Citrulline and intestinal fatty acid-binding protein as biomarkers for gastrointestinal dysfunction in the critically ill

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Abstract

Currently there is no reliable tool available to monitor gastrointestinal function in the critically ill. Biomarkers are therefore of great interest in this field as the lack of monitoring tools impedes any interventional studies. The potential biomarkers citrulline and intestinal fatty acid-binding protein (I-FABP) are the present focus. Targeted literature searches were undertaken for physiology and pathophysiology, sampling, measurement methods and clinical use of citrulline and I-FABP as biomarkers of intestinal function and injury. Physiology and pathophysiology, specific aspects of sampling and different laboratory assays are summarized and respective pitfalls outlined.

Studies in animals and patients outside the ICU support the rationale for these biomarkers. At the same time, evidence in critically ill patients is not yet convincing, several specific aspects need to be clarified, and methodology and interpretation to be refined. We conclude that there are good physiological rationales for citrulline as a marker of enterocyte function and for I-FABP as a marker of intestinal injury, but further studies are needed to clarify whether and how they could be used in daily practice in caring for critically ill patients.

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Citrulline and intestinal fatty acid-binding protein (I-FABP) have been increasingly studied since the turn of the millennium as potential biomarkers of intestinal dysfunction [1–5]. Citrulline has been proposed as a marker of enterocyte (dys)function, whereas I-FABP is thought to be a promising biomarker of intestinal cellular damage reflecting ischaemia.

The present narrative review summarizes pathophysiological aspects and existing evidence evaluating clinical usage of these two biomarkers in critical illness based on explorative literature searches. Additionally, important details and pitfalls in sampling, laboratory measurement and interpretation of values are discussed.

CITRULLINE AS A MARKER OF ENTEROCYTE FUNCTION
Physiology and pathophysiology

Citrulline (CIT) is a non-essential amino acid which is not present in proteins, but which is intimately involved in the urea cycle as an intermediary of both glutamine and arginine. Citrulline is produced from glutamine in the mitochondria of mature enterocytes. Although important in hepatic metabolism, its net production is almost exclusively in the small intestine, from where (as enterocytes lack argininosuccinate synthase) it is released into the portal circulation. Unless liver function is profoundly disturbed, citrulline enters the systemic circulation at a very similar concentration, only being removed in the kidney by conversion to arginine. There is sparse evidence that amino acid levels are increased in acute liver disease as a result of catabolic breakdown of endogenous protein, from gluconeogenesis and from a failure of new hepatic protein synthesis. In animal models of acute liver failure plasma citrulline is elevated [6], this being most pronounced in animals with hepatic encephalopathy. In humans with hepatic coma, dramatically high citrulline levels in cerebrospinal fluid have been observed [7]. The changes in chronic liver disease are less pronounced, but circulating levels are high in animal models [8] and generally appear to be a little

elevated in stable human disease [9, 10]. In acute on chronic disease – at any rate in alcoholic hepatitis – the levels may then fall [11].

The vast majority of citrulline released by the intestine is taken up in the proximal convoluted tubules of the kidneys as a precursor for *de novo* arginine synthesis [12]. Therefore expectedly, renal failure is associated with higher circulating citrulline levels [13–16] with the highest values observed in patients with end-stage renal failure. Compared to controls with normal kidney function, citrulline levels are significantly higher in patients with kidney dysfunction with a creatinine clearance of less than 50 mL min^{-1} [14, 15]. Similar to other amino acids, citrulline is removed with renal replacement therapy. Citrulline plasma concentrations decrease more than 50% during an intermittent haemodialysis session [13, 17]. Little is known of citrulline loss in continuous renal replacement therapies, but it seems to be lower than that of other amino acids in paediatric patients treated with continuous venovenous haemodialysis [18].

Accordingly in patients with normal renal and hepatic function the circulating citrulline level may be considered a reproducible marker of enterocyte function. However, there are several steps in metabolism influencing its plasma concentration: 1) availability of glutamine as a precursor of citrulline; 2) function of enterocytes; 3) liver function; 4) metabolism in kidneys.

This may be the reason why plasma citrulline levels, successfully used in the monitoring of patients following small bowel transplantation, are not yet of proven value for wider application in patients with acute severe illness with an unstable metabolic state. Unfortunately the concentrations of citrulline in blood do not follow a clear bimodal pattern so as to permit confident diagnosis or exclusion of intestinal failure from the citrulline level alone.

Urinary citrulline levels have been shown to correlate with serum citrulline levels, but have not been investigated in critical illness [19].

Arguably the most important physiological role of citrulline is as a precursor for arginine, a nitric oxide (NO) donor. The importance of NO in sepsis and in critical care in general is acknowledged and supplementation with arginine has been considered logical in critically ill patients in order to raise nitric oxide levels and thus to combat oxidative damage. Although intracellular arginine concentrations are generally sufficient to saturate available NO synthase (NOS), the addition of extrinsic arginine can nonetheless enhance NO production, in part via activation of the CAT-2 arginine transporter, which is closely associated with NOS in cell membranes [20].

Therapeutic use of arginine has, after initial optimism [21–24], not been proven to be beneficial in the critically ill. Notably, most of the studies used arginine as a part of a package of 'immunonutrients', making it difficult to determine a specific effect of this amino acid [25]. Nonetheless, evidence overtly in favour of parenteral arginine is lacking, making it difficult to dismiss the trials of combination immunotherapy that appear to show harm in a context where intravenous monotherapy proved fatal to dogs [26].

Despite this, the concept of the NO donor is still valid and more recently citrulline has received attention as a source for arginine, it being known that, unlike arginine, which is largely extracted by the liver, citrulline passes on into the hepatic vein and thus into the systemic circulation and can arguably constitute a better arginine source than arginine itself [27, 28].

There is also reason to believe that citrulline might be of therapeutic potential in the critical care context. In vitro citrulline has positive effects on the immune response and reduces the effects of oxidative stress [29]. In studies of trained athletes improvements in performance and enhanced recovery can be elicited [30]. An anabolic effect in sarcopenia has been shown in animals [31] and this is also supported by recent human studies [32]. Citrulline as a therapeutic agent has been shown to be beneficial in pulmonary hypertension, improving left ventricular ejection fraction, improving endothelial function [33] and in the rare MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes syndrome) [34].

Sampling and measurement

In biological matrices, citrulline is most frequently measured as part of an amino acid profile. However, time-efficient and comprehensive quantification, of typically well in excess of the 21 proteinogenic amino acids, from complex biological matrices, continues to be a challenge due to their poor chromophore and fluorophore response, zwitterion functionality and diversity. Different methods for analysing amino acid profiles have been proposed, utilising pre- and post-column derivatisation of either their amine or carboxyl functional groups with ninhydrin, o-phthalaldehyde, phenyl isothiocyanate, alkyl chloroformate, dansyl chloride or butanolic hydrochloric acid, and more recently methods requiring no derivatization and detection of the native analytes.

The dedicated amino acid analyser, employing ion exchange and ninhydrin post-column derivatization, is still considered the established standard methodology for clinical diagnostic use. A major disadvantage of this measurement procedure is the long run time of typically 120 minutes and therefore the high total costs per sample [35].

However, in recent years much effort has been spent on developing alternative robust high-throughput methods [36–39]. Several analytical high performance liquid chromatography (HPLC) pre-column derivatization methods have been developed for amino acid quantification, coupling liquid chromatography with optical detection (ultra violet [UV], fluorescence) or mass spectrometry (MS) [39]. Liquid chromatography mass spectrometry (LC-MS) plays an important and increasing role as the detector of choice for HPLC, due to the selectivity which can distinguish analytes by their mass-to-charge ratio (m/z). LC-MS/MS has afforded gains of selectivity and sensitivity by utilising multiple reaction monitoring (MRM), whilst some investigators have utilised the specificity of high resolution time of flight (ToF) mass spectrometry [36]. Capillary electrophoresis (CE) with or without derivatization addition of ion-pair reagents to HPLC mobile phases and hydrophilic interaction chromatography (HILIC) [39] are other options to separate derivatized amino acids. Gas chromatography mass spectrometry (GC-MS), NMR and direct infusion have also been used for amino acid analysis [40]. No significant differences between blood samples collected into heparin or ethylenediaminetetraacetic acid (EDTA) or serum have been observed when stored for up to 7 days at 4°C [40]. For longer term storage, plasma samples precipitated with perchloric acid followed by neutralisation with potassium carbonate prior to storage at −80°C showed an undetectable reduction in concentration after 6 months and a 4.5% reduction after 5 years [41]. Storage of frozen plasma without any additional preparation may however result in significant changes due to ongoing hydrolysis of proteins [42]. Available studies have used different handling of samples, possibly leading to different results. However, currently there is no consensus preferring one method over the other. Additionally to described methods, commercially available enzyme-linked immunosorbent assay (ELISA) kits are available for the specific quantitation of citrulline. However, whilst requiring less expensive analytical instrumentation to perform the analysis, the requirement to perform the analysis in duplicate can make this an expensive alternative for large studies.

Discussion of existing evidence

Studies in patients with gastrointestinal diseases have shown that plasma citrulline levels are correlated with bowel length in short bowel syndrome patients but also with disease severity in enteropathies such as Crohn's or coeliac disease [1, 3, 5]. Moreover, citrulline levels may increase with treatment in coeliac disease [3, 5]. These observations suggest that citrulline may reflect enterocyte function which is not limited to enterocyte mass, and give a rationale to study citrulline in critically ill patients with relatively unaffected enterocyte mass but impaired enterocyte function. This rationale is also supported by experimental and clinical data showing that citrulline levels decrease with toxic or radiation-induced injury to the small intestine [43–45].

Importantly, assessment of post-absorptive levels of citrulline is suggested in patients with gastrointestinal diseases [1, 46, 47], showing also a correlation with absorption capacity measured with tracer ingestion methods [46]. In critical illness, specific assessment of a post-absorptive state is difficult, because patients are either not fed or receive enteral nutrition in a continuous manner.

A recent study in healthy men shows that plasma citrulline levels increase during exercise in a normal hydrated state, but not during exercise when dehydrated [48].

To overcome the problem of elevated citrulline levels in renal failure patients (see also 2.1) adjustment of the thresholds for acceptable citrulline has been suggested for patients with intestinal transplant and chronic renal failure [14].

Studies in critically ill patients have generally shown low citrulline levels [4, 49–54]. Piton described initially (during the first 48 h after ICU admission) decreasing plasma levels that recover towards the end of the first week [4].

Several studies have shown an association between citrulline levels and the outcome of critical illness [4, 51, 52], whereas the direction of association (causality) remains unclear. However, the most remarkable decreases in citrulline levels have been observed in the most severely ill patients, including those with septic shock or receiving catecholamines for another reason, having a poor neurological outcome after cardiac arrest, and in those who ultimately do not survive [4, 51, 52].

A few small studies have shown that low citrulline levels correlate with clinical signs of intestinal dysfunction [54, 55]. At the same time, citrulline levels were not found to correlate with small bowel capacity to absorb glucose in the critically ill [53] or with overall intestinal energy absorption capacity [56]. A very small observation on metabolomics demonstrated that initially very low (compared to healthy) plasma citrulline levels increased during enteral nutrition but did not change during parenteral nutrition in critically ill patients [57].

Based on current evidence it is not possible to clearly conclude whether citrulline is suitable for specific monitoring of GI (dys)function in critical illness. The main remaining questions are: 1) whether low levels largely reflect metabolic derangement related to a severe condition and not necessarily

absorption capacity in critical illness; 2) how to create a scoring system (using citrulline as a marker of GI function) in the absence of a gold standard for monitoring GI function and absorption in critical illness; 3) how to deal with elevated citrulline levels due to renal dysfunction, often present in critical illness and also insufficiently described with currently available parameters (serum creatinine and urine output); 4) which other factors (e.g. fluid status, adrenergic state, concomitant diseases) influence citrulline levels; 5) how to optimize rather complicated and expensive measurement of citrulline (see 2.2) for larger studies to answer the previous questions.

The theory about GI dysfunction being a part and possibly also a motor of multiple organ dysfunction syndrome has been widely discussed [58, 59]. Existing studies show that critical care patients demonstrating signs of GI dysfunction are more severely ill than patients without GI dysfunction [60–62]. However, clinical presentation of several concomitant GI symptoms and/or feeding intolerance during critical illness is independently associated with impaired outcome [60–62]. Despite several attempts, there is still no reliable tool to monitor GI (dys)function in critical illness [59]. Descriptive grading of acute GI injury has been proposed [63], but is 1) based on subjective evaluation of GI symptoms and tolerance of enteral nutrition, 2) not applicable retrospectively. Therefore, citrulline as a possible measurable and quantifiable marker for GI dysfunction is of great interest.

Taken together, current evidence supports the hypothesis that citrulline may reflect enterocyte function in critical illness, but does not confirm its role as a marker of GI (dys)function in daily clinical practice. Further studies are needed to establish whether citrulline could be used to test the effect of any interventions on GI function or to help in guiding clinical decisions (e.g. on nutrition). Citrulline dynamics need to be better related to clinical signs of GI dysfunction but also studied together with methods evaluating absorption of different nutrients.

I-FABP AS A MARKER OF CELLULAR DAMAGE
Physiology and pathophysiology

I-FABP is a small (15 kDa) cytosolic protein that is exclusively present in mature epithelial cells of the small and large intestine. More specifically, I-FABP is found mainly in enterocytes of the duodenum and jejunum and, to a much lesser extent, in the ileum and colon [64]. At least 8 more proteins in the FABP family have been discovered and numbered – I-FABP is also known as FABP2 [65]. FABPs participate in the uptake, metabolism and transport of long-chain fatty acids [66].

In health, enterocytes undergo programmed apoptosis and are shed into the intestinal lumen with no extracellular release of intracellular contents. Therefore, I-FABP plasma levels in apparently healthy humans have been found to be undetectable or very low [64, 67, 68] and reflect the natural enterocyte turnover. Due to their low molecular weight, fatty acid binding proteins are excreted rapidly (plasma half-life = 11 min for L-FABP [69]) unchanged via the kidneys.

Plasma I-FABP levels are expected to be elevated in renal dysfunction. A pilot study by Okada *et al.* demonstrated a twofold increase in I-FABP in patients with end-stage renal disease compared to controls with normal renal function [70]. Being a small water soluble protein, I-FABP was readily removed during haemodialysis, yielding post-dialysis levels similar to controls [70]. More data are needed on the effects of renal failure and extracorporeal therapies on I-FABP levels to aid interpreting its levels in critically ill patients.

When enterocyte membrane integrity is breached, I-FABP is released into the extracellular space and enters the circulation. An additional mechanism of I-FABP uptake to the vasculature was proposed by Schellekens *et al.*: after an ischaemic insult that has caused substantial necrosis and shedding of enterocytes from villi, causing a disruption in the gut barrier, I-FABP may be taken up from the lumen through an incomplete mucosal barrier [71]. This is suggested by their findings that after longer durations of ischaemia, I-FABP release still continues hours after cessation of ischaemia while there is histological proof of continued mucosal barrier disruption [71].

Mature enterocytes form the epithelium along and on top of intestinal villi, a location known to be highly susceptible to ischaemia. As such, I-FABP has for long been of interest as a marker of ischaemic intestinal epithelial damage. Indeed, elevations in systemic I-FABP levels have been shown in various settings of intestinal ischaemia: in healthy adults undergoing submaximal physical effort [68, 72]; after major but non-abdominal surgery [73–75]; trauma with and without abdominal lesions [67, 76]; after cardiac arrest [50, 52]; sepsis [77]; acute mesenteric ischaemia of both occlusive and non-occlusive types [78–80]. Previous research suggests that systemic levels of I-FABP become elevated after minor ischaemic events with reversible injury of questionable clinical significance as well as in cases with full-thickness necrosis of the intestine and associated complications.

Elevated I-FABP plasma levels have also been observed in conditions with other mechanisms of enterocyte damage, e.g. immune-mediated epithe-

lial inflammation and villous atrophy in coeliac disease [81, 82] and the complex process of mucositis seen with some radiotherapy and chemotherapy schedules [83, 84].

Sampling and measurement

Measurement of I-FABP by enzyme-linked immunosorbent assay (ELISA) is the most commonly used analytical technique. This assay involves the use of a monoclonal antibody specific for human I-FABP pre-coated onto the microtitre plate wells to detect endogenous I-FABP in samples, then by the addition of an enzyme-labelled polyclonal antibody to capture and form a sandwich complex. Following a series of washing to remove any unbound antibody, a substrate solution is used to activate colour development that is in proportion to the amount of bound I-FABP. The intensity of the colour (absorbance) is then measured by a spectrophotometer at a specific wavelength. Immunoassays are reliable, provided sufficient quality control measures are in place. The typical assay reproducibility (CV: coefficient of variation) is < 15%, which can be further improved by the use of a fully automated plate processor to increase throughput and minimise human error. However, the accuracy of I-FABP measurements has been reported to be suboptimal [85]; the quality of the antibody employed in immunoassays is highly dependent on its originating animal species and the purification process; variability in antibody production can affect the specificity of the antibody and reduce the ability of the assay to distinguish between the subtypes of FABP. For these reasons, I-FABP measurement is not routinely performed for diagnosis. Commercial immunoassays can nonetheless provide adequate detection of I-FABP in serum and urine. It is important to understand the limitations of the immunoassays employed, in particular their sensitivity and specificity, and to use them only for the predetermined purposes. Batch consistency can be ensured by using the same lot of antibody, with uniform procedures for operational processes, equipment and detection instruments. Importantly, different measurement kits with very different reference values have been used in the available studies of critically ill patients [86].

Summary of existing evidence

I-FABP plasma or serum levels have been studied in a very broad range of clinical conditions, summarized below.

Elevated I-FABP in plasma, serum or urine has been demonstrated in the presence of intestinal ischaemia including necrotizing enterocolitis in neonates, and in several other abdominal pathologies (abdominal trauma, abdominal surgery, abdominal sepsis, acute pancreatitis, intestinal malignancies, coeliac disease, sclerosing cholangitis).

Intestinal ischaemia

Rapid and non-invasive diagnosis of intestinal ischaemia remains challenging, so there is great interest in studying I-FABP in this context.

In a human translational ischaemia-reperfusion model (resected jejunum studied in vitro), I-FABP release into blood stream occurred early during small bowel ischaemia [71]. I-FABP changes appear to occur very fast, an obvious increase occurring within 30 minutes of ischaemia, declining within the next 30 minutes of reperfusion, with full recovery within 120 minutes [87].

Such fast changes make it perfect for research, but possibly less perfect for clinical use, where the exact time of development of ischaemia cannot be captured. Indeed, sufficient diagnostic value of serum and urinary I-FABP was only confirmed in the early stages of mesenteric ischaemia in one study in 43 critically ill patients with suspected mesenteric ischaemia [88]. Moreover, it was hypothesized that if ischaemia is progressing transmurally and (re)perfusion of the mucosa does not occur, I-FABP is possibly no longer flushed out, leading to 'falsely' low concentrations [88].

Another small study in patients with suspected acute mesenteric ischaemia demonstrated significantly higher urinary I-FABP in patients with confirmed ischaemia ($n = 13$) vs. internal controls (ischaemia not confirmed, $n = 5$), whereas the difference in serum I-FABP was not significant [89].

In the third study comparing patients with confirmed vs suspected but not confirmed intestinal ischaemia, serum I-FABP levels were not significantly different between these two groups whereas lactate levels were [90].

There is increasing evidence suggesting I-FABP as a possible diagnostic tool in infants with suspected necrotizing enterocolitis (NEC) [91–93]. The magnitude of the I-FABP response correlates with disease severity and the extent of surgical resection required [94], but perhaps surprisingly no association can be confirmed between I-FABP levels and the incidence of bloodstream infection in these patients [95]. Two systematic reviews support a role for I-FABP in diagnosis of I-FABP, but recognize heterogeneity of data collection and relatively low study quality measures [96, 97].

Other abdominal conditions

Patients with intestinal malignancy are found to have elevated levels of I-FABP pre-operatively, and regardless of the underlying diagnosis major

abdominal surgery is responsible for its (further) elevation [98]. In patients with abdominal sepsis, significantly higher levels of I-FABP are found compared to those with a comparable severity of sepsis caused by pneumonia [98]. Unfortunately this distinction was insufficiently binary to be clinically useful in differentiating abdominal from pulmonary infection [98]. This interpretation is also muddied somewhat by another study which showed that urinary I-FABP is predictive of disease severity and poorer survival in patients with pneumonia as the primary diagnosis [99].

Two studies of patients with abdominal trauma reached some similar conclusions, with clearly raised I-FABP levels in these patients compared to controls or those with non-abdominal injuries, and indeed a positive correlation with the day 0 and day 1 SOFA scores [100] and Injury Severity Scores [76], but failed to show a correlation with sepsis or mortality [100].

In children with intestinal intussusception I-FABP levels are strongly associated with the presence of intestinal necrosis [92]. The authors were appropriately reserved about the clinical value of this given that a cut-off of 1538 ng mL^{-1} yielded sensitivity and specificity of only 64% and 88% respectively. Importantly, the group of normal controls had a value of 478 ng mL^{-1}, suggesting a major difference in calibration from other investigators' assays [101].

I-FABP is elevated in acute pancreatitis and significantly more so in patients with more severe forms of acute pancreatitis (0.74 vs. 0.40 ng mL^{-1}: $P = 0.03$), but this observation is insufficient alone to provide robust prognostic information [102]. The I-FABP levels were however highly informative in the now infamous study of probiotics in acute pancreatitis, in which elevation was strongly associated with the later onset of bacteraemia, infected pancreatic necrosis and multi-organ failure (P values all under 0.05), and increased I-FABP appeared strongly linked to more extensive enterocyte damage and to more serious bacterial translocation [103].

In ICU patients with acute gastrointestinal injury (AGI) [55] there was a close correlation between the severity of AGI and the level of I-FABP, whereas a prognostic association was not found [104]. Piton and Capellier [105] suggested I-FAPB as a marker of intestinal mucosal damage to identify patients at the highest risk of bacterial translocation and the systemic inflammatory response syndrome.

Septic shock and endotoxaemia

Elevated I-FABP was associated with significantly higher 28-day mortality in septic shock ($P < 0.05$), but the odds ratio of 1.036 suggests only very limited clinical value [106]. The authors propose however a threshold of 19 ng mL^{-1}, above which their data predict a 28-day mortality of 29%, compared to only 2% with lower levels ($P = 0.011$) [106].

Positive correlations with endotoxin levels have been demonstrated for urinary I-FABP levels after successful resuscitation from cardiac arrest [50] and for plasma I-FABP levels in heat shock [107]. The urinary I-FABP levels peaked 24 hours or more before those of the endotoxin, suggesting that there may be some predictive value [50].

In a study of 19 children with meningococcal septicaemia, patients in whom I-FABP returned to normal within 12 hours survived, whereas in non-survivors I-FABP remained elevated through to the time of death [77].

Cardiovascular conditions

In cardiogenic shock or severe acute cardiac failure patients in the highest quartile (> 0.59 ng mL^{-1}) of serum I-FABP on admission day had 2.5 times the 30-day mortality of those in the lowest quartile, independently of demographics or use of inotropes [108]. Moreover, if the initial level exceeded 10.2 ng mL^{-1} the mortality rate was 88.9% [108]. This supports earlier data from Kitai *et al*. [109], who showed similar numerical linkage in acute decompensated cardiac failure but in whose patients the changes were not statistically significant. After cardiac surgery those with higher I-FABP had significantly more complications, including higher frequencies of multiple organ dysfunction ($P < 0.01$), infectious complications ($P < 0.01$) and a longer ICU stay ($P < 0.01$): in each case the median I-FABP was roughly twice as high in the adversely affected group [74]. I-FABP rose in all of a series of patients undergoing aortic surgery (thoracic in 55 and exclusively abdominal in 25), and 4 patients with the highest levels died from intestinal ischaemia on day 2 or day 3 (sensitivity and specificity both in excess of 98%) [80].

Other observations

Blood transfusion in premature infants is followed by a reproducible rise in I-FABP [110], whereas the mechanism remains unclear.

Taken together, despite some promising results, available studies have not been able to confirm reliability of I-FABP plasma, serum or urinary levels as a marker of intestinal injury in everyday clinical practice at the time. Different laboratory assays and reference levels (see also 3.2) are complicating synthesis of available evidence [86]. I-FABP is a promising biomarker, but future studies need to establish whether it will be usable as a marker of intestinal injury.

SUMMARY OF STRENGTHS AND WEAKNESSES

Strengths and weaknesses of citrulline and I-FABP as biomarkers for gastrointestinal function/injury are summarized in Table 1.

TABLE 1. Strengths and weaknesses of citrulline and I-FABP as biomarkers for gastrointestinal function

	Strengths	Weaknesses	Pitfalls in interpretation
Citrulline as a marker of enterocyte function in critically ill	– (Patho)physiological rationale – Non-invasive diagnostic tool – Numerical value permitting dynamics to be followed	– Not clear how to interpret the results (could a specific value trigger a specific therapeutic approach?) – Influenced by several factors: • glutamine availability • liver function • renal function • sample handling and measurement method – Expensive labour-intensive assay	Pooling results with different: – material (plasma vs. serum) – sample preparation (deproteinization before or after freezing) – laboratory assay
I-FABP as a marker of cellular damage (included due to mesenteric hypoperfusion)	– (Patho)physiological rationale – Non-invasive diagnostic tool – Numerical value permitting dynamics to be followed	– Not clear how to interpret the results (could a specific value trigger a specific therapeutic approach?) – Influenced by several factors: • timing of the measurements • renal function • measurement method – Values are increased in many conditions that may occur concomitantly in critical illness – Expensive labour-intensive assay – Use in urgent clinical settings, e.g. acute mesenteric ischaemia is hindered by the turnover time of current kits	Pooling results with different: – material (plasma vs. serum) – laboratory assay Interpreting one value may be misleading due to magnitude of ischaemia (no flush-out) and/or timing of the measurement (fast changes with ischaemia-reperfusion)

FUTURE STUDIES

Unification of sample handling and measurement methods is desirable to achieve comparable results in different studies. Studies comparing different methods in repeated measurements should be undertaken to identify the methodology least prone to mistakes. Studies with concomitant measurement of mesenteric perfusion and GI absorption should confirm the physiological rationale and measurement methods for these biomarkers. The role of renal dysfunction in dynamics of these biomarkers should be investigated in detail to allow correct interpretation of the results and selection of patients who may profit from measurements.

Thereafter, studies need to be undertaken to assess dynamics of biomarkers targeted to specific patient groups (e.g. critically ill patients with shock), specific events (e.g. GI symptoms and/or complications) and interventions (e.g. enteral nutrition). Only then can cut-off values for specific events or interventions be validated. Taken together, there is still a long way for these biomarkers to advance to daily practice.

CONCLUSIONS

There is a good physiological rationale for citrulline as a marker of enterocyte function and for I-FABP as a marker of intestinal injury. This rationale is supported by animal studies (mainly for I-FABP) and in patients outside the ICU (mainly for citrulline), but less so in critically ill patients. In the light of the profound need for tools to assess GI function and GI injury in critically ill patients, these biomarkers deserve further investigations.

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