INTRODUCTION

Liver cirrhosis is defined histologically as a diffuse process with liver cell necrosis/apoptosis, fibrosis and regenerative nodules.[1] There are several causes of liver cirrhosis, the most common being high alcohol consumption, hepatitis C, hepatitis B, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, and non-alcoholic steatohepatitis.[1]

Cirrhosis, apart from other features peculiar to the cause, results in two major events: hepatocellular failure and portal hypertension. Important complications of liver cirrhosis include, but are not limited to, esophageal varices, ascites, hepatic encephalopathy, hepatic failure with jaundice, hepatocellular cancer, and cholangiocarcinoma.[1]

In recent years it has become widely recognized that liver cirrhosis may affect several organ systems such as the cardiovascular system,[2,3] the respiratory system,[4] the kidneys,[5,6] and the skeletal system.[7,8]

Cirrhosis has also been associated with varying degrees of malnutrition[9] as well as with alterations in the gastrointestinal (GI) tract.[10] Apart from liver transplantation, no specific cure exists for liver cirrhosis to date. The effects of liver cirrhosis on the GI tract have been considered to be mainly associated with portal hypertension.

A major endoscopic finding is varices most commonly located in the esophagus and/or the fundus of the stomach. Mucosal changes are also frequently encountered upon endoscopic examination of the GI tract in patients with liver cirrhosis.[10,11]

Portal hypertensive intestinal vasculopathy is a term used to describe the fundamental structural change in the intestine, a vasculopathy due to changes in the intestinal microcirculation secondary to longstanding portal hypertension.[10]

Signs of portal hypertensive intestinal vasculopathy may be observed in all parts of the GI tract.[10] The prevalence of portal hypertensive gastropathy, with its characteristic mosaic appearance, has been reported in 11 - 94% of cirrhotic patients.[10] The stomach has also been found to be significantly thickened on ultrasound examination in patients with cirrhosis and portal hypertension.[12]

Ascites is a very common manifestation of decompensated cirrhosis and represents a pathologic accumulation of fluid within the peritoneal cavity.[13,14] The term “ascites” is derived from the Greek term “askos” in reference to its similar appearance to a wine bag or sac.

Cirrhotic ascitic fluid accumulation results from a number of factors broadly defined in terms of hormonal and cytokine dysregulation and related volume overload in the setting of portal hypertension.[13]

The manifestation of ascites is an important landmark in the progression of cirrhosis; (1) It is the most common cause for hospital admissions and thus contingent costs, (2) It portends increased 1-year mortality, and (3) Functions as a risk stratification marker for orthotopic liver transplantation (OLT).[13,15,16]

The genesis and perpetuation of ascites is a common complication, occurring in more than 50% of patients within 10 years of the diagnosis of cirrhosis.[17]

The normal hepatosplanchnic lymph formation is about 1 ml/min In patients with cirrhosis, this rate can increase up to 10 ml/min.[18,19]When the production of lymphatic fluid exceeds the lymphatic transport capacity, ascites develops. According to the amount of ascites, this can be divided into grades 0-3.[20]

Grade 3 represents the gross and tense ascites with vast discomfort to the patient (Table I). However, the presence of even lower grades of ascites is not just a cosmetic problem since it is associated with poor survival with a 50% mortality rate within 3 years.[21,22]

Table(1): Grades of ascites according to the International Club of Ascites.[20]

Grade of ascites	Explanation
Grade 0	No detectable ascites
Grade 1	Mild ascites that is only detectable by ultrasound examination
Grade 2	Moderate ascites with moderate distension of the abdomen
Grade 3	Large or tense ascites with marked distension of the abdomen

Pathogenesis of the ascites syndrome:

Major factors involved in the complex pathogenesis of ascites are portal and sinusoidal hypertension, arterial vasodilatation, and neurohumoral activation, all leading to sodium and water retention.[24,25]

According to the peripheral arterial vasodilatation theory, development of systemic and splanchnic vasodilatation results in a decrease in the effective arterial blood volume and a hyperdynamic circulation.[26]

This theory has lately been modified into what has been termed ‘‘the forward theory of ascites formation’’ (Figure 1) which combines arterial underfilling with a forward increase in splanchnic capillary pressure and filtration with increased lymph formation.[19]

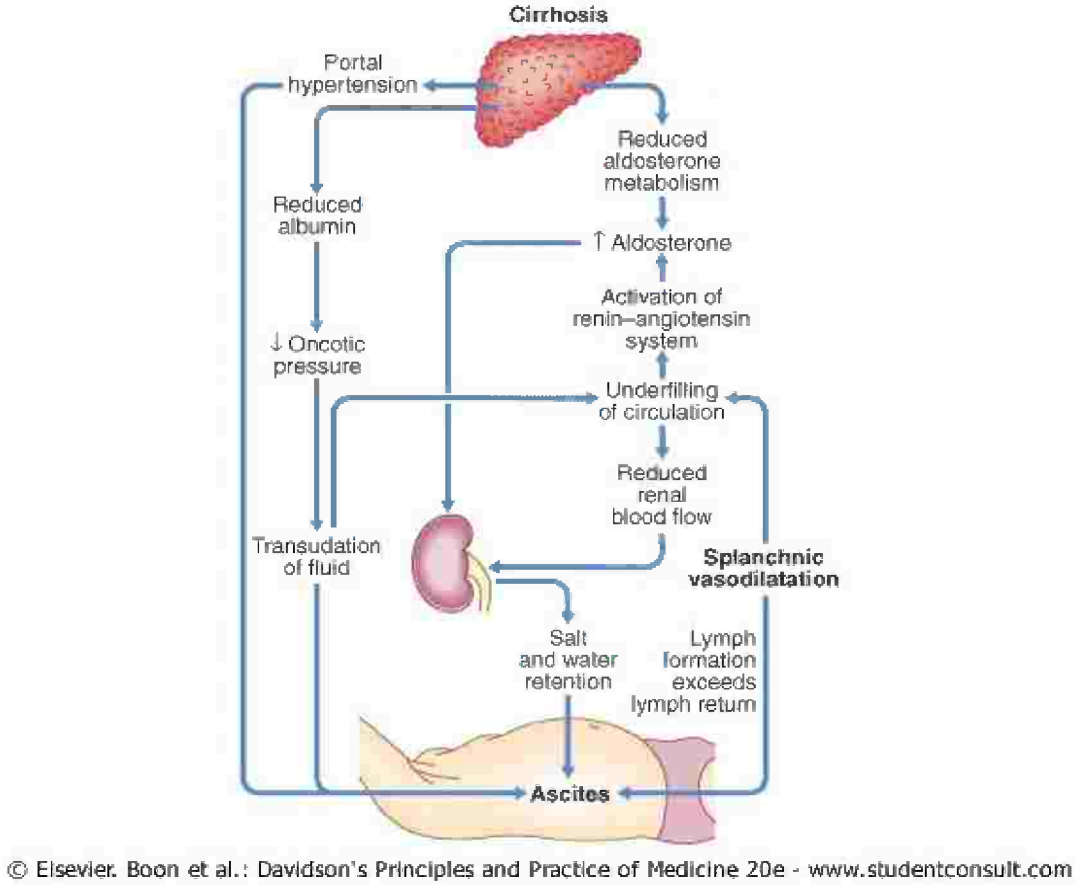

Figure 1: Pathophysiology of the development of ascites in cirrhosis and potential targets for treatment.

Portal hypertension

In cirrhosis, portal and sinusoidal hypertension is a prerequisite for the development of ascites. The hydrostatic pressure within the hepatic sinusoids favours transudation of fluid into hepatic lymphatics and the peritoneal cavity.[18,27]

The topographic site of the lesion is therefore important. Patients with post-hepatic portal hypertension with hepatic vein thrombosis, as in Budd-Chiari syndrome, often develop ascites which is difficult to treat.[28]

On the other hand, development of ascites is considered a late-phase manifestation in patients with portal hypertension due to portal vein thrombosis..[29]

The hepatic vascular resistance and portal venous inflow together with the development of portosystemic collaterals determine the height of the portal pressure. Factors that determine hepatic vascular resistance include both structural and dynamic components.[30]

Among the structural components are fibrosis and regeneration nodules.

Dynamic structures include hepatic stellate cells, myofibroblasts, and other cells with contractile properties. A preferential sinusoidal constriction in the liver seems to be attributed to a defective nitric oxide (NO) production but also to endogenous vasoconstrictors like endothelin-1 (ET-1), angiotensin II, catecholamines, and leukotrienes may all increase the hepatic sinusoidal resistance.[30,31]

The haemodynamic imbalance with a predominant sinusoidal constriction contributes significantly to the development of portal hypertension and thereby is an important target for treatment. Moreover, the formation of ascites depends on the balance between the increased local transvascular filtration and augmented lymph drainage.[18] Thus, the amount of ascitic fluid produced is governed by increased transsinusoidal filtration of protein and fluid and by accelerated transperitoneal hydrostatic and oncotic dynamics.

However, in contrast to earlier assumptions, the decreased plasma oncotic pressure may be of minor importance for the generation of ascites and low plasma concentrations of albumin have little influence on the rate of ascites formation (Figure 2).[18,32,33]

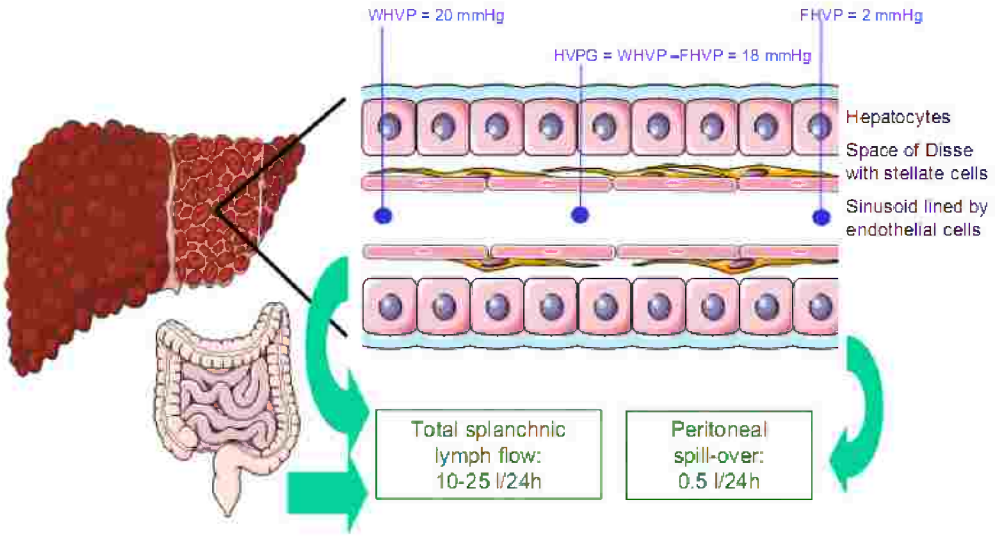

Figure 2. Hydrostatic pressures and transperitoneal fluid dynamics in cirrhosis: An increased hepatic venous pressure gradient (HVPG) generates increased transsinusoidal fluid filtration with an overall increased hepatosplanchnic lymph flow of 10–25 l/24 h. As long as the transsinusoidal filtration keeps pace with lymphatic drainage, no surplus protein-rich fluid will spill over into the peritoneal cavity. When filtration exceeds the lymphatic drainage, protein-rich fluid will accumulate in the peritoneal cavity as ascites. However, this spillover fraction (0.5 l/24 h) is relatively small compared to the overall transsinusoidal filtration and lymphatic drainage. WHVP =wedged hepatic venous pressure; FHVP =free hepatic venous pressure.

Pathophysiology of arterial vasodilatation and neurohumoral activation:

The pathophysiological coupling between early portal hypertension and the development of the systemic and splanchnic vasodilatation and the hyperdynamic syndrome is still obscure. It may be brought about either by overproduction of circulating vasodilators induced by shear stress in the splanchnic circulation or by direct neurohumoral signals from the liver to the brain.[31,35]

Several findings indicate that the splanchnic vasodilatation precedes renal sodium and water retention.[36]

In portal hypertension, splanchnic vasodilatation leads to reduced systemic vascular resistance, decreased effective blood volume, and a reduction in arterial blood pressure with activation of potent vasoconstricting systems such as the sympathetic nervous system (SNS), the renin-angiotensin-aldosterone system (RAAS), and non-osmotic release of vasopressin.[19,25,31]

The haemodynamic consequences of the development of a hyperdynamic circulation with an increased heart rate and cardiac output have previously been described as a mediator of the effective blood volume, and underfilling of the arterial circulation occurs in these patients as a result of diminished systemic vascular resistance.[26]

However, at a much later stage of the disease, underfilling of the arterial circulation may also occur secondary to a reduction of the increased cardiac output, as described in patients with renal failure and SBP.[37]

NO (nitric oxide) is among the vasodilators that have been implicated in the systemic vasodilatation, and is primarily synthesized in the systemic vascular endothelium by NO synthase.[38,39]

In portal hypertension, there seems to be a diminished release of NO from sinusoidal endothelial cells in the cirrhotic liver, whereas in the systemic circulation there is evidence of an up-regulation of the NO synthesis.[40]

Calcitonin gene-related peptide (CGRP) and adrenomedulin are potent vasodilatating neuropeptides, which have been found increased especially in patients with ascites and the hepatorenal syndrome (HRS).[31,36]

The increase in circulating vasoactive substances is mainly due to increased production and, to a lesser extent, to a decrease in hepatic clearance.[41]

It is likely that these peptides play a role as neurotransmitters, both in the initiation of the haemodynamic changes and in the perpetuation of the hyperdynamic circulation and the formation of ascites. Systemic vasodilatation has also been related to resistance to pressor substances, such as noradrenaline, angiotensin II, and vasopressin.

An impaired response to these vasoconstrictors is most likely related to changes in receptor affinity, down-regulation of receptors, and to post-receptor defects related to increased NO expression.[31,42,43]

Recently, alterations in arterial and total vascular compliance have also been considered.[44,45]

Renal dysfunction

In the early phases of cirrhotic portal hypertension, the renal sodium excretion capacity is impaired, with reduced natriuretic response to acute administration of sodium chloride or to changes in posture.[45,46]

These early events are seen before the development of ascites, but in most of the patients they represent the initiation of a more pronounced renal dysfunction. This includes progressively increased sodium and water reabsorption and decreases in renal perfusion and the glomerular filtration rate (GFR) often in parallel with reduced liver function.[47]At later stages, there is a progressive fall in GFR and renal blood flow (RBF).[23]

According to the sequence of the development of the functional renal abnormalities, genesis of ascites has been divided into successive pathophysiological phases (Table II).

Table II. Pathophysiological phases in the development of ascites and the hepatorenal syndrome.

Term	Phase	Sodium and water retention	Activated RAAS and SNS	Impaired RBF and GFR
Pre-ascitic cirrhosis	Phase 1	No/Yes	No	No
Mild–moderate ascites	Phase 2	Yes	No/Yes	No
Moderate–tense ascites	Phase 3	Yes	Yes	No/Yes
HRS type 2	Phase 4	Yes	Yes	Yes
HRS type 1	Phase 5	Yes	Yes	Yes

Abbreviations: RAAS = renin-angiotensin-aldosterone system; SNS = sympathetic nervous system; RBF = renal blood flow; GFR = glomerular filtration rate; HRS = hepatorenal syndrome.
Modified from Arroyo et al. [3].

The early phase 1 is also called the pre-ascitic phase, because ascites is not present, but the renal metabolism of sodium is impaired, despite normal RBF, GFR, and free water clearance.[48,49]

The second phase denotes a negative sodium balance despite decreased urinary sodium excretion, and the absence of ascites in this phase can be achieved by reducing the dietary intake of sodium. At this stage there is only activation of the RAAS and SNS in some patients, and the GFR and RBF may be normal.[50]

In phase 3, sodium excretion is often below 10 mmol/day and there is immense activation of the RAAS and SNS, but the RBF and GFR are still normal or low normal.[51,52]The arterial blood pressure is often low or low normal, despite activation of RAAS and SNS, and therefore these patients are highly susceptible to the hypotensive effects of angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor inhibitors, and V1-vasopressin antagonist.[23]

Phases 4 and 5 ascites denote the development of the HRS type 2 and type 1, respectively. Type-2 HRS is characterized by moderate renal failure with a slow progressive course and it is typically associated with refractory ascites. Type -1HRS is characterized by a rapid decrease in renal function which is often precipitated by SBP.[53]The new diagnostic criteria for HRS according to The International Club of Ascites are presented in Table II.[53]

The microbiota in the pathogenesis of liver disease and its complications:

Because of various body surfaces, such as skin, oral cavity, vaginal mucosa, respiratory passages, and, most importantly, the gastrointestinal (GI) tract are colonized by a wide variety of microorganisms.

These surfaces provide a favorable habitat for these organisms to reside and thrive. The term "gut microbiota" refers to a complex mixture of diverse microbes present in the GI lumen of an individual. It consists of approximately 1014 microbial cells, that is a number nearly 10-fold larger than that of human cells in an adult.[54]

Density, diversity, and relative composition of bacterial species vary along the length of the GI tract, being the most numerous in oral cavity and colon. Acidic environment in the stomach and rapid motility of the small intestine ensure that bacterial density in these organs is very low. In contrast, colonic contents contain nearly = 1011–1012 bacteria per gram of feces, with obligate anaerobes dominating over aerobes and facultative anaerobes in a ratio of 100–1000:1

Nearly 99% of microbes in the human gut belong to 1000–1200 bacterial species, with the remainder being contributed by archaea, viruses, and prokaryotes.[55]

These bacterial species belong mostly to genera that are placed in one of the four phyla, namely Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with little representation from the other bacterial phyla.[56]

GI tract is sterile at birth. Colonization begins soon thereafter, initially with flora acquired from the mother's vaginal canal and thereafter from the surrounding environment. An individual's gut microbiota is generally well established by 1 year of age and remain unchanged through life except for minor temporary fluctuations.[57]

The composition of adult gut microbiota varies widely between individuals and depends on several factors, including host genetics, diet, and other environmental factors. Thus, a particular individual's gut harbors a subset of about 150–200 bacterial species. [55]

Gut microbiota plays several important roles in the host's health. It supplements the host's nutritional needs through breakdown and absorption of complex dietary carbohydrates, which human enzymes cannot digest, as also synthesis of some essential substances, for example vitamin K.

In addition, it helps maintain the integrity of intestinal epithelial barrier through production of short-chain fatty acids, particularly butyrate, that are trophic for colonic epithelial cells and help epithelial restitution[58] and contributes to maturation of host immune system, including development of Peyer's patches, mucosal lymphoid follicles, and antibody secreting plasma cells.[59]

Small intestinal bacterial overgrowth (SIBO):

With regard to intestinal permeability, changes in intestinal tight junctional proteins have been described in cirrhosis and, though the precise pathophysiology of barrier dysfunction is unclear, roles for metabolites of alcohol and/or pro-inflammatory cytokines have been postulated.[60,61,62,67,68]

Alterations in gastrointestinal motility in cirrhosis have been variably ascribed to the effects of autonomic dysfunction, altered levels of circulating neuropeptides and the effects of inflammatory mediators on gut muscle and nerve.[63]

Among the various potential contributions of the microbiota to liver disease, small intestinal bacterial overgrowth (SIBO) has been one of the most extensively studied. Indeed, an altered gut microbiota was first noted by Hoefert in chronic liver disease over80 years ago;[64] since then SIBO has been documented to be common in liver disease, to correlate with its severity and to be linked to minimal and overt encephalopathy and an increased risk for SBP through translocation across the gut wall.[65,66]

Impaired antimicrobial defense mechanisms may further contribute to the development of bacterial translocation in portal hypertension and cirrhosis.[67]

Spontaneous bacterial peritonitis (SBP):

Is the most frequent and life-threatening infection in patients with liver cirrhosis requiring prompt recognition and treatment.

It is defined by the presence of >250 polymorphonuclear cells (PMN)/mm^3 in ascites in the absence of an intra-abdominal source of infection or malignancy.[69,70]

Epidemiology and Prognosis of SBP

SBP is the most frequent bacterial infection in cirrhosis, accounting for 10–30% of all reported bacterial infections in hospitalized patients.[71,72] In outpatients without symptoms the prevalence is low (3.5%[73] or lower[74,75]), but the prevalence increases in the nosocomial setting, ranging from 8% to 36%.[76,77]

Bacterascites, defined as positive culture results but no increase in the PMN count in the ascitic fluid, occurs with a prevalence of 2–3% in outpatients[73,75] and in up to 11% in hospitalised patients.[76,78] In-hospital mortality for the first episode of SBP ranges from 10% to 50%, depending on various risk factors.[72,79,82]

One-year mortality after a first episode of SBP has been reported to be 31% and 93%.[75,84,86,88]

In fact, the occurrence of SBP or other severe bacterial infections markedly worsens the prognosis in patients with cirrhosis and it has been proposed that a new prognostic stage of cirrhosis not reflected in current staging systems should be defined, the so-called 'critically ill cirrhotic'.[86]

Patients at this late stage have to be evaluated for the possibility of liver transplantation. Predictive factors reported for a poor prognosis in various cohorts of patients with SBP are summarised in (figure 3) and include age,[80,84] Child score,[85,87,90] intensive care,[80,82] nosocomial origin,[82, 88] hepatic encephalopathy,[48] elevated serum creatinine and bilirubin,[49] lack of infection resolution/need to escalate treatment and culture positivity[89,90] as well as the presence of bacteraemia[91] and variants as a genetic risk factor.[92]

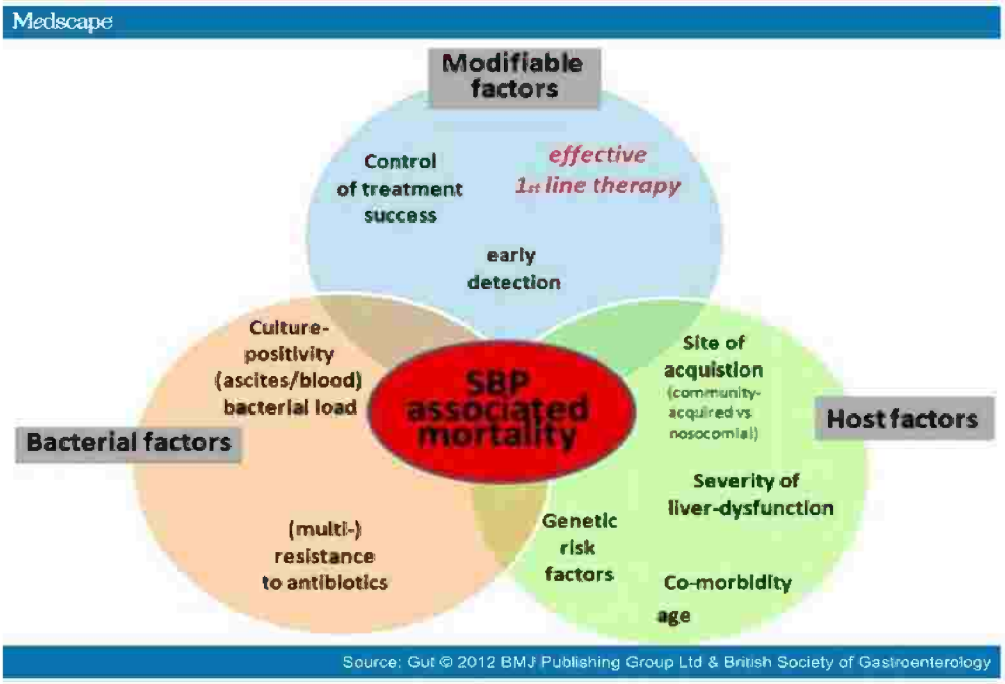

Figure 3: Spontaneous bacterial peritonitis (SBP)-associated mortality

Bacterial Translocation (BT) and Pathophysiology:

Bacterial translocation (BT) is the most common cause of SBP.[81,82] However, particularly in nosocomial SBP, other sources such as transient bacteraemia due to invasive procedures can lead to SBP.

Limited BT to mesenteric lymph nodes (MLN) is a physiological phenomenon, whereas any increase in the rate and severity of BT may be deleterious for the patient and thus should be termed 'pathological BT'. Only a few intestinal bacteria are able to translocate into MLN, including Escherichia coli, Klebsiella pneumoniae and other Enterobacteriaceae.[94]

Interestingly, these species most frequently cause SBP, and DNA sequencing studies reveal genotypic identity of bacteria in MLN and ascites in the vast majority of cases.[95,96] This suggests that pathological BT is the underlying cause and source of SBP in cirrhosis and supports the view that the route of pathological BT leading to SBP is largely lymphatic. Three factors have been implicated in the development of pathological BT in liver cirrhosis:[92]

(1) alterations in gut microbiota.

(2) increased intestinal permeability.

(3) impaired immunity.

Microbiota in Liver cirrhosis is associated with distinct changes in faecal microbial composition[97,98] including an increased prevalence of potentially pathogenic bacteria such as Enterobacteriaceae. Moreover, small intestinal bacterial overgrowth (SIBO), defined as >105 colony forming units/ml jejunal aspirate and/or colonic-type species, is frequently present in advanced stages of liver cirrhosis and has been linked with pathological BT, SBP and endotoxinaemia.[99,101]

Cirrhosis is associated with structural and functional alterations in the intestinal mucosa that increase permeability to bacteria and bacterial products. In particular, changes in enterocyte mitochondrial function and increased oxidative stress of the intestinal mucosa have been identified.[102,103]

Local Ascitic-peritoneal Host Defence in Peritonitis:

The peritoneal cavity probably has the most severe lack of host defence compared with other compartments in decompensated cirrhosis. In fact, ascites per se may be considered a risk factor for the development of peritonitis. In healthy conditions, peritoneal host defence mechanisms are very efficient and intraperitoneal injection of various numbers of single organisms does not cause peritonitis unless adjuvant substances or ascites are present.[104]

Monocyte chemotactic protein 1 is one of the most potent chemokines, and a functional polymorphism has been proposed as a risk factor for SBP in alcoholic cirrhosis.[105] A chemotactic gradient is necessary to achieve appropriate neutrophil recruitment into the peritoneal cavity.

In fact, PMN chemoattractants such as zymosan are very effective in preventing the death of animals with E coli-induced peritonitis when administered locally but not systemically.[106]

Besides influx of PMN, bacterial clearance is determined by the overall killing capacity which is dependent on opsonisation, burst activity and inflammatory response. A marked reduction in opsonic and bactericidial activity is well-known in cirrhosis. In particular, low C3 levels in cirrhotic ascites correlate strongly with opsonic activity[107]and have been shown to predispose to SBP.[108]

The total protein content also mirrors opsonic activity and has been shown to be predictive of the development of SBP.[109]At a protein level of >1.5 g/dl ascitic fluid, the incidence rates of SBP have been consistently reported to be lower than 1%. In contrast, at protein levels <1.5 g/dl ascitic fluid, the risk of SBP increases, parallelling the decrease in protein content and reaching incidence rates of 27–41% at levels <1.0 g/dl.[83,110,111]

In fact, significant levels of, for example, adiponectin, visfatin and resistin are observed in ascites and the latter is increased in the presence of SBP.[112,115]

Other factors that may contribute but have not been addressed thoroughly include compartmentalisation via activation of coagulatory systems or the omentum (called the 'abdominal policeman') and visceral fat. The latter is a relevant source of adipokines known to modulate the inflammatory response. [116]

Liver Dysfunction and Systemic Risk Factors

Cirrhosis is accompanied by deficits in innate and adaptive intrahepatic, intestinal and systemic immunity. Patients with cirrhosis with decreased reticuloendothelial system (RES) activity develop SBP at a higher rate than those with close to normal RES activity.[87].

A bilirubin level of >3.2 mg/dl and platelet count of <98 000/mm^3 significantly increase the likelihood of SBP,[113] and each model for end-stage liver disease (MELD) point increases the risk of SBP by about 11%.[114]However, circulating mononuclear cells also present with alterations in Toll-like receptor (TLR)[122]and HLA expression[116,117] as well as reduced chemotactic, opsonic, phagocytic and killing capacity.[118,119]

CARD15/NOD2[92,120] and Toll-like receptor 2(TLR2)[121] have been reported to be associated with an enhanced probability of acquiring SBP. TLR2polymorphisms and NOD2variants seem to represent supplementary risk factors since the simultaneous presence of both unfavourable polymorphisms markedly increases the risk of SBP.[121]This underlines the known interaction of NOD2 and TLRs, in particular the modulation of TLR2-dependent cytokine responses by NOD2.[122]

Medication can also affect the chances of developing SBP. The use of proton pump inhibitors (PPI) has been proposed to facilitate SIBO and thus to contribute to pathological BT. In fact, retrospective case–control studies reveal a potential association between the use of PPI and development of SBP.[123,124]

Considering the frequently inadequate overuse of PPI in patients with cirrhosis, we therefore recommend restricting their use to indications of proven benefit. In contrast, non-selective B-blockers (NSBB) may prevent SBP.[125,126]It is tempting to speculate that this benefit relates to an improvement in chemotaxis, proinflammatory cytokine release and killing capacity reported for B-adrenergic antagonists in various experimental settings.[127,128]

Diagnosis of SBP

Symptoms and signs are frequently absent in patients with SBP,[129] so a diagnostic paracentesis should be performed in all patients with ascites admitted to hospital regardless of whether or not there is clinical suspicion. Diagnosis should be prompt and treatment must not be delayed until the microbiology results are available. Thus, in all the available guidelines, diagnosis is based on a fixed defined cut-off PMN count in the ascitic fluid.[121,122] .

Owing to the short life span of PMN, their ascitic count is independent of diuretics and/or other modulations of ascites volume. In contrast, lymphocytes which have a long life span increase in concentration during diuresis.[130]

Moreover, differential diagnoses of predominant lymphocytosis in ascitic fluid include tuberculous peritonitis, neoplasms, congestive heart failure, pancreatitis and myxedema, but not usually SBP. PMN are therefore used to define SBP, and the greatest sensitivity is reached at a cut-off value of 250 PMN/mm^3, although the best specificity has been reported with a cut-off of 500 PMN/mm^3.[131,132]

However, since it is important not to miss a case of SBP, the most sensitive cut-off value is used. Nonetheless, this upper limit has been set quite arbitrarily since it was tested in the setting of culture-positive peritonitis. Thus, the range of PMN in truly non-infected ascites that is, the ascitic PMN count that is clinically relevant for the patient is not known. Moreover, SBP caused by Gram-positive cocci has been reported frequently to have a PMN count below the threshold of 250/mm^3.[133] Interestingly, bact DNA from Gram-negative bacteria in ascitic fluid is associated with a higher ascitic PMN count than bact DNA from Gram-positive bacteria,[134]

New Methods for diagnosis of SBP

LERS IN SBP

The leukocyte Esterase Reagent Strips (LERS), commonly used in every day practice for the rapid diagnosis of urinary tract infections (UTIs), were certainly featuring as a promising candidate

LERS had already been successfully evaluated in the diagnosis of infection in other sterile body fluids i.e. synovial, pleural, cerebrospinal fluid and peritoneal dialysate.[135,136] Butani et al[137] were the first to present their results on the use of LERS in SBP diagnosis.

Gut Wall Integrity

The gut wall forms a physical/anatomical and immunological barrier. The physical/anatomical barrier of the gut is formed by a monolayer of epithelial cells, originating from multipotent stem cells present in the crypt. The epithelial cells together with the lamina propria form the mucosa of the intestine [138,139,144].The epithelial stem cells give rise to four major epithelial cells: 1) the absorptive enterocytes, which make up >80% of all small intestinal epithelial cells, 2) the goblet cells, which produce a variety of mucins and trefoil peptides, 3) the enteroendocrine cells, which export peptide hormones and 4) the Paneth cells, which secrete a wide variety of antimicrobial peptides (Figure 1)

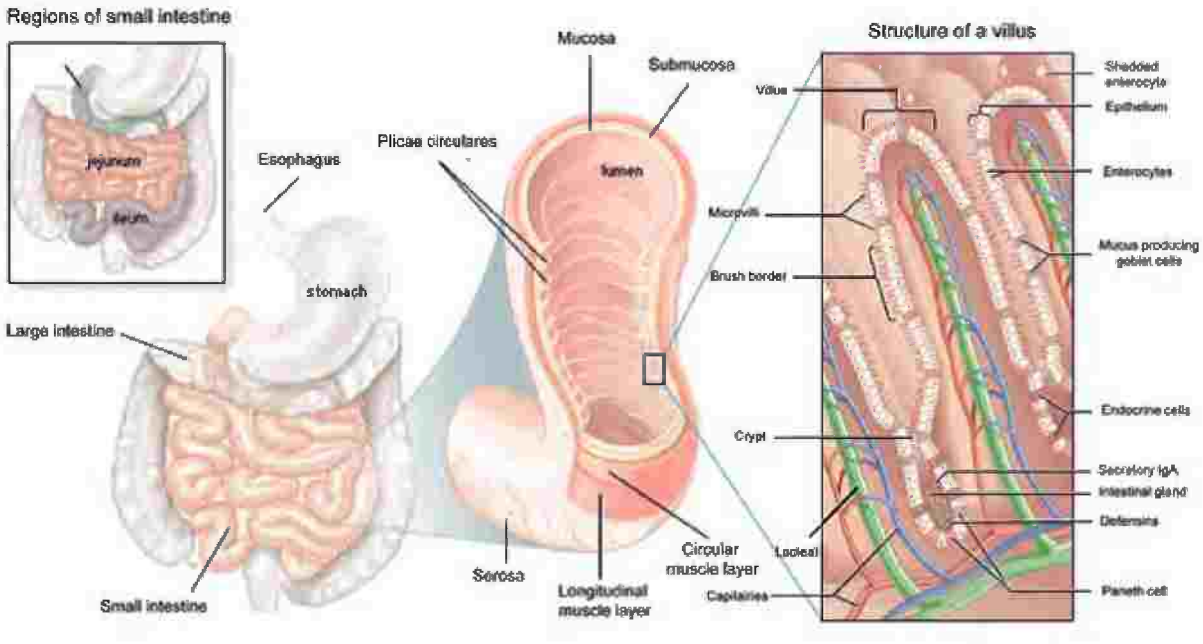

Figure 4: the composition of the small intestine

Tight junctions (TJ) are the major complexes responsible for the adherence of intestinal epithelial cells to each other and are in this context an important part of the intestinal barrier (Figure 5).[144,145] TJ are built up by multiple proteins: occludins and members of the claudin family are the major sealing proteins.

The sealing proteins interact with cytoplasmic proteins, including zonula occludin proteins (ZO), functioning as adaptors between the TJ proteins andactin and myosin contractile elements within the cells.[145]

Breakdown of this barrier potentially leads to the translocation of luminal antigens, microbiota and their toxic products into the circulation. The layers next to the mucosa, the submucosa, muscularis and serosa, are also of importance, although they are not in direct contact with the lumen.

Injury of these layers can result in transmural damage of the intestine and lead to a serious complication and passage of the luminal content into the abdominal cavity.

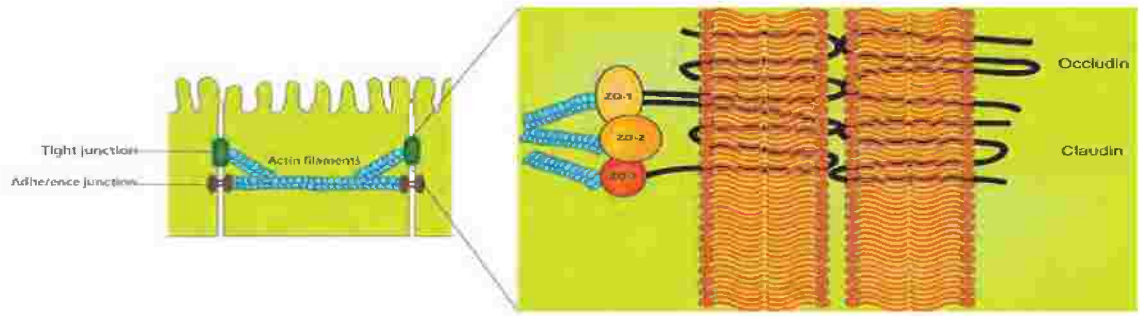

Figure 5: Composition of tight-junctions (in green (left) and in detail (right)) between neighbouring enterocytes (in yellow)

Next to the physical/anatomical barrier, there is also an immunological barrier The intestinal epithelium is not merely a static barrier, but also has a number of specialized protective adaptations.[138,139]

Enterocytes are considered to actively participate as innate immune sensors of microbial pathogens and commensal organisms.[146] Host recognition of microbial components is achieved by so-called pattern recognition receptors (PRRs), such as the NOD-like (NLRs) and Toll-like receptors (TLRs).

Muramyl dipeptide (MDP), derived from peptidoglycan, is present in the cell wall of virtually all bacteria and recognized by NOD2, a PRR expressed in intestinal epithelial cells[146]including Paneth cells.[147]

Paneth cells secrete defensins, antimicrobial peptides, in the villous crypt, maintaining its sterility. Moreover, we recently reported that Paneth cells are equipped with the proper molecules to recognize and signal endotoxin, one of the most potent immunostimulatory products derived from Gram-negative bacteria.[147].

Continuous antimicrobial protection of the crypt is of crucial importance, since the pluripotent stem cells are located there. Damage to stem cells would have severe consequences for the maintenance of the homeostasis of normal gut epithelium[148].

Goblet cells secrete mucus, a composition of glycoproteins and water, which provides a filter overlying the intestinal epithelium. Additionally goblet cells secrete trefoil peptides, small proteins needed for epithelial growth and repair.

Furthermore, gut-associated lymphoid tissue (GALT) is present in the lamina propria and provides immune surveillance. Sampling of luminal antigens occurs by M-cells and dendritic cells (DC), which present antigens to T and B cells, thereby inducing an effector immune response. This response includes secretion of large amounts of IgA by plasma cells.

This secretory IgA covers the mucosal surface and has a major role in excluding antigen from passing the epithelium [138,139].

ASSESSMENT OF GUT WALL INTEGRITY

I))Physical/anatomical components of the gut wall

a-Condition of enterocytes:

Measurement of endogenous cytosolic enterocyte proteins in urine or plasma have been shown to be useful to estimate enterocyte damage.

Fatty Acid BindingProteins (FABP): comprise a class of low molecular weight (14-15 kDa) cytosolic proteins found in high concentrations in tissues involved in the uptake and consumption of fatty acids.

Intestinal Fatty Acid Binding Protein (I-FABP): is primarily limited to mature enterocytes of the small and large intestine.[149,150] It circulates in low amounts in the blood stream of healthy individuals. I-FABP is a useful plasma marker for early enterocyte cell death and levels rise rapidly after episodes of acute intestinal ischaemia and inflammation.[149,150]

The level of circulating I-FABP has been reported to correlate with the histological status of the epithelium after intestinal ischaemia-reperfusion in experimental studies.[151,152]

A second gut-specific FABP is Ileal-Bile Acid Binding Protein (I-BABP), which is exclusively present in mature enterocytes of the jejunum and ileum.[153] Enterocytes also contain Liver-FABP (L-FABP), which is localized in small amounts in the mature enterocytes of the small and large intestine but in abundance in the liver.[149]

Glutathione S-Transferases (GST): are a family of more or less tissue specific cytosolic enzymes. These proteins are involved in the detoxification of a range of xenobiotic compounds by conjugation to glutathione and grouped into species-dependent families based on their isoelectric point.[154]

Alpha and pi GST are found in the small and large intestine.[155] However, they are not organ-specific, since they are also expressed in liver and kidney.[156]Plasma levels of alpha GST are elevated upon ischemic intestinal damage.[157]

b- Functional enterocyte mass:

Functional enterocyte mass is reflected by levels of circulating **citrulline**, anamino acid not incorporated into proteins.[158,159]

Differentiated small intestinal enterocytes specifically produce citrulline from glutamine and are responsible for the major part of the total amount of circulating citrulline.[159,160]

Declined circulating levels of citrulline provide evidence for the loss of small bowel epithelial cell mass, as is shown in haemopoietic stem cell transplant recipients suffering from severe oral and gastrointestinal mucositis following intensive myeloablative therapy.[158,159]

c- Condition of tight junctions:

Currently, invasive intestinal biopsies provide the only possibility to detect tight junction breakdown. Endoscopy for biopsy is a time consuming and invasive procedure.

d- Intestinal permeability:

Intestinal permeability is frequently assessed using oral ingestion of various relatively small to large-sized probe molecules and measurement of their urinary excretion.[140,141,142]

Disaccharides (lactulose) or Poly-ethylene-glycol (PEG)-3350 are frequently used as orally ingested large molecules and monosaccharides **(mannitol, Lrhamnose)or PEG-400** are used as small molecular probes.[142]

Subsequently, the renal excretion of the two probes is monitored over a defined interval (mostly 5 hours), and permeability is then expressed as the ratio of the urinary recovery of the large molecule divided by the small molecule.[142]

e- Translocation of bacteria and their products:

Breakdown of the mucosal barrier potentially leads to translocation of microbiota or their toxic products. Two promising plasma markers, reflecting translocation of bacteria or their products, are **D-lactate and endotoxin(lipopolysaccharide, LPS),** which are metabolic products or components of the commensal bacteria of the gastrointestinal tract.

D-lactate is only produced by bacteria as a product of bacterial fermentation.[161]Baseline levels of D-lactate in healthy subjects are very low, but the exact mechanism by which D-lactate enters the blood stream is unknown. Increased levels of D-lactate have been correlated with conditions in which the number of bacteria elevates rapidly,including in patients with bacterial overgrowth due to infection, short bowel syndrome and mesenteric ischaemia.[162,163]

LPS, the major constituent of the outer membrane of Gram-negative bacteria, is released from bacteria when replicating or dying.[164] Increased circulating LPS levels have been related to an impaired mucosal barrier. The presence of LPS can be measured directly in blood, e.g. by the Limulus Amoebocyte Lysate (LAL) assay.[165]

In addition, anti- LPS antibodies can be measured by endotoxin-core antibody (EndoCAb), an indirect measurement of LPS leakage into the circulation.[166]

f- Transmural damage:

Any part of the gastro-intestinal tract may undergo damage to all layers of the GIwall from a variety of cause, releasing gastric or intestinal contents into the peritoneal cavity, which potentially cause peritonitis. Symptoms develop suddenly,with severe pain followed shortly by signs of (septic) shock. If a perforation is noted, immediate surgery is necessary, because mortality from peritonitis increases rapidly.

The diagnosis of transmural damage (i.e. perforation) of a gastro-intestinal organ usually depends on the detection of free intraperitoneal air, which is most often located in the right subphrenic space. Traditionally, a chest X-ray and a plaina bdominal X-ray in the upright position, or, more recently, ultrasonography are the diagnostic tools used to detect free air.

However, the sensitivity of these tools is<80%. Currently, a computed tomography (CT) scan is sometimes performed,which may detect free intraperitoneal air as well as small fluid collections and subtle tissue-infiltration at different locations.

g- Splanchnic perfusion:

Numerous clinical conditions are accompanied by a reduced splanchnic blood flow, including vascular disease, major surgery and various types of shock.Prolonged hypoperfusion of the splanchnic region will inevitably lead to hypoxic tissue injury. Furthermore, the splanchnic region is an important source and target of inflammatory mediators, which have a major impact on both systemic and regional blood flow and tissue function.[167]

Gut mucosal perfusion can be invasively measured by **gastric tonometry**. Gastric tonometry assesses the pCO2 in the gastric mucosa, taking into account that an increase in tissueCO2 production accompanies anaerobic metabolism, signifying the effectiveness of regional splanchnic perfusion.[168]

II)) Immunological components of the gut wall

Gut wall inflammation:

A broad range of pathologies can lead to intestinal inflammation, neoplasia, inflammatory bowel disease (IBD), infections, auto-immune diseases (e.g.celiac disease), ischemia-reperfusion, intestinal hypoperfusion, and e.g. the use of non-steroidal anti-inflammatory drugs.

Generally, defects or increased permeability of the mucosal barrier will cause intestinal inflammation in response to the enormous number of bacteria present in the bowel. The pathogenesis of inflammatory intestinal diseases implies the recruitment of leukocytes into the intestinal wall.[169]

Activated neutrophils infiltrate the mucosa and their products can be detected in faeces due to release into the intestinal lumen.[169]

Therefore, faecal markers of neutrophils are specific for the detection of inflammatory intestinal diseases. Numerous neutrophil derived proteins present in stool have been studied, including **calprotectin, lactoferrin and elastase.**[169]

The most promising marker is **calprotectin**, because of its remarkable resistance to proteolytic degradation and its stability in stool kept on room temperature for at least 7 days.[170]

Calprotectin is a 36 kDa calcium and zinc binding protein that plays a regulatory role in the inflammatory process. It constitutes about 60% of the soluble proteins in human neutrophilic cytosol and is also found in monocytes, macrophages and ileal tissue eosinophils. It is released during cell activation or cell death and has antiproliferative, antimicrobial and immunomodulating functions.[169,170]

Fecal calprotectin is nowadays used in clinical practice to evaluate disease activity in the follow-up of patients treated because of active IBD.[169]

D-lactate

Breakdown of the mucosal barrier potentially leads to translocation of microbiota or their toxic products promising plasma marker reflecting translocation of bacteria or their products, is D-lactate which is a metabolic product or component of the commensal bacteria of the gastrointestinal tract D-lactate is essentially a product of bacterial metabolism due to bacterial fermentation of unabsorbed carbohydrates in the colon and absorption of this metabolite from the intestinal lumen.[171]

D-lactate is usually present in human blood at very low concentrations as a product of methyl glyoxal metabolism, which is produced in small amounts from fat, protein and carbohydrate metabolism.

D-lactate is also produced by bacteria of the gastrointestinal tract and is absorbed in the small intestine and colon. D-lactate is thought to be metabolized in the liver to pyruvate by the enzyme D-a-hydroxy acid dehydrogenase at one-fifth the rate at which L-lactate dehydrogenase (L-LDH) metabolizes L-lactate.10 About 10% of D-lactate is excreted in the urine and it has been reported that L-lactate interferes in its renal reabsorption.[172] Thus, metabolism and excretion of D-lactate and L-lactate are closely related.

D-lactate concentration may be of value as a clinical diagnostic tool in a variety of disorders:

(1) D-lactate might contribute to the development of osteomalacia in patients with malabsorption receiving long-term parenteral nutrition.[173]
(2) Measurement of plasma D-lactate concentration has a lower false-negative rate than C-reactive protein or leukocyte count when used as a marker in the diagnosis of appendicitis.[174]
(3) Serum D-lactate concentration is increased in critically sick patients with septic shock.[175]
(4) Plasma D-lactate is a reliable marker of colonic ischaemia and a very sensitive marker for gut failure.[176]
(5) Plasma and urine D-lactate might be used as a biochemical marker to support the diagnosis of neonates suspected of necrotizing enterocolitis (NEC).[177]
(6) Plasma D-lactate and urine excretion of D-lactate are markedly increased in triosephosphate isomerase deficiency and can be used to support its diagnosis.[178]

Several methods have been reported to measure D-lactate in blood, plasma, urine or biological fluids.[178,179] The most widely accepted method is based on conversion of D-lactate to pyruvate in the presence of nicotinamide-adenine dinucleotide (NAD) and D-lactate dehydrogenase (D-LDH) and monitored by a spectrophotometric technique.

Baseline levels of D-lactate in healthy subjects are very low. Increased levels of D-lactate have been correlated with conditions in which the number of bacteria elevates rapidly, including in patients with bacterial overgrowth due to infection, short bowel syndrome, and mesenteric ischaemia.[180]

Fatty acid binding proteins (FABP)

Measurement of endogenous cytosolic enterocyte proteins in plasma has been shown to be useful to estimate enterocyte damage . Fatty acid binding proteins (FABP) Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins that bind long chain fatty acids.

They are intracellular proteins, with a low molecular weight of approximately 15 kDa, that plays important roles in the transportation and metabolism of long-chain fatty acids with a high degree of tissue specificity. They are abundantly present in various cell types.

FABP family proteins could be used as tissue specific injury marker based on the following characteristics of FABP:

(1) A soluble protein in the cytoplasm,
(2) High tissue specificity
(3) Abundance in the tissue
(4) Low molecular weight

There are at least nine distinct types of FABP, each showing a specific pattern of tissue expression

Among the FABP family proteins, intestinal fatty acid-binding protein(I-FABP) is protein derived from the human FABP2 gene and is specifically and abundantly present in epithelial cells of the mucosal layer of the small intestinal tissue. Three isoforms of FABP are present in the intestine: intestinal(I)-FABP, liver (L)-FABP, and ileal-bile acid binding protein (I-BABP).[181] The presence of FABP on the tops of the villi, the initial site of destruction in numerous intestinal diseases, makes circulating FABP potentially useful plasma markers in early stages of intestinal diseases.

Intestinal FABP (I-FABP) is primarily limited to mature enterocytes of the small and large intestine.[181,182,183] It circulates in low amounts in the blood stream of healthy individuals. I-FABP is a useful plasma marker for early enterocyte cell death and levels rise rapidly after episodes of acute intestinal ischaemia and inflammation.[181,182,183,184]

The level of circulating I-FABP has been reported to correlate with the histological status of the epithelium after intestinal ischaemia-reperfusion in experimental studies.[184,185] Based on this mechanism, many investigators have already reported the relationship between serum I-FABP concentration and small intestinal diseases from early 1990s.

Recently, a sandwich ELISA system for measuring human I-FABP concentration is available by using the combination of antibodies highly specific to I-FABP. This ELISA system did not show any cross-reactivity with other types of FABP and indicated excellent quantitative characteristics such as reproducibility, dilution linearity, and recovery.

I-FABP is the most promising endogenous enterocyte protein (marker) to assess enterocyte injury, as this protein is specifically expressed in the gut and released immediately into the circulation upon cell damage.