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RESEARCH LETTER

Prolonged endothelial-dysfunction in human arterioles following infection with SARS-CoV-2

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection has a major global impact and is responsible for over 230 million cases and 4.7 million deaths worldwide.[1]

More than 200 different chronic symptoms have been identified to occur following recovery from the virus.[2] Impaired endothelial function, especially in the microcirculation plays a crucial role in regulating tissue perfusion and is strongly predictive of future cardiovascular adverse events.[3] In conduit arteries, impaired flow-mediated dilation (FMD) persists in COVID-19 patients for 3–4 weeks after a positive SARS-CoV-2 test compared with healthy controls.[4] However, the effects of SARS-CoV-2 infection in resistance microvessels remain unknown. The present study aimed to evaluate the magnitude and duration of microvascular-endothelial-(dys) function post-infection with SARS-CoV-2.

2. Methods

Discard surgical specimens were collected (no consent necessary) as approved by Institutional Review Board of the Medical College of Wisconsin and in accordance with the *Declaration of Helsinki*. Fresh human microvessels (average internal-diameter 152 ± 7 μm) were isolated from adipose or atrial appendages. All patients tested negative for SARS-CoV-2 prior to surgery. To avoid undetected prior COVID-19 infection, historic data pre-October 2019 were used, classified for cardiovascular risk factors (RF) as previously published[5] and grouped as historic-controls (n = 19, 2 males/17 females, 44 ± 3 years, 14 white/5 non-white, risk-score 0.2 ± 0.1); historic-control with similar in cardiovascular RF (Historic-CTRL+Similar RF; n = 28, 5 males/23 females, 42 ± 1 years, 20 white/8 non-white, coronary artery disease (CAD) 4, hypertension 7, hyperlipidaemia 8, diabetes mellitus 1, ≥60 years 0, risk-score 3.8 ± 0.6); controls with more severe cardiovascular RFs (historic-CTRL+RF++; n = 13, 10 males/3 females, 64 ± 2 years, 10 white/3 non-white, CAD 10, hypertension 9, hyperlipidaemia 8, diabetes mellitus 7, age ≥60 years 8, risk-score 6.4 ± 0.7) and compared to patients with prior SARS-CoV-2 positive test (p-SARS-CoV-2+; n = 26, 3 males/23 females, 48 ± 3 years, 14 white/12 non-white, CAD 2, hypertension 13, hyperlipidaemia 9, diabetes mellitus 9, age ≥60 years 7, risk-score 3.2 ± 0.7). Among p-SARS-CoV-2+ subjects, the time between positive and negative PCR test results was stratified to ≤3 months (n = 9), or 4–16 months (n = 17). Microvascular dilation was assessed *in vitro* using videomicroscopy as previously described.[6] Data are presented as %Maximal dilation at given dose ± SEM. Statistical analysis was performed for %Maximal dilation by 2-way-ANOVA vs. pressure gradient (cmH_2O, *Figure 1A*) and one-way-ANOVA with multiple comparisons accounting for time since SARS-CoV-2+ (months, *Figure 1B*).

3. Results

Endothelial-dependent dilation to acetylcholine (at 10^{-5} M, p-SARS-CoV-2+: 71 ± 4%*, n = 9 vs. historic-CTRL: 95 ± 3%, n = 16, *P < 0.05), and FMD (at 100 cmH_2O, p-SARS-CoV-2+: 46 ± 3%*, n = 26 vs. historic CTRL: 86 ± 2%, n = 6, Historic-CTRL+Similar RF: 88 ± 2%, n = 28, and Historic-CTRL+RF++: 86 ± 2%, n = 13, *P < 0.05, *Figure 1A*) were markedly reduced in p-SARS-CoV-2+ subjects. Smooth-muscle mediated, endothelial-independent dilation to sodium nitroprusside (at 10^{-4} M, p-SARS-CoV-2+: 84 ± 6% vs. historic-CTRL: 92 ± 2%, n = 5/group) was not altered by SARS-CoV-2 exposure. Stratification by time since p-SARS-CoV-2+, patient age, and race revealed no impact of age or racial/ethnic background on endothelial function. Reduced endothelial dilator capacity persisted several months after SARS-CoV-2 exposure (*Figure 1B*). Decreased endothelial function was observed in p-SARS-

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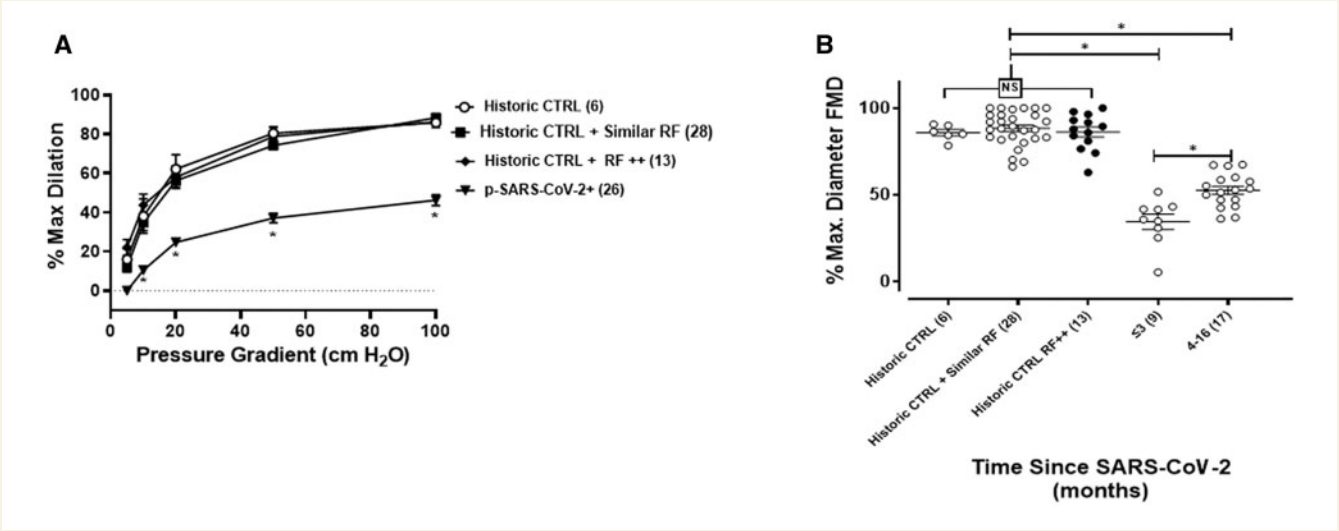

Figure 1 Previous SARS-CoV-2 infection causes prolonged reduction in flow-mediated dilation (FMD). (*A*) Endothelial dependent FMD was significantly reduced in arterioles from p-SARS-CoV-2+ subjects compared to controls that were otherwise healthy (Historic CTLR) or had underlying established cardiovascular RFs to similar level or additional comorbidities (Historic CTRL + Similar RF or Historic CTRL + RF ++). (*B*) Maximal FMD was compared to Historic CTLR, Historic CTRL + Similar RF and CTRL-RF++ in relation to time since p-SARS-CoV-2+. *P<0.05 2-way ANOVA RM vs. all historic CTRLs (*A*); one-way ANOVA RM (*B*). NS, not significant. Ns (isolated vessels/group) indicated in figure legend.

CoV-2+ patients when compared to CTRL-RF controls independent of the degree of pre-existing cardiovascular RFs.

4. Discussion

Our findings indicate endothelial-dependent dilation in human arterioles is impaired for months after SARS-CoV-2 exposure. This finding might contribute to long-lasting symptoms of post-COVID-19 infection and could directly increase risks for future cardiovascular events. The degree of endothelial dysfunction is inversely related to time after SARS-CoV-2 infection. Further studies are needed to confirm these findings and define the impact of disease progression/severity, biological predisposition, social, and environmental influences on endothelial function following infection with SARS-CoV-2.

4.1 Limitations

Data were collected in isolated arterioles from subjects with a variety of cardiovascular RFs that might affect vasodilator responses. However, compared to historical pre-COVID era data, no significant difference between controls and patients with similar risk profile as p-SARS-CoV-2+ patients were observed, suggesting that SARS-CoV2 can incite endothelial dysfunction. Due to the de-identified nature of sample collection, we could not assess the role of systemic changes (e.g. inflammation) or determine if the severity of COVID-19 symptoms or different treatment regimens contribute to the degree of endothelial-dysfunction observed. However, endothelial dysfunction was observed in a subset of patients when the severity of infection was reported (symptomatic: FMD at 100 cmH_2O, 54 ± 9%, time since SARS-CoV-2 + 8 - 12 months, n = 3 vs. asymptomatic: 38 ± 8%, time since p-SARS-CoV-2+ ≤3 months, n = 3), suggesting the phenotype links to the infection itself rather than treatment or severity of the disease.

In conclusion, we observed significantly reduced endothelial-dependent dilation months after exposure to SARS-CoV-2 that may lead to future cardiovascular complications.

Conflict of interest: none declared.

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