/2); normal EF (55±2.9%) without signs of LV diastolic dysfunction and regular NT-pro BNP levels.
2. Symptomatic patients with classical signs of HFpEF, LV diastolic dysfunction, and abnormal NT-proBNP levels, served as a group where a viral presence and / or a significant cardiac inflammation were excluded (n=3; age (54±7.0); male/female (2/1); preserved EF (55±3.2%)).
3. Symptomatic patients, who recovered from COVID-19 (n=3; 5±2 weeks post-COVID-19; age (44±13); male/female (2/1); preserved EF (59±0.88%) and regular right and left ventricular wall motions, absence of SARS-CoV2 genome in EMB). In detail, they suffered from exercise intolerance (NYHA II-III), and atypical angina but showed regular NT-pro BNP levels. Spiral CTs excluded lung embolization. Cardiac MRI analyses were positive for post-inflammatory abnormalities (positive T2 mapping), pericardial inflammation or microvascular dysfunction, respectively. None of these patients had an increase in extracellular volume, indicating absence of cardiac fibrosis.

Presence of other viruses was excluded, whereas subacute lymphocytic myocarditis was diagnosed in 1 out of the 3 patients.

Histological analysis

All antibodies and concentrations are listed in **Supplemental Table S2**. EMB were fixed in 10% neutral buffered formalin overnight at 4°C (classical protocol) or at room temperature to avoid potential SARS-CoV2 infection. Four µm sections were stained with anti-CD34 antibodies, αSMA-Cy3 antibody, and NG2 antibody. Epitope retrieval was performed for CD34 and NG2 stainings by using citrate buffer (0.1M Target Retrieval Solution; DAKO S1699, Agilent). Amplification of CD34 was performed using a Tyramide Superboost kit (Alexa Fluor 488 Tyramide SuperBoost™ Kit, B40922, Invitrogen).

For hamster cardiac tissue, four µm paraffin sections were cut and deparaffinized. For laminin staining, proteinase K epitope retrieval was used (1/500 in PBS, 3115828001, Sigma Aldrich). For NG2, PDGFR-β, and αSMA staining citrate buffer (0.1M Target Retrieval Solution, DAKO S1699, Agilent) was used.

***In vitro* stimulation of pericytes**

Human immortalized pericytes (CL 05008-CLTH, Celther) were cultured in DMEM high glucose medium (41965039, Thermofisher) supplemented with penicillin/streptomycin. Cells were exposed to 50 ng/ml Recombinant SARS-CoV-2 S Protein RBD Fc Chimera, CF (10499-CV, R&D Systems) for 24h to induce ACE2 internalization. Three samples per condition were stained with Annexin V (1/9; A13202, Thermofisher) to assess the number of apoptotic cells. Sequences of qPCR primers are given in **Supplemental Table S3**.

Statistical analysis

Results are presented as mean $\pm$ SEM. Statistical analysis was performed using GraphPad 8 Prism. Variance was tested with F-test ($p < 0.05$) and normality was tested with Shapiro-Wilk test ($p < 0.05$). Biological outliers were picked up based on Grubb's test ($p < 0.05$). *In vivo* data were analyzed using a one-way ANOVA with Dunnett's post hoc test ($p < 0.05$ *, 0.01 **, 0.001 ***).

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AUTHOR CONTRIBUTION

MD performed experiments, designed experiments, analysed data and wrote the manuscript. LL performed experiments, designed experiments, analysed data and edited the

manuscript. IC analysed data and edited the manuscript. RB performed experiments and edited the manuscript. JR analysed data and edited the manuscript. SJS performed experiments and edited the manuscript. NG performed experiments. ML performed experiments. PV designed experiments and edited the manuscript. SVL designed experiments, and edited the manuscript. SH designed experiments and edited the manuscript. CT performed experiments, designed experiments, and edited the manuscript. EAVJ designed experiments, analysed data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

All data are available upon request.

COMPETING INTERESTS

The authors declare no competing or financial interests.

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FIGURES

Figure 1. SARS-CoV-2 infected hamsters lose their NG2⁺ pericytes at 4 days post infection.

Hamsters were infected with SARS-CoV-2 virus and sacrificed 4 days post infection, at the peak of viral load. **(a)** Viral RNA levels were quantified by RT-qPCR in lung, heart, and muscle from infected and uninfected hamsters. **(b)** SARS-CoV-2 infection was analyzed by *in situ* hybridization techniques **(c)** Heart sections of control and infected hamsters were stained for isolectin (green), NG2 (red), and PDGFR β (blue) to analyze percent pericyte positive

capillaries (**d**). HFD, high fat diet; L-NAME, N γ Nitro L arginine methyl ester; NG2, neuronal antigen 2; PDGFR β , platelet-derived growth factor receptor beta. Values are presented as mean $\pm$ SEM with n = 7 (control) and 5 (SARS-CoV-2 infected hamsters). Statistical analysis was performed using a two-tailed students t-test, with * p < 0.05 and ** p < 0.01. Scalebar represents 20 μ m (**b, c, d**).

Figure 2. SARS-CoV-2 spike protein exposure makes immortalized pericytes more susceptible for relaxation, without inducing apoptosis. Immortalized pericytes were exposed to 50 ng/ml SARS-CoV-2 spike protein for 24h. **(a-b)** Pericytes were stained for Annexin V (red) and ToPro (blue), with double positive cells indicating apoptosis. **(c-f)** RNA levels for *ADORA1* (**c**), *ADORA2* (**d**), *PTGER4* (**e**) and *ADRA2A* (**f**) were analyzed by RT-qPCR. *ADORA1*, adenosine A1 receptor; *ADORA2*, adenosine A2A receptor; *PTGER4*, prostaglandin E2 receptor 4; *ADRA2A*, Adrenoceptor Alpha 2A receptor. Values are presented as mean $\pm$ SEM with n = 2-3. Statistical analysis was performed using a two-tailed students t-test, with * p < 0.05. Scalebar represents 100 μ m (**a-b**).

Figure 3. Hamsters on HFD + L-NAME diet do not recover their pericyte coverage after SARS-CoV-2 infection. (a) Twelve-week-old hamsters received chow diet or HFD + L-NAME diet one week prior to SARS-CoV-2 infection. Hamsters were subjected to a cardiac echography and sacrificed two weeks after SARS-CoV-2 infection. (b) Viral RNA levels were quantified by RT-qPCR in lung and heart of all hamster groups. (c-d) Body weights were followed up over the course of the experiment and normalized to tibia length. (e) Heart sections of control and infected hamsters were stained for isolectin (green), NG2 (red), and PDGFR β (blue) to analyze pericyte coverage (f-i). GC, genome copies; HFD, high fat diet; L-NAME, N γ Nitro L arginine methyl ester; PDGFR β , platelet-derived growth factor receptor

beta; TL, tibia length; W, weight. Values are presented as mean $\pm$ SEM with n = 6. Statistical analysis of body weights was performed using a two-way ANOVA with a Dunnett's multiple comparison post hoc test with * p < 0.05, ** p < 0.01. All other data was analyzed using a one-way ANOVA followed by a Dunnett's multiple comparisons test, with * p < 0.05, comparing HFD + L-NAME hamsters or infected hamsters with or without HFD + L-NAME diets to control hamsters. Scalebar represents 20 μ m (**e**).

Figure 4. SARS-CoV-2 infected hamsters present with a small cardiomyocyte hypertrophy and increased cardiac fibrosis. (a) Heart sections of control and infected hamsters, either on regular chow diet or HFD + L-NAME diet, were stained for laminin and Topro3 to quantify cardiomyocyte hypertrophy **(b)**. **(c)** Heart sections of control and infected hamsters, either on regular chow diet or HFD + L-NAME diet, were stained with Picro Sirius Red to analyze cardiac fibrosis **(D)**. HFD, high fat diet; L-NAME, N γ Nitro L arginine methyl ester. Values are presented as mean ± SEM with n = 6. Statistical analysis was analyzed using a one-way ANOVA followed by a Dunnett's multiple comparisons test, with * p < 0.05, comparing HFD + L-NAME hamsters or infected hamsters with or without HFD + L-NAME diets to control hamsters. Scalebar represents 20 μm **(a)**, 50 μm **(c)**.

Figure 5. Echocardiography demonstrates an extremely mild diastolic dysfunction in SARS-CoV-2 infected hamsters on HFD + L-NAME diet. Cardiac function of hamsters was investigated using echocardiography two weeks after SARS-CoV-2 infection. HR (a), FS (b), E wave (c), IVRT (d), E/E' (f), and E/A (g) were analyzed. A, late mitral inflow peak velocity; E, early mitral inflow peak velocity; E', early diastolic mitral annulus peak velocity; FS, fractional shortening; HFD, high fat diet; HR, heart rate; IVRT, isovolumic relaxation time; L-NAME, N γ Nitro L arginine methyl ester. Values are presented as mean $\pm$ SEM with n = 6. All data were analyzed using a one-way ANOVA followed by a Dunnett's multiple comparison test with * p < 0.05, ** p < 0.01, comparing HFD + L-NAME hamsters or infected hamsters with or without HFD + L-NAME diets to control hamsters.

Figure 6. Recovered COVID19 patients lose the same amount of α SMA^{low} pericytes as patients that develop HFpEF independently of SARS-CoV-2 infection. (a) Heart biopsies from control, HFpEF patients, and recovered COVID19 patients were stained for H&E, CD34 (green), and α SMA (red). Microvascular density (b) and the amount of α SMA^{low} vessels (c) were analyzed. H&E, hematoxylin, and eosin; α SMA, alpha smooth muscle actin. Values are presented as mean $\pm$ SEM with n = 3 per group. Statistical analysis was performed using one-way ANOVA followed by a Dunnett's multiple comparisons test, with * p < 0.05, comparing HFpEF patients or recovered COVID19 patients to controls. Scalebar represents 20 μ m.

Figures

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