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Histological Damage of Colonic Epithelium is Associated with Clinical Severity and Outcome in Colectomized Critically Ill Patients

S. Sipola · T. I. Ala-Kokko · J. J. Laurila · M. Vakkala · P. Ohtonen · J. Saarnio · T. J. Karttunen · H. Syrjälä

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Abstract
Background Severe intestinal mucosal damage and organ failure has been associated in experimental models. Our purpose was to determine whether there is any association between histopathological findings and postoperative mortality among ICU patients undergoing emergency colectomies for various illnesses.
Methods In a retrospective case control study, total colectomy specimens from 50 patients in a mixed ICU were analysed: 18 had sepsis, 11 vascular operations, and 21 *Clostridium difficile* colitis. Overall thickness, the width of epithelial defects, and presence of cryptal damage were assessed. Extent of necrosis and amount of neutrophils were separately evaluated in the layers of the colonic wall. Clinical features, including sequential organ failure assessment (SOFA) scores and survival, were registered.
Results The histopathological findings for the three clinical entities were similar, except for the abundance of characteristic pseudomembranes in the Clostridium group. Mucosal height (maximum) showed a negative correlation with SOFA score on admission ($\rho = -0.296$, $P = 0.037$), and with preoperative blood lactate level ($\rho = -0.316$; $P = 0.027$). The nonsurvivors had wider enterocyte defects (60 vs. 40.8, $P = 0.002$) and more severe crypt damage (61 vs. 27 %; $P = 0.024$) than the survivors.
Conclusions The histopathological damage involves all layers of the colon wall among ICU patients being largely similar in sepsis, *C. difficile* infection, and ischemia after vascular operations. Mucosal epithelial damage is associated with clinical severity of the illness and mortality.

T. J. Karttunen and H. Syrjälä have contributed equally to this study.

S. Sipola (✉) · T. I. Ala-Kokko · J. J. Laurila · M. Vakkala · P. Ohtonen
Division of Intensive Care Medicine, Department of Anaesthesiology, Oulu University Hospital and Medical Research Center, PO Box 21, 90029 Oulu, Finland
e-mail: seija.sipola@ppshp.fi

J. Saarnio
Department of Surgery, Oulu University Hospital and Medical Research Center, PO Box 21, 90029 Oulu, Finland

T. J. Karttunen
Department of Pathology, Oulu University Hospital and Medical Research Center, PO Box 21, 90029 Oulu, Finland

T. J. Karttunen
Department of Pathology, Institute of Diagnostics, University of Oulu, Oulu, Finland

H. Syrjälä
Department of Infection Control, Oulu University Hospital and Medical Research Center, PO Box 21, 90029 Oulu, Finland

Introduction

Mucosal damage leading to high mortality rate may be precipitated by several types of critical illness, including sepsis, pseudomembranous colitis, as well as systemic or local hypoperfusion. The histological changes in ischaemic colitis are considered to be nonspecific and have been reported to include oedema, distorted crypts, mucosal and submucosal haemorrhage, inflammatory infiltration, granulation tissue, intravascular platelet thrombi, and necrosis [1]. Different from animal studies, the link between intestinal mucosal damage causing gut origin sepsis, multiorgan dysfunction, and mortality via bacterial translocation has not been clearly shown in clinical studies [2–5]. Previous studies on histopathology of colitis have largely focused on specific etiological types of colon wall damage and often limited on using biopsy specimens, which do not provide

information of alterations in the submucosal layers of the intestinal wall. As far as we are aware, there are no studies comparing detailed histological findings and clinical parameters and prognosis in critically ill patients. Thus, we performed a detailed retrospective analysis of histological findings of the colons to evaluate the association between histopathological findings and postoperative mortality among ICU patients undergoing unplanned colectomies for various illnesses.

Materials and methods

Patients

The study was approved by the Ethics Committee of Oulu University Hospital. This is a histological substudy of our previous larger material of 77 colectomies [6, 7]. This current study consisted of 50 patients with total colectomy. There were three groups of clinical conditions leading to the need for colectomy: (1) sepsis; (2) severe *Clostridium difficile* colitis; or (3) ischaemic damage of the colon after cardiac or vascular surgery. The control group consisted of 34 patients (mean age 65 years) who provided tissue samples of histologically normal colon. The indication for colectomy among the control patients was a colon tumour less than 4 cm in size to avoid cases with any changes due to tumour-related obstruction.

Clinical parameters were retrospectively retrieved from the ICU clinical data management system database and clinical patient charts (Centricity Critical Care Clinisoft; GE Healthcare, Helsinki, Finland). The Acute Physiology and Chronic Health (APACHE II) score [8] as a score of severity of illness was determined at the time of admission to the intensive care unit, and the sequential organ failure assessment score (SOFA) as a measure of organ dysfunction was recorded daily 3 days before and on the day of surgery [9]. Similarly, plasma lactate levels, norepinephrine dose (mg/day), and the white blood cell count (WCC) were determined daily 3 days before surgery and on the day of surgery. The worst value in each 24-h period was identified. Mortality on day 28 was recorded.

Histological examination

The colon samples were taken for routine histopathological analysis, fixed in neutral 10 % formalin, and embedded in paraffin. Due to the retrospective nature of this study, there were different numbers [1–5] of segments available for analysis in each patient. The sections were stained with haematoxylin and eosin. A total of 362 colon tissue samples from the 50 patients and 72 samples from the 34 controls were analysed. Histological features were evaluated blindly without any clinical information, including patient category. Samples were taken from each colonic section available for analysis: ascending colon ($n = 75$), right transverse colon ($n = 80$), left transverse colon ($n = 77$), descending colon ($n = 75$), and rectosigmoid ($n = 55$). All assessments were made by two investigators (SS, MV) with an experienced pathologist (TJK). The histological features were assessed separately for the surface epithelium and other structures in the mucosa, submucosa, muscularis propria, and serosal layers. The amount of inflammatory cells, including neutrophilic and eosinophilic granulocytes and mononuclear inflammatory cells, was evaluated and scored separately in the surface epithelium, and mucosa, submucosal, muscular, and serosal layers. In addition, a neutrophil score was calculated for mucosal neutrophils. Scoring was based on visual analogue scales [10].

The height of enterocytes and crypt were measured by using a calibrated ocular micrometer. The thickness of the mucosa, submucosa, and muscularis propria were measured similarly. The extent of degenerative changes in the surface epithelium was estimated, including the proportion (%) of surface area showing defects and signs of degeneration and necrosis. The criteria for degeneration included vacuolisation and/or partial enterocyte detachment. In addition, the proportion (%) of area showing necrosis (depth and width) was estimated in the mucosa, submucosa, muscularis propria, and serosal layers by assessing both the depth (proportion of the thickness) and width of the lesion. In each case, the segment of the colon exhibiting the most severe damage was used for the analysis.

Statistical analysis

The statistical analyses were performed using SPSS for Windows (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA: IBM Corp.). The data were expressed as a percentage or as a median with 25th–75th percentiles. Categorical data were analysed using Fisher's exact test. The Kruskal–Wallis test was used when three clinical groups were compared. The Student's t test was applied (either equal variance assumed or not assumed) to analyse differences between survivors and nonsurvivors. Spearman's correlation coefficient (ρ) also was calculated. Differences were considered significant when two-tailed $P < 0.05$.

Results

The 28-day mortality rate in the patient group was 56 % (28/50). Table 1 summarises the essential clinical data for survivors and nonsurvivors. No differences were found between survivors and nonsurvivors in the mean age,

Table 1 Patient demographic and clinical data and the distribution of histological specimens from different segments of the colon

Characteristic	Survivors $n = 22$	Nonsurvivors $n = 28$	P
Sex, *n* (%)			>0.9
Male	13 (43 %)	17 (57 %)	
Female	9 (45 %)	11 (55 %)	
Age (25th–75th percentile)	70.5 (62–77)	73.5 (65–76)	0.36
APACHEII admission (25th–75th percentile)	24.5 (21–31.5)	30 (23–34)	0.077
SOFA admission (25th–75th percentile)	6.5 (3–9)	9.5 (7.5–13.5)	0.006
SOFA operative day (25th–75th percentile)	10.5 (7–13)	12.5 (10.5–14)	0.027
WCC (10^9/l) operative day (25th–75th percentile)	14.9 (12.3–25.3)	17 (10.3–31.1)	0.55
Worst Surgical findings, *n* (%)			0.69
Perforation	2 (9 %)	3 (11 %)	
Peritonitis	5 (23 %)	6 (21 %)	
Colon ischemia	7 (32 %)	5 (18 %)	
Colon necrosis	8 (36 %)	14 (50 %)	
Disease leading to colectomy, *n* (%)			>0.9
Ischemia	4 (18 %)	6 (21 %)	
Cl. colitis	10 (45 %)	11 (39 %)	
Sepsis	8 (36 %)	11 (39 %)	
Number of colon segments analysed, *n* (%)	$n = 180$	$n = 182$	>0.9
Ascending	38 (21 %)	37 (20 %)	
Right transverse	41 (23 %)	39 (21 %)	
Left transverse	39 (22 %)	38 (21 %)	
Descending	34 (19 %)	41 (23 %)	
Rectosigmoid	28 (16 %)	27 (15 %)	

gender, or APACHE II score. The nonsurvivors had more severe organ dysfunctions according to SOFA score at admission (9.5 vs. 6.5; $P = 0.006$). The clinical conditions leading to colectomy were similar for survivors and nonsurvivors, and *Clostridium colitis* was the most common condition in both (Table 1). At the time of surgery, all patients had oedemic and dilatated colon, usually with ascites. Necrosis or ischaemia of the colon were the most common surgical findings but were not associated with survival (Table 1).

Control samples exhibited normal bowel wall structure with no epithelial degenerative changes, necrosis, or neutrophil infiltration (Fig. 1). The histopathological findings for the three clinical entities (colectomy due to severe sepsis, vascular ischaemia, or *Clostridium colitis*) were similar (Fig. 1; data not shown), except for the abundance of characteristic pseudomembranes in the Clostridium group.

Intra-abdominal operative findings did not show any association with histological findings in the resected colons (data not shown). No significant correlations were found between enterocyte defects and maximal lactate level, given noradrenaline dose and operative day SOFA score. However, a negative correlation was found between organ failures (SOFA score) on admission and the mucosal maximum height ($\rho = -0.3$; $P = 0.037$) and the minimum height of crypts ($\rho = -0.33$; $P = 0.041$).

A significant negative correlation also was found between the blood leucocyte count and the width of submucosal necrosis ($\rho = -0.31$; $P = 0.027$) and between the depth of submucosal necrosis ($\rho = -0.321$; $P = 0.023$). Moreover, the blood lactate level before surgery correlated negatively with the mucosal maximum height ($\rho = -0.32$; $P = 0.027$). There were no correlations between maximum preoperative noradrenaline dose (mg/day) and the histopathological findings.

The histological findings in the patient group were compared between survivors and nonsurvivors (Table 2). There were two statistical significant differences between the groups: enterocyte defects were wider in nonsurvivors (40.8 vs. 60, $P = 0.002$) and the presence of complete crypt damage at least in one segment available for analysis was more common among the nonsurvivors (61 %) than the survivors (27 %; $P = 0.024$).

Neutrophil infiltration was present in 40 % (145/362) of specimens. No significant differences were found between survivors and nonsurvivors in regard to the amount of neutrophils in the mucosa or other layers of the bowel wall (Table 2). Necrosis was present in the muscle layer and subserosal adipose layer of 63/362 (26/50 patients) and 153/362 (33/50 patients) histological samples. No significant differences were found between survivors and nonsurvivors in regards to the extent and depth of necrosis in the submucosal layer (Table 2).

Discussion

Our study is the first detailed analysis of whole colon wall histopathology of colitis due to various illnesses in colectomized ICU patients, where histopathological findings were compared to clinical features and outcome. We found an association between serum lactate and admission SOFA score with the histological damage of colon wall. Moreover, the presence of epithelial defects and complete crypt damage was associated with high mortality.

Thus far, the effect of intestinal epithelial damage on patient mortality has been shown in studies using circulating levels of intestinal fatty acid binding protein as a marker of enterocyte damage in early phase of abdominal sepsis and acute pancreatitis [3, 11]. In our series, the width

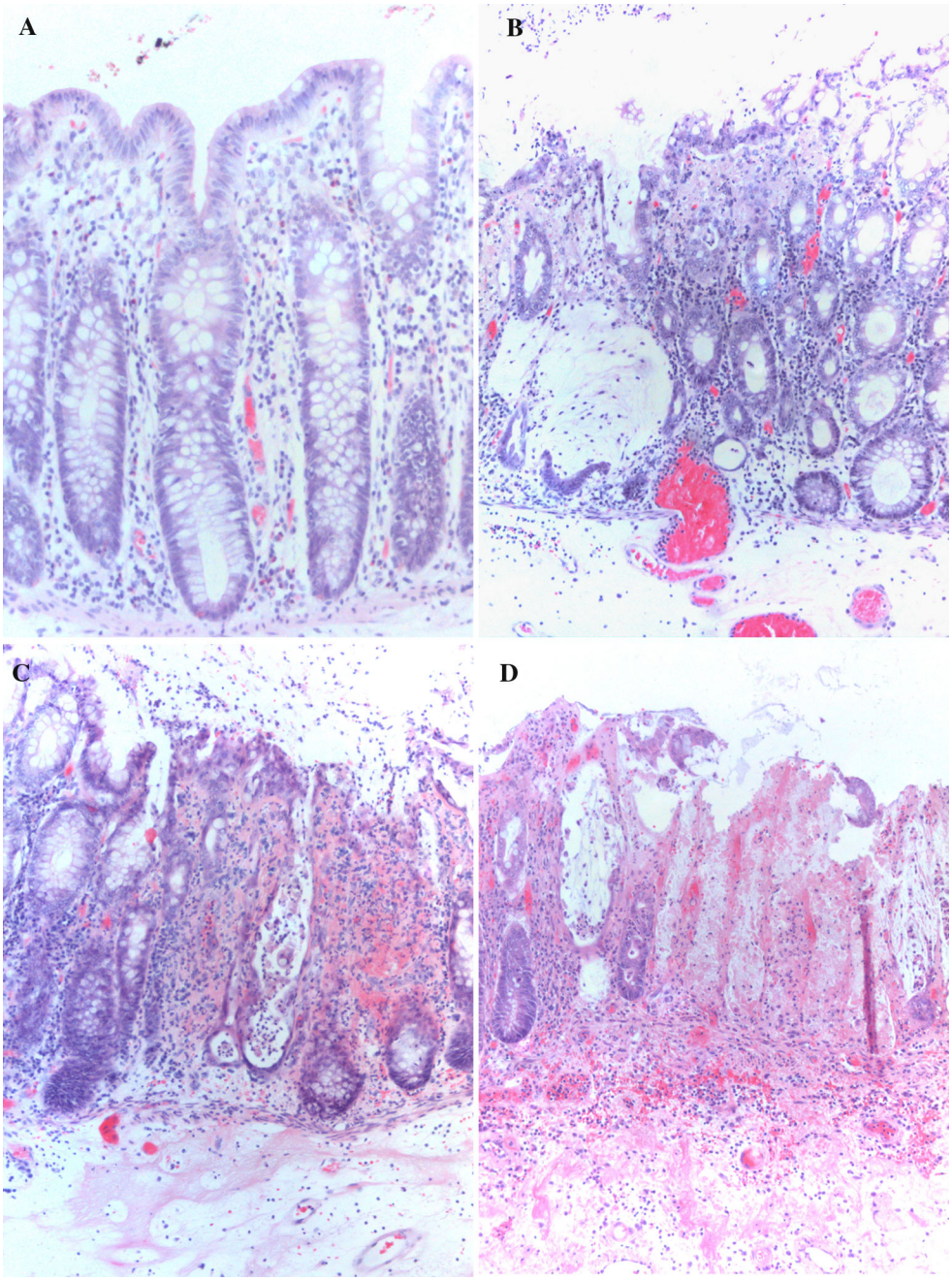

Fig. 1 **a** Histologically normal colonic mucosa from a control patient. **b** Severe enterocyte degeneration and neutrophilic infiltration in a colectomized patient. **c** Crypt abscess with destruction of the crypt epithelium. **d** Detachment of superficial epithelium and a focus of mucosal necrosis

of enterocyte defects and the presence of severe crypt damage were more common among the nonsurvivors. According to animal models, such a disruption in the intestinal mucosal barrier may aggravate bacterial translocation [2, 5]. On the other hand, the depth and width of submucosal necrosis did not associate with mortality. These findings highlight the importance of mucosal integrity in critically ill patients. Moreover, we found that mucosal damage was associated with the maximum preoperative lactate level, an indicator of tissue hypoxia, whereas the same was not seen between maximum preoperative noradrenaline dose. This is line with studies that show that norepinephrine has minimal impact on mesenteric blood flow [12].

Neutrophil infiltration was present in 40 % in our study. In patients with severe ischaemic necrosis, histopathological evaluation of the resected colonic segment has revealed mucosal to transmural necrosis with neutrophilic

Table 2 Histological features of the colon based on 28-day survival

Histological feature	Survivors $n = 22$	Nonsurvivors $n = 28$	P value
Epithelial changes			
Enterocyte defects	40.8 (30–60)	60 (41.3–67.6)	0.02
Minimal mucosal height (micrometer)	56 (40–80)	50 (0–67)	0.11
Minimal enterocystic height (micrometer)	7 (6.5–9)	9 (6–10)	0.36
Neutrophilic infiltration			
Neutrophil score (VAS) in the surface epithelium, crypts, and lamina propria	1 (0.67–1.33)	1 (0.67–1.33)	0.66
Neutrophil score (VAS) in the submucosa, muscularis, and subserosal fat	1.67 (1–1.67)	1 (0.67–1.67)	0.23
Submucosal necrosis			
Lateral width (%)	46.3 (27.5–53.8)	40 (23.1–57.5)	0.7
Depth (%)	47.5 (28.8–77.5)	46.3 (22.5–65)	0.21

The mean value of analysed samples in each segment was calculated for each patient. The resected section of the colon showing the most severe pathological changes was used for analysis. Data are expressed as median and 25th and 75th percentiles

VAS visual analogy scale

infiltration [13]. In early ischaemic lesions, mild to moderate neutrophil infiltration has been shown [14]. In our series, all of our patients presented with severe colon damage, but there were no differences in neutrophil infiltrations in colon wall between survivors and nonsurvivors.

All our patients had pancolic disease with mortality of 56 %. In another retrospective analysis of mostly conservatively treated patients with ischemic colitis, only 7.3 % had pancolic disease basing on biopsies and they had a mortality of 22 % [15]. In contrast to our study, no information was given on the condition of the epithelia or severity of mucosal damage. In another retrospective database, two or more segments of the colon was affected in more than half of the patients in conservatively treated ischaemic colitis [16].

Our study arise the question whether precolectomy biopsies would help in timing of surgery in critically ill patients. Our results showed that the histopathological damage is heterogenic varying from nearly normal findings to severe necrosis depending on the sampling site. Furthermore, in the case of deteriorating critically ill patient there is no time to wait for the results of histopathological analysis.

Our study has some limitations. The study was retrospective and involves a single centre with a relatively small number of patients. In addition, not all of the colon segments could be evaluated to a similar extent due to nonsystematic sampling in some cases. Our control group consisted of normal colons. To further reveal disease processes would require colonic biopsies from less severe cases in a prospective manner. The histological damage of colon as a cause of mortality would need further studies.

In patients with total colectomy, epithelial mucosal height was associated with blood lactate levels, whereas epithelial defect and severe crypt damage associated with mortality. There were no differences in the histopathological findings between different underlying causes except the abundance of pseudomembranes in clostridium group. Our results highlight the association of colonic epithelial mucosal damage with the severity of illness and outcome.

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