

Seija Sipola

COLECTOMY IN AN ICU PATIENT POPULATION

CLINICAL AND HISTOLOGICAL EVALUATION

UNIVERSITY OF OULU GRADUATE SCHOOL;
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**COLECTOMY IN AN ICU PATIENT
POPULATION**

Clinical and histological evaluation

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of the Doctoral Training Committee of Health and
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Abstract

Colectomy is performed in critically-ill patients who, for example, experience colonic ischemia following cardiac surgery or reconstruction of a ruptured aortic aneurysm, nonocclusive mesenteric ischemia with severe sepsis, or toxic megacolon due to *Clostridium difficile* infection.

The present retrospective study was conducted in the mixed intensive care unit (ICU) of the Oulu University Hospital to clarify the clinical picture, effects of surgical treatment on organ functions and outcome in critically-ill patients treated with colectomy during 2000-2009. Their histologic and immunohistologic findings were compared with histologically normal colon walls of 34 controls operated for colon tumors.

The annual incidence of colectomy in our ICU varied from 0.08% to 0.4%. The mean age of the study patients was 68.8 (sd 9.7) yrs. They had multiple organ failure in 60% and one-year mortality was 62%. One-year survival from the hospital discharged patients was 91% (29/32). During preoperative period, increasing levels of serum lactate, an increase in the need for higher doses of norepinephrine, and neurologic SOFA subscore were associated with mortality. The histopathologic damage involves all layers of the colon wall being largely similar in sepsis, fulminant *clostridium difficile* infection and in ischemia after cardiovascular operations. The extent of epithelial damage of colonic epithelium correlated with clinical severity and outcome in the patients. Tight junction protein claudin-1 was down-regulated thoroughly of colonic epithelium, whereas claudin-2 was up-regulated only in the least affected areas. The number of proliferating epithelial cells of colonic epithelium, analyzed by Ki-67 expression, was higher in the worst affected areas in the study patients as compared to results of controls. The proportion of apoptotic cells analyzed by expression of M30 was larger in the worst damage area than in controls. Up-regulation of Toll-like receptor 9, as a part of innate immunity mechanism, in worst areas of colonic epithelium was higher in the surface epithelium compared with least affected areas and in crypts compared with control specimens.

Colon ischemia in critically-ill patients is a pancolic phenomenon with life-threatening consequences. Histologic damage in the colon wall was similar irrespective of the underlying cause. Immunohistochemical characteristics resembled those described earlier in inflammatory bowel disease.

Keywords: colectomy, critically-ill patients, histologic findings, immunohistologic findings, multiple organ failure, pancolic

Sipola, Seija, Leikkauksella hoidettujen tehohoitopotilaiden koliitti. Kliininen ja histologinen tutkimus

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Anestesiologia, Tehohoidon yksikkö; Diagnostiikan laitos, Patologia; Kliinisen lääketieteen laitos, Kirurgia; Medical Research Center Oulu; Oulun yliopistollinen sairaala, Infektioiden torjuntayksikkö

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Tiivistelmä

Leikkaukseen johtavaa tehohoitopotilaan koliittia esiintyy esimerkiksi sydän- ja verisuonileikkauksen jälkeen, yleistyneessä tulehdusreaktiossa sekä *Clostridium difficile*-infektiossa.

Takautuvasti kerätyn tutkimuksen tavoitteena oli selvittää vuosina 2000–2009 Oulun Yliopistollisen sairaalan päivystysteho-osastolla hoidettujen potilaiden koliitin kliininen taudinkuva, kirurgisen hoidon vaikutus elinvaurioihin, ennuste ja histologiset ja immunohistologiset löydökset. Leikkauksella hoidettujen potilaiden histopatologisia ja immunohistologisia tutkimustuloksia verrattiin 34:ään histologisesti normaaliin suolinäytteeseen, jotka oli otettu paksusuolisyövän vuoksi tehdyissä leikkauksissa.

Päivystysteho-osaston vuosittainen tehohoitopotilaan koliitin esiintyvyyden vaihteluväli oli 0.08 %–0.4 %. Tutkimuspotilaiden keski-ikä oli 68.8 (sd 9.7) vuotta. 60 %:lla heistä todettiin monielinvaurio, ja 62 % heistä menehtyi ensimmäisen vuoden aikana. Sairaalasta kotiutetuista potilaista 91 % oli elossa vuoden kuluttua.

Leikkausta edeltävä kohonnut valtimoveren laktaattipitoisuus, verenpainetta tukeva noradrenaliinitarpeen nousu sekä neurologisen toimintakyvyn heikkeneminen olivat yhteydessä potilaiden kuolleisuuteen. Histopatologiset muutokset ulottuivat kaikkiin paksusuolen kerroksiin ja olivat samankaltaisia eri koliiteissa. Epiteelivaurion laajuus oli yhteydessä potilaiden kliiniseen taudinkulkuun ja ennusteeseen. Immunohistologisissa tutkimuksissa paksusuolen epiteelin klauidiini-1:n esiintyminen oli alentunut, kun taas klauidiini-2:sta oli runsaammin vähemmän vaurioituneella alueella. Vaikeimmin vaurioituneilla suolen alueilla solujen uudistumista kuvaavan merkkiaineen, Ki-67:n, määrä oli suurempi kuin kontrollipotilaiden värjäyksissä. Samanlainen ero vaikeimmin vaurioituneiden alueiden ja kontrollinäytteiden välillä todettiin myös M30-värjäyksen perusteella apoptoosin osalta. Välittömään puolustusmekanismiin kuuluvan Tollin kaltaisen reseptori (TLR) 9:n värjäytyvyys oli vaikeimmin vaurioituneilla epiteelialueilla voimakkaampi kuin vähemmän vaurioituneella alueella. Myös kryptan alueella oli enemmän TLR 9 värjäytyvyyttä kuin kontrollinäytteissä.

Tehohoitopotilaan koliittia esiintyy koko paksusuolen alueella. Histopatologiset muutokset ovat samankaltaisia eri tautitilojen aiheuttamissa koliiteissa. Immunohistokemialliset tutkimuslöydökset vastaavat aikaisemmin tulehduksellisten suolistosairauksien yhteydessä kuvattuja muutoksia.

Asiasanat: histologiset löydökset, immunohistologiset löydökset, koliitti, monielinvaurio, paksusuoli, päivystysteho-osasto

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Oulu April 2014

Seija Sipola

Abbreviations

ACS	Abdominal Compartment Syndrome
ADP	Adenosine diphosphate
AGI	Acute Gastrointestinal Injury
APACHE II	Acute Physiology and Chronic Health Evaluation II score
ATP	Adenosine triphosphate
CNS	Central nervous system
CO ₂	Carbon dioxide
CRP	C-reactive protein
CT	Computed tomography
ESICM	the European Society of Intensive Care
ESPEN	the European Society for Clinical Nutrition and Metabolism
FAE	Follicle associated epithelium
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
Hif-1	Hypoxia inducible factor
HIF-1 α	Hypoxia inducible factor alpha
IAP	Intra-abdominal pressure
ICU	Intensive care unit
Ig	Immunoglobulin
IL	Interleukin
ILFs	Isolated lymphoid follicles
IMA	Inferior mesenteric artery
IR	Ischemia-reperfusion
IRI	Ischemia-reperfusion injury
JAM	Junctional adhesion molecule
LA	Least affected
MODS	Multi organ dysfunction syndrome
MOF	Multiple organ failure
NADPH	Oxidase nicotinamide adenine dinucleotide phosphate-oxidase
NOMI	Nonocclusive mesenteric ischemia
PAMP	Pathogen-associated molecular patterns
RRT	Renal replacement therapy
SIRS	Systemic inflammatory response syndrome
SMA	Superior mesenteric arteries
SOFA	Sequential organ failure assessment score

TJ	Tight junction
TLR	Toll-like receptor
WA	Worst affected
WCC	White cell count
ZO	Zonula occluden

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I-IV):

- I Sipola, Syrjälä H, Koivukangas V, Laurila JJ, Ohtonen P, Saarnio J & Ala-Kokko TI (2013) Colectomy in intensive care patients: operative findings and outcome. *World J Surg* 37: 333–338.
- II Sipola S, Syrjälä H, Koivukangas V, Laurila JJ, Ohtonen P, Saarnio J & Ala-Kokko TI (2013) Impact of preoperative organ failures on survival in intensive care unit patients with colectomy. *World J Surg* 37: 1647–1651.
- III Sipola S, Ala-Kokko TI, Laurila JJ, Vakkala M, Ohtonen P, Saarnio J, Karttunen TJ & Syrjälä H (2013) Histological damage of colonic epithelium is associated with clinical severity and outcome in colectomized critically ill patients. *World J Surg*. DOI: 10.1007/s00268-013-2388-8.
- IV Sipola S, Ala-Kokko TI, Laurila JJ, Saarnio J, Ohtonen P, Syrjälä H, Karttunen T (2014) Expression of Toll-like receptors, hypoxia inducible factor, tight junction proteins, apoptosis and regeneration in severe colitis among critically ill colectomized patients. Manuscript.

This study also includes so far unpublished data.

Table of contents

Abstract	
Tiivistelmä	
Acknowledgements	7
Abbreviations	9
List of original publications	11
Table of contents	13
1 Introduction	15
2 Review of the literature	17
2.1 Structure and function of the colon.....	17
2.1.1 Basic functions and gross anatomy	17
2.1.2 Blood supply.....	17
2.1.3 Neurologic network.....	19
2.1.4 Normal histology of the colon.....	20
2.1.5 Regulation of blood flow.....	22
2.2 Role of the colon in the host response.....	23
2.2.1 The intestinal microbiota.....	23
2.2.2 Mucus layer.....	24
2.2.3 Immune surveillance	24
2.2.4 Nutrition	25
2.3 Colonic mucosal injury as a promoter of systemic inflammation and organ failure	26
2.3.1 Microbial translocation in the gut.....	26
2.3.2 The three-hit model of gut origin in sepsis.....	27
2.3.3 Ischemia-related histopathological alterations in the colon.....	28
2.4 Mucosal immunity and protection mechanisms.....	30
2.4.1 Toll-like receptors.....	30
2.4.2 Tight junction proteins.....	31
2.4.3 Hypoxia, apoptosis and proliferation.....	32
2.4.4 Effects of barrier dysfunction	33
2.5 Colitis in the critical care setting.....	34
2.5.1 Etiology and pathophysiology of the main types of colitis in the critical care setting.....	35
2.5.2 Incidence	38
2.5.3 Clinical presentation.....	38
2.5.4 The diagnosis of colitis.....	40

2.5.5	The clinical management of colitis in the critical care setting	41
2.5.6	Outcome of patients with colitis	43
3	Aims of the present research	45
4	Patients and methods	47
4.1	Patients and study setting	47
4.2	Clinical management	48
4.3	Clinical data	48
4.4	Histologic examination	49
4.5	Immunohistochemical examination	50
4.6	Statistical methods	53
5	Results	55
5.1	Patients	55
5.2	Incidence of colectomy in the ICU	55
5.3	Surgical findings in colectomy	56
5.4	Occurrence of organ system dysfunctions and outcome	56
5.5	Histologic findings	59
5.6	Immunohistochemistry	60
6	Discussion	61
6.1	Incidence and overall outcome of colectomy in ICU	61
6.2	Evaluation of organ dysfunction and outcome	61
6.3	Histopathologic characterisation	62
6.4	Immunohistochemical characteristics	63
6.4.1	The innate immunity	63
6.4.2	Tight junctions	64
6.4.3	Hypoxia	65
6.4.4	Proliferation and apoptosis	65
6.5	Role and mechanisms of gut wall damage in the pathogenesis of multiorgan failure (MOF)	65
6.6	Strengths and limitations of the study	66
7	Clinical implications and future studies	69
8	Conclusions	71
	References	73
	Original publications	91

1 Introduction

A variety of names have been used to describe colonic ischemia including necrotizing enterocolitis, hemorrhagic enterocolitis, ischemic colitis, ischemic ulcerative colitis, ischemic megacolon, obstructive colitis, non-occlusive ischemia and toxic megacolon. These names reflect the variety of clinical pictures in which ischemic damage of the colon wall occurs (Reintam Blaser *et al.* 2012b). In intensive care unit (ICU) patient population, intestinal function is a crucial determinant as a part of multiple organ failure (MOF), mortality and overall prognosis (Rombeau & Takala 1997). The treatment strategies for GI problems have been based on experience, rather than evidence, because the pathophysiology of GI damages is limited and there is a lack of clinically relevant measurements for gastrointestinal (GI) function. There is a lack in the overall understanding of GI dysfunction's systemic significance and its effects on the overall prognosis of the ICU patient population (Reintam Blaser *et al.* 2012a). For example, the GI dysfunction assessment is not a part of the widely-used sequential organ failure assessment score (SOFA) (Vincent *et al.* 1996, Vincent *et al.* 1998).

In 2012, the Working Group on Abdominal Problems of the European Society of Intensive Care (ESICM) developed a four grade severity scale as well as recommendations for acute gastrointestinal injury (AGI) for clinical and research purposes. According to these recommendations, secondary AGI develops as a consequence of the host response in critical illness without a primary pathology in the GI system, and stages III and IV are life-threatening (Reintam Blaser *et al.* 2012a).

Several conditions in critical illness may lead to life-threatening intestinal complications: colonic ischemia following vascular or cardiac surgery, non-occlusive mesenteric ischemia in severe sepsis, or colitis or toxic megacolon due to *clostridium difficile* infection. Colectomy in the ICU patient population is an infrequent operation associated with a high mortality and the final decision to perform an emergency colectomy remains largely empirical without clearly defined guidelines for patient selection.

There are no studies concerning the histopathologic changes and their role in the pathophysiology of ischemic colitis and MOF in the ICU patient population. The histologic changes in such intestinal mucosal damage have been reported to be non-specific and include edema, distorted crypts, mucosal and submucosal hemorrhage, inflammatory infiltration, granulation tissue, intravascular platelet

thrombi, and necrosis (Price 1990). Intestinal epithelial cells together with dynamic junctional complexes and pattern recognition receptors such as Toll-like receptors form the backbone of the innate immune system in the colon (Bowie & O'Neill 2000, Medzhitov & Janeway 2002). Several clinical studies have shown that intestinal mucosal epithelial damage, intestinal epithelial barrier dysfunction and response to the pathological host defence may play a role in remote organ failures and mortality (Besselink *et al.* 2009, Deitch 2012, Doig *et al.* 1998b). Furthermore, intestinal mucosal damage and abnormal intestinal permeability may play a role in remote organ failures and mortality via bacterial translocation and cytokine response (Antonsson & Fiddian-Green 1991a, Balzan *et al.* 2007).

The present study was conducted to evaluate the clinical picture, diagnostic and histopathological characteristics, as well as the outcome of ischemic colitis in an ICU patient population treated with colectomy.

2 Review of the literature

2.1 Structure and function of the colon

2.1.1 Basic functions and gross anatomy

The colon is about 1.50 m long and has a diameter of around 6 cm. It absorbs 400–1000ml of fluid each day (Fig.1) (Cohn et al 2009). The species of intestinal bacterial flora reside symbiotically with the host and perform a number of functions: fermentation of indigestible carbohydrates and lipids, conversion of bilirubin to urobilinogen as well as synthesising certain vitamins (K, B-12, thiamine, riboflavin) (Cummings *et al.* 1986, Didierlaurent *et al.* 2002, Mortensen & Clausen 1996, Sumi *et al.* 1977, Topping & Clifton 2001). The colon performs a mixing (haustration) and propulsive movement to squeeze and roll the faecal material into a form which permits the absorption of water and electrolytes as well as emptying the portion of the proximal colon.

2.1.2 Blood supply

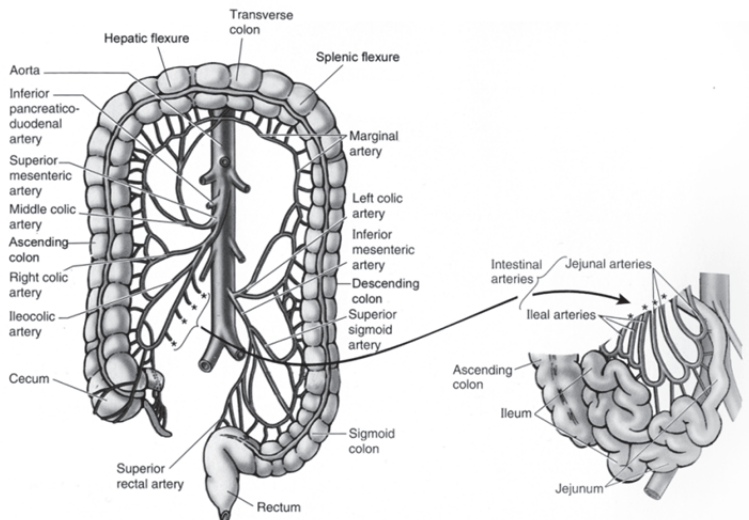


Fig. 1. The arterial supply to the intestines. Modified from Moore K.L, Dalley A.F. (2005) in the textbook of Clinically Orientated Anatomy (fifth edition), page 274.

The splanchnic area receives 10–35% of cardiac output, and accounts for 20–35% of total oxygen consumption (Antonsson & Fiddian-Green 1991b). The arterial blood supply of the gut comes via three major supply trunks originating off the aorta; the celiac, superior mesenteric (SMA) and inferior mesenteric arteries (IMA) (Fig.1). Significant individual variations exist, however, and the sources of collateral flow between the mesenteric vessels, as well as between the mesenteric vessels and the nonmesenteric systemic circulation, are numerous. The primary potential pathway of collateral flow between the celiac axis and SMA is through the gastroduodenal and pancreaticoduodenal arteries. Three major anastomotic pathways are also found between the SMA and IMA (Rosenblum *et al.* 1997). The colon is protected from ischemia by a network of collateral arteries via the marginal artery of Drummond, a system of arcades connecting the major arteries. The anatomy is highly variable (Gandhi *et al.* 1996, Greenwald *et al.* 2001).

Venous drainage of the gut takes place via the portal venous system. The small bowel and the proximal colon through the splenic flexure drain via the superior mesenteric vein. The remainder of the colon drains via the inferior mesenteric vein. Collateral venous vessels are present between each major area (Sise 2010).

In the histologic view, the mesenteric blood flow is divided into two parallel circuits consisting of five series-coupled components that serve the muscularis and submucosa/mucosa respectively. In combination with the mesenteric collecting veins (portal tributaries), they contain up to 30% of the body's total blood volume (Fig.2) (Ceppa *et al.* 2003).

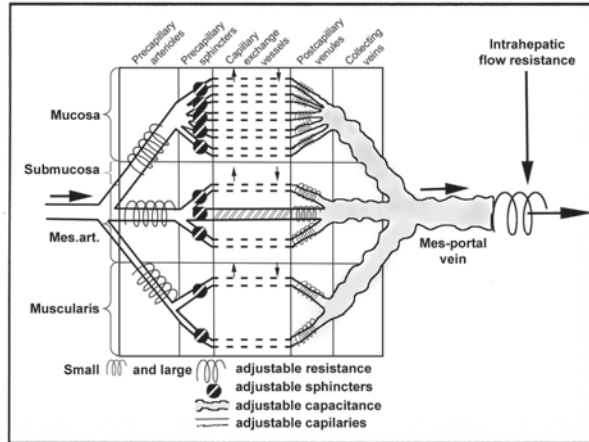


Fig. 2. The anatomic arrangement of the intestinal microvasculature. Modified from Ceppa E.P, Fuh K.C, Bulkley G.B. (2003), Mesenteric hemodynamic response to the circulatory shock Current Opinion Critical Care 9: 127–132.

2.1.3 Neurologic network

The intestine has a complex nerve network comprising autonomic motor and sensory neurones and a separate enteric nervous system. The mesenteric vessels are reactive. Their blood flow is thought to arise from multiple levels of intrinsic and extrinsic control mechanisms. The tone of these vessels depends on a complex balance between neurally mediated sympathetic vasoconstriction, the local action of vasoregulatory substances, the parasympathetic cholinergic nerve supply, the enteric nervous system and endothelial-derived agents (Rosenblum *et al.* 1997). Nerve fibers from the sympathetic nervous system innervate gut mucosa and lymphoid tissue (Straub *et al.* 2006). Experimental models of critical illness have described an inflammatory reflex, whereas the parasympathetic vagus nerve inhibits cytokine release (Tracey 2007). Additionally, host-derived regulators from the enteric nervous system have been reported to take part in the crosstalk between the immune system and the epithelium (Haller 2006). There are still minimal data, however, on the specific role of the enteric nervous system in critical illness.

2.1.4 Normal histology of the colon

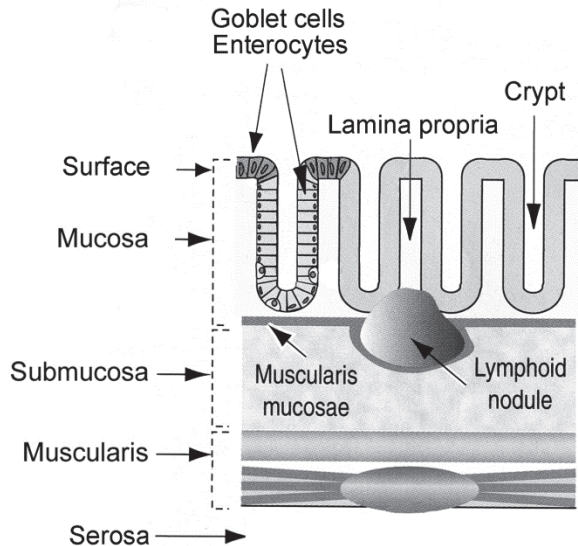


Fig. 3. Histology of the colon. Modified from Kierszenbaum AL. (2002) in the textbook of Histology and Cell Biology page 439.

Mucosa of the colon

The intestinal mucosal barrier is comprised of enterocyte membranes, tight junctions, secreted mucus and immunologic factors such as tissue macrophages, which interact with the external environment (Fig.3) (Lambert 2009). Enterocytes are columnar cells, whose function is the absorption, regulation and secretion of nutrients and ions. At the apical surface, epithelial cells form a brush border and contain many of the digestive enzymes and transporter receptors essential to metabolism (Salim & Soderholm 2011). Enterocytes are capable of producing cytokines following inflammation also in the absence of bacteremia (Mainous *et al.* 1995). Antigens are introduced to mucosal immune responses by the specialized follicle-associated epithelium (FAE), of which M cells are a unique epithelial cell type containing a transepithelial transport system for molecules and particles M cells are also responsible for the induction of mucosal immunity (Jepson & Clark 1998, Mach *et al.* 2005, Neutra *et al.* 1996). In the lamina propria, T cells, B cells, eosinophils, mast cells, dendritic cells and macrophages

comprise the mucosal immune system (Garside *et al.* 2004, Salim & Soderholm 2011). In the normal colon, the number of eosinophils and basophils is variable and neutrophils are not normally present. The submucosal stroma includes a few lymphocytes, plasma cells, fibroblasts, macrophages and fat cells. Vascular structures contain arterioles, venules and lymphatics, which infrequently appear large and tortuous in an otherwise normal colon. In the submucosa, blood vessels and lymphatics are normally free of inflammatory cells (Levine & Haggitt 1989).

The smooth-muscle layers of the colon (muscularis propria) are consisted with blood and lymphatic vessels, including a circular inner layer and a longitudinal outer layer (Fraser *et al.* 1981). The muscularis mucosa is transversed by lymphoglandular complexes, vascular channels and a neural arch referred to as Auerbach's plexus. These separate the mucosa from the deeper submucosa (Levine & Haggitt 1989).

Epithelial intercellular junctions

Nerve processes and a thin layer of fibroblasts are intimately associated with the epithelial basement membrane (Moon 1997). Two major routes for epithelial penetration are the transcellular route and the paracellular route. Molecules pass through the intestinal layer via either the transcellular or the paracellular route (Salim & Soderholm 2011). The presence of healthy epithelial cells and a functionally normal paracellular pathway modify the integrity of the epithelial barrier function. The paracellular pathway is controlled by tight junctions between epithelial cells. (Krug *et al.* 2009).

The epithelial cells are bound together by a junctional complex comprised of tight junctions, adherens junctions, gap junctions and desmosomes (Fig.4.). This structure restricts the passage of macromolecules through the intestinal epithelial monolayer. Claudins, occludins and a junctional adhesion molecule (JAM) are the molecules associated with the tight junction transmembrane proteins. Claudins and occludins interact directly with the peripheral membrane proteins. The JAM (immunoglobulin superfamily) is not involved in intestinal barrier function; but rather participates in the cell-cell adhesions of epithelial cells (Martin-Padura *et al.* 1998). The intracellular component of tight junctions also consists of zonula occluden (ZO) proteins, which are connected to intracellular actin filaments (Van Itallie *et al.* 2013). Intraepithelial lymphocytes restrict the passage of pathogens and improve transepithelial resistance by modulating tight junction proteins,

which affects the functional integrity of the mucosal barrier (Dalton *et al.* 2006). Tight junction proteins are described in more detail in chapter 2.4.2.

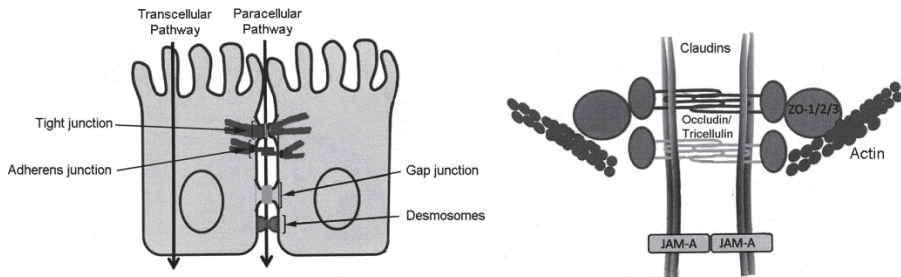


Fig. 4. The tight junctional complex. Modified from Salim S.Y., Söderholm J.D. (2011) Importance of distributed intestinal barrier in inflammatory bowel diseases. Inflammatory Bowel Disease 17(1): 362–381.

2.1.5 Regulation of blood flow

The organs served by the mesenteric circulation account for only 5% of the total body weight yet command a substantial proportion (20–30%) of the total cardiac output under normal hemodynamic conditions (Guyton AC in the Textbook of Medical Physiology 1981: 344–356). The anatomy of the vasculature gets a distribution of total intestinal blood flow between the mucosal, submucosal and muscularis layers and the factors which affects blood flow distribution between these layers. The mucosa and submucosa receive approximately 50–90% of the blood flow to the gut (Granger *et al.* 1980, Kvietys & Granger 1982).

The intestinal circulation is controlled by systemic blood pressure as well as by local autonomic mechanisms. Circulating native and exogenous catecholamines induce vasoconstriction and regulate splanchnic vascular volume. Autonomic factors produce both vasoconstriction and vasodilatation. Intense persistent vasoconstriction (induced by renin, angiotensin, vasopressin, thromboxanes, leukotrienes) is considered to be a cause of intestinal necrosis (Fändriks Lars 2010, Yasuhara 2005).

Autoregulation of circulation

The mesenteric organ is autoregulated, which is defined as a disproportionately smaller decrease in tissue perfusion and blood flow in response to a decrease in

systemic perfusion pressure. These autoregulatory compensatory responses are mediated by arteriolar vasodilatation. Two mechanisms can trigger this response: The myogenic response is a direct reflexive response to a decrease in perfusion pressure. This is believed to be dependent on the endothelial generation of nitric oxide (Alemany *et al.* 1997, Myers *et al.* 1996). The metabolic response is to increase levels of adenosine in response to a decrease in pH and in oxygen tension (Jacobson & Pawlik 1994, Shepherd 1978). Autoregulation is also manifested as a postischemic reactive hyperemia, which is the attenuation of the decrease in blood flow immediately after a fixed decrease in perfusion pressure and by autoregulatory escape. During ischemia, perfusion is redistributed favoring the more metabolically active areas with relatively smaller decreases in flow (Folkow 1967). As blood flow decreases, the tissues respond by increasing oxygen consumption. The autoregulation of oxygen consumption is protective for the gut under mild to moderate ischemia (Granger *et al.* 1980).

Matching of metabolism and perfusion

The balance between oxygen demand and delivery in the GI-tract is determined not only by the blood flow through the main arteries, blood distribution in the bowel wall and blood oxygen and hemoglobin content, but also by oxygen exchange within the mucosa from the base to luminal side, matching the metabolism and perfusion with the mucosa and the cell's capacity to utilize oxygen. Mesenteric vasoconstriction occurs even before any hemodynamic instability arises (Dubin *et al.* 2001, Hamilton-Davies *et al.* 1997, Knichwitz *et al.* 1998, Kolkman & Mensink 2003).

2.2 Role of the colon in the host response

2.2.1 The intestinal microbiota

Immediately after birth the intestine is colonized by beneficial bacterial communities, which facilitate the digestion, absorption and storage of nutrients. The innate recognition of commensal bacteria is essential for the normal function of the gut mucosa (Artis 2008). Alterations in the intestinal microbiota can create a proinflammatory environment or may lead to an overgrowth of a pathogenic strain (Loh & Blaut 2012, Morgan *et al.* 2012, Pant *et al.* 2013). The optimal

functioning of the host immune system is maintained when commensal bacteria assert their effects by forming a resistance to pathogen colonization by producing antimicrobial substances, altering luminal pH, and directly competing with pathogens for nutrients (Artis 2008, Lebeer *et al.* 2010). It has been demonstrated that patients with a systemic inflammatory response syndrome (SIRS) have a lower number of anaerobic bacteria and a higher number of pathogenic bacteria in their gut. Less is known, however, about how critical illness affects the commensal bacteria in the gut or how commensal bacteria affect the gut epithelium and immune system during critical illness (Shimizu *et al.* 2006). Hyperpermeability, antibiotic usage and immunosuppression, especially cytotoxic drugs, can also alter the balance of intestinal microbiota.

2.2.2 Mucus layer

The mucus layer is the first anatomical structure, which serves as a physical and chemical barrier against pathogens and their toxins to the gut. The mucus layer is mainly composed of mucin glycoproteins. Mucins are essential for growth, epithelial renewal, differentiation and intestinal integrity (Corfield *et al.* 2001). The mucins are synthesized and secreted by goblet cells, which are found in the colonic epithelium. The mechanisms mediating the goblet cell response to intestinal insults are poorly defined, but alterations in mucin secretion and function are a response to the intestinal defence mechanism (Deplancke & Gaskins 2001, Grootjans *et al.* 2013). The majority of microorganisms in the colonic lumen can be found in the outer mucus layer, whereas the inner mucus layer is directly adjacent to the epithelium and relatively sterile. Alterations to, or the absence of, the mucus layers allows bacteria to come into direct contact with the epithelial cells. Loss of the mucus layer results in bacteria penetrating the colonic barrier and adhesion to epithelial cells leads to the activation of inflammatory signaling cascades (Cerf-Bensussan & Gaboriau-Routhiau 2010, Johansson *et al.* 2008, Johansson & Hansson 2013).

2.2.3 Immune surveillance

The major targets of innate immune recognition are pathogen-associated molecular patterns (PAMPs), which are structurally and functionally heterogeneous proteins shared by large groups of microorganisms. The recognition of these patterns allows the innate immune system to detect the

presence of a harmful infectious microbe and to determine the type of infecting pathogen. Pattern recognition receptors activate conserved host defense signaling pathways. The survival of the host organism depends on its ability to recognize infectious microbes and to induce appropriate defense responses (Medzhitov & Janeway 2000).

Immune surveillance occurs at specific sites in the mucosa and is composed of organized lymphoid tissues and specializations of the epithelium (Neutra *et al.* 2001). Gut-associated lymphoid tissue (GALT) with isolated lymphoid follicles (ILFs) plays a central role in the colon's inflammatory conditions and in colonic mucosal regeneration and these ILFs are composed of a specialised FAE (O'Brien *et al.* 2008, Salim & Soderholm 2011, Spahn & Kucharzik 2004). The FAE contains antigen sampling cells, called M cells, which continuously transport luminal material transepithelia to the underlying tissue (Neutra *et al.* 2001, Niedergang & Kraehenbuhl 2000). After induction of ILFs, immune cells are distributed throughout the lamina propria. In the lamina propria, the immune system which responds to foreign antigens consists of T cells and B cells, eosinophils, mast cells, dendritic cells and macrophages (Garside *et al.* 2004, Salim & Soderholm 2011). Intestinal dendritic cells and macrophages travel to the mesenteric lymph nodes and present antigens to T cells and B cells. Dendritic cells also selectively induce IgA production and help to protect against mucosal penetration. The secretion of IgA is an essential factor in the regulation of luminal bacteria and in limiting bacterial penetration (Macpherson *et al.* 2000, Macpherson & Uhr 2004, Macpherson *et al.* 2008).

2.2.4 Nutrition

Active function in the gastrointestinal tract is essential for maintaining the integrity of the immune system and gut barrier function. Nutritional support therapy is generally recommended via enteral nutrition, but there is a lack of evidence that bacterial translocation is reduced with the use of enteral nutrition (Alexander 1998, Alpers 2002, Jeejeebhoy 2001, Lipman 1995). On the other hand, total parenteral nutrition in humans induces atrophy and remodeling of the intestinal mucosa (epithelium and lamina propria) with a decrease in the absorbing surface. The response of the mucosa to parenteral nutrition is immediate (Groos *et al.* 1996). Nutritional support is challenging in the ICU patient population. According to The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines on nutrition in the ICU, enteral nutrition via

tube-feeding is the preferred way of feeding the critically-ill patient and in patients who cannot be given sufficient enteral nutrition; the deficit should be supplemented parenterally. ESPEN guidelines for enteral and parenteral nutrition are widely used in daily practice (Kreymann et al 2006).

2.3 Colonic mucosal injury as a promoter of systemic inflammation and organ failure

2.3.1 Microbial translocation in the gut

The term translocation is usually used to describe the passage of viable bacteria from the GI tract across the mucosa to normally sterile tissues such as the mesenteric lymph nodes or other internal organs. Although bacterial translocation and its complications have been clearly demonstrated in animal studies, this has been difficult to prove in patients (Balzan *et al.* 2007, Berg & Garlington 1979, Zanoni *et al.* 2009). Translocation is also used in the passage of inert particles and macromolecules across the intestinal mucosal barrier. Translocation may be a normal phenomenon which allows the gut to assemble a controlled local immune response helping to keep harmful antigens out of the circulation (Balzan *et al.* 2007, Besselink *et al.* 2009, Deitch 2012, Doig *et al.* 1998a).

Gut mucosal injury has been suggested to permit the translocation of bacteria and their toxins and the associated release of mediators and cytokine response while collectively they have been thought to be responsible for the progression of multiple organ failure and increased mortality (Fig.5) (Antonsson & Fiddian-Green 1991a, Balzan *et al.* 2007, Besselink *et al.* 2009, Deitch 2012, Doig *et al.* 1998a, Doig *et al.* 1998b).

Two models of the origin of translocation in sepsis have been described: Deit's three-hit model (see 2.3.2) (Das *et al.* 2012, Deitch 2002) and Moore's theory (Deitch 2012, Koike *et al.* 1994, Moore *et al.* 1991). Moore's theory attempts to demonstrate bacterial translocation via the portal vein as a major factor in the pathogenesis of MODS, but this could not be confirmed (Moore *et al.* 1991). The translocation of bacteria, their antigenic components or of cytokines generated in the gut, and the role of these in causing sepsis and MODS remains unclear. (Gatt *et al.* 2007, Kanwar *et al.* 2000, MacFie *et al.* 1999, MacFie *et al.* 2006).

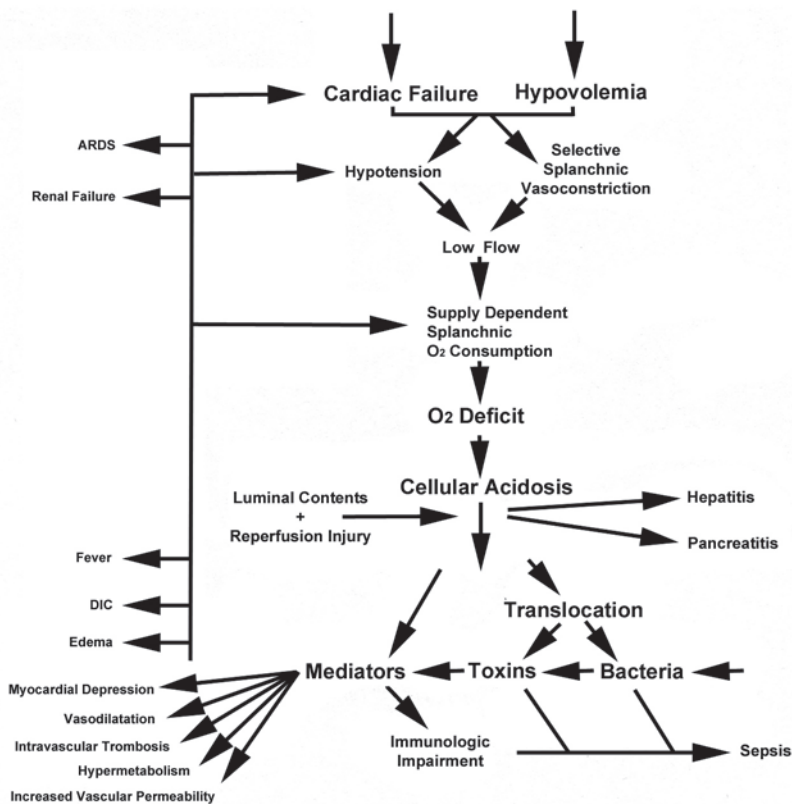


Fig. 5. Causes and effects of splanchnic ischemia. Modified from Antonsson J.B, Fiddian-Green R.G (1991) the role of the gut in shock and multiple system organ failure; Eur J Surg 157: 3–12.

2.3.2 The three-hit model of gut origin in sepsis

In Deitch's three-hit model on the pathogenesis of gut inflammation leading to sepsis, an initial insult results in splanchnic hypoperfusion (first hit) resulting in the production of proinflammatory factors and intestinal cytokine production. Resuscitation then leads to an ischemia-reperfusion injury (second hit), with a concomitant loss of gut barrier function and is followed by translocation of

microbes and an inflammatory response. The gut becomes a proinflammatory organ, which affects the local and systemic immune systems (third hit) resulting in systemic inflammatory response syndrome (SIRS) and multi organ dysfunction syndrome (MODS). The hypothesis of gut-lymph theory proposes that macrophages and other immune cells in the submucosal lymphatics trap the majority of the translocating bacteria. After that, the surviving bacteria or protein components of the dead bacteria, along with cytokines in the gut, travel via the mesenteric lymphatics, through the thoracic duct into the left subclavian vein to reach the right side of the heart. The activation of alveolar macrophages via the pulmonary circulation leads to acute lung injury and may progress to acute respiratory dysfunction syndrome and MODS. The loss of the gut barrier, even in the absence of bacteremia and endotoxemia, can cause a septic state, which leads to organ dysfunction. It has been recently emphasised that gut-derived sepsis and MODS do not require bacterial translocation, and that bacterial translocation itself may not lead to MODS and sepsis. These conditions may also occur together (Deitch 1990a, Deitch 1990b, Deitch *et al.* 1992, Deitch 2002, Deitch *et al.* 2006, Deitch 2012). Although numerous modifications on the gut origin of sepsis hypothesis have been published, the biological mechanisms in the gut which may lead to sepsis and MODS remain unclear.

2.3.3 Ischemia-related histopathological alterations in the colon

Histopathology in total ischemia and necrosis

Ischemia of the intestine may cause necrosis and infiltration of eosinophilic granulocytes. Inflammatory cells infiltrate the mucosa and submucosa within 12 to 24 h and the mucosa loses its normal structural details. Mucosal and submucosal inflammation, hemorrhage and edema increase between 24 to 48 h and necrotic changes proceed to mucosal breakdown. Necrosis of the muscularis propria may lead to perforation. On microscopy, typical changes are mucosal degeneration and superficial necrosis, with hemorrhage and various degrees of neutrophilic infiltration. Edema, hemorrhage and neutrophilic infiltration with fibrin thrombi in vessels may contribute to an extension of ischemia and lead to submucosal necrosis. In severe ischemic injury, inflammation may be transmural and necrosis of the muscularis occurs. The

glands adjacent to the areas of ulceration and healing inflammatory changes may manifest as cryptitis or crypt abscesses (Mitsudo & Brandt 1992).

In ischemic enterocolitis the earliest lesion appears to be thrombosis involving the capillary plexus high up in the intestinal mucosa. The surface epithelium is progressively lost and the upper half of the crypts shows varying degrees of degeneration. Necrosis of the superficial layers of the mucosa occurs later, with edema and hemorrhages and an acute mild to moderate inflammatory infiltrate in the lamina propria. The submucosa is edematous. Plaques of membranes consist of fibrin, inflammatory cells, mucus and necrotic epithelium. Ulceration may extend into the submucosa and may involve the inner muscle layers and - very rarely - full-thickness necrosis of the bowel wall (Whitehead 1971).

Histopathology in pseudomembranotic colitis

In pseudomembranous colitis, histopathologic changes in the colonic mucosa have been demonstrated to include lamina propria hyalinization, atrophic and/or necrotic mucosa, crypts with a withered appearance, neutrophilic inflammation, lamina propria hemorrhage, mucosal necrosis, and/or intravascular microthrombi (Dignan & Greenson 1997). Pseudomembranotic formation and “ballooned crypts” containing neutrophilic abscesses were categorized as *Clostridium difficile* colitis (Mosli *et al.* 2013, Wiland *et al.* 2013). In colonic epithelial cells, *clostridium difficile* toxins lead to fluid secretion, edema, increased mucosal permeability and mast cell degranulation. Toxin A *in vitro* has induced apoptosis in macrophages, eosinophils and T cells in lamina propria (Mahida *et al.* 1998). The toxins also induce the production of proinflammatory cytokines and chemokines and activate surface and intracellular immune sensors and signaling pathways (Castagliuolo *et al.* 1998, Hasegawa *et al.* 2011, Ishida *et al.* 2004, Mykoniatis *et al.* 2003, Ng *et al.* 2010, Ryan *et al.* 2011). Toxin B is a potent cytotoxin capable of inducing enzyme-independent necrosis in both cells and tissue. Toxin B-induced necrosis is mediated by the host epithelial cell NADPH (nicotinamide adenine dinucleotide phosphatase) oxidase complex (Farrow *et al.* 2013). A hypervirulent strain, which is referred to as NAP1/B1/027, was first observed at the beginning of the twenty-first century. This strain produces type C toxin, which are the most virulent strains of the bacteria (Kazanowski M *et al.* 2013).

Ischemia-reperfusion injury in the colon

In colonic ischemia/reperfusion injury (IRI), subepithelial spaces have been observed after 60 minutes of ischemia at the surface epithelium of the colon. During reperfusion, IR-damaged epithelial cells have been shed into the lumen. After 60 minutes of reperfusion, the epithelium has been shown to have an irregular appearance, but extensive damage has not been observed. After 60 minutes of ischemia, massive mucus secretory activity of the goblet cells has been observed as a response to bacterial colonisation. A disorganised damaged mucus layer allows for the penetration of bacteria to colonic crypts and adherence to epithelial cells. IR-induced epithelial damage has similar features in human and rat studies (Grootjans *et al.* 2010, Grootjans *et al.* 2013).

2.4 Mucosal immunity and protection mechanisms

2.4.1 Toll-like receptors

Toll-like receptor (TLR) activation has been shown to play an important role in the maintenance of intestinal epithelial barrier function (Kawai & Akira 2006).

The Toll-like receptor family plays a central role in innate immune responses against pathogens as well as following induction of the adaptive immune response. Regulation of the Toll-like receptor family is essential for the maintenance of the intestinal luminal microbiota. Dysregulation (up or down- regulation) of the response leads to a pathologic host defense reaction. Direct interaction of TLRs with the intestinal epithelium could lead to the development of intestinal inflammation. Sub-epithelial and circulatory leucocyte activation may also lead to the initiation and propagation of mucosal inflammation. After the recognition of microbial pathogens, TLRs trigger intracellular pathways which culminate in the introduction of inflammatory cytokines such as IL-6, IL-1beta, IL-12, tumor necrosis factor alpha, as well as alternative pathways that induce responses against different pathogens. The signaling from TLRs induces antigen-presenting cells called dendritic cells (Kawai & Akira 2006).

Colonic damage in mice was associated with more severely decreased epithelial proliferation and increased intestinal epithelial cell apoptosis compared to controls in an experimentally induced colitis model with Toll-like receptor knock-out mice (Fukata *et al.* 2006). An aggravated Toll-like receptor mediated immune response has been suggested to be central in the pathogenesis of

inflammatory bowel disease such as ulcerative colitis. In human studies up-regulated Toll-like receptor family expression (TLR-2, 4, 5, 8, 9) has been shown to be associated with the severity of intestinal inflammation, disease activity and cytokine release in colonic biopsies in patients with inflammatory bowel disease (Frolova *et al.* 2008, Sanchez-Munoz *et al.* 2011, Szebeni *et al.* 2007).

The intensity of TLR signaling within the epithelium varies: Under basal conditions TLRs attend to the regulation of barrier integrity, such as epithelial migration, proliferation and apoptosis. During states of systemic stress, such as hypoxia or remote infection, the extent of TLR signaling within the epithelium is amplified. The extent of inflammation is compounded by the contribution of TLR activation in leukocytes and the release of proinflammatory molecules. If the balance of TLR signalling within the epithelium can be regulated to a homeostatic state, mucosal inflammation may not develop. Increased TLR expression leads to impaired epithelial function, increased injury, and decreased repair, resulting in mucosal inflammation. TLR signaling also plays a role in the GI tract during sepsis, as a part of sepsis-induced ileus. (Buchholz & Bauer 2010, Kawai & Akira 2006).

2.4.2 Tight junction proteins

Tight junctions are static components attending to innate immunity against bacteria. Tight junctions are located horizontally around the entire cell, and create and maintain epithelial cell polarity by regulating the movement of proteins and lipids within the plasma membrane (Farquhar & Palade 1963, Turksen & Troy 2004). Tight junctions are the most apical component of intracellular junctions with two classic functions: a barrier function, which regulates solute and water flow through the paracellular space (homeostasis) and a fence function, separating the apical from the basolateral cell surface domains to maintain cell polarity. In the literature, inflammatory cytokines such as tumor necrosis factor alpha and interferon-gamma have been shown to have considerable influence on TJ protein alterations (Bruewer *et al.* 2003, Wang *et al.* 2005). In vitro interleukin 4 and interleukin 13 have been shown to increase permeability using intestinal epithelial monolayers (Ceponis *et al.* 2000). Various viruses and bacteria are able to disturb the tight junction's barrier function and increased inflammation and proinflammatory cytokine expression are associated with various illnesses such as inflammatory bowel diseases (Su *et al.* 2013, Zeissig *et al.* 2007).

The first integral membrane protein discovered in tight junction strands was occludin. It is the most reliable immunohistochemical marker for tight junctions (Furuse *et al.* 1993). Occludin is directly involved in the tight junction barrier itself as well as in the fence functions of the tight junction system. It is also involved in the transepithelial migration of inflammatory cells such as neutrophils. (Huber *et al.* 2000, Sawada 2013).

The claudin family, composed of 27 members, is necessary for the formation and function of tight junctions and is responsible for alterations in paracellular permeability (Sawada 2013, Turksen & Troy 2004). The expression of claudin-1 and claudin-2 are increased in inflammatory bowel disease when inflammatory activity is present. In colitis ulcerosa and in Crohn's disease down-regulation in the expression of claudin-1 was revealed in the epithelial cells immediately adjacent to the transmitting neutrophils. Claudin-2 is a leaky protein and increased claudin-2 in epithelial cells correlates with cell permeability (Kucharzik *et al.* 2001, Weber *et al.* 2008, Zeissig *et al.* 2007). Epithelial tight junctions have been demonstrated to be compromised in critical illness due to increased permeability and persistent activation of systemic inflammation (Fink 2003, Fink & Delude 2005).

2.4.3 Hypoxia, apoptosis and proliferation

Ischemia response

Inadequate blood flow in the colon leads to colonic inflammation. There is a close association between inflammation and hypoxia at the tissue level. In colitis, the presence of colonic hypoxia is a significant factor in the disease process (Karhausen *et al.* 2004). Cellular hypoxia induces a cascade of molecular responses capable of affecting multiple cellular processes. The redox-sensitive transcription factor hypoxia inducible factor (HIF-1) plays a critical role in the physiologic and pathophysiologic response to hypoxia and ischemia. HIF-1 regulates the pathophysiological response to hypoxia and ischemia by increasing the transcription of proteins which are involved in angiogenesis, erythropoiesis, glycolysis and cell survival in low –oxygen conditions (Iyer *et al.* 1998, Pugh & Ratcliffe 2003, Semenza 2001). The HIF-1 complex consists of HIF-1 α and HIF-1 β subunits and HIF-1 α protein levels are markedly up-regulated in hypoxic cells (Semenza 2000). In murine experimental colitis, down regulation of HIF-1

expression correlated with more severe clinical symptoms, whereas increased levels were associated with a protection of mucosal epithelial integrity (Karhausen *et al.* 2004). In colonic biopsies of patients with ischemic colitis, HIF-1 α was also over-expressed in the epithelial and intestinal cells in ischemic regions compared to normal regions or normal colon controls (Okuda *et al.* 2005). In vivo, gut ischemia induces a prolonged mucosal HIF-1 response which continues during reoxygenation. A prolonged intestinal HIF-1 response is part of the pathogenesis of IRI (Feinman *et al.* 2010). HIF-1 α expression was also suppressed in sepsis and was inversely associated with severity of the illness (Schafer *et al.* 2013).

Epithelial apoptosis and regeneration

Intestinal epithelium apoptosis at the luminal surface is a programmed, well-coordinated event with well-defined morphological features, and is followed by the removal of dying cells (Kerr *et al.* 1972). Intestinal epithelium proliferation is a renewing process at the crypt bottom. A disruption in the balance between epithelial cell apoptosis and proliferation is associated with inflammatory bowel diseases (Araki *et al.* 2010). An increase of apoptotic cells has been reported in the colonic epithelia in active ulcerative colitis. In an experimental acute mice colitis model both increased apoptosis and decreased proliferation of the colonic epithelial cells were found (Araki *et al.* 2010, Iwamoto *et al.* 1996, Souza *et al.* 2005, Yukawa *et al.* 2002). It has been demonstrated that gut epithelial apoptosis is elevated during sepsis and endogenous bacteria may mediate intestinal apoptosis and the local inflammatory response (Fox *et al.* 2012). The prevention of epithelial apoptosis during inflammation also partially decreases sepsis-induced proliferation (Coopersmith *et al.* 2003).

2.4.4 Effects of barrier dysfunction

Alterations in the mucosal architecture or alterations in intestinal permeability occur in humans, but its relationship to the prevalence of bacterial translocation is uncertain (Gatt *et al.* 2007). In experimental endotoxemia using polyethylene glycol as a permeability marker, an increase in intestinal permeability was observed. This was most likely caused by inflammation-induced changes in paracellular permeability, rather than ischemia-mediated enterocyte damage (Hietbrink *et al.* 2009).

As an index of gut barrier dysfunction increased intestinal permeability has been observed in human studies after burns (LeVoyer *et al.* 1992), major trauma (Pape *et al.* 1994) and sepsis (Johnston *et al.* 1996). Endotoxemia may also be present in the patient with shock (Rush *et al.* 1988), or after burns (Winchurch *et al.* 1987) and sepsis (van Deventer *et al.* 1988).

The assumption that intraoperative splanchnic hypoperfusion is associated with a down-regulation of monocyte function, increased intestinal permeability, and an exaggerated acute phase response, supports the idea that the gut plays a central role in the inflammatory response of major surgery (Holland *et al.* 2005). In contrast, Kanwar *et al.* demonstrated that gut barrier dysfunction occurs after surgery, but that the magnitude of change does not differentiate between patients who will develop sepsis or not. An increased preoperative intestinal permeability was not shown to have a predictive value for sepsis (Kanwar *et al.* 2000).

In the literature, there are findings which support the idea that the intestine plays a crucial role in the pathophysiology of sepsis (Deitch *et al.* 1992). On the other hand, Clark *et al.* have focused on the theory, that the intestinal epithelium, the intestinal immune system and the intestine's endogenous bacteria may all play vital roles in driving MODS. The complex crosstalk between these three interrelated portions of the GI tract has been suggested to form a gut a "motor" for critical illness (Clark & Coopersmith 2007).

2.5 Colitis in the critical care setting

Several situations may cause a breakdown of the intestinal barrier allowing bacterial translocation and a cytokine-mediated systemic inflammatory response syndrome, sepsis, multiorgan dysfunction syndrome and death (Gatt *et al.* 2007). Ischemia and reperfusion injury after cardiac or vascular surgery, fulminant toxic megacolon due to *clostridium difficile* infection and sepsis with severe abdominal infection cause morbidity and mortality in the critically-ill patient population. Non-occlusive mesenteric ischemia is characterized by gastrointestinal ischemia with normal vessels, whereas early mucosal ischemia leads to increased permeability, possible bacterial translocation, and further mucosal hypoperfusion (Kolkman & Mensink 2003). The diagnosis of intra-abdominal sepsis is challenging because there will usually be alternative sources of sepsis, such as lungs, urine etc. Determining whether or not an unstable ICU patient has an abdominal process which requires intervention may be challenging as the signs

that usually are sought after may be absent due to sedation and paralysis (Weledji EP & Ngowe MN 2013).

2.5.1 Etiology and pathophysiology of the main types of colitis in the critical care setting

Ischemia

The etiology of ischemic colitis is multifocal. Many patients have underlying atherosclerosis. Hypoperfusion states due to congestive heart failure and transient hypoperfusion during the perioperative period may impair colonic perfusion leading to colonic ischemia (Elder *et al.* 2009). Mesenteric arterial emboli or thrombosis, venous thrombosis or trauma may lead to occlusive vascular disease causing ischemic colitis (Mitsudo & Brandt 1992). Nonocclusive ischemia may be due to cardiac failure, shock, abdominal compartment syndrome, dehydration and drugs with vasoconstrictive effect (Glauser *et al.* 2011). Ischemia may also be due to radiation injury, volvulus, stricture or internal or external herniation (Sun & Maykel 2007).

In ischemia-reperfusion injury (IRI) there is an interruption of blood supply to the gut or in the construction of free intestinal grafts followed by a reperfusion period. The events that occur during IRI are complex and are mediated by reactive oxygen metabolites and an activation of polymorphonuclear neutrophils. IRI can diminish the barrier function of the gut, and can promote an increase in intestinal permeability or bacterial translocation (Kong *et al.* 1998). Clinically, IRI may manifest as continued organ dysfunction in the postreperfusion period (e.g. myocardial stunning, reperfusion arrhythmias) (Collard & Gelman 2001).

Pseudomembranous colitis

The cause of pseudomembranous colitis in critically-ill patients is *Clostridium difficile* infection, which is associated with a disruption of the endogenous gut flora; most frequently after or during antibiotic treatment (Thibault *et al.* 1991). Well known and emerging risk factors for *clostridium difficile* colitis are the administration of antibiotics, such as clindamycin, penicillin, cephalosporins and fluoroquinolones, age over 65 years, severe co-morbidity, hospitalisation, cytostatics and treatment with prokinetic drugs or acid suppressive drugs

(Khanafar N *et al.* 2013, Kazanowski M *et al.* 2013). Spore-forming gram-positive *Clostridium difficile* bacteria produce toxins which lead to an inflammatory response. This includes damage to intestinal epithelial cells, neutrophilic infiltration and local chemokine and cytokine secretion resulting in life-threatening systemic toxicity in some patients, which can further develop into sepsis and multi-organ failure (MOF) (Voth & Ballard 2005). The spectrum of *Clostridium difficile* –associated disease can range from mild to life-threatening fulminant colitis. *Clostridium difficile* causes fulminant colitis in 3–8% of patients (Adams S & Mercer D 2007). The hypervirulent strain of the bacteria leads to the development of more systemic symptoms and multiple organ failure and increased mortality (Wanahita A *et al.* 2003).

Septic and other shock states

Splanchnic hypoperfusion and the resultant mucosal compromise are central events in the development of systemic immune response (SIRS) and multiple organ dysfunction syndrome (MODS) in critical care patients. Changes in intestinal flora, overgrowth of pathogens, colonization and translocation are significant events in the GI tracts associated with sepsis and MOF (Fiddian-Green 1988, Marshall *et al.* 1993, Rush *et al.* 1988).

Mucosal damage in shock, trauma and sepsis is due to various combinations of intracellular hypoxia as a consequence of ischemia and tissue injury (Fink 1991). In septic patients, ischemic colitis during circulatory stress and vasoconstriction leads to intramucosal acidosis or mucosal hypoperfusion (Kolkman & Mensink 2003). Diminished oxygen utilization decreases adenosine triphosphate (ATP) formation causing ATP depletion. This results in a distorted cell homeostasis causing cell swelling, which leads to necrosis. The intestinal epithelial barrier function is reduced by a weakening of the tight junctions, and bacterial translocation may be an early event (Wattanasirichaigoon *et al.* 1999). In ischemia/reperfusion injury, oxygen enters the ischemic tissue, the damage in microvasculature leads to intestinal edema and membrane integrity is lost (Kolkman & Mensink 2003). The activation of poly (adenosine diphosphate (ADP)-ribose) synthetase or poly (ADP-ribose) polymerase, which were identified as major intermediates in ATP formation, leads to severe energy depletion of cells and necrotic type cell death IRI (Szabo & Dawson 1998). The onset of reperfusion results in an outflow of ischemic and reperfusion waste products (mediators, cytokines and activated neutrophils) into the systemic

circulation (Syk *et al.* 1998) Colonic ischemia can result from alterations in the systemic circulation, or anatomic or functional changes in the mesenteric vasculature. In critically-ill patients, 15% of acute mesenteric ischemia may be caused by arterial thromboembolism, venous thrombosis or splanchnic vasoconstriction, which is termed nonocclusive mesenteric ischemia (NOMI). Cardiac function is usually impaired in patients with NOMI, which exacerbates the ischemia, and generally results in a very poor prognosis (Acosta 2010). Hypotension can be due to sepsis, impaired left ventricular function and hypovolemia due to bleeding or dehydration. These conditions lead to systemic hypoperfusion, which triggers a mesenteric vasoconstrictive reflex. Colonic ischemia can be associated with many medical and surgical conditions, where the specific cause of ischemia cannot be identified. Local hypoperfusion and reperfusion injury are both thought to contribute to the disease process (Elder *et al.* 2009). In an experimental pig model, changes in cardiac index and flow of the superior mesenteric artery were linearly correlated during hypodynamic and hyperdynamic septic shock. The microcirculatory blood flow in the mucosa of the colon decreased significantly during hypodynamic shock. Intravenous fluid administration increased microcirculatory blood flow in the colon to baseline levels (Hiltebrand *et al.* 2000). In endotoxin shock, SMA blood flow was maintained whereas celiac trunk blood flow was compromised and epithelial injury to the gut developed in the mid-colon after 12 hours. In histologic findings mucosal epithelial cellular integrity revealed cryptal epithelial injury in the colon (Tenhunen 2004).

A fall in oxygen delivery below the tissues' requirements due to impairment of myocardial function or hypovolemia may cause gut mucosal ischemia during cardiac or vascular surgery. Perfusion during cardiopulmonary bypass is a potent stimulus for the release of endogenous vasoconstrictors (angiotensin II). Non-pulsatile perfusion activates platelets and leucocytes to form cellular aggregates capable of occluding vessels within the microcirculation. These micro aggregates release a variety of mediators, which can develop gut mucosal ischemia by causing endothelial damage, increasing permeability and thereby causing tissue edema, vasoconstriction, and an increase in the metabolic demand for oxygen (Fiddian-Green 1988). An increase in gut mucosal permeability occurs within 30 minutes of developing mucosal hypoxia.

Complete mucosal necrosis occurs within about 2 hours of the onset of intramucosal acidosis (Fiddian-Green 1990).

2.5.2 Incidence

A meta-analysis of the incidence of ischemic colitis showed that, in the general population, the incidence varies from 4.5 to 44 cases per 100 000 person-years and the risk of ischemic colitis was increased in women and in individuals older than 65 years of age (Higgins *et al.* 2004). Although up to 85% of the cases of ischemic colitis can improve conservatively within the first few days and resolve completely within a few weeks, nearly one-fifth of the patients develop peritonitis and require surgery (Brandt *et al.* 1992).

After aortic reconstruction (endovascular prostheses), ischemic colitis has been reported to occur in 2–3% of the cases (Menegaux *et al.* 2006). The incidence of gastrointestinal complications after cardiac surgery is relatively low at 0.8–3.7% (Musleh *et al.* 2003).

Some 20% of critically ill patients with fulminant *Clostridium difficile* colitis required surgery (Byrn *et al.* 2008, Grundfest-Broniatowski *et al.* 1996). The indications for surgery include peritonitis, perforation, ileus with toxic megacolon and shock or organ dysfunction (Adams S & Mercer D 2007).

In a general surgical ICU the incidence of intra-abdominal sepsis was 63%, where intra-abdominal infection was the primary source in 64% of the patients with septic shock, and 67% of them required an emergency operation (Moore *et al.* 2011). Peritonitis was the main source of severe sepsis in 63% of the cases in another surgical intensive care unit (Weiss *et al.* 2007). In a large, prospective pan-European study the incidence of abdominal infection in a septic ICU patient population was 21% (Volakli *et al.* 2010b), whereas in Australia and New-Zealand the incidence of abdominal sepsis was 19% in an adult patient population (Finfer *et al.* 2004). The incidence of abdominal sepsis in the elderly varies. In elderly patients with severe sepsis or septic shock, an intra-abdominal focus was demonstrated in 17% of cases (Nasa *et al.* 2012), whereas in another study the abdomen was the source of sepsis in 52% in patients over 65 years of age and in 36% of patients under 65 years (Carbajal-Guerrero *et al.* 2013).

2.5.3 Clinical presentation

In colonic infection and ischemia the clinical presentation varies, depending on the severity of colitis. Most patients present with abdominal pain and tenderness, melena and an urge to defecate followed by mild rectal bleeding (Brandt LJ *et al.* 1992). An associated ileus may manifest itself as nausea and vomiting. Clinical

examination reveals tenderness of the abdomen. In *Clostridium difficile* infection diarrhea usually occurs 48–72 h after infection. A significant number of stools per day are typical for *Clostridium difficile* infection. Pyrexia, leucocytosis and a positive fecal occult blood test may be present in fulminant *Clostridium difficile* infection. Patients with severe infection may develop a toxic megacolon, rapidly progressive symptoms with multiple organ failure and sepsis (Khanfer *et al.* 2013).

Patients with intra-abdominal infections present with rapid-onset abdominal pain and symptoms of GI dysfunction with or without signs of inflammation, such as pain, tenderness, fever, tachycardia or tachypnea. The clinical picture varies; patients with fecal peritonitis are more severely ill with signs of septic shock than those with purulent peritonitis with or without perforation (Weledji EP & Ngowe 2013).

In severe ischemia with transmural infarction and necrosis, marked tenderness with peritoneal signs may be present. Respiratory failure, myocardial, hepatic, renal, gastrointestinal and central nervous system (CNS) dysfunction together can result in multi-organ failure and sepsis. Ischemic colitis should be considered in patients presenting with leukocytosis, metabolic acidosis with lactatemia and septic shock.

In sepsis, the need for massive fluid resuscitation and transfusion has been recognized as a risk factor for abdominal compartment syndrome (ACS) and is a predominant cause of increased intra-abdominal pressure (IAP) (Sugrue & Buhkari 2009). Intra-abdominal hypertension is defined as an IAP repeatedly greater than 12 mmHg and ACS is defined as a sustained IAP of greater than 20 mmHg. Increased IAP is associated with new organ dysfunction and is reflected in an increased mortality (Keskinen *et al.* 2007). Hypomotility and swelling of the intestinal walls also occur and, in extreme cases, ischemic necrosis of the gut develops (Scheppach 2009). Acute colonic pseudo-obstruction characterized by massive colonic dilatation without mechanical blockage can lead to complications such as ischemia and perforation. In acute pseudo-obstruction the risk for colon perforation increases when the cecal diameter exceeds 12 cm and when distension is present for more than 6 days. Mortality is approximately 40% in acute pseudo-obstruction if ischemia or perforation is present (Saunders 2007).

2.5.4 The diagnosis of colitis

The diagnosis of intestinal ischemia depends on the ability of the clinician to suspect and recognize it. The presence of abdominal pain, a white blood cell count (WCC) greater than $20 \times 10^9/l$, metabolic acidosis and increased lactate production may be indicative of severe colitis (Pepin 2004). No laboratory tests have been found to be sufficiently specific to diagnose ischemic colitis, but in clostridium colitis diagnostic stool samples detect the presence of toxins. Polymerase chain reaction for the detection of stool *Clostridium difficile* toxins is the newest and most practical microbiological method for identifying *Clostridium difficile*. The result of the test is available within 40 minutes and it has a sensitivity of 85%. The most reliable test is a stool culture, but the results are not available for approximately 4–5 days (Kazanowski M et al 2013).

The role of plain films in the rapid diagnosis of perforation or bowel obstruction is to expedite surgical treatment. Axial computed tomography (CT) is often the examination of choice in the diagnosis of ischemic colitis. It is performed in cases of acute abdomen with an indeterminate cause (Romano S *et al.* 2006). Findings include non-specific ileus, focal or segmental bowel wall thickening, submucosal edema or hemorrhage, pneumatosis or portal venous gas. Contrast enhanced CT detects ischemic colon with sensitivity rates exceeding 90% (Thoeni & Cello 2006). Doppler ultrasonography will be limited by overlying bowel gas, operator-dependent quality and poor sensitivity for low-flow vessel diseases. Ultrasonography may be of interest in follow-up or for monitoring a pathologic condition not requiring immediate surgery (Romano S *et al.* 2006). In patients in whom colonic ischemia is suspected but no signs of peritonitis are present, a sigmoidoscopy may be considered to identify mucosal changes (Sreenarasimhaiah 2003).

Sigmoidoscopy or colonoscopy directly visualizes mucosal changes and can be used to obtain biopsy specimens. In *clostridium difficile* colitis it may provide an immediate diagnosis with specific changes, such as erythematous mucosa with characteristic yellow plaque (pseudomembrane) can be observed during endoscopy. Lymphocytes are found in the biopsies of the lesions (Kazanowski M et al 2013). In severe ischemia, cyanotic mucosal nodules appear in the company of hemorrhage and hemorrhagic ulcerations. The biopsy features are non-specific and include many findings which can be seen in other conditions, such as inflammatory bowel disease (Mitsudo & Brandt 1992, Price 1990).

It is important to differentiate between ischemic colitis and mesenteric ischemia. Most patients with acute mesenteric ischemia are profoundly ill, suffering a sudden onset of severe abdominal pain without bloody stools. Patients with chronic mesenteric ischemia suffer severe postprandial abdominal pain, leading to a fear of food and weight loss (Elder *et al.* 2009). Differential diagnosis also includes inflammatory bowel disease, pseudomembranous colitis, colonic pseudo-obstruction, diverticulitis and colon carcinoma.

2.5.5 The clinical management of colitis in the critical care setting

For the medical treatment of the *Clostridium difficile* colitis metronidazole, vancomycin and fidaxomicin can be used, but some patients develop fulminant *Clostridium difficile* colitis requiring ICU treatment. Patients with severe infection may develop toxic megacolon, rapidly progressive symptoms with multiple organ failure and sepsis (Adams & Mercer 2007). *Clostridium difficile* infection spreads easily by the fecal-oral route and by direct contact with the patient; in particular through the hands of hospital personnel, clothing and stethoscopes. Therefore a patient with suspected or confirmed *clostridium difficile* infection should be placed in isolation. Patients should be well hydrated and their electrolyte balance should be carefully monitored. When there is evidence of circulatory dysfunction (eg, hypotension refractory to volume resuscitation, or evidence of end-organ hypoperfusion, such as elevated blood lactate), the condition is termed as septic shock (American College of Chest Physicians/Society of Crit.Care Med. 2007). Consideration should be given to early ICU administration for patients with significant co-morbidity, as ICU treatment can then play a prophylactic role before sepsis (Adams & Mercer 2007). Effective antimicrobial administration within the first hour of hypotension associated with septic shock has been demonstrated to increase survival to hospital discharge in adult patients (Kumar *et al.* 2006).

In septic shock and sepsis, cardiac function, hypoperfusion and oxygenation should be optimized. A pulmonary artery catheter may assist in guiding fluid therapy and cardiac function in the hemodynamically unstable septic patient. The use of resuscitation fluids is one component of a complex hemodynamic resuscitation strategy. Fluids should be used with the same caution that is used with any intravenous drug. Crystalloids and albumin are the fluids recommended to be used (Myburgh *et al.* 2013). After adequate volume expansion, catecholamine should be initiated among other things to augment cardiac

contraction and venous return in support of the failing circulation (Persichini *et al.* 2012). The secondary effects of vasoactive drugs on the splanchnic perfusion, however, are minor compared to their systemic effects and it must be kept in mind that, after optimal fluid resuscitation, vasopressor support may still be necessary for the optimization of cardiac function and oxygenation (Woodsley CA *et al.* 2006). Vasopressor therapy is necessary, to maintain perfusion in the face of life-threatening hypotension. In the international Surviving Sepsis Campaign guidelines norepinephrine is recommended as the first-choice vasopressor for septic patients (Dellinger *et al.* 2013b). If any additional agent is necessary to maintain adequate blood pressure, epinephrine is suggested as an addition or substitute for norepinephrine. Vasopressin (up to 0.03U/min) can be added to norepinephrine to raise the mean arterial pressure up to 65 mmHg. In states of hemodynamic shock, a dobutamine infusion should be added to the vasopressor when myocardial dysfunction is present or if there are ongoing signs of hypoperfusion, despite achieving adequate intravascular volume and adequate mean arterial pressure (Dellinger *et al.* 2013a). A nasogastric tube should be placed if ileus is present. Empiric broad-spectrum antibiotics are given to cover aerobic and anaerobic bacteria and to minimize translocation and sepsis. The use of systemic corticosteroids may potentiate ischemic damage and predispose to colonic perforation (Beck *et al.* 1950).

Endoscopy may be a potential diagnostic tool for identifying ischemic changes in the bowel wall, but ischemic colitis may mimic ulcerative colitis or Crohn's disease, in which cases a histopathology study can be helpful (Elder *et al.* 2009). The anatomy of the major vessels and their flow patterns can be estimated with angiography, duplex ultrasound, magnetic resonance angiography, or CT angiography. Various diagnostic techniques are available to assess the perfusion of the mucosa: mucosal laser Doppler flowmetry, endoluminal pulse oximetry, endoscopy, and intravital microscopy, but none of them are useful in clinical practice (Kolkman *et al.* 2000). Actual ischemia irrespective of flow can be measured by tonometry, the probe for which can be positioned in the stomach, jejunum or colon. Unfortunately, tonometry is very sensitive to large measurement errors and carbon dioxide (CO₂) production by the microflora in the colon can cause false-positive readings (Kolkman & Mensink 2003). Surgery is restricted to patients with gangrene or irreversible intestinal ischemic damage or when bowel decompression is needed for treatment. Patients with ACS should undergo urgent decompressive laparotomy (Diaz *et al.* 2010).

2.5.6 Outcome of patients with colitis

The mortality rate for ischemic colitis after aortic reconstruction has been as high as 90% (Brewster *et al.* 1991). Gastrointestinal complications following cardiac surgery can have severe consequences, with mortality rates up to 71–100% (Musleh *et al.* 2003). Fulminant *Clostridium difficile* infections result in mortality rates of 35–80% (Byrn *et al.* 2008, Grundfest-Broniatowski *et al.* 1996). Patients undergoing colectomy for a *clostridium difficile* infection had an overall death rate of 57% in one study (Dallal *et al.* 2002). In a large systematic review and meta-analysis on emergency colectomy for *clostridium difficile* colitis the overall rate of emergency surgery was 1.1% with a range from 0.2–7.6% for individual studies and the 30-day postoperative mortality rate was 41.3% with a range from 19–71% (Bhangu *et al.* 2012).

The mortality in patients with abdominal sepsis has remained as high as 20–60% (Bosscha *et al.* 2000, Christou *et al.* 1993, Lamme *et al.* 2004, van Ruler *et al.* 2007).

In the United Kingdom, the hospital mortality in patients with abdominal sepsis treated in an ICU was 63% (McLauchlan *et al.* 1995). On the other hand, surgical ICU patients with abdominal sepsis, treated in a Swedish University hospital (Lund), had an in-hospital mortality of 28% and a total mortality of 50% (Haraldsen & Andersson 2003). The significance of intra-abdominal hypertension and abdominal compartment syndrome has varied from no association, to an up to 53% mortality rate (Kim *et al.* 2012, Vidal *et al.* 2008). In an elderly patient population with severe sepsis or septic shock with an intra-abdominal focus, the mortality rate was 46% among those aged below 60 years as compared to 61% among those age between 60 and 80 years and 79% in patients older than 80 years (Nasa *et al.* 2012). In a recent, large, prospective pan-European study on abdominal infection in an ICU patient population ICU mortality was 29% and hospital mortality was 38% (Volakli *et al.* 2010a)

3 Aims of the present research

Specific study questions and hypothesis

The treatment of colitis requiring colectomy in an ICU population is a challenge, as there are no general guidelines for optimal therapy. In this study we attempted to describe the clinical and histologic picture of ischemic colitis and its association with multiple organ failure and outcome in this patient population. The following questions were of particular interest:

1. What kind of diagnostic clinical, surgical and histologic findings are associated with colectomy and what is the outcome in such a colectomized ICU patient population (I)?
2. Are there any preoperatively prognostic changes in organ failure score, need for medication or in laboratory values which may be useful as a predictive markers for evaluating the need for laparotomy (II)?
3. What histologic findings of the colon are observed in critically-ill patients who underwent colectomy and is there any association between histologic findings and clinical and laboratory parameters or organ failure scores predicting favourable outcome (III)?
4. To assess the mechanisms of epithelial damage and permeability we investigated the expression of markers of ischemia, innate immunity, intercellular junctions, apoptosis and cell proliferation rate. What are the immunohistologic characteristics of epithelial damage in colitis among critically-ill patients (IV)?

4 Patients and methods

The present study consisted of a retrospective, observational cohort study (Study I and II). The use of tissue specimens obtained after colectomy in studies III and IV was approved by the National Institute for Health and Welfare. The studies were conducted in the medical-surgical Intensive Care Unit at the Oulu University Hospital in collaboration with the Department of Pathology, University of Oulu. The study protocol was approved by the Ethics Committee of the Oulu University Hospital (65/2007) and the Northern Ostrobothnia Hospital District (179/2007).

4.1 Patients and study setting

A total of 77 ICU patients who underwent emergency colectomy as registered in the hospital's operative database during the period 2000–2009 were included in the studies I and II.

The clinical data were collected from the electronic patient data management system and patient charts (Centricity Critical Care Clinisoft, GE Healthcare, Helsinki, Finland). The long-term outcome data were updated from Statistics Finland on April 30, 2011. Statistics Finland is a public authority, which produces official national statistics.

The histologic series is a subpopulation of patients during the years 2000–2006, in whom emergency colectomy was performed for 50 of the critically-ill patients described in studies I and II. In study III the total histologic database included 362 tissue samples from 50 patients and 53 samples from 34 controls.

In the immunohistologic study IV, in total, 78 tissue samples from 39 patients and 25 samples from 25 controls were sectioned, stained and analyzed.

The control group in studies III and IV consisted of 34 colonic resection patients, mean age 65 years, who provided tissue samples of histologically normal colon. The control material consisted of ten samples from 1) ascending, 2) right transverse, 3) left transverse, 4) descending colon and 5) recto-sigma. The indication for colonic resection among the control patients was a colonic tumor. The inclusion criteria were a tumor size of less than 4 cm and no sign of tumor-related obstruction.

4.2 Clinical management

Between 2000 and 2009, colectomy was performed in our mixed ICU on 82 patients. Five of these patients were excluded due to a preoperative diagnosis of a malignant disease; four had acute myeloid leukemia and one had pancreatic cancer.

All patients were treated by the same multidisciplinary team, which included intensivists, a surgical gastroenterologist, and an infectious disease specialist. The decision to perform colectomy had been based on the patient's clinical status, deteriorating organ function, imaging findings (ultrasound, computed tomography), case-by-case sigmoidoscopy findings, and laboratory test results. On admission, all patients were severely ill (APACHE II score >25).

All patients had total colectomy and ileostomy as surgical management. The patients were categorized into one of three clinical groups according to his or her dominant medical problem before colectomy: 1) sepsis, 2) fulminant *Clostridium difficile* colitis, or 3) colonic ischemia following cardiovascular surgery (I). The majority of the sepsis patients had an intra-abdominal focus (26/31) before surgery.

4.3 Clinical data

The following data were collected: age, gender, admission diagnosis, and scores for disease severity, including Acute Physiology and Chronic Health Evaluation II score (APACHE II) on admission and the day of surgery. The degrees of organ dysfunction were evaluated using SOFA scores recorded at admission and for the three days before colectomy. The SOFA score describes and quantifies the dysfunction of six organ systems (lung, liver, kidney, coagulation, cardiovascular, and central nervous system), using grades from 0 (normal) to 4 (the most severe dysfunction) with a maximum score of 24 (Vincent *et al.* 1996, Vincent *et al.* 1998). The presence of MOF during ICU treatment and the need for renal replacement therapy (RRT) were recorded. White cell count (WCC), serum lactate, C-reactive protein (CRP), and the need for vasopressors were evaluated from the three days before operation until operation. The length of ICU stay, calculated as hours divided by 24 hours, and the length of the hospital stay were recorded.

Treatments given in the ICU, such as the need for mechanical ventilation or for renal replacement therapy, were recorded, and the numbers of organ failure-

free days (patient is alive and without a particular organ failure) were calculated (Bernard *et al.* 1997). Intraoperative findings and the severity of abdominal cavity contamination were also reviewed. The classification was based on the surgeons' observations of the colon during the operation and on visual examination of the resected colon. Routine histologic findings were recorded including the presence of histologic signs of ischemia, necrosis, and pseudomembranous colitis.

4.4 Histologic examination

Due to the pioneering nature of the histopathological analysis in this type of case series, a review of a respective subset of histological samples was first performed to get an overview of the histopathological characteristics and about the range of their variation. Next we considered which features would provide clinically relevant information to possibly explain the macroscopic operative findings, intestinal dysfunction, dysfunction of other organ systems and the overall prognosis. Based on this evaluation and consideration, the histological features to be assessed and the criteria for their assessment were determined. In the assessment, the aim was to systematically evaluate severity and the extent of alterations in the different tissue components of the bowel wall. Quantitative measures were used as much as possible to minimize investigators bias. Finally, all samples were re-evaluated prospectively using this systematic and detailed structured proforma.

The routine 362 histologic tissue samples from the 50 patients were re-evaluated and all samples were included (Study III). The sections were stained with haematoxylin and eosin.

The studied samples represented all the sections of the colon: including (number of specimens) ascending colon ($n=75$), right transverse colon ($n=90$), left transverse colon ($n=77$), descending colon ($n=75$) and recto-sigma ($n=60$). Histologic features were assessed separately in the surface epithelium, crypts, lamina propria, submucosa, muscularis propria and serosal layers.

The extent of changes in enterocytes and in the surface epithelium was estimated, including the proportion (%) of surface area displaying any epithelial defect and degeneration or necrosis. The criteria for degeneration included cytoplasmic vacuolization or partial detachment. The proportion (%) of area showing necrosis (depth and width) was estimated in the M cell area, mucosa, submucosa, muscularis propria, and the serosal layers by assessing both the depth

(proportion of the thickness) and the width of the lesion. In each case, the segment of the colon exhibiting the most severe damage was used for the analysis.

The histopathologic evaluation under light microscope at 40× and 100× magnification included measurements of the height of the enterocytes, crypts, villi, mucosa, submucosa and muscularis using a calibrated ocular micrometer. The quantification of the amount of inflammatory cells, including neutrophilic and eosinophilic granulocytes, and mononuclear inflammatory cells was scored separately in the M cell area, mucosa, and submucosal, muscular, auerbach and serosal layers. The scoring was based on visual analogue scale (Laurila *et al.* 2005). In each case, the score was determined according to the dominant pattern as ranging from absent (score 0) through slight (1) and moderate (score 2) to heavy increase (score 3) (see the original manuscript III). The dilatation of lymphatic vessels in the submucosa, and the presence of leukocytes margination, hemorrhage and thrombosis in blood vessels were scored as being absent or present in the mucosa, submucosa, muscularis and serosal layers.

The histologic features were analyzed blindly, without any clinical information using sections stained with hematoxylin and eosin. All assessments were made by two investigators, after a subset of specimens had been analysed together with an experienced pathologist (study III).

4.5 Immunohistochemical examination

The sample selection for the immunohistochemical examination (Study IV) was based on an assessment of the extent of epithelial damage in the evaluation of hematoxylin-eosin-stained sections in study III. Samples with mucosa were included and were taken from each colonic section available for analysis. To gain further insight into the mechanisms of the epithelial damage in colitis associated with critical illness, the intestinal expression of Ki-67 as a measure of proliferation as well as occludin, claudin-1 and claudin-2 as elements of tight junctions were investigated. The proportion of cells expressing the apoptosis marker M30 and the hypoxia marker HIF-1alpha were also investigated. The intestinal expressions of proteins' Toll-like receptors 2, 4, 5 and 9 were investigated as markers of innate immunity.

For each patient, two specimens were selected for the immunohistochemical stainings. One specimen represented the least affected segment and the second specimen represented the most severely affected segment of the colon in each patient. The selection was based on the assessment of the extent of epithelial

damage in the tissue section from the primary histologic examination. In total, 78 tissue samples from 39 patients and 25 samples from the controls were sectioned, stained and analyzed. The samples were taken as follows (least vs. worst): right colon 9 vs. 6, transverse 18 vs. 16, left colon 5 vs. 11 and rectosigmoid 7 vs. 6. For the control samples this information was not available.

The immunohistochemical stainings were conducted in accordance with the manufacturer's recommendations, and for each antibody a set of samples was used for the optimization of the dilution of the primary antibodies (Table 1).

Each immunostaining was analyzed as the percentage of the epithelial cells showing the extent of epithelial damage expression in 10 high-power fields with a 40 time magnification of each sample.

The immunohistologic features were evaluated blindly without any clinical information, not even the patient category. All assessments were made by one investigator and a subset of specimens was also evaluated by a pathologist.

Table 2. The antibodies used in the immunohistochemical stainings (Study IV) and their detection.

Antigen	Type of antibody	Code/Manufacturer	Pretreatment	Dilution	Incubation time	Detection kit/Manufacturer
Ki-67	mouse monoclonal	NCL-Ki67- MMI/Novocastra Newcastle Upon Tyne, UK	TRIS/EDTA	1:25	30 min	Envision/Dako Copenhagen, Denmark
M30	mouse monoclonal	CytoDEATH™ antibody/Roche Enzo Life sciences, Switzerland	TRIS/EDTA	1:1 000	30 min	Envision/Dako
HIF-1alfa	mouse monoclonal	Neo Markers Termon CA	TRIS/EDTA	1:500	60 min	Novolink/ Leiga
Occludin	polyclonal rabbit	Invitrogen Carporation 542 Flynn Camarillo: CA 93012	pronase E	1:800	60 min	Envision/Dako
Claudin-1	polyclonal rabbit	Invitrogen Carporation 542 Flynn Camarillo: CA 93012	TRIS/EDTA	1:100	60 min	Envision/Dako
Claudin-2	mouse monoclonal	Zymed Laboratories INC San Francisco CA 94080	TRIS/EDTA	1:50	60 min	Envision/Dako
TLR2	mouse monoclonal	Abonova 9FNo108 Jhouzin St. Taipei, Taiwan	TRIS/EDTA	1:100	60 min	Envision/Dako
TLR4	mouse monoclonal	Abonova 9FNo108 Jhouzin St. Taipei, Taiwan	TRIS/EDTA	1:1000	60 min	Envision/Dako
TLR5	mouse monoclonal	IMGENEX Bio Site San Diego CA USA	TRIS/EDTA	1:50	60 min	Envision/Dako
TLR9	mouse monoclonal	IMGENEX Bio Site	TRIS/EDTA	1:150	60 min	Envision/Dako

4.6 Statistical methods

The analyses were performed in co-operation with a professional medical biostatistician. All analyses were performed using SPSS for Windows (versions-18.0, 20.0 and 21.0 Chicago, IL, USA) and SAS for Windows (versions 9.2 and 9.3). The data are expressed as percentage and as median with 25th - 75th percentiles, unless otherwise stated. Kappa statistics were calculated between the operative and routine histologic findings. For cross-sectional between-group comparisons, Student's *t*-test or Mann-Whitney U test were used with continuous variables and Fisher's exact test for categorical variables. The Kruskal-Wallis test was used when more than two groups were compared. Spearman's correlation coefficient (ρ) was calculated. Repeatedly measured data were analyzed using the Linear Mixed Model (continuous variables) or the Generalized Linear Mixed Model (categorical variables). The SOFA subscores were dichotomized as organ failure (organ-specific score ≥ 3) vs. no organ failure (organ-specific score 0–2). The following *p*-values were reported for repeatedly measured data: *p*-time, the overall change over time; *p*-group, the average between-group difference; and *p*-time \times group, the interaction between time and group. Two-tailed *p*-values are reported. A *p*-value less than 0.05 were considered to be statistically significant.

5 Results

5.1 Patients

The final case series consisted of 77 patients, including 31 patients in the sepsis group, 21 in the cardiovascular surgery group (12 after cardiac surgery and 9 after vascular surgery), and 25 in the fulminant *Clostridium difficile* colitis group. The classification of operative findings was based on the surgeons' observations of the colon during operation and on visual examination of the resected colon.

There were no statistically significant differences in age, gender, BMI, admission APACHE II, admission SOFA or previous conditions before admission to hospital between survivors and non-survivors or between the three groups of patients. The most common admission diagnoses in the sepsis group were sepsis (48.4%), pancreatitis (25.8%), and pneumonia (12.9%). Patients belonging to the cardiovascular surgery group had undergone previous cardiac or aortic surgeries. Four of nine heart surgery patients and eight of twelve vascular surgery patients underwent emergency surgery before ICU admission. In the fulminant clostridium colitis group, pseudomembranous clostridium colitis was diagnosed on admission in 56% of patients and during ICU treatment in the remaining 44%.

A preoperative CT scan was performed for 43 patients (56%) and in 65% of the cases it showed some pathological finding: bowel dilatation in 7 cases (16%), colitis in 8 cases (19%), perforation in three cases (7%), pancreatitis in 7 cases (16%) and aortic aneurysm in two cases (5%).

Colectomy was performed within 3 days of ICU admission in 19/31 (61.3%) patients with sepsis, 13/25 (52%) patients with *clostridium difficile* and 16/21 (76.2%) cardiovascular patients. At laparotomy, all patients had an edematous and dilated colon, and usually accompanied with some degree of ascites. Seven patients underwent a second-look laparotomy due to persistent intra-abdominal sepsis during their ICU stay.

5.2 Incidence of colectomy in the ICU

Colectomy was performed in our unit in 2 to 11 cases per average of 2294 annual admissions (0.08–0.4%) during the 2000–2009 study period (Table 2).

Table 3. Incidence and 28-day survival of colectomized ICU patients during 2000–2009 in the Oulu University Hospital.

Year	Main underlying condition			28-day survival		Total
	Sepsis	Cardiovascular	<i>Cl.difficile</i> colitis	Alive n (%)	Dead n (%)	
2000	2	4	5	7 (64)	4 (36)	11
2001	7	2	2	7 (64)	4 (36)	11
2002	1	3	2	4 (67)	2 (33)	6
2003	4	2	1	2 (29)	5 (71)	7
2004	6	2	1	2 (22)	7 (78)	9
2005	5	3	3	2 (18)	9 (82)	11
2006	4	2	5	6 (55)	5 (45)	11
2007	1	1	0	1 (50)	1 (50)	2
2008	1	2	1	2 (50)	2 (50)	4
2009	0	0	5	3 (60)	2 (40)	5
total	31	21	25	36 (47)	41 (53)	77

5.3 Surgical findings in colectomy

The operative findings had a wide overlap between the groups (Study I). Necrotic or ischemic colon was the most common surgical finding with no association with survival. Necrotic lesions were frequent in the cardiovascular and sepsis groups, whereas distension and thickening of the colonic wall were typical in the patients with fulminant clostridium difficile colitis. The correlation between ischemic lesions observed during operation and the demonstration of necrosis in the routine histologic examination was relatively good; a kappa value of 0.641 (95% CI 0.49–0.79) was determined.

5.4 Occurrence of organ system dysfunctions and outcome

When the six different organ failures were separately evaluated, increasing numbers of respiratory, cardiovascular, renal, and coagulation organ failures were observed preoperatively of time in the entire study population, with no difference observed between the survivors and non-survivors (Fig 6). Only the increasing neurological SOFA subscore as a consequence of decreasing GCS score was associated with mortality during time between survivors and nonsurvivors ($p=0.029$) (Study II). In our series 46/77 patients (60%) had MOF. The major cause of death was MOF in 30 cases (73% II), but none of the surviving MOF

patients died during the one-year follow-up period. Two cases (5%) died of irreversible shock without MOF. In 11 patients, treatment withdrawal preceded death. MOF was more common in sepsis 21/31 (68%) and in cardiovascular cases 15/21 (71%) than in cases with *clostridium difficile* 10/25 (40%). Necrosis in the routine histologic examination was as common in patients with (72%, $n=46$) and without MOF (61%, $n=31$) ($p=0.46$) (Study II).

The patients in the cardiovascular surgery group required renal replacement therapy more often than the patients in the other groups ($p=0.01$) (Study I). A preoperative increase in the serum lactate level and the need for higher doses of noradrenalin was associated with mortality. Non-survivors had higher admission and maximum SOFA scores, although the differences were not statistically significant between survivors and nonsurvivors (Fig 7) (Study II).

Twenty-nine of the 77 patients (38%) died during their ICU stay. The hospital mortality rate was 47% (36/77) and 28-day mortality rate was 53% (41/77). One-year mortality was 62% (48/77). There was no difference in ICU, hospital or one-year mortality between the three patient groups. Only three of the 32 hospital survivors had died after hospital discharge at the one-year follow-up (I).

The median hospital stay was significantly longer for patients with sepsis than for the other groups; cardiovascular and fulminant *clostridium difficile* colitis (18 vs.14 vs.13) (I). Hospital mortality in case of widespread histological colonic necrosis was 64% (27/42) and 51% (18/35) without necrosis ($p=0.35$).

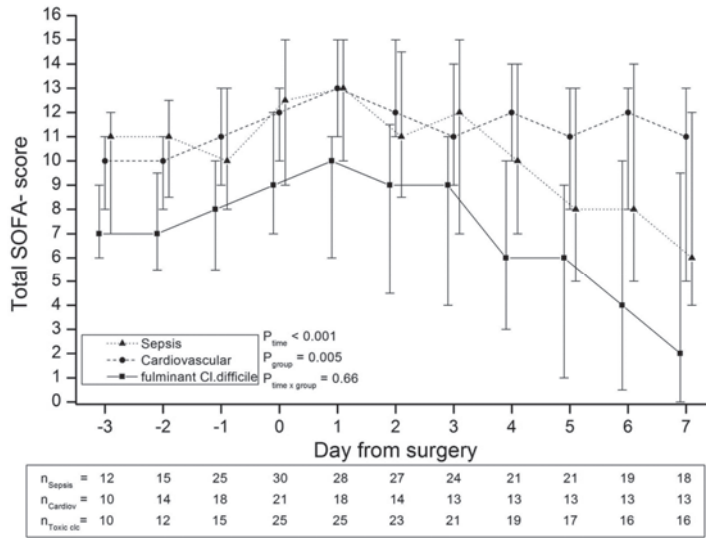


Fig. 6. Total SOFA score in colectomized ICU patients between groups. P-values are reported as follows: P_{time} indicates change over time, P_{group} indicates change between groups, $P_{\text{time} \times \text{group}}$ indicates interaction between the groups and time.

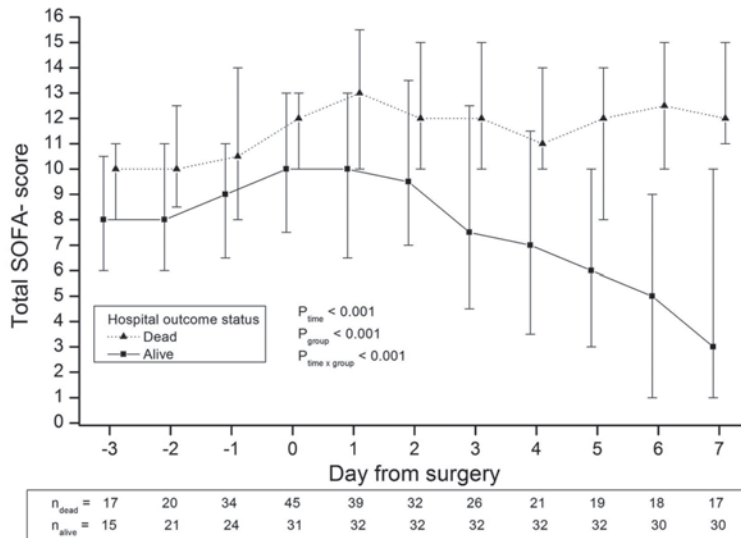


Fig. 7. Total SOFA score in colectomized ICU patients between survivors and nonsurvivors. P-values are reported as follows: P_{time} indicates change over time, P_{group} indicates change between groups, $P_{\text{time} \times \text{group}}$ indicates interaction between the groups and time.

5.5 Histologic findings

The basic epithelial findings by colon segments are described in table 3.

In the histologic specimens, necrosis was common in sepsis patients (81%) and in the group of cardiovascular surgery patients (95%). In the *Clostridium difficile* colitis patients ten (40%) of them had necrosis. Also, two patients in the cardiovascular surgery group as well as five patients in the sepsis group had pseudomembranous colitis on histologic examination, although no clostridium toxins had been detected. There was good agreement between the ischemic lesions observed during surgery and the demonstration of necrosis in the histologic examination, as determined by a kappa value of 0.641 (95% CI 0.49–0.79). The histopathologic findings of the colon in the three clinical entities (colectomy due to severe sepsis, ischemic or clostridium colitis) were quite similar (Table 3). In the detailed histologic analysis there were two significant differences between survivors and non-survivors: the enterocyte defects were wider in the non-survivors ($p=0.006$) and the finding of complete crypt damage at least one of the segments available for analysis was more common among the non-survivors (61%) than the survivors (27%, $p=0.024$). No significant differences were found between the survivors and the non-survivors, in regard to the amount of neutrophils in any layer of the bowel wall, or in regard to the extent and depth of necrosis in the submucosal layer. A negative correlation was found between the blood leucocyte count and the width of submucosal necrosis ($\rho=-0.31$; $p=0.027$) and between the blood leucocyte count and the depth of mucosal necrosis ($\rho=0.321$; $p=0.023$). Also, blood lactate level before surgery correlated negatively with the mucosal height ($\rho=-0.32$; $p=0.027$).

Table 4. The histological findings by colon segments (median, 25th - 75th percentiles).

Variable	Ascending colon	Right transverse colon	Left transverse colon	Descending colon	Recto-sigma	p-value
Enterocyte						
minimum μm	10.0 (7.0–3.0)	10.0 (8.0–13.0)	12.0 (10.0–15.0)	11.0 (9.0–14.5)	11.0 (9.5–14.5)	0.14
maximum μm	12.5 (9.0–15.0)	13.0 (10.0–15.0)	13.0 (10.0–15.0)	12.0 (10.0–15.0)	12.0 (10–15.0)	0.24
Crypt						
minimum μm	32.0 (24.0–57.5)	40.0 (26.0–57.5)	40.0 (24.0–60.0)	32.0 (24.0–56.0)	34.4 (24.0–55.0)	0.48
maximum μm	36.0 (28.0–73.5)	40.0 (29.0–72.5)	46.0 (28.0–60.0)	40.0 (28.0–64.0)	34.4 (24.0–56.0)	0.45
Mucosa						
minimum μm	75.0 (56.0–104.0)	80.0 (60.0–96.0)	88.0 (64.0–120.0)	88.0 (64.0–110.0)	88.0 (62.5–111.0)	0.059
maximum μm	95.0 (70.0–120.0)	96.0 (80.0–133.0)	109.0 (80.0–140.0)	100.0 (84.0–128.0)	94.0 (70.0–130.0)	0.16
Necrosis						
minimum μm	20.0 (0.0–80.0)	20.0 (5.0–72.5)	30.0 (10.0–90.0)	20.0 (5.0–70.0)	20.0 (7.5–55.0)	0.37
maximum μm	30.0 (10.0–90.0)	50.0 (20.0–85.5)	50.0 (20.0–95.0)	30.0 (10.0–100.0)	30.0 (10.0–70.0)	0.81

5.6 Immunohistochemistry

The expression of tight junction protein claudin 1 was decreased in the epithelium in least affected (LA) and worst affected (WA) areas compared to controls (10 and 0 vs. 40; $p=0.002$ for LA, $p<0.001$ for WA) and similarly in crypts (50 and 40 vs. 80; $p<0.001$ for both). The expression of leaky protein claudin 2 was increased in LA areas compared to controls (10 vs. 0, $p=0.03$). Toll-like receptor 9 was most intensive both in the surface of epithelium of LA areas 10.0(0.0–30.0) $p=0.03$ and WA area of crypt ($p=0.02$) compared to controls. The depth of expression of the proliferation factor Ki-67 was more intensive in the crypts compared to controls ($p=0.03$). The proportion of cells showing expression of apoptosis marker M30 was larger in the worst damage area than in controls ($p<0.001$).

6 Discussion

6.1 Incidence and overall outcome of colectomy in ICU

The annual incidence of colectomy among our ICU patients was very low 0.08–0.4%. Similarly, a low incidence of colectomy has been reported following aortic reconstruction (Menegaux *et al.* 2006), cardiac surgery (Sakorafas & Tsiotos 1999), and fulminant *clostridium difficile* infection (Byrn *et al.* 2008, Grundfest-Broniatowski *et al.* 1996). In the fulminant *clostridium colitis* group surgical intervention was unavoidable due to progressive multiorgan dysfunction and the mortality rate was high, highlighting the need for clearly defined guidelines in the treatment of fulminant *clostridium difficile* colitis (Wang *et al.* 2005).

Our study showed that colectomy in ICU patients is associated with a high hospital mortality. It also showed that patients surviving after discharge from the hospital have good one-year outcomes. The outcome results in this study are in agreement with earlier studies and there were no difference in ICU, hospital or one-year mortality between the three patient groups studied (Sakorafas & Tsiotos 1999, Volkert *et al.* 2008). In the ICU population, the treatment of colitis requiring colectomy is a challenge and there are no widely accepted guidelines regarding the surgical management of critically-ill patients with suspected severe intestinal complications. Earlier recognition of ischemic colitis and optimal therapy before the occurrence of irreversible multi-organ failure may, however, be lifesaving.

6.2 Evaluation of organ dysfunction and outcome

An increasing degree of preoperative organ dysfunction (SOFA score) objectively described the severity of multiorgan dysfunction (II). This is in agreement with findings previously presented in the literature (Vincent *et al.* 1996, Vincent *et al.* 1998). Although organ failures increased preoperatively, there were no statistically significant differences between the three study groups' populations or between survivors and non-survivors. Similar findings have been demonstrated in intra-abdominal sepsis and in acute acalculous cholecystitis (Laurila *et al.* 2006, van Ruler *et al.* 2011). It has earlier been demonstrated that the neurologic SOFA score was associated with outcome in colectomized patients with fulminant *Clostridium difficile* colitis (Byrn *et al.* 2008), we saw the same phenomenon in

all three patients groups. These findings suggest that neurologic SOFA has a better prognostic value than other determinants of MOF.

Non-surviving patients had statistically significantly higher serum lactate levels and daily norepinephrine doses (NE) on the day of surgery, and both increasing lactate levels and a greater need for NE were associated with mortality (II). These findings support the idea presented in previous studies that increasing preoperative lactate levels and higher need for NE treatment may be associated with mortality among *Clostridium difficile* colitis and in acute ischemic colitis (Lamontagne *et al.* 2007, Reissfelder *et al.* 2011). Previous studies have demonstrated that a high CRP level and a WBC count of over 20 are independent risk factors for a complicated course of the disease and are predictors for 30-day mortality in a *clostridium difficile* patient population (Bhangu S., Bhangu A., Nightingale P., Michael A. 2010, Lamontagne *et al.* 2007, Pepin *et al.* 2009). In this study, the preoperative WBC count increased significantly but did not differ statistically between survivors and nonsurvivors. This might be explained by an association with a phenomenon specific especially to the *clostridium difficile* disease. This hypothesis requires further evaluation to confirm these findings.

6.3 Histopathologic characterisation

Study III is the first detailed analysis of the entire colon wall histopathology in cases of colitis due to various illnesses in colectomized ICU patients. Earlier studies on histologic changes during colitis have largely focused on specific etiological types of colon wall damage and are often limited to using biopsy specimens (Brandt *et al.* 2010, Menegaux *et al.* 2006, Pepin *et al.* 2009, Pepin *et al.* 2009, Price 1990). This detailed histologic analysis of the colon wall showed that the breadth of enterocyte defect and severe crypt damage are the most significant factors associated with mortality. A similar effect of intestinal epithelial damage on patient mortality has been shown in the early phase of abdominal sepsis and acute pancreatitis (Besselink *et al.* 2009, Derikx *et al.* 2007). According to animal models, such a disruption in the intestinal mucosal barrier may aggravate bacterial translocation (Balzan *et al.* 2007, Besselink *et al.* 2009). The depth and width of submucosal necrosis was not associated with mortality in this study.

According to the histopathologic findings in the epithelium and crypt, the more severe the damage and permeability disorder are, the more severe is the disease, whereas simple submucosal necrosis is a more localized phenomenon.

Translocation may also be involved in progressive multiorgan dysfunction. When the histopathologic findings were compared to clinical features and outcome, this study determined that mucosal epithelial damage is associated with the clinical severity of the illness and mortality. To date, similar findings have not been reported in the literature.

In this study, an increase in eosinophils was infrequent. In equine studies, it has been reported earlier, that ischemia of the intestine produces eosinophilic infiltration of the superficial mucosal epithelium with increased eosinophils and nitrotyrosine production (Grosche *et al.* 2011). One explanation for the disparity in these findings could be the nature of pancolic disease in our series, where the inflammation has proceeded to a life-threatening stage and eosinophilic activation has passed its maximal activation. Another explanation could be the association between the eosinophilic reaction and the visual numbers of mucosal eosinophil, where the eosinophilic activation is obvious without a numerical increase. Eosinophilic activation was not qualified. Steroid therapy is, on the other hand, a common treatment in the septic patient population and steroids are the mainstay of treatment of eosinophilic gastroenteritis, with 90% responding to this therapy (Ingle & Hingle Ingle 2013). This treatment does, however, diminish the eosinophilic reaction.

6.4 Immunohistochemical characteristics

6.4.1 The innate immunity

In our series there was an up-regulation of TLR 5 and TLR 9 (IV), indicating an up-regulation of the innate immunity activation cascade. The expression of TLR 2 and TLR 4 was similar, however, when compared to the controls. In inflammatory bowel disease up-regulated Toll-like receptor family expression (TLR-2, 4, 5, 8, 9) has been associated with the severity of intestinal inflammation, disease activity and cytokine release observed in colonic biopsies (Frolova *et al.* 2008, Sanchez-Munoz *et al.* 2011, Szebeni *et al.* 2007). Previous studies have shown an inflammation-dependent induction of TLR 2 and TLR 4 expression in colonic macrophages and epithelial TLR 4 cell expression was usually stronger than TLR 2 expression (Frolova *et al.* 2008). In cases with strong positivity, crypts were equally or more involved (Frolova *et al.* 2008, Hausmann *et al.* 2002). According to results published in previous studies, there was only a mild expression of TLR

2, but a heavy expression of TLR 4. A previous study reported that TLR 5 induction potentiated cytokine production in human T cells. This response required at least 24 hours of TLR 5 induction and lasted for approximately 24–36 hours after removal of a TLR 5 ligand. The time-frame when TLR 5 functions are active is significantly longer than has previously been appreciated (Tremblay *et al.* 2013). It has been reported that TLR9 signaling influences and regulates the severity of the mucosal inflammation and TLR 9 agonists have been demonstrated to be effective therapeutic agents (Furi *et al.* 2013). In our study, TLR 9 immunoreactivity was higher in the worst damaged area compared to least-damaged area, probably reflecting the host response to inflammation or translocation of microbes. Taken together, our results suggest that ischemic damage has progressed so long that the expression of TLR 2 and 4 have already faded. Moreover, up-regulated TLR 9 expression may reflect the severity of the colon damage as has been earlier shown in association in chronic inflammatory bowel disease (Furi *et al.* 2013). The observed differences in TLR expression requires further studies to confirm the relevance of these results.

6.4.2 Tight junctions

Claudin-1 was significantly down-regulated in both the epithelium and crypt compared to the controls (IV). Similarly, down-regulation of tight junction proteins has been demonstrated in ulcerative colitis (Gassler *et al.* 2001). The leaky protein claudin-2 was up regulated in the least affected areas compared to controls. A similar finding has been reported earlier in inflammatory bowel disease (IBD) (Heller *et al.* 2005, Prasad *et al.* 2005). As the functional consequence of both claudin-2 up-regulation and claudin-1 down-regulation is an increase in epithelial permeability, our findings support the concept of a severe aberration in epithelial permeability in ICU colitis. As claudin-2 up-regulation was present in even the least affected area, where the basic histology was normal or close to normal, the permeability aberration appears to affect the entire colon and not only those segments in which the morphology had been severely affected. Our results suggested that in critical illness, the damage of the colon is a pancolonial phenomenon which urgently underscores the need for better approaches for assessing the severity of colon damage than is thus far available.

6.4.3 Hypoxia

HIF-1 α expression was significantly up-regulated in the crypts of the most-damaged area compared to control tissue; there was also an increased expression in the least-affected area compared to the control tissue (IV). HIF-1 α has been shown to be overexpressed in colonic biopsies of patients with ischemic colitis in the epithelial and interstitial cells of the ischemic regions as compared to normal regions of the colon or normal controls (Okuda *ym* World J Gastroenterol 2005). Another study found that there is an intense but focal expression of HIF-1 α in inflammatory bowel disease (Fink 2003). These findings may indicate that both hypoxia and active inflammation could also explain increased HIF-1 α expression. Due the marginal differences in these findings, however their importance remains an open question and need further studies are required.

6.4.4 Proliferation and apoptosis

Expression of the cell-proliferation marker Ki-67 was more extensive in the crypts of the worst-affected areas compared to controls. The proportion of cells expressing the apoptosis marker M30 in the surface epithelium was higher in studied samples than in controls (IV). An increase in apoptotic cells has been reported in the colonic epithelium in patients with active ulcerative colitis (Iwamoto *et al.* 1996, Souza *et al.* 2005, Yukawa *et al.* 2002). In an earlier study in patients with acute cholecystitis the proliferation to apoptosis ratio has been shown to be significantly greater in the group with intense HIF-1 α expression than in the group with weak HIF-1 α expression (Vakkala *et al.* 2007). An increased rate of epithelial apoptosis and abnormal proliferation may aggravate intestinal barrier dysfunction and mucosal ischemia. Increased cellular turnover, as seen in our results, may also be related to ischemia–reperfusion damage, as described in an earlier animal model (Shima *et al.* 2006). Our results suggested that even in these critically-ill conditions both active apoptosis and proliferation are maintained, as a host response to overcome this critical situation.

6.5 Role and mechanisms of gut wall damage in the pathogenesis of multiorgan failure (MOF)

In cases of severe sepsis mucosal damage in the gut is considered to be an important mechanism underlying multiple organ failure (Carrico *et al.* 1986,

Deitch *et al.* 1992, Marshall *et al.* 1993, Wilmore *et al.* 1988). In this study, 84% of sepsis patients had an intra-abdominal focus and 71% of sepsis patients had necrosis as their main histologic diagnosis. These findings support the idea that gastrointestinal mucosal injury is involved in multiple organ failure and sepsis. Furthermore, in histologic microscopic examination, necrosis was observed in all three groups and necrosis did not have a significant impact on hospital mortality or on the presence of MOF. Future studies are necessary, however, to clarify intestinal ischemic and inflammatory mechanisms in the gut which can lead to MOF and sepsis.

6.6 Strengths and limitations of the study

This was a retrospective, single center study with a relatively small ICU patient ($n=77$) population, thus limiting the generalizability of the results. The same multidisciplinary team treated all studied patients, however, using a similar protocol during the entire study period. Patients were selected retrospectively according to their medical history and the hospital operative database. A prospective, multicenter study would have been a more exact method for the purposes of reliability, but the collection of this data was deemed to be unfeasible within a reasonable time period in Finland. In general, there is a paucity of data regarding the surgical management of colectomized ICU patients and most published studies published have been comprised of small numbers of patients (Darras *et al.* 2011, Longo *et al.* 2004, Perera *et al.* 2010).

The histopathologic investigation was blinded by using normal colons as controls. All tissue samples were examined by at least two investigators, and a subset of samples with a third investigator, an experienced gastrointestinal pathologist. This method is widely accepted as a reliable method of assessing tissue examination. The immunohistologic assessments were made by one investigator blinded for clinical data and guided by an experienced pathologist.

The categorization of patients into three groups (sepsis, cardiovascular, clostridium colitis) was retrospectively based on information in the electronic patient clinical data management system and in the hospital's operative database. A patient's retrospective categorization might be questionable, but interestingly, the histopathologic findings for the three clinical entities were similar. The categorization of the cardiovascular group and clostridium difficile colitis groups were clear as the dominant medical problem was evident. In contrast, the sepsis group was more heterogenous, with variability in the septic focus. From that point

of view, the categorization of groups was logical for identifying the histologic features of colitis in different clinical entities. The study population was quite small and the groups were non-equivalent; if the patient population had been larger, the reliability of the results would have been emphasized. The classification of operative findings was based on the surgeons' observations of the colon during operation and on visual examination of the resected colon, therefore the interpretation was not systematic and a wide overlap was reported between the groups. A structured examination of the abdominal cavity during the operation would have been more informative.

In the original studies I and II, analyses were made of all cases treated in the Department of Surgery at the Oulu University Hospital during the years 2000–2009. The histologic and immunohistologic studies (III, IV) examined all patients whose tissue specimens were available during 2000–2006. The histologic database included 362 tissue samples, which can be considered an acceptable number to provide sufficient information on the phenomena studied

In studies III and IV, tissue samples represented routine pathologic sampling performed for diagnostic purposes. Accordingly, all colon segments were not sampled in all cases, and it is possible that pathologists have taken fewer samples from the colon segments with a normal macroscopic appearance. This may be one reason for the absence of expected correlations between some clinical and histopathologic parameters, such as between hospital mortality and the extent of colon wall necrosis. The segment location for each tissue specimen was also based on investigator's best estimation, as this was not always recorded by the pathologist. A prospective, more systematic, macroscopic, histologic and immunohistologic examination would permit more detailed and reliable results in the histologic analyses. No statistically significant differences were observed in mucosal neutrophil levels between the study groups (III), perhaps owing to the small sample size.

The sample size was also small in study IV, which can be observed in the wide percentile range and borderline p-values. This is, however, the first detailed immunohistologic study of ICU colitis. Due to the small sample size, the comparison of three different patient groups loses statistical power. Earlier studies on ischemic colitis were performed by biopsy specimens in less severely ill non-ICU patients (Brant *et al.* 2010, Mosli *et al.* 2013) and control colon samples were retrieved from cancer patients, not healthy patients. In our series, resected colons were obtained from the macroscopically healthy margin of the tumor. Inflammation in the margin cannot be excluded.

The patients were severely ill on admission to the ICU, having a mean (SD) APACHE II over 25 and admission SOFA over 9.0. Although the SOFA score was not developed to predict patient outcome, the ability of the daily maximum SOFA score and the total maximum SOFA score to distinguish between survivors and nonsurvivors was relatively good. While the total SOFA score changed significantly during the preoperative period, this change was not statistically significant between non-survivors and survivors. The absence of a hepatic SOFA score may have caused an underestimation of liver failure and this might have had a considerable impact on the total SOFA score. Daily bilirubin values are not performed in clinical practice, however, if liver dysfunction is not suspected.

Increases in serum lactate levels and in the need for norepinephrine were observed in this study. Unfortunately, the perfusion and flow volume of splanchnic vessels or perfusion of the entire gut wall cannot be reliably measured. We still lack any practical method to monitor splanchnic perfusion or oxygenation in a clinical setting. For example, gastrointestinal tonometry is available as a diagnostic technique capable of detecting early mesenteric ischemia, but it is very sensitive to large measurement errors and - especially in the colon- CO₂ production by the microflora can cause false-positive readings (Kolkman *et al.* 2000). Sublingual tonometry may avoid these persistent drawbacks and the correlation between sublingual and gastric tonometry with lactate acidosis, indicating severe shock, has been confirmed in ICU patients (Kolkman & Mensink 2003, Marik 2001). Future prospective studies are needed to evaluate whether these parameters are indeed surrogate markers for gut hypoperfusion.

7 Clinical implications and future studies

Colitis in the critically-ill ICU patient population is a pancolic disease associated with multi-organ failure. Markers indicating the need for earlier laparotomy may therefore be life-saving. This invites the implementation of full intensive care and aggressive supportive therapy before the vicious circle of MOF develops. The treatment of abdominal sepsis includes source control as soon as possible after the diagnosis of an intra-abdominal infection has been made. Laparotomy remains the cornerstone of care, but the decision to perform laparotomy is often challenging to make in the face of an unstable patient in symptoms which are often subtle. According to the results of this study, increasing lactate levels and an increasing need for higher NE doses in ICU patients with colitis could be a marker for the need for early laparotomy. Further studies are needed to determine guidelines for optimizing management strategy in the abdominal sepsis patient population.

The mechanism and the histopathologic findings of mucosal damage were similar regardless of the etiology of colitis. Although the entire colon wall was affected, the presence of epithelial defects and complete crypt damage was shown to be associated with a high mortality rate. These findings highlight the importance of mucosal integrity in the critically-ill patient and an association between the colonic epithelial mucosal damage and the severity of illness and outcome. Gastrointestinal hypoperfusion and mesenteric vasoconstriction may occur even before systemic hemodynamic instability is apparent. Therefore, the principle aim in the initial management of this situation is to provide cardiorespiratory stabilization, with optimal hemodynamic control. Intensive hemodynamic monitoring is essential with a view to achieve adequate tissue perfusion and oxygenation, which also improves mucosal perfusion. Early source control with empiric broad-spectrum antibiotics minimizes the possibility of translocation and sepsis. Intra-abdominal pressure monitoring is needed to recognise intra-abdominal hypertension and abdominal compartment syndrome and in which case a decompressive laparotomy may be life-saving.

Further studies on how to help preserve intestinal integrity are clearly needed: First, the increased intestinal permeability measured by using oral ingestion of small to large-sized probe molecules and the measurement of their urinary excretion as an index of gut barrier dysfunction has been reported in the literature. Further studies are needed to evaluate the turning point at which a normal phenomenon is exaggerated into an acute phase response leading to gut dysfunction and multiorgan failure. Second, the mechanism and the consequences

of epithelial damage in colitis related to critical illness should be investigated using intestinal failure biomarkers such as Intestinal Fatty Acid Binding proteins, which have been used as indicator of intestinal ischemia, and to correlate these with endotoxemia. Third, future prospective studies should evaluate the clinical parameters, as an example, the role of splanchnic perfusion as measured via gastric tonometry together with intestinal failure biomarkers as indicators for the need for earlier laparotomy. Fourth, the relationship of microbial translocation and mucosal permeability in the pathogenesis between critical illness is poorly documented and this phenomenon should be clarified.

8 Conclusions

1. Critically-ill patients ending up in colectomy are severely ill, with an APACHE II score over 25 and admission SOFA score over 9.0. They suffered MOF in 60% of cases. Necrosis is present both macroscopically and microscopically, except in cases with clostridium colitis. Colectomy in ICU patients is associated with a high ICU (38%), hospital (47%) and one-year mortality, but most patients surviving to discharge from hospital are alive one year later (94%).
2. In critically-ill patients ending up in colectomy, none of the preoperative characteristics displayed any association with the extent or depth of necrosis or other signs of colonic damage. Preoperative serum lactate level, increasing need of norepinephrine and increasing neurologic SOFA subscore were, however, associated with mortality. These signs may be useful when evaluating the need for laparotomy.
3. The magnitude of mucosal damage in the colonic epithelium was associated with clinical severity and outcome in colectomized, critically-ill patients. Broader enterocyte defects were found in the non-survivors' colons ($p=0.006$), the presence of complete crypt damage was more common among the non-survivors (61%) than the survivors (27%, $p=0.024$). Tissue damage involving all the layers of the colon and histological findings of the colon in the three patient groups were mainly similar.
4. The immunohistologic characteristics of the epithelial damage in colitis among critically-ill patients were an increase in the expression of tight junction protein claudin-1, which was significantly increased in the epithelium ($p=0.05$) and crypt ($p=0.02$), between the patients compared to controls. The expression of leaky protein claudin-2 was also increased compared to controls ($p=0.03$). Increased expression of HIF-1 α and TLR 9 suggests that both ischemia and activation of innate immunity are involved in the pathogenesis of colitis in critically-ill patients.

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Original publications

- I Sipola S, Syrjälä H, Koivukangas V, Laurila JJ, Ohtonen P, Saarnio J & Ala-Kokko TI (2013) Colectomy in intensive care patients: operative findings and outcome. *World J Surg* 37: 333–338.
- II Sipola S, Syrjälä H, Koivukangas V, Laurila JJ, Ohtonen P, Saarnio J & Ala-Kokko TI (2013) Impact of preoperative organ failures on survival in intensive care unit patients with colectomy. *World J Surg* 37: 1647–1651.
- III Sipola S, Ala-Kokko TI, Laurila JJ, Vakkala M, Ohtonen P, Saarnio J, Karttunen TJ & Syrjälä H (2013) Histological damage of colonic epithelium is associated with clinical severity and outcome in colectomized critically ill patients. *World J Surg*. DOI: 10.1007/s00268-013-2388-8.
- IV Sipola S, Ala-Kokko TI, Laurila JJ, Saarnio J, Ohtonen P, Syrjälä H, Karttunen T (2014) Expression of Toll-like receptors, hypoxia inducible factor, tight junction proteins, apoptosis and regeneration in severe colitis among critically ill colectomized patients. Manuscript.

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