Is the Intestine a Portal of Entry for the Serious COVID-19 Complications of Endotoxemia and Thrombosis?

David H. Alpers, MD

Abstract: Severe acute respiratory syndrome coronavirus 2 infection has been associated with both endotoxemia and thrombosis of small and large vessels, but the relationship between these 2 phenomena has not been pursued. Oliva et al. in this issue of *Clinical and Translational Gastroenterology* demonstrate an association between the 2 findings and suggest that increased intestinal permeability is a possible mechanism to explain the endotoxemia. Although the evidence to support this hypothesis is only suggestive, the role of the small intestine in the illness produced by the virus needs to be further explored.

An amazing amount of data on coronavirus 2019 (COVID-19) infection has been published over the past year, and of necessity, much of it is fragmented. The infection is known to induce endotoxemia measured activity in critically ill patients, nearly all of which had evidence of 16S RNA amplification in serum, with proteobacteria the most frequent phylum (1). Moreover, lipopolysaccharide (LPS) is elevated in patients with obesity and diabetes, known risk factors for COVID-19 infection, and LPS levels are high also in infected patients with cardiac toxicity, and in other viral illnesses, such as human immunodeficiency virus (2). COVID-19 infection is also associated with thrombotic complications through activation of platelets, endothelial injury from the virus, and abnormal blood flow dynamics (3). The major mechanism leading to lung thrombosis is dysregulation of angiotensin signaling, leading to inflammation, with subsequent increased signaling through thrombin and purinergic receptors to cause platelet activation (4). This latter mechanism may lead to thrombosis in other organs and be complicated by coagulopathy from comorbid conditions. About one-third of severely infected patients have macrovascular complications, including venous thromboembolism, stroke, and acute myocardial infarction (5). Both LPS and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause endothelial damage and alter platelet function, and D-dimer levels can be very high, so the relative importance of endotoxemia in the pathogenesis of the thrombosis is not entirely clear. Despite the occurrence of both endotoxemia and thrombosis in COVID-19–infected patients, studies have not previously focused on the association between these phenomena in the same patients.

The current study by Oliva et al. (6) measured LPS levels and activity and recorded macrothromboses in a series of patients with SARS-CoV-2–related pneumonia who were hospitalized and in some cases required mechanical ventilation. Among 81 patients, 11 (14%) had clinical thrombosis, 5 arterial and 6 venous, although no other clinical details are provided. LPS levels were higher in the entire group compared with control outpatient subjects matched for demographics and clinical conditions. LPS activity (TLR4 activation) was also increased, as were D-dimer and high-sensitivity C-reactive protein levels. LPS and D-dimer independently predicted thrombotic events for each increasing quartile, but LPS and D-dimer values were not associated. To evaluate the possible role of the intestine as the entry point for endotoxin into the body, the authors also measured serum zonulin as a biomarker for intestinal permeability. Zonulin levels were higher in the patients with COVID-19, and these levels were correlated with LPS levels. The authors suggest that enhanced gut permeability may be an underlying mechanism to explain the observation of endotoxemia as a risk factor for thrombotic events.

The demonstration of the association of LPS and thrombotic events is a potentially important observation, albeit in a small sample, as it fits with the previous observations that both endotoxemia and thrombosis are part of the spectrum of COVID-19 disease in critically ill patients. Whether this association is causative or confounded by comorbid conditions is not clear from this report. Regarding the presence of endotoxemia, whether or not causative, the suggestion that endotoxin might be entering by increased gut permeability is not strongly supported by the evidence. Neither LPS nor zonulin are validated biomarkers for intestinal permeability. LPS enters the body by various mechanisms, of which increased permeability is a minor one, based on existing data (7). LPS has a much larger radius (10–15 Å) than the mean paracellular intestinal pathway (3.6 Å), and the small bowel is more permeable than the colon and accounts for most of the effect of tests used to measure gut permeability. Most of the microbiome, however, resides in the colon in humans. Perhaps because of this physiology, or because LPS can itself alter gut

Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri, USA. **Correspondence:** David H. Alpers, MD. E-mail: dalpers@im.wustl.edu.

permeability, there is poor correlation between serum LPS and the gold-standard measurements of gut permeability, most commonly lactulose-mannitol or lactulose-rhamnose absorption (8). Similarly, there is a poor correlation of serum zonulin with gold-standard measurements of gut permeability (9). There are also 2 other possible explanations for increased LPS in COVID-19 infection, other than that of altered gut permeability. First, LPS might be induced in susceptible individuals already harboring low levels of endotoxemia (2). Second, organisms superimposed on SARS-CoV-2 infection, in the lung or elsewhere in the body, could produce LPS (1). The hypothesis that the gut might be an important portal of entry for gut organisms has been suggested because of the high concentration in the small bowel of the ACE-2 receptor, required for entry of SARS-CoV-2 into cells (10). In fact, SARS-CoV-2 has been shown to infect human gut organoids, but the intracellular virus is positioned to be apically secreted, so entry into the body through enterocytes has not yet been demonstrated (11). Moreover, the virus is rapidly inactivated by gastric fluid, and functional virus has not usually been detected in feces (11). Thus, it is still uncertain whether the intestine functions as a site of active SARS-CoV-2 replication and entry into the body.

Despite these questions about the role of the small intestine, there is some evidence in other systems that SARS-CoV-2 infection might be capable of altering gut permeability. In a study using multiple human intestinal cancer cell lines, SARS-CoV-2 increased the epithelial-to-mesenchymal transition, breaking down the alveolar epithelial barrier, and resulting in downregulation of genes associated with tight junctions (12). No physiological or morphological demonstration of altered permeability was demonstrated, and because altered epithelial-to-mesenchymal transition is a heightened pathway in cancer cells, there is need to demonstrate this phenomenon in nonmalignant intestinal cells. Alternatively, it is possible that the effects of SARS-CoV-2 infection in the lung or the virus itself might spread from the lung to the intestine, thus altering barrier function (13). However, the possibilities are speculative at present, and the zonulin data in this article suggest only the possibility of altered gut barrier function. Further work will be needed to determine whether the intestine plays an important role in the pathogenesis of COVID-19, either by providing a portal of entry for the virus itself, or by allowing bacterial or LPS translocation into the body of severely infected patients.

CONFLICTS OF INTEREST
Guarantor of the article: David H. Alpers, MD.

REFERENCES

1. Sirivongrangson P, Kulvichit W, Payungporn S, et al. Endotoxemia and circulating bacteriome in severe COVID-19 patients. Intensive Care Med 2020;8(1):72.
2. Kruglikov IL, Scherer PE. Preexisting and inducible endotoxemia as crucial contributors to the severity of COVID-19 outcomes. PLOS Pathogens 2021;17(2):e1009306.
3. Gasecka A, Borovac JA, Guerrero RA, et al. Thrombotic complications in patients with COVID-19: Pathophysiological mechanisms, diagnosis, and treatment. Cardiovasc Drugs Ther 2021;35(2):215–29.
4. Sriram K, Incel PA. Inflammation and thrombosis in COVID-19 pathophysiology: Proteinase-activated and purinergic receptors as drivers and therapeutic candidate targets. Physiol Rev 2021;101:545–67.
5. Piazza G, Monroe DA. Diagnosis, management, and pathophysiology of arterial and venous thrombosis in COVID-19. JAMA 2020;324:3548–9.
6. Oliva A, Cammisotto V, Cangemi R, et al. Low grade endotoxemia and thrombosis in Covid-19. Clin Trans Gastroenterol 2021 [in press].
7. Guerville M, Boudry G. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. Am J Physiol Gastrointest Liver Physiol 2016;311:G1–15.
8. Chakaroun RM, Massier M, Kovac P. Gut microbiome, intestinal permeability, and tissue bacteria in metabolic disease: Perpetrators or bystanders. Nutrients 2020;12:1082.
9. Tatucu-Babet OA, Forsyth A, Owen E, et al. Serum zonulin measured by enzyme-linked immunosorent assay may not be a reliable marker of small intestinal permeability in healthy adults. Nutr Res 2020;78:82–92.
10. Cardinale V, Capurso G, Ianiro G, et al. Intestinal permeability changes with bacterial translocation as key events modulating systemic host immune response to SARS-CoV-2: A working hypothesis. Dig Liver Dis 2020;52:1383–9.
11. Zang R, Gomez Castro MF, et al. TMPRSS2 and TMPRSS4 promotes SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol 2020;5(47):eabc3582.
12. Stewart CA, Gay CM, Rankemar K, et al. Lung cancer models reveal SARS-CoV-2 induced EMT contributions to COVID-19 pathophysiology. bioRxiv 2020. doi: 10.1101/2020.05.28.122291.
13. Stanifer ML, Kee C, Coretese M, et al. Critical role of type III interferon in controlling SARS-CoV-2 infection in human intestinal epithelial cells. Cell Rep 2020;32:107863.