(Patho)physiology of gut wall integrity in health and disease in man

The intestinal mucosa is responsible for the absorption of nutrients from the lumen on the one hand and for the separation of the potentially toxic luminal content (external environment) from the host (internal environment) on the other hand. Disruption of this delicate balance at the mucosal interface occurs in numerous (intestinal) diseases. Assessment of gut wall integrity loss in clinical practice is still a challenge, since it is difficult to evaluate gut condition non-invasively with the currently available diagnostic tools. Moreover, non-invasive, rapid diagnostic means to assess intestinal condition are also needed in the follow-up of many intestinal diseases for evaluation of the effects of treatment on the recovery of the disorder. Furthermore, primarily experimental animal studies have shown that gut wall integrity loss is involved in the development of various inflammatory syndromes, including post-operative or post-traumatic systemic inflammatory response syndrome (SIRS), sepsis and multiple organ failure (MOF) [1,2].

In this thesis, markers for human gut wall integrity were evaluated as tools for diagnosis and follow-up in patient studies. Next, the development of gut wall integrity loss in critically ill patients and patients undergoing major surgery was investigated to study whether gut barrier loss is of importance in the development of post-operative complications. Finally, as intestinal ischemia-reperfusion was proven to be present in critically ill patients and in patients undergoing major surgery and was related to the onset of gut wall integrity loss, the sequelae of intestinal ischemia-reperfusion were investigated in a novel human model.

The **first aim** of this thesis was to gain more insight in characteristics of markers that are potentially useful for the assessment of gut wall integrity loss, namely tests for enterocyte damage and tight junction loss.

This thesis focussed on markers for enterocyte damage, more specifically on the family of fatty acid binding proteins (FABP), since these proteins are tissue specific [3]. Three isoforms of FABP are present in the intestine: intestinal (I)-FABP, liver (L)-FABP, ileal-bile acid binding protein (I-BABP) [3]. Results of previous experimental animal studies and some human (pilot-)studies have shown that FABP are potentially useful as plasma- and/or urinary markers to detect enterocyte damage, as occurs after ischaemia or inflammation [4,5]. To gain insight in FABP plasma and urinary concentrations, the exact localization of these proteins in the gut was investigated. The expression of I-FABP and L-FABP in the different segments of the human intestine, obtained during routine surgery, was evaluated (in the second paragraph of chapter 2). I-FABP and L-FABP are expressed in the mucosa of the entire small intestine and have a

predominant expression in the jejunum. I-BABP is specifically present in the ileum with a mean (±SEM) concentration of 303 (±113) µg I-BABP/gr tissue (data not shown). I-FABP, L-FABP and I-BABP are in particular highly expressed in cells present on the top of the villi. The presence of FABP on the top 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. Subsequently, the release of I-FABP and L-FABP into the circulation of patients with obvious intestinal epithelial damage, i.e. patients with celiac disease, was investigated. This pilot-study showed that I- and L-FABP circulating levels were significantly increased in untreated celiac disease compared to healthy volunteers. Furthermore, I- and L-FABP concentration normalized after implementation of the therapy for celiac disease (gluten-free diet) in all but one patient. However, one has to bear in mind that L-FABP shows a multi-tissue expression, in contrast to I-FABP. Besides the expression in the intestine, L-FABP is also present in hepatocytes and tubular cells of the kidney [3]. Increased circulating and/or urinary L-FABP levels can therefore be derived from other organs than the intestine.

Subsequently, the release and clearance of FABP was investigated in patients undergoing liver resection for secondary tumors in an otherwise healthy liver (as is described in paragraph 3, chapter 2). Plasma levels of L-FABP increased early during surgery, namely during liver manipulation. Other classical liver injury markers (alpha glutathione s-transferase (GSTa) and aspartate amino transferase (AST) showed a similar rise. L-FABP and GSTa levels decreased immediately postoperatively, whereas AST gradually kept increasing. As a consequence, L-FABP and GSTa are probably more sensitive for detecting ongoing hepatocyte injury and impending liver failure than AST. To prove this assumption, however, a large prospective study is needed.

Next, the release and clearance of FABP was investigated in the same study by collection of blood from efferent and afferent vessels of the intestine, liver and kidney during surgery. Arterial renal venous concentration gradients showed that the kidneys remove approximately 30% of L-FABP in a single pass, leading to a calculated L-FABP half-life of 11 minutes. In conclusion, the immediate postoperative decline of L-FABP is a result of its rapid renal clearance. This further emphasizes that FABP is an accurate marker for actual cell damage and that urinary concentration are potentially useful in reflecting this damage, since FABP are rapidly cleared by the kidneys.

As the epithelial barrier is constituted by both enterocytes and tight junctions, the next study was directed at finding a marker for the assessment of tight junction damage. In chapter 2, paragraph 4 was studied whether an

important tight junction protein, i.e. claudin-3 [6], can be used as a non-invasive marker to study the condition of tight junctions. In a translational study, using both a rat hemorrhagic shock model and a human setting of patients with active inflammatory bowel disease (IBD), the immunohistochemically visualized loss of claudin-3 from intestinal tissue resulted quickly in the appearance of this protein into urine. This is the first study to report that measurement of the status of tight junctions can be performed non-invasively.

In conclusion, the results of chapter 2 showed that non-invasive (i.e. urine based) assessment of the condition of the epithelial layer of the gut, which is responsible for the barrier between external environment and host, is possible with the use of proteins originating form cytoplasma of damaged enterocytes (FABP) and from disrupted tight junctions (claudin-3) (Figure 1).

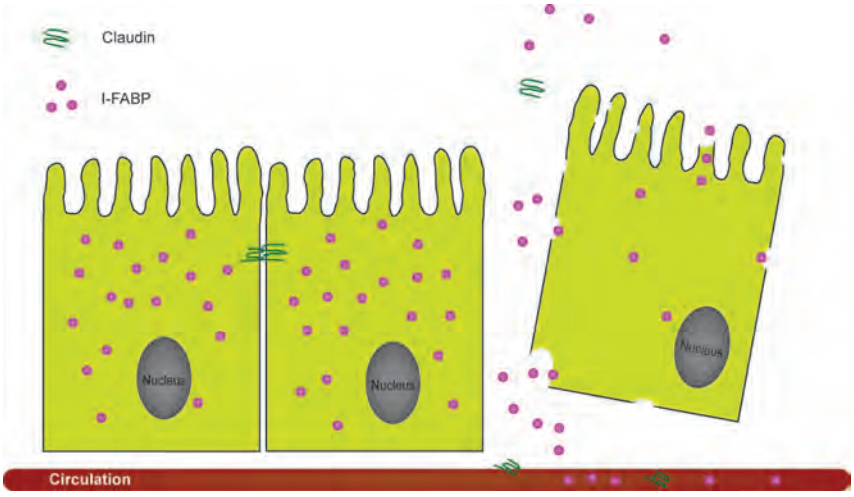


Figure 1: the leakage of FABP and claudin, reflecting the condition of the epithelial layer of the gut.

The **second aim** of this thesis was to analyse the usefulness of markers for compromised gut wall in patients with overt gut damage and in patients with suspected intestinal disease to distinguish the target disease from other pathologies.

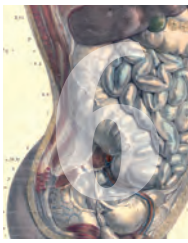
Diagnostic tests aim at discriminating between clinically "normal" and "abnormal". New diagnostic tests should subsequently answer the following 4 questions before implementation of the new test in daily clinical practice can be contemplated [7].

Question 1: Do patients with the target disorder have different test results from normal individuals? The answer requires comparison of test results among individuals with the disease and individuals that are disease-free. In chapter 3, paragraph 2, a 2-year-old girl with rhabdomyosarcoma is presented, who suffered from three episodes of gastrointestinal mucositis and bacteraemia following two cytostatic treatments consisting of ifosfamide, vincristine, and actinomycin D (IVA). This is a well-known and potentially harmful complication of chemotherapy, which is difficult to diagnose and of which the pathophysiology is poorly understood. Gastrointestinal mucositis presented as abdominal complaints, fever, which was accompanied by raised C-reactive protein levels, and positive blood culture for coagulase-negative staphylococci. The signs and symptoms and laboratory results were preceded by increased plasma levels of intestinal fatty acid binding protein (I-FABP), reflecting enterocyte damage. Next, the relationship between enterocyte mass and enterocyte loss was explored by examining citrulline serum levels (reflecting functional enterocyte mass) [8,9] and by assessing circulating I-FABP and I-BABP (assessing enterocyte loss) in adult patients with haematological malignancy receiving allogeneic HSCT and preceding myeloablative conditioning, consisting of idarubicin, cyclophosphamide and total body irradiation (chapter 3, paragraph 3). This regimen is known to induce oral and intestinal mucosal barrier injury [10]. A decline in circulating citrulline concentrations was found with the nadir 19 days after the start of the myeloablative regimen. This is in line with previous studies and shows a clear transient reduction in the small intestinal epithelial cell mass. Interestingly, an almost simultaneous reduction in circulating levels of I-FABP and I-BABP were observed. The baseline circulating levels of FABP are considered to represent the continuous loss or, in other words, the turnover of enterocytes, since the main loss of enterocytes in the healthy intestine consists of senescent enterocytes which release FABP upon detachment or death into the gut lumen at the end of their lifespan. Surprisingly, these data showed that less cytosolic content of enterocytes is released into the circulation after intensive myeloablative conditioning than under normal, healthy circumstances, indicating a decreased rate of dying enterocytes. This, in turn, suggests a reduced turnover of enterocytes after myeloablative conditioning. Furthermore, this is the first study to report on specific changes in I-BABP levels, representing specific jejunal and ileal enterocyte loss, using a newly developed ELISA for measurement of serum and plasma I-BABP.

Question 2: Are patients with certain test results more likely to have the target disorder than patients with other test results? This question is also answered by comparison of test results among patients with the targetdisease and healthy

individuals, but now test characteristics as sensitivity, specificity and likelihood ratios are estimated. As discussed above in this chapter, the potential usefulness of assessing circulating concentrations of FABP in a pilot study of patients with celiac disease, which is characterized by intestinal mucosal damage, was reported (chapter 2, paragraph 2). The results of the study suggest that besides a possible additional role for FABP in the diagnosis of celiac disease, assessment of plasma/urinary concentration of FABP may provide a new important non-invasive tool to monitor the short-term response to a gluten-free diet and in this way contribute importantly to the follow-up of celiac patients. However, further studies are necessary to determine whether assessment of intestinal epithelial cell damage by FABP analysis can replace the invasive procedure of endoscopy and mucosa biopsy. Next, the usefulness of plasma markers for neutrophil activation products was explored, since gut damage irrevocably leads to gut wall inflammation, which implies the recruitment of leukocytes into the intestinal wall. Acute appendicitis (AA) is an intestinal disease, characterized by gut damage and massive infiltration of neutrophils into the gut wall, for which the diagnosis is often delayed due to its aspecific clinical presentation and lack of adequate laboratory markers. The circulating neutrophil activation product calprotectin showed the most promising results; it was found to be significantly increased in 51 patients with proven AA compared to 9 patients without proven AA and 27 healthy volunteers (chapter 3, paragraph 4). This yielded an overall accuracy of the test, summarized using area under the receiver operating characteristics curve (AUC), of 0.91. This is considered to be clinical relevant.

Question 3: Among patients in whom it is clinically sensible to suspect the target disorder, does the test result distinguish those with and without target disorder? To obtain the appropriate answer, a consecutive series of such patients should be studied. This phase in the evaluation of diagnostic tests was applied to the potential improvement of the diagnostic accuracy of necrotizing enterocolitis (NEC), a serious intestinal neonatal disease with high morbidity and mortality rates, as is reflected in chapter 3, paragraph 6 and 7. In urine and faeces of 29 consecutively included neonates suspected for NEC proteins representing important (histo-)pathological factors of NEC were measured: epithelial integrity loss (urinary I-FABP and urinary claudin-3) and gut wall inflammation (faecal calprotectin). In the 12 neonates suspected for NEC, who ultimately developed NEC, the levels of the studied markers were significantly higher than in the neonates who finally had other diagnoses (mainly sepsis). Areas under the receiver operating characteristic curve of urinary I-FABP, urinary claudin-3 and faecal calprotectin were 1.0, 0.84 and 0.94, respectively. These data are considered to be clinically relevant.



Question 4: Do patients who undergo the diagnostic test fare better (in their ultimate health outcomes) than similar patients who do not? The question has to be answered by randomizing patients to undergo the test of interest or another (or no) test. However, it is disputable whether unravelling phase 4 questions is valid and efficient with this design (randomised comparison of the test OR: randomised assignment of patients to the test), since this study design evaluates both the test and different treatment strategies following the tests [11]. Furthermore, a large number of patients is needed. Therefore, an alternative study design for phase 4 questions is to move the point of randomisation from the decision point whether or not to test, to what to do with the test result, i.e. to apply the test to all patients before randomisation, irrespective of the result [11]. If the costs of the test are low compared with the costs of following up patients and monitoring outcome, randomising only test-negative patients potentially offers a more efficient design. Moreover, if the trial is sufficiently powered, assessment of the effectiveness separately in test-positive and test-negative patients can lead to evidence-based practice recommendations.

In conclusion, the potential usefulness of new markers (FABP, claudin-3, calprotectin) in the early diagnosis of intestinal diseases was shown. It is of importance that most of the tests can be performed in non-invasively collected material, such as urine and faeces. Especially in neonates and children this is a great advantage, since blood collection for diagnostic purposes is traumatic for all children and a major cause for anaemia in neonates. All studies have to be continued into the following phase of the evaluation of new diagnostic tests. For the neonates suspected for NEC, question 4 has to be answered for which the considerations regarding the design have been described.

The **third aim** of this thesis was to evaluate the development of gut wall integrity loss in critically ill patients and in people undergoing major (non-abdominal) surgery.

Experimental animal studies have generated the hypothesis that the intestines are central in the origin of post-operative and post-traumatic sequelae [1,2,12-14]. It is hypothesized that major surgery or trauma leads to a redistribution of blood to the vital organs (brain, heart) at the expense of the intestines. Especially the mature intestinal mucosal cells are susceptible to hypoperfusion and ischemia. Damage of intestinal mucosa can lead to local inflammation, impaired barrier function, and thereupon potentially to translocation of micro-organisms [1,2]. Moreover, cell injury leads to the release of immunostimulatory proteins or nucleotides, so-called danger signals that activate the immune system and induce systemic inflammation [15]. The translocation can result in a

derailment of local inflammation and subsequently amplification of a systemic inflammatory response, as is mainly shown in animal studies. With the use of the markers for gut wall integrity, which were characterized in previous studies, the involvement of the gut in patients undergoing major surgery was investigated. This is of utmost importance as different opinions exist. First, the development of intestinal hypoperfusion, objectivated by gastric mucosal $PiCO_2$, was explored and its relationship with intestinal epithelial cell damage, assessed by circulating levels of I-FABP, in patients admitted to the intensive care unit (ICU) because of postoperative abdominal sepsis (chapter 4, paragraph 2). Splanchnic hypoperfusion in the early phase of abdominal sepsis correlated strongly with intestinal mucosal damage. Moreover, elevated plasma I-FABP values on admission to the ICU were associated with a poor outcome in patients with abdominal sepsis. Next, in children with meningococcal sepsis it was shown in chapter 4, paragraph 3 that half of the patients presented with intestinal epithelial cell damage at admission to the paediatric ICU and that the children who died were characterized by continued presence of gut damage, while in all survivors this injury came to an end within 12 hours after starting intensive treatment.

In addition to these two clinical retrospective studies showing intestinal epithelial cell damage in a large number of patients immediately upon admission to the ICU, the development of gut wall integrity loss during surgery in two clinical prospective studies was investigated (chapter 4, paragraph 4 and 5).

Two of the most extensive and complex surgical procedures are open surgical repair of thoracic aortic aneurysms (TAA) and thoracoabdominal aortic aneurysms (TAAA) that are associated with significant postoperative morbidity and mortality. In order to prevent peroperative ischemic injury of visceral organs, extracorporeal circulation (ECC) technique has been implemented in open TAA repair to provide (retrograde) distal aortic perfusion (DAP) via the femoral artery. In case of TAAA repair involving the origin of visceral arteries, ECC with DAP is combined with selective organ perfusion catheters (DAP and SP) to provide designated visceral arteries directly with blood. In chapter 4, paragraph 4 it was shown that intestinal mucosal cell injury, but not hepatic or renal tubular cell injury, occurred in patients undergoing elective open TAA (n=8) or TAAA (n=22) repair, despite artificial visceral perfusion. The extent of intestinal injury and following pro-inflammatory reaction was more pronounced in patients undergoing DAP and SP. The most likely explanation for the difference in intestinal injury is a more prominent hypoperfusion of the intestines during ECC with DAP and SP. Furthermore, intestinal injury correlated positively with markers of systemic inflammation (IL-6 and IL-8). Next, the development

of gut wall integrity loss in patients undergoing major spinal fusion surgery was studied, which is characterized by long duration of surgical procedure, significant blood loss, prolonged systemic hypotension and the potential development of postoperative complications (chapter 4, paragraph 5). This type of surgery was chosen because it does not directly compromise the intestines by intestinal manipulation or the use of extracorporeal circulation. In a significant number of patients increased circulating levels of I-FABP and I-BABP and elevated urinary values of claudin-3 were observed, indicating the development of gut wall integrity loss. Furthermore, a significant inverse association between mean arterial pressure and succeeding plasma levels of I-FABP and I-BABP was found, which indicates that systemic hypotension is correlated with intestinal mucosal cell injury, detected 1/2 hour later. The systemic hypotension is mainly caused by anaesthetics, causing a decreased systemic vascular resistance. Finally, splanchnic hypoperfusion (gastric mucosal $PiCO_2$ and $P_{r\text{-}a}CO2$-gap) correlated strongly with intestinal mucosal damage (plasma I-FABP) at all observed time-points during surgery.

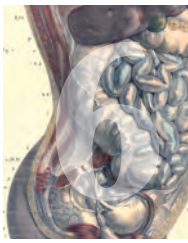
In conclusion, the results of these studies in patients undergoing TAA(A)-repair and spinal fusion repair show for the first time the relation between altered splanchnic perfusion and the development of intestinal mucosal cellular damage in patients undergoing major surgery. Collectively, these findings shed a new light on the potential role of intestinal barrier compromise during major surgery, which was deduced from numerous animal studies, but now for the first time reported in relatively healthy children and adolescents undergoing major (non-abdominal) surgery. Furthermore, these results indicate a need to re-examine currently accepted criteria of haemodynamic parameters, both regarding the use of ECC and accepted systemic hypotension, in patients undergoing major surgery.

True, the presence of intestinal damage does not show any cause-and-effect relationship with the development of sepsis or multiple organ failure (MOF). Moreover, intestinal damage may be part of more generalized tissue damage with epithelial barrier dysfunction in lung, liver and kidney [16,17]. These studies are however the basis for further research to clarify the onset of intestinal damage, since direct measurement of intestinal damage has shown interesting results. In addition, assessment of intestinal epithelial damage in patients with sepsis or undergoing major surgery may have important clinical implications. Evaluation of intestinal tissue damage in the early phase of sepsis is an adequate predictor for survival. Furthermore, the adequacy of treatment of circulatory failure in sepsis is currently monitored using indirect parameters of tissue perfusion and oxygenation. However, these parameters do not reflect the

actual defects in (peripheral) tissue perfusion and subsequent tissue damage. Assessment of plasma I-FABP levels offers the possibility to monitor the presence of intestinal epithelial cell damage as a consequence of splanchnic hypoperfusion, which could help to monitor treatment directed at restoration of peripheral perfusion and prevention of organ damage. Further studies are needed to clarify the diagnostic potential of assessment of plasma I-FABP in monitoring the treatment of sepsis in the acute phase and during follow-up.

As reported in the chapter 4, altered splanchnic perfusion was observed during major surgery and in critically ill patients, which was related to the development of gut wall integrity loss. Therefore, the **final aim** of this thesis was to set up a model, which enabled us to study the pathophysiological consequences of controlled intestinal ischemia-reperfusion (IR) in detail in man. Moreover, acute mesenteric ischemia is a disease entity itself, which is accompanied by high morbidity and mortality rates because of its aspecific presentation and the lack of accurate markers. A human model for intestinal IR potentially would also give the opportunity to study new diagnostic tests. A pancreatico-duodenectomy is a procedure in which a variable length of jejunum is removed. This enabled us to study IR induced cell damage in a harmless human jejunal IR model. Isolated jejunum (6 cm) was subjected to 30 minutes ischemia followed by reperfusion, which is described in chapter 5, paragraph 2.

Mean (SEM) arteriovenous concentration gradients of I-FABP across the jejunum revealed rapid epithelial cell damage, which was dependent on the mannose-binding lectin (MBL) genotype of the patient (chapter 5, paragraph 3 and 4). Overall, I-FABP release significantly increased from 290 (46) pg/ml before ischemia towards 3997 (554) pg/ml immediately after ischemia ($p<0.001$) and declined gradually to 1143 (237) pg/ml within 1 hour reperfusion ($p<0.001$). However, in patients with MBL null alleles no mucosal cell damage, assessed by arteriovenous concentration gradients of I-FABP, was observed immediately after 30 minutes of ischemia, while the highest increase of I-FABP was found in the MBL homozygous wildtype individuals. Directly after ischemia the intestinal epithelial lining was microscopically normal in all patients, while subepithelial spaces appeared at the villus top, caused by contraction of the basement membrane, which demarcates epithelium from lamina propria. However, after 25 minutes reperfusion, apoptosis, visualized by M30 immunostaining, was observed at the villus top accompanied by shedding of mature epithelial cells into the lumen and loss of I-FABP staining of epithelial cells, indicating membrane integrity loss. Interestingly, within 60 minutes



reperfusion the epithelial barrier resealed, while debris of apoptotic, shedded epithelial cells was observed in the lumen. At the same time, both apoptosis and inflammation were absent in intact epithelium (chapter 5, paragraph 5).

In conclusion, this is the first human study to clarify jejunal IR induced cell damage and repair and its direct consequences. It reveals a unique, endogenous clearing mechanism for injured enterocytes upon 30 minutes of ischemia: rapid detachment of damaged apoptotic enterocytes into the lumen. This process is quickly followed by repair of the epithelial continuity, resulting in a normal epithelial lining, which is hypothesized to be a key factor in the prevention of the development of inflammation. Our results might explain why the gut can tolerate 30 minutes of ischemia in events as exercise, trauma, sepsis, repair of a (thoraco-)abdominal aneurysm of the aorta, and cardiopulmonary bypass. It is of importance to understand the maximal time of ischemia, which the gut can bear by utilization of its repair mechanism for injured cells.

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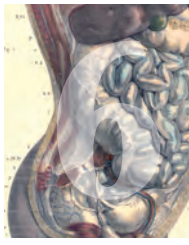

Nederlandse samenvatting

Dit proefschrift gaat ten eerste over het herkennen van darmziekten en ten tweede over de rol van de darm tijdens grote chirurgische ingrepen en bij ernstig zieke patiënten op de intensive care unit.

De voornaamste functie van de darm is de opname van voedingsstoffen. Daarom moet de darmwand doorlaatbaar zijn voor voeding, nodig voor energie en als bouwstof. De inname van voedsel gaat echter ook gepaard met de blootstelling van de darm aan een grote hoeveelheid lichaamsvreemde voedingsstoffen en micro-organismen. De tweede functie van de darm is dan ook om een effectieve barrière te vormen tegen deze potentieel schadelijke inhoud van de darm. Er bestaan vele mechanismen om deze barrière in stand te houden, welke besproken worden in hoofdstuk 1, paragraaf 3. Er zijn situaties waarin de barrière verstoord wordt en dit leidt dan vaak tot darmziekten en mogelijk tot andere gevaarlijke aandoeningen als bloedvergiftiging. Het is voor artsen soms lastig om vast te stellen of een darmziekte aanwezig is bij een patiënt die zich presenteert met buikklachten. Dit komt, doordat veel ziekten gepaard gaan met buikklachten, ook al is de darm er niet bij betrokken. Hierdoor is de diagnostiek van darmaandoeningen vertraagd en dientengevolgde ook de correcte behandeling, hetgeen leidt tot hogere morbiditeit en mortaliteit. Er is derhalve behoefte aan laboratoriumtesten die duidelijk maken of de darm beschadigd is. In aansluiting op deze diagnostische uitdaging, wordt de nabehandeling van darmziekten soms ook gehinderd door de afwezigheid van een niet-invasieve, snelle diagnostische test, die kan vaststellen of de therapie, gericht op herstel van de darmschade, succesvol is. In dit proefschrift werd een aantal potentiële testen geëvalueerd, die met name schade aan de darmcellen die de barrière vormen aantonen. Onderzoek naar markers voor myocardischemie gaf aanknopings-punten waaraan markers voor darmschade moesten voldoen. Troponine T is daarbij het meest treffende voorbeeld. Het is een klein eiwit dat karakteristiek voorkomt in het doelorgaan (hart) en snel de cel verlaat bij schade. Analoog hieraan komen in de darm eiwitten tot expressie, die behoren tot de familie van fatty acid binding proteins (FABP's). FABP's hebben normaliter een functie in het transcellulaire transport van langeketenvetzuren en galzouten, zijn in het cytosol gelokaliseerd, in water oplosbaar en hebben een laag moleculair gewicht (14-15 kDa). Deze eigenschappen zorgen ervoor dat ze de cel gemakkelijk verlaten na verlies van de integriteit van de celmembraan en terechtkomen in de circulatie. In de darm komen de isovormen *intestinal* (I)-FABP, *liver* (L)-FABP en ileal-bile acid binding protein (I-BABP) voor. (Pilot-)studies van andere onderzoekers wezen uit dat necrose van de darm vroegtijdig vastgesteld kan worden door in plasma en/of urine eiwitten te

bepalen die afkomstig zijn uit het cytosol van darmepitheel. Om de plasma en/of urine concentraties van deze eiwitten te begrijpen, was inzicht nodig in de exacte lokatie van deze eiwitten in de darmwand. In hoofdstuk 2, paragraaf 2 wordt beschreven dat FABP's in de hele darm tot expressie komen en met name in de dunne darm. Microscopisch zijn deze eiwitten gelokaliseerd in de epitheelcellen. Bij patiënten met coeliakie, een ziekte die onder andere gekenmerkt wordt door epitheelschade door glutenovergevoeligheid, werd gevonden dat I-FABP en L-FABP concentraties in het bloed bruikbare markers zijn voor het vaststellen van met histologisch onderzoek geobjectiveerde darmschade. Ook bleken deze markers potentieel waardevol zijn in de evaluatie van het dieeteffect. Teneinde de klaringsdynamiek van FABP's te bepalen werd vervolgens peroperatief bij chirurgische patiënten bloed verzameld uit de aan- en afvoerende vaten van darmen, lever en nieren. Na analyse van pre- en postrenaal plasma werd vastgesteld dat FABP's door de nier werden geklaard (fractionele renale extractie: 28%); berekend werd dat de plasma halfwaardetijd elf minuten bedroeg (hoofstuk 2, paragraaf 3).

Hieruit wordt geconcludeerd dat er sterke aanwijzingen zijn dat circulerend en urinair FABP's waardevolle markers zijn om darmschade aan te tonen.

Vervolgens werden deze testen gebruikt om zuigelingen, verdacht van de ernstige darmontsteking necrotiserende enterocolitis (NEC), vroegtijdig op te sporen. NEC komt voornamelijk voor bij te vroeg geboren, fles-gevoede kinderen. Deze aandoening kent een hoog sterftecijfer: 20-40% van de kinderen met NEC overlijdt aan deze aandoening. Gebrek aan goede, betrouwbare (vroeg)diagnostische mogelijkheden is mede de oorzaak van de hoge mortaliteit en morbiditeit. Therapeutische modaliteiten worden daardoor te laat geïnitieerd. De belangrijkste bevinding is dat deze testen, die uitgevoerd kunnen worden in materiaal dat op niet-invasieve manier werd verkregen (te weten urine en faeces), in staat blijken om vast te stellen of zuigelingen die verdacht werden van NEC deze ziekte, daadwerkelijk hadden of niet (hoofdstuk 3, paragraaf 6 en 7). Dit is klinisch erg relevant, omdat zuigelingen zodoende voortaan sneller een adequatere behandeling kunnen krijgen.

Vervolgens werd onderzoek gedaan naar de hypothese, die voortkomt uit proefdierstudies en die stelt dat verlies van de darm barrière betrokken is bij het ontstaan van ontstekingsreacties, zoals kan plaatsvinden bij postoperatieve of posttraumatische complicaties, bloedvergiftiging (sepsis) en meervoudig orgaanfalen (multiple organ failure (MOF)). Deze proefdierstudies laten zien dat grote operaties of ernstig trauma leiden tot een verminderde doorbloeding van de darm, waarbij ontstekingsfactoren vrijkomen en de barrière van de

darmwand verloren gaat. Bovendien komen door celschade alarmsignalen uit het cytsoplasma van de cel vrij, die een ontstekingsrepons opwekken. Verlies van de darm barrière kan resulteren in de passage van schadelijke voedingsstoffen en micro-organismen en hun toxinen door de darmwand heen en het vrijkomen ervan in het lichaam. Dientengevolge kan een ongecontroleerde hevige ontstekingsreactie ontstaan, waardoor ziektebeelden als sepsis kunnen ontstaan. Voorgaand onderzoek naar bovenstaande hypothese in patiëntgebonden studies liet uiteenlopende resultaten zien. Daarom werd in dit proefschrift de vraag bestudeerd of de darm aangedaan is als gevolg van grote chirurgische ingrepen; de resultaten worden in hoofdstuk 4 gepresenteerd. Patiënten die op de intensive care unit terechtkwamen vanwege sepsis als complicatie na een grote buikoperatie bleken darmschade te hebben. Deze schade was gerelateerd aan de mate van doorbloeding van de darm. De hoeveelheid darmschade was een goede voorspeller voor de mortaliteit in deze groep patiënten. Twee studies waarin achtereenvolgens patiënten geïncludeerd werden die een langdurige operatie aan hun aorta of aan hun scoliose ondergingen, lieten zien dat darmschade en barrière verlies ontstond bij deze patiënten. De darmschade was gecorreleerd met doorbloedingsveranderingen van de darm en de erop volgende ontstekingsreactie.

Aangezien aangetoond werd dat de doorbloeding van de darm een belangrijke factor is voor de instandhouding van de barrière, werd een experimenteel model ontwikkeld waardoor de gevolgen van doorbloedingsstoornissen (ischaemie) van de darm in de mens bestudeerd konden worden (hoofdstuk 5). Daarenboven is darmischaemie een ziekte-entiteit, die ontstaat door thromboembolie, en die gepaard gaat met hoge morbiditeit en mortaliteit door de aspecifieke klinische presentatie en een gebrek aan accurate diagnostische mogelijkheden. In het nieuw ontwikkelde model voor darmischaemie kunnen derhalve ook nieuwe testen voor de vroegdiagnostiek van darmischaemie worden geevalueerd. In patiënten, die een pancreatico-duodenectomie ondergingen, werd gebruik gemaakt van het feit dat in deze procedure een variabele lengte dunne darm (jejunum) wordt verwijderd. Hierdoor ontstond een voor de patiënt risicoloos humaan jejunum ischaemie-reperfusie (IR) model. Geïsoleerd jejunum (6cm) werd onderworpen aan 30 minuten ischaemie, gevolgd door reperfusie. Arterioveneuze concentratiegradiënten van I-FABP over bestudeerd jejunum liet het ontstaan van epitheliale celschade zien, die afhankelijk was van het mannose-bindend lectine genotype van de patiënt. Direct na ischaemie was de intestinale epitheliale bekleding microscopisch intact, terwijl subepitheliale ruimtes ontstonden in de villus top door terugtrekking van de basaal membraan. Echter, na 25 minuten reperfusie werd massale afstoting van de mature epitheliale cellen in

het lumen waargenomen, gepaard gaand met apoptose. Vervolgens was de epitheliale continuïteit hersteld binnen 60 minuten reperfusie en apoptose afwezig. Darmbarrièrefalen, gemeten door de concentraties van afbraakprodukten van bacteriën over het geïsoleerde stuk darm, en een ontstekingsreactie werden niet waargenomen. Concluderend; dit is de eerste humane studie die intestinale IR geïnduceerde celschade en de directe consequenties opheldert, waarin een uniek, endogeen beschermingsmechanisme beschadigde cellen elimineert en het ontstaan van barrièreverlies of inflammatie voorkomt.