

# Ischemia-induced intestinal de-epithelization and consequent cholangitis lenta after usage of extracorporeal membrane oxygenation in COVID-19 patients: an autopsy series

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## Abstract

Since its discovery in late 2019, severe acute respiratory syndrome coronavirus 2 has spread around the world, causing millions of deaths due to coronavirus disease 2019 (COVID-19). Numerous clinical and post-mortem investigations of COVID-19 cases have found myriad clinical and pathological manifestations of the disease. In this report, we present three autopsy cases in which, despite weaning from extracorporeal membrane oxygenation (ECMO), extensive intestinal epithelial shedding, probably due to ischemia, was followed by massive watery diarrhea and the spread of infection via the portal vein due to bacterial translocation, which resulted in cholangitis lenta. Thrombophilia was attributed to ECMO usage and COVID-19-related vascular endothelial damage. These cases provide instructive findings showing that the loss of the intestinal barrier may be the underlying cause of severe watery diarrhea and liver failure in COVID-19 patients, especially with ECMO usage.

## INTRODUCTION

Since its discovery in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide, causing millions of deaths. Genetically similar to SARS-CoV-1, which became epidemic in 2002–03, SARS-CoV-2 causes coronavirus disease 2019 (COVID-19), which commonly causes symptoms such as fever, cough and shortness of breath [1]. Acute respiratory distress syndrome (ARDS), which is histopathologically characterized by diffuse alveolar damage (DAD), is the most frequent cause of death which is common to both infections [2, 3]. However, a variety of extrapulmonary complications, such as diarrhea, heart failure, acute myocardial infarction and arrhythmia, have been reported in COVID-19 cases [4]. It is worth investigating such complications because they can be fatal in COVID-19. In particular, endothelial damage and thromboinflammation are considered to be the major mechanisms underlying multiorgan damage in COVID-19 patients [4].

We performed autopsies in five cases of COVID-19 at Sapporo Medical University Hospital (Sapporo, Japan) between 2020 and 2021 (i.e. before the outbreak of the Omicron strain). Here, we report three autopsy cases showing unusual findings. In all cases,

patients were treated with extracorporeal membrane oxygenation (ECMO) for respiratory failure and subsequently weaned off ECMO. However, all three patients had a common clinical presentation and eventually died. Autopsy findings revealed common findings in the alimentary tract and liver. We believe that these findings provide additional insights into non-pulmonary complications caused by ECMO usage for COVID-19 patients.

## CASE REPORT

### Remarkable common observations across the three cases

The clinical characteristics of the patients in this report are presented in Table 1. Total/direct bilirubin, aspartate aminotransferase/alanine aminotransferase and serum total protein/serum albumin levels shown are the results obtained from the last blood test that was taken 1–2 days before each patient's death. Increased blood bilirubin and liver enzyme levels, as well as decreased serum total protein and albumin levels, were observed in these patients. All three patients required blood oxygenation with ECMO. In Cases 1 and 3, histopathological examination at

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**Table 1.** Clinicopathological characteristics of the reported cases

Case		1	2	3
Clinical characters	Age (year)	68	62	71
	Sex	M	M	M
	AST/ALT (U/l)	71/30	53/34	86/86
	T-Bil/D-Bil (mg/dl)	11.8/8.3	9.1/6.3	2.0/1.6
	TP/Alb (g/dl)	2.9/1.4	3.3/1.9	2.3/0.6
	ECMO	+ (41 days)	+ (17 days)	+ (8 days)
	Onset of diarrhea	Day 25 (during ECMO)	Day 32 (1 day after weaning off ECMO)	Day 18 (8 days after weaning off ECMO)
	Diarrhea (maximum)	3000 ml/day	5000 ml/day	5400 ml/day
	Duration	82 days	144 days	61 days
	Autopsy findings	Jaundice	+	+
Ascite		+ (N/A)	+ (4600 ml)	+ (2200 ml)
Pleural effusion		+ (N/A)	+ (L: 400 ml, R: 500 ml)	+ (L: 600 ml, R: little)
DAD		+	–	+
Thrombus		+	+	+
Intestinal de-epithelialization		+	+	+
Cholangitis lenta		+	+	+
ATN		+	+	+

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; D-Bil, direct bilirubin; TP, total protein; Alb, albumin; ATN, acute tubular necrosis; N/A, not acquired.

autopsy revealed DAD in the organizing stage. Thrombus formation was observed in small pulmonary vessels in all cases (data not shown).

In addition, all three patients presented with a large amount of watery stool during the clinical course. In Cases 1 and 3, biopsy specimens of the colon revealed withering of the superficial mucosal, suggesting intestinal ischemia (Fig. 1A). Although typical findings for ischemia were not apparent in Case 2, edematous granulation tissue, which can be indicative of ischemia, was observed in the mucosal layer (Fig. 1A). In all cases, autopsy specimens showed that the mucosal layer was replaced by the granulation tissue (Fig. 1B). In the liver, proliferation of dilated bile ductules with presence of bile plugs at the periphery of portal tract were commonly observed (Fig. 1C). These characteristics indicated cholangitis lenta [5]. Centrilobular zonal necrosis was also found in the liver in all three cases (data not shown).

Although the ultimate cause of death differed in each case, circulatory insufficiency due to severe watery diarrhea and consequent protein-losing enteropathy were underlying contributors in all cases, as evidenced by the presence of acute tubular necrosis in the kidney (data not shown). Further details of the characteristic findings in each case are summarized below.

### Case 1

A 68-year-old man was diagnosed as having COVID-19. He was placed on a ventilator on Day 4 and was put on ECMO on Day 8. From Day 25, the patient had watery diarrhea. He was weaned off ECMO on Day 48. Trans-arterial embolization was performed on Day 51 and resection was performed on Day 52 to control the intestinal bleeding. He had persistent liver failure and uncontrollable gastrointestinal bleeding and died on Day 82.

Autopsy revealed a splenic infarction. In the kidney, microthrombi and acute tubular necrosis were observed within the glomerulus. Multiple organ failure due to disseminated intravascular coagulation caused by putative COVID-19-related vascular disease and sepsis was presumed to be the cause of death.

### Case 2

A 62-year-old man was intubated and put on ECMO on Day 14 of COVID-19 due to worsening of respiration. He was weaned

from ECMO on Day 31. Watery stools increased from Day 32, reaching a maximum of 5000 ml/day. Colonoscopy confirmed marked shedding of the intestinal mucosa. Blood transfusion was continuously repeated because the persistent intestinal bleeding could not be controlled. He also had liver failure and died on Day 144.

Autopsy revealed a subacute myocardial infarction. The cause of death was presumed to be circulatory failure due to gastrointestinal hemorrhage and the large amount of watery stool as well as liver failure due to extensive liver necrosis.

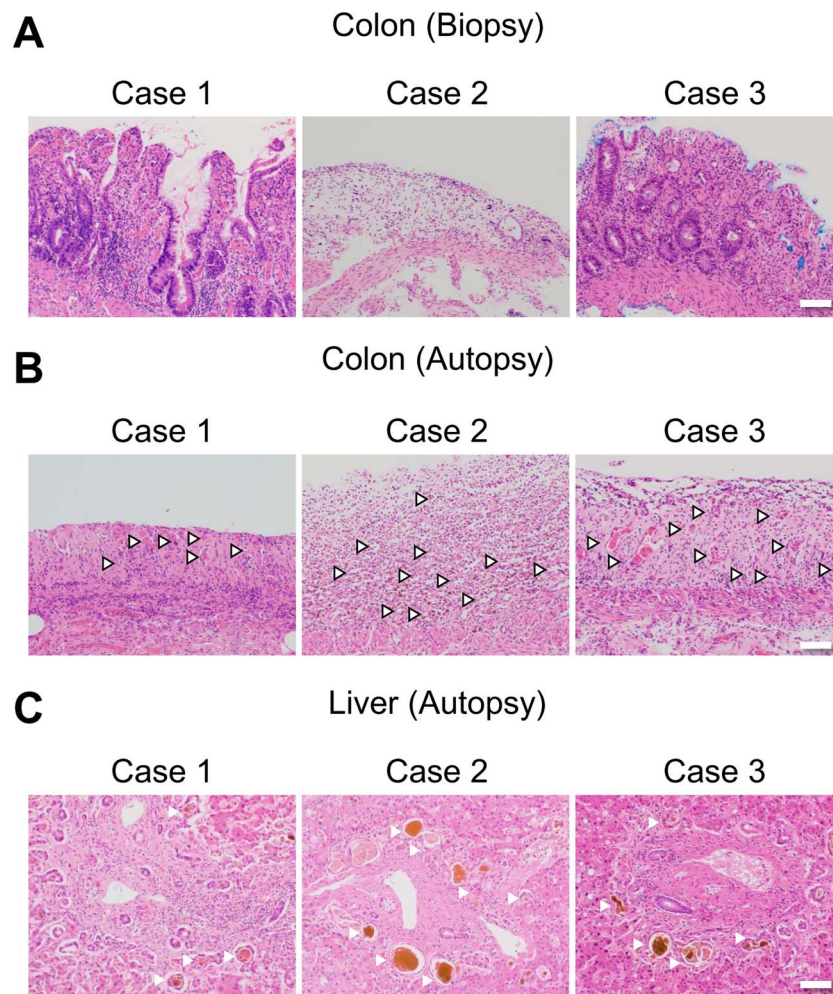
### Case 3

A 71-year-old man was diagnosed as having COVID-19-related pneumonitis. He was put on ECMO on Day 3 and was weaned off it on Day 10. He was confirmed as SARS-CoV-2 antigen-negative on Day 14, but the severe watery diarrhea persisted. He died on Day 61 due to circulatory and respiratory failure.

Autopsy revealed the formation of micro-abscesses in the liver and myocardium. The cause of death was presumed to be circulatory failure due to severe watery diarrhea and ascites in combination with DAD-induced respiratory insufficiency. In addition, sepsis attributed to bacterial translocation resulting from the loss of the intestinal barrier was also suspected.

## DISCUSSION

The epithelial tight junction, which is located at the interface separating the inner tissue from the outside environment, serves as a physical barrier [6]. When the epithelial tight junction is compromised, for example, when the skin barrier is lost because of extensive burns, dehydration and infection often lead to death. In all three COVID-19 cases in this report, the patient had severe watery diarrhea during the clinical course. Autopsy findings revealed intestinal epithelial shedding, which is frequently observed as a post-mortem change. However, such findings have not received much attention in previous autopsy reports on patients who died of COVID-19. Curiously, in all three cases reported here, granulation tissue, consisting of micro-vessel proliferation and immature fibrosis, was observed in the mucosa, indicating that



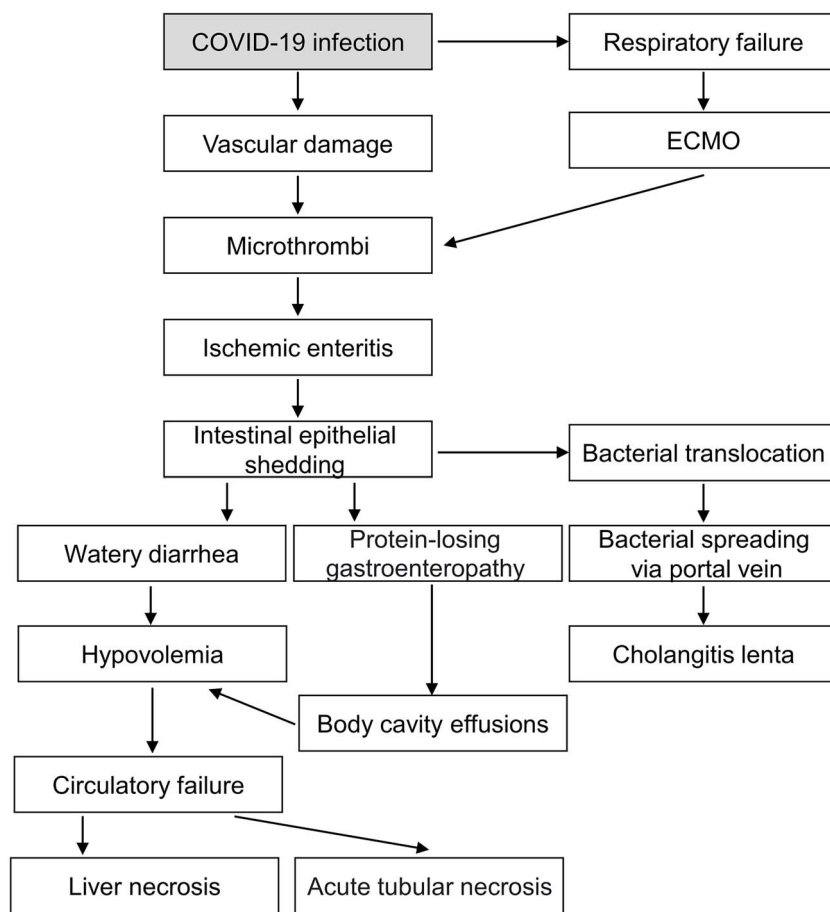
**Figure 1.** Histopathological images of biopsy and autopsy cases by hematoxylin and eosin staining; **(A)** histopathological image of colon biopsy specimen; In Case 1 (obtained at Day 34) and Case 3 (obtained at Day 21), withering of the superficial mucosal was observed, suggesting ischemia; edematous granulation tissue in the mucosal layer was observed in Case 2 (obtained at Day 41); **(B)** histopathological image of a colon specimen obtained during autopsy; the mucosal layer has been replaced by granulation tissue in all cases; arrowheads indicate micro-vessels in the granulation tissue; **(C)** histopathological image of liver specimen obtained at autopsy; cholangiolar proliferation with neutrophil infiltration and dilated bile ductular cholestasis (arrowheads) are commonly observed in the periphery of Glisson's capsule, indicating cholangitis lenta; (A–C) bar = 100 μm.

the epithelial shedding occurred before death. We speculate that this disruption of the intestinal epithelial barrier was the cause of the severe watery diarrhea and protein-losing gastroenteropathy that led to circulatory failure. Epithelial regeneration likely did not occur because the extensive epithelial shedding damaged even the epithelial stem cells of the intestine. In addition, loss of the epithelial barrier would have led to bacterial translocation from the intestine, resulting in the spread of bacterial infection via the portal vein. This is supported by the fact that all three cases showed evidence of cholangitis lenta, which is characterized by a bile ductule reaction at the edge of the portal canals as well as their dilatation and biliary stasis [5].

The putative mechanisms by which SARS-CoV-2 infection causes enterocolitis are intestinal ischemia due to microthrombus or direct infection of the intestinal epithelium [7–11]. In all three cases, severe epithelial shedding in the intestinal mucosa without transmural necrosis was observed. In biopsy specimens from Cases 1 to 3, although the basal intestinal epithelial cells remained, the luminal sides of the epithelia were de-epithelialized. In Case 2, although the typical ischemic change was not observed, it was nevertheless consistent with

ischemia-induced changes. Despite no evidence of thrombi in the intestinal tissues, the presence of thrombi in the pulmonary vessels in all cases would support a systemic thrombotic tendency. Although SARS-CoV-2 can infect the intestinal epithelium, we did not evaluate SARS-CoV-2 infection in the intestine. Also, we did not observe any apparent apoptotic bodies together with lymphocyte infiltration in the intestinal epithelium, which would indicate direct infection or epithelial injury caused by an antiviral immune response [10]. Therefore, we concluded that the intestinal ischemia was mainly the result of microthrombi in the peripheral vessels of the intestine in these cases. A schematic diagram summarizing the common findings in the three cases is shown in Figure 2.

Increasing reports have indicated that SARS-CoV-2 infection affects the coagulation system and promotes thrombus formation. Endothelial injury and endothelitis caused by SARS-CoV-2 infection leads to excessive thrombin production, inhibition of fibrinolysis and activation of the complement pathway, resulting in thrombotic inflammation and eventually microthrombus deposits and microvascular dysfunction [4]. Although gastrointestinal ischemia has been reported in only 0.7% of COVID-19



**Figure 2.** Schematic diagram representing the common findings of our cases; loss of the intestinal barrier may be an underlying cause of severe gastrointestinal symptoms and/or liver failure in COVID-19 patients, possibly leading to death.

patients, it has a mortality rate of 39% [12]. We suspect that COVID-19-related coagulopathy may have been involved in the pathophysiology of our cases. However, considering the clinical course of these cases, the fact that watery diarrhea was observed during or after ECMO management suggests the profound contribution of ECMO in the pathogenesis of microthrombi. Indeed, thrombophilia is a notorious complication of ECMO [13]. Furthermore, an animal study suggested that ECMO may reduce the integrity of the intestinal barrier [14]. Thus, the thrombophilia observed in our cases may be at least partly attributable not only to COVID-19-related coagulopathy but also to adverse events directly and/or indirectly related to ECMO. Although a growing body of evidence is accumulating to demonstrate that COVID-19 complicate sometimes ischemic enterocolitis, this is the first report of an autopsy series suggesting that the use of ECMO for COVID-19 has affected intestinal ischemia. Ischemic enteritis has been recognized as a complication of ECMO in recent years. We believe that our cases were instructive in the use of ECMO in patients with vascular injuries, probably not just those related to COVID-19.

Although DAD resulting in ARDS tends to be commonly regarded as both symptom and cause of death in COVID-19, we reported three cases in which common gastrointestinal symptoms led to death even after the patients were weaned off ECMO. We should bear in mind that the loss of the intestinal barrier may be an underlying cause of severe gastrointestinal symptoms and/or liver failure in COVID-19 patients, especially with ECMO usage.

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## CONFLICT OF INTEREST STATEMENT

None declared.

## CONSENT

Written informed consent was obtained from the bereaved families in all cases.

## GUARANTOR

Terufumi Kubo.

## DATA AVAILABILITY

The data or figures used in the current study are available from the corresponding author on reasonable request.

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