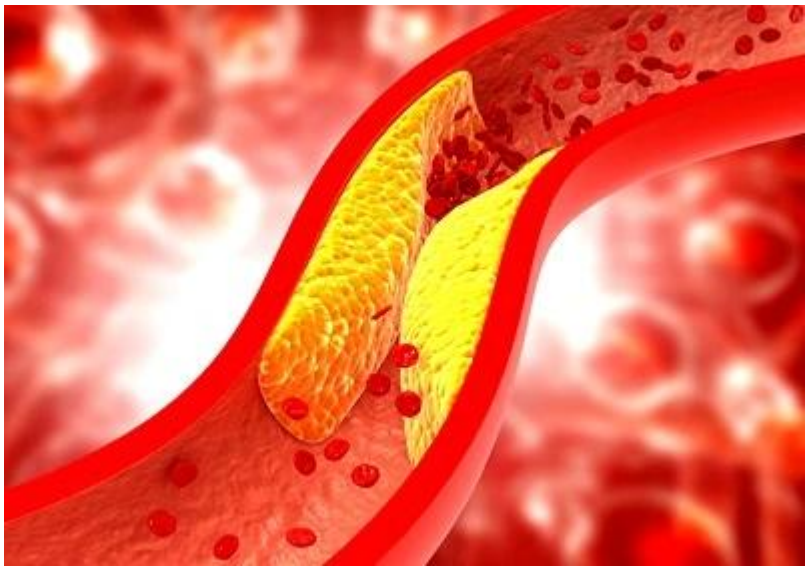
The clinical complications of acute COVID-19 and post-COVID syndrome include, among others, ischemic cardiovascular events such as acute myocardial infarction and stroke due to the disruption of chronically inflamed atherosclerotic plaques. The authors from the United States performed an autopsy study to investigate whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can directly infect atherosclerotic plaques in coronary vasculature samples.

Atherosclerosis is characterized by the accumulation of cholesterol-loaded macrophages (foam cells) at all stages of the disease, from early pathological intimal thickening to late fibroatheroma lesions. The authors assumed that SARS-CoV-2 can potently activate macrophages that infiltrate arterial vessels, leading to plaque inflammation and increased risk of acute ischemic cardiovascular events.



About the study

The authors performed an autopsy study on coronary artery samples obtained from eight deceased individuals who had been diagnosed with severe COVID-19, as confirmed by reverse transcription polymerase chain reaction. The mean age of individuals was 69.6 (59-84), and 75% were men. All autopsy cases had preexisting atherosclerosis (all had coronary artery disease, one had myocardial infarction, and one had ischemic stroke). They also had numerous cardiovascular risk factors (all had hypertension, seven had obesity or hyperlipidemia, six had type 2 diabetes, and four had chronic kidney disease).

The clinical cardiovascular pathologist classified histopathological findings of *postmortem* coronary artery specimens as follows: adaptive intimal thickening, pathological intimal

thickening, fibrocalcific plaque, and fibroatheroma. To identify the macrophage infiltration, the authors employed immunohistochemical staining for CD68. A neural network artificial intelligence approach was used to distinguish the coronary arterial wall from the perivascular fat in each sample.

Results

The virus was present and replicated in atherosclerotic lesions of coronary arteries. The RNA encoding the SARS-CoV-2 spike (S) protein was found in all lesions of the coronary arterial wall, including adaptive intimal thickening, pathological intimal thickening, fibrocalcific plaque, and fibroatheroma.

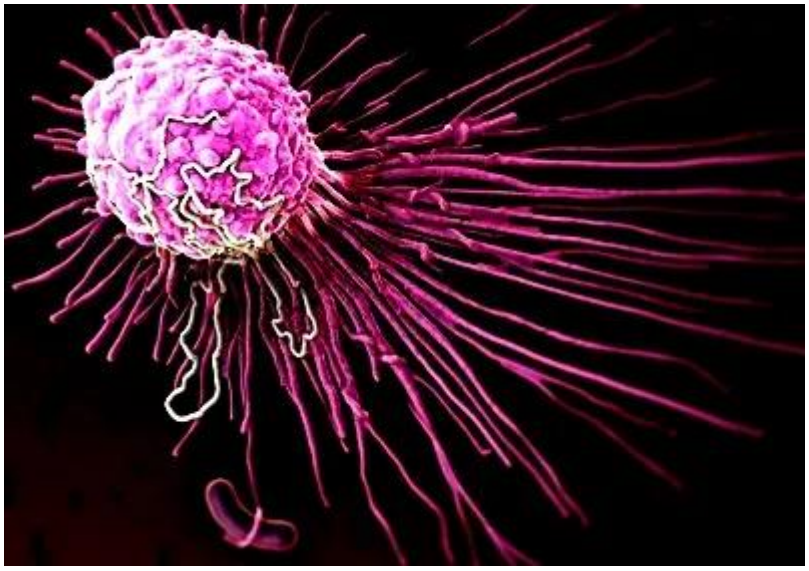
The highest rate of SARS-CoV-2 replication was observed in the pathological intima thickening, which is the early-stage lesion that progresses to more advanced atherosclerotic plaque. Across all samples, a significantly higher amount of the S protein was detected in the arterial wall than in the corresponding perivascular fat. However, vascular tissue and perivascular fat from coronary artery samples of COVID-19 patients with acute cardiovascular manifestations accumulated more viral RNA encoding S protein than those of COVID-19 patients without cardiovascular complications.

The expression of ACE2 was lower in the aorta and the tibial artery, but higher in the coronary artery. The expression of ACE2 in the coronary arteries was comparable to that in the lungs. Consequently, coronary vasculature may be more susceptible to SARS-CoV-2 infection than other vascular beds.

The macrophage infection with SARS-CoV-2

The subsequent analysis demonstrated that SARS-CoV-2 infected and replicated in macrophages within the coronary artery wall. The foam cells exhibited a greater susceptibility to infection than other macrophages, with a significant accumulation of SARS-CoV-2 nucleoprotein.

Pathological intimal thickening contained a higher number of CD68+ cells expressing the SARS-CoV-2 S protein than other types of lesions or perivascular fat. This number was 4.8-fold higher in the pathological intimal thickening than in the corresponding perivascular tissue. Importantly, viral RNA in infected macrophages and foam cells decreased over 48 hours, confirming that macrophages and foam cells were susceptible to SARS-CoV-2 infection but could not sustain a productive viral infection.



Despite abortive viral replication in macrophages and foam cells, the infection of these cells with SARS-CoV-2 resulted in a robust inflammatory response, characterized by the release of cytokines and chemokines, such as interleukin (IL)-6, IL-1β, CCL7, CXCL-9, β-NGF, IL-3, LIF, MIF, interferon (IFN)-α and IFN-γ. The pro-atherogenic cytokine IL-18 was released from infected macrophages, but not from foam cells. The infected foam cells, not macrophages, released IFN-α2, a type I IFN response cytokine that inhibits viral replication. Several pro-inflammatory and pro-atherogenic cytokine and chemokine genes, including CCL3, CCL7, CXCL10, and TNFSF10 were up-regulated in both, infected macrophages and foam cells. Genes involved in lipid metabolism were also up-regulated, suggesting a reprogramming of lipid metabolism in response to SARS-CoV-2.

Additionally, neuropilin-1 (NRP-1) blocking reduced the infection of foam cells and other macrophages *in vitro* and viral replication, confirming that viral entry into cholesterol-loaded macrophages was NRP-1-dependent. This effect was two-fold greater in foam cells. These results revealed a critical role of NRP-1 in the SARS-CoV-2 infection of these cells.

Lastly, the authors examined whether SARS-CoV-2 can directly infect atherosclerotic plaques using an *ex vivo* viral infection model in human vascular explants. The results revealed that SARS-CoV-2 infected the vascular tissue, triggered an inflammatory response, and induced key pro-atherogenic cytokines IL-6 and IL-1β release, already identified in cultured macrophages and foam cells. Over time, the viral titer decreased, with no detectable infectious particles, indicating an abortive replication in the vascular explants. This result is consistent with findings observed *in vitro* in foam cells and macrophages.

Conclusion

This study provided the first evidence that SARS-CoV-2 was detected and replicated in the coronary arteries sampled at autopsy of severe COVID-19 cases. SARS-CoV-2 targeted plaque macrophages and demonstrated a stronger tropism for arterial wall lesions than for perivascular fat. This was correlated with macrophage infiltration levels. SARS-CoV-2 entry was increased in cholesterol-loaded primary macrophages and was partially dependent on neuropilin-1. The SARS-CoV-2 further induced a robust inflammatory response in cultured macrophages and human atherosclerotic vascular explants and the production of cytokines.

These findings revealed the molecular basis of SARS-CoV-2 infection in coronary lesions and its contribution to the acute cardiovascular manifestations of COVID-19. Coronary vessel infection and plaque inflammation can result in plaque rupture and acute cardiovascular complications. However, the authors emphasize that this study focused on a small group of older individuals with COVID-19 who had pre-existing atherosclerosis and numerous cardiovascular risk factors. Therefore, these observations cannot be extrapolated to younger, healthy individuals.

The study has been published in Nature Cardiovascular Research.

Journal Reference

Eberhardt, N., Noval, M.G., Kaur, R. *et al.* SARS-CoV-2 infection triggers pro-atherogenic inflammatory responses in human coronary vessels. *Nat Cardiovasc Res* 2023; 2, 899–916. https://doi.org/10.1038/s44161-023-00336-5