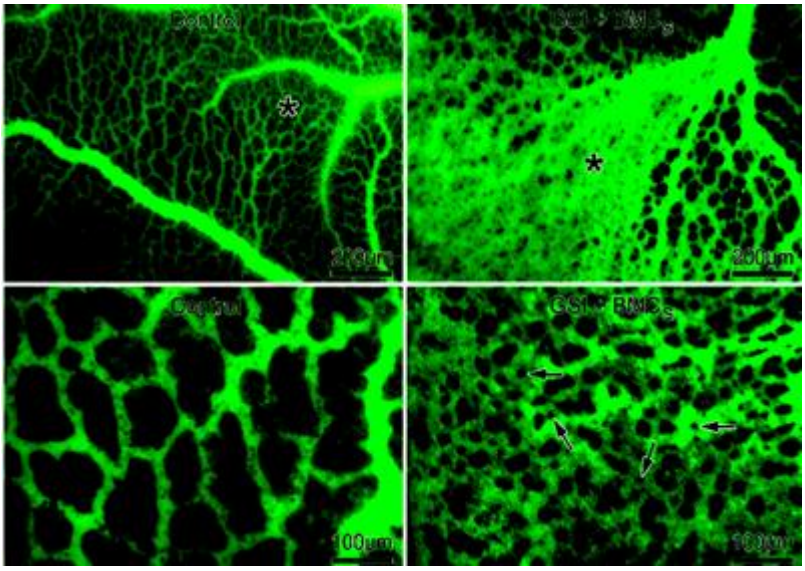
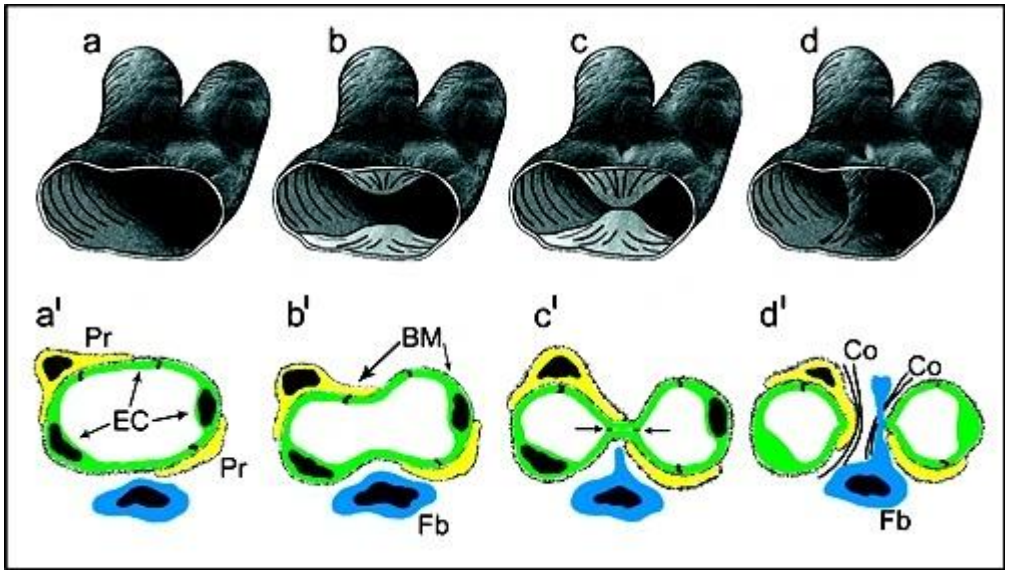
A wide range of cardiac symptoms has been observed in COVID-19 patients, often significantly influencing the clinical outcome. Current hypotheses include direct and indirect injury to the myocardium by viral infection. Cardiomyocytes express significant levels of the angiotensin-converting enzyme 2 receptor for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but the lack of transmembrane serine protease 2, which cleaves the spike (S) protein, allowing this binding to take place, likely hampers direct cardiomyocyte infection. Another hypothesis suggests that the systemic release of pro-inflammatory cytokines increases vascular wall permeability, leading to myocardial edema. The German authors in this multicenter autopsy study used histology, immunohistochemistry, electron microscopy, and gene expression analysis to evaluate, for the first time, the molecular, morphological, and ultrastructural alterations of cardiac tissue from patients who died of COVID-19, influenza A virus subtype H1N1, and non-influenza lymphocytic (e.g., coxsackievirus) myocarditis.

The authors emphasized that findings of conventional heart histopathology in COVID-19 cases are variable, from typical lymphocytic myocarditis and thrombotic microangiopathy to the absence of significant lymphocytic infiltrates and myocyte damage. The most frequently reported morphological finding is a slight increase in perivascular macrophages, often referred to as borderline myocarditis. Furthermore, the majority of cases did not meet the established Dallas criteria for myocarditis.



Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. According to the current consensus, there are two types of angiogenesis: sprouting and splitting (intussusceptive) angiogenesis. Intussusceptive (non-sprouting) angiogenesis was first described in 1986 by Caduff (Caduff JH et al. Scanning electron microscope study of the developing microvasculature in the postnatal rat lung. Anat Rec. 1986;216:154 64.) Intussusceptive angiogenesis is a highly dynamic process of microvascular growth observed within minutes to hours after a stimulus. The formation of a transluminal pillar is crucial for quick capillary plexus development.



Intussusceptive angiogenesis

Intussusceptive angiogenesis was observed during the formation and remodeling of vascular beds in numerous organs, including the mammary gland, bone, lungs, glomeruli, skeletal muscle, ovaries, and others. This vascular expansion is particularly beneficial in tissues with high metabolic demand (ischemia or hypoxia) because it enables the rapid delivery of nutrients and oxygen and the rapid removal of metabolic products. However, intussusceptive angiogenesis was observed not just as a physiological mechanism, but also in non-neoplastic diseases (atherosclerosis, inflammatory diseases, malignancies, and viral infections) and neoplastic diseases. Intussusceptive angiogenesis, for example, plays a pivotal role in fibrotic remodeling in interstitial lung disease.

Ackermann *et al.* were the first to describe intussusceptive angiogenesis in the pathogenesis of COVID-19. They examined the lungs of seven people who died from COVID-19 and found a significant distortion of the lung angioarchitecture with prominent variations in the caliber of small vessels. Surprisingly, the lung capillaries showed cylindrical microstructures in the capillary lumina. In addition, they registered that the level of intussusceptive angiogenesis was increasing with the increasing duration of hospitalization. (Ackermann M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. N Engl J Med. 2020; 383: 120–128. https://www.nejm.org/doi/full/10.1056/NEJMoa2015432)

Stromal cell-derived factor-1 (SDF-1)/CXC motif chemokine receptor 4 (CXCR4) signaling is involved in blood vessel formation and remodeling by intussusception. Growing evidence

implicates a pivotal role of the SDF-1/CXCR4 axis in myocardial repair, especially in myoangiogenesis after acute myocardial infarction and cardiomyopathy. Also, family members of vascular-endothelial growth factor (VEGF) and their pathways play a critical role in both sprouting and intussusceptive angiogenesis. Previous studies demonstrated that signs of enhanced intussusceptive angiogenesis, the number of small holes in primordial capillary plexuses, are increased by overexpression of VEGF and angiopoietins-1/2. These findings suggest an important role of VEGF in intussusceptive angiogenesis associated with COVID-19. (Madureira G & Soares R. The misunderstood link between SARS-CoV-2 and angiogenesis. A narrative review. Pulmonology, 2023, 29:4, 323-331. https://doi.org/10.1016/j.pulmoe.2021.08.004).

About the study

The researchers used conventional histopathology, immunohistochemistry, microvascular corrosion casting, scanning electron microscopy, phase-contrast synchrotron radiation tomographic microscopy, and gene expression analysis to assess morphological and molecular changes in the heart samples of patients who died of SARS-CoV-2 infection, pneumonia caused by influenza A virus subtype H1N1, and non-influenza/non-SARS-CoV-2 myocarditis (e.g., coxsackievirus). The study also included the heart samples of non-infected control cases. SARS-CoV-2 spike and nucleocapsid proteins were detected by immunohistochemistry, and the presence of RNA was detected using an RNA-FISH probe and RT-PCR.

Myocarditis was defined according to the established Dallas criteria.

Results

Cardiac specimens were obtained from deceased patients: 24 COVID-19 cases (nine women and fifteen men), 16 influenza A subtype H1N1 cases (seven women and nine men), 8 non-influenza/non-SARS-CoV-2 myocarditis cases (three women and five men), and 9 control individuals (seven women and two men). COVID-19 patients were significantly older (73.7 ± 10.8 years) compared to influenza cases (52.3 ± 15.3 years), common myocarditis cases (54.8 ± 21.3), and non-inflamed control patients (54.9 ± 18.0).

Echocardiography data and serum levels of CK-MB, troponin, and NT-proBNP were available for 16/24 COVID-19 cases, 12/16 influenza cases, and 3/8 non-influenza

myocarditis cases, respectively. Elevated serum markers, e.g., CK-MB, troponin, NT-proBNP, and/or abnormalities in echocardiography, e.g., reduced LVEF/RVEF or dyskinesia, defined cardiac involvement.

From all available heart specimens, 13 from COVID-19 cases, 3 from non-influenza myocarditis cases, and 3 from control individuals were suitable for vascular corrosion casting and 3D scanning electron microscopy, the gold standard for detailed analysis of the microvasculature, angiogenesis, and vascular occlusions.

Light microscopy findings and cell infiltrates

Based on histopathologic findings, none of the hearts from patients who died of COVID-19 met the established diagnostic criteria for viral myocarditis. 17/24 analyzed heart samples of COVID-19 patients were positive for SARS-CoV-2 RNA.

Cardiac specimens of all infectious groups showed a marked increase in macrophages (CD68+) compared to controls. The number of macrophages was two-fold higher in the hearts of patients who died of influenza A virus than in those who died of COVID-19 or non-influenza/non-SARS-CoV-2 myocarditis (e.g., coxsackievirus). In 18/24 cardiac specimens of COVID-19 cases, the perivascular connective tissue displayed a diffuse infiltration with CD11b+ macrophages, and higher levels of TIE2-expressing macrophages (TieMs) than in cardiac specimens of patients who died of influenza A virus and control cases. In 10/24 COVID-19 hearts, TIE2 was upregulated up to ten-fold.

All hearts of those who died of non-influenza/non-SARS-CoV-2 myocarditis showed lymphocytic infiltrates (severe inflammatory infiltrate in 3/8 cases, minor inflammatory infiltrate in 4/8 cases, and minor inflammatory infiltrate with single-cell necrosis in 1/8 cases). These marked lymphocytic inflammatory infiltrates were mixed (CD4, CD8, CD20). In contrast, lymphocytic infiltrates in COVID-19 and influenza cases were scarce.

Necrosis was not detected in the hearts of patients who died of COVID-19 or influenza, or in control cases. Cardiomyocyte hypertrophy was most pronounced in the hearts of COVID-19 patients, followed by the controls, the influenza cases, and the cases with non-influenza/non-SARS-CoV-2 myocarditis.

The level of interstitial fibrosis did not differ between the four groups.

Vascular remodeling

Conventional light microscopy and immunohistochemical staining for fibrin showed only scarce small vessel thrombi and no large vessel thrombi in the cardiac tissue of deceased COVID-19 patients. Two hearts from the patients who died of influenza had large vessel thrombi without small vessel thrombi. In contrast to the results of light microscopy, SEM imaging of corrosion casts revealed a significantly higher quantity of ultrastructurally detectable thrombi in COVID-19 hearts, which are too small to be detected by conventional light microscopy, as indicated by abrupt breakoff of capillaries with a diameter of 1–3 µm.

Altered vascular architecture, a loss of vascular hierarchy, a tortuous arrangement, irregular sinusoidal vessel networks, and frequent alterations of vessel diameter were all seen in heart samples from COVID-19 cases. Additionally, transluminal intussusceptive pillars were observed in the hearts of individuals who died of COVID-19, as evidenced by small holes in vascular corrosion casts at numerous vessel branches.

Importantly, the presence of microthrombi positively correlated with the occurrence of intussusceptive pillars in COVID-19 hearts. This was not found in non-influenza myocarditis cases. Intussusceptive pillars, disturbed, feathered courses of heart fibers, and mild interstitial fibrosis were also identified using phase-contrast synchrotron radiation tomographic microscopy.

Vascular changes in COVID-19 hearts were observed at the level of the afferent, large-caliber arteries.

Gene expression analysis

In the cardiac tissue of deceased COVID-19 patients, gene expression analysis identified significant upregulation of pro-inflammatory genes (IL1B, IL-6, IL8, and the toll-like receptor TLR2), hypoxia- and angiogenesis-related genes (VEGFC, FT1, and NOS3), and genes associated with monocyte recruitment (CXCR4, SDF-1, MMP9, CCR2, CXCR2, and MYD88). According to the authors, a marked increase in tissue macrophages and the upregulation of CCR2, CXCR4, and MYD88 in the hearts of COVID-19 cases suggest activation of local macrophages and upregulation of transcriptional profiles linked to the recruitment of circulating monocytes.

According to the analysis of 797 genes, there was a clear difference between heart samples from those who died of COVID-19 and those who died of influenza A, but not between heart

samples from COVID-19 cases and those who died of non-influenza/non-SARS-CoV-2 myocarditis. Heart samples of COVID-19 patients had twice as many differentially expressed genes as heart samples from influenza cases (152 genes in COVID-19 *versus* 72 genes in influenza). According to comparative gene analysis, in COVID-19 hearts were upregulated genes for angiogenesis, cell migration, and epithelial-mesenchymal transition (EMT) pathways, as signs of endothelial activation. The main characteristic of the hearts of those who died of influenza was the expression of genes for pro-inflammatory signaling and the antiviral response.

Conclusion

This study demonstrated that cardiac involvement in COVID-19 manifested as perivascular infiltration with a subpopulation of CD11b+/TIE2+ macrophages rather than as conventional viral myocarditis defined by mononuclear infiltrates and myocyte damage. The most remarkable finding in cardiac remodeling triggered by the SARS-CoV-2 infection was marked neovascularization in the form of intussusceptive angiogenesis, which was associated with a significant infiltration of CD11b+/TIE2+ macrophages, drivers of intussusceptive angiogenesis. These findings support the view that macrophages are the main responders, from the recruitment of circulating monocytes to irreversible tissue remodeling in intussusceptive angiogenesis.

Researchers concluded that cardiac involvement in COVID-19 is an angiocentric macrophage-driven inflammatory process, distinct from classical anti-viral inflammatory response. These findings are underestimated by conventional histopathologic analysis. Because the alterations in the cardiac vasculature are irreversible, systematic follow-up studies of long COVID cases are strongly recommended to identify potential fibrotic remodeling.

Journal Reference

Werlein C, Ackermann M, Stark H et al. Inflammation and vascular remodeling in COVID-19 hearts. Angiogenesis (2023) 26:233–248. (Open Access)
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