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Review

Plasma citrulline level as a biomarker for cancer therapy-induced small bowel mucosal damage

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Regimen-related mucosal toxicity is extremely common following cytotoxic chemotherapy and radiotherapy. Mucositis is as an important determinant of the inflammatory response and infectious complications in cancer treated patients. Most assessment scales for mucosal damage are focussed on oral mucositis, since it is easy to evaluate. Measuring gastrointestinal musocal damage objectively remains difficult because it cannot be seen directly or readily detected. One of potential non-invasive biomarkers of gastrointestinal mucosal damage is plasma citrulline level. Citrulline is an amino acid produced by small bowel enterocytes. Low concentration of free circulating citrulline signifies severe intestinal mucosal damage in humans with nonmalignant disorders, such as villous atrophy-associated diseases, short bowel syndrome, Crohn's disease, and is used in follow-up after small bowel transplantation. The plasma citrulline level is a reliable and objective biochemical marker of enterocyte mass and function in humans, and therefore can be used to monitor enterocyte toxicity resulting from chemotherapy and radiotherapy during anticancer therapy in patients with severely disturbed gut integrity.

Key words: biomarker, citrulline, mucositis, mucosal injury, toxicity

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INTRODUCTION

Mucositis is a frequent, clinically significant and sometimes dose-limiting event elicited by chemo- or radiation cancer therapy. Mucous membrane damage means a disruption of the body's natural barrier against infection. Inflammation and loss of mucosal integrity increase the risk of local bacterial, fungal, and viral infections, which predisposes immunosuppressed patients to developing sepsis (Rubenstein *et al.,* 2004). Mucositis is the culmination of a series of biologically complex and interactive events that occur in all tissues of the mucosa. Understanding the pathobiology of mucositis, its incidence, and scoring is essential for archiving progress in research on and care of this common side-effect of anticancer therapies. Based on anatomical and functional differences between segments of the gastrointestinal tract, two types of mucositis have been identified: oral mucositis (OM) and gastrointestinal mucositis (GIM). The events that take place in the gut are almost certainly more complicated than those occurring in the oral cavity, since the gastrointestinal tract is intrinsically more complex in terms of its function. There is a striking lack of reliable data on the incidence of gastrointestinal mucositis. Cancer therapy-induced damage to the intestinal mucosa results in intestinal crypt cell apoptosis, villous atrophy, and enterocyte mass reduction (Keefe *et al.,* 2000). The clinical presentation of gastrointestinal mucositis includes nausea, vomiting, watery diarrhea with blood or mucus, and abdominal cramps. This leads to impaired absorption of digestion products. Clinical consequences of mucositis include dehydration, malnutrition, potentially life threatening infections, and even increased mortality (van der Velden *et al.,* 2010). The small intestine comprises three functionally distinct segments: the duodenum, jejunum, and ileum. The structure of the mucous membrane lining the intestine is similar in all three parts. The entire intestinal surface is covered with finger-like protrusions called intestinal villi. Their role is to increase the absorptive surface of the intestine. The cells covering the villi — enterocytes — include intestinal absorptive cells. Enterocytes are suited for contact digestion at the level of microvilli and absorption of the end products (Helander & Fändriks, 2014). The small-intestinal mucosa is a hierarchical tissue that consists of three types of cells (Potten, 1998): stem cells of high proliferative activity, incompletely differentiated transitional cells, and mature, fully differentiated cells of the mucous membrane (Schofield, 1983). The intestinal epithelium undergoes continuous regeneration. From the sites of cell renewal in intestinal crypts, the cells move along the lateral surface of the villi and exfoliate at the top (Potten & Loeffler, 1987). The endothelial renewal cycle lasts approximately 3–5 days. Anti-cancer treatment both damages and depletes stem cells and transient cells, with a consequent inability of the body to compensate fully for the deficiency resulting from exfoliation of differentiated cells. This results in morphological changes of the mucosa in the form of inflammation.

Citrulline is an endogenous non-protein amino acid. This amino acid, naturally occurring in the body, has been known since 1930, when Wada first isolated it from watermelon juice (the term citrulline derives from *citrullus*,

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Abbreviations: ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; CPS, carbamoyl phosphate synthase; GALT, gut-associated lymphoid tissue; GIM, gastrointestinal mucositis; HTs, hierarchical tissues; MBI, mucosal barrier injury; NOS, nitric oxide synthase; OCT, ornithine carbamoyltransferase; OM, oral mucositis; P5CS, pyrroline-5-carboxylate synthase; PO, proline oxidase; ROS, reactive oxygen species

the Latin word for watermelon) (Wada, 1930). Diet is a poor source of citrulline and its main source in the body is endogenous synthesis. *De novo* formation of citrulline takes place in epithelial absorptive cells of the small intestine — enterocytes (Windmueller and Spaeth, 1981; Crenn *et al.,* 2000; Curis *et al.,* 2005). The substrates for intestinal citrulline synthesis are amino acids derived from the diet. Citrulline formed in the intestine is released into the bloodstream, increasing the amount of blood-borne amino acids. The tissue distribution of the enzymes involved in citrulline metabolism demonstrates three metabolic pathways of free circulating citrulline: arginine biosynthesis, arginine–citrulline–nitric oxide cycle and urea cycle. A total of 97.5% of normal adult Caucasians with healthy intestinal mucosa function and no renal dysfunction have citrulline concentrations between 30 and 50 μmol/L, with a mean of 40 μmol/L (Crenn *et al.,* 2003). Because citrulline is synthesized almost exclusively by the intestine, its plasma level has been identified as a biomarker of the functional small bowel enterocyte mass (Crenn *et al.* 2003). Injury to the small intestine can be measured by the decline in circulating citrulline levels, with lower values corresponding to more severe small intestinal damage. Plasma citrulline assays have recently emerged as the best diagnostic tool, regardless of the etiology of the intestinal mucosal disease. The earliest clinical evidence of this idea was obtained in 2000 by Crenn and oworkers. Since that pioneering study, other investigators have demonstrated clinical correlation between the serum citrulline level and the degree of small-intestinal mucosal damage in the course of various diseases: short bowel syndrome and bowel surgery, villous atrophy syndrome, Crohn's disease, intestinal toxicity of chemotherapy (Blijlevens *et al.,* 2004) and radiotherapy (Lutgens *et al.,* 2003). Plasma citrulline levels are a quantitative biomarker of enterocyte mass and functional enterocyte metabolic mass but not of the digestive function *per se* (Curis *et al.,* 2007; Crenn *et al.,* 2008). Regular assessments of citrulline levels allow for monitoring of small-intestinal function. The only limitation of this correlation is significant renal failure (creatinine clearance <30 mL/min) (van de Poll *et al.,* 2004; Crenn *et al.,* 2008).

THE STRUCTURE OF SMALL INTESTINAL MUCOSA

Stretching from the pyloric orifice at the junction with the stomach to the ileocecal valve at the junction with the colon, the small intestine is the longest segment of the gastrointestinal tract. The length of the small intestine can be divided in to three functionally separate parts: the duodenum, jejunum, and ileum. The duodenum can be distinctly differentiated, while there is no distinct boundary between the jejunum and the ileum. The functions of the small intestine are:

— digestion — exposing chyme to digestive enzymes

— absorption — transporting the digested foods across the epithelium

— moving chyme towards more distal parts of the gastrointestinal tract

These functions are supported by digestive secretions of the liver and pancreas, whose efferent ducts open into the small intestine.

The small intestinal wall is composed of four layers: the mucosa, submucosa, muscularis externa and serosa. The mucosa is responsible for the chemical, while the muscularis mucosa — for the mechanical component of digestion. To ensure chyme exposure to digestive enzymes and to facilitate nutrient absorption, the functional area of the intestine should be as large as possible. This is best achieved with a tubular shape, with the length of the tube proportionally increasing its resorptive area. The length of the intestine is not the only factor determining its absorptive surface area, with the mucosal structure of the small intestinal wall facilitating an increase of its active surface for the purpose of absorption. The inner surface of the small intestine is covered with circular folds, or plicae circulares. They have the form of long flaps of the mucosal and submucosal layers, positioned perpendicularly to the long axis of the intestine, and projecting into the intestinal lumen. Increasing the absorptive surface area 2–3-fold, the plicae circulares are the first component of small intestinal absorptive functionality. The entire surface of the intestinal mucosa is covered with finger-like protrusions, or intestinal villi. The purpose of the intestinal villi is to further increase the absorptive surface area of the intestine, by a factor of approximately 10 (yielding a 30-fold increase in total). There are 20–40 villi per 1 mm^2 of intestinal surface. The epithelium covering the villi consists predominantly (95%) of tall, columnar, brush-bordered epithelial cells called enterocytes, or intestinal absorptive cells. The luminal surface area of the small intestine is covered with minute, densely and regularly distributed microvilli. Enterocytes are adapted for contact digestion within the microvilli and absorption of the end products of digestion. Microvilli covering the surface of enterocytes are a structure most suited for this purpose. The presence of microvilli increases the mucosal surface area of the intestine further by a factor of approximately 20–30 (400–900-fold increase in total) (Gray, 2000; Sherwood, 2006; Helander & Fändriks, 2014).

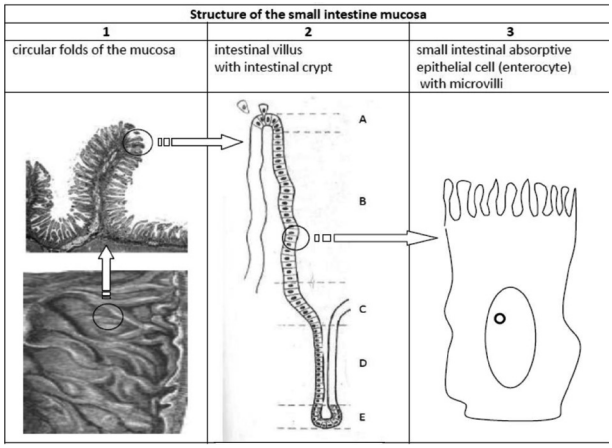

Figure 1. Structure of the small intestinal mucosa
Schematic representation of the small intestinal mucosa. Left column (**1**) — the circular folds (also, plicae circulars) — large valvular flaps projecting into the lumen of the small intestine covered with intestinal villi. This scheme incorporates fragments of figures from a public domain edition of Gray's Anatomy from the classic 1918 publication. Middle column (**2**) — longitudinal croos-section of an intestinal villus with the intestinal crypt, with five functional zones of the intestinal epithelium: **A** — exfoliating epithelial cells, **B** — functional, **C** — maturation, **D** — proliferative, **E** — stem cells. Right column (**3**) — entrocyte with microvilli.

Numerous crypt outlets can be found at the base of intestinal villi. Intestinal crypts are lined with simple columnar epithelium continuous with that of the intestinal villi. The simple epithelium lining the gut is organized into millions of contiguous crypts of Lieberkühn (Potten, 1998) organized in crypt/villus units in the small intestine. It is at the same time one of the most important

tissue barriers in the body, the site of efficient absorption of nutrients and water and one of the most actively renewing tissues. The control of cell-cell adhesion during cell migration, division and morphogenesis is crucial for its maintenance in health, disease and regeneration (Solanas & Batlle, 2011). The homeostasis of these remarkable stem cell driven multicellular proliferative units (Potten & Loeffler, 1987) requires the regulation of gene networks, signaling pathways, and many dynamic processes (Huynh *et al.*, 2013; Vanuytsel *et al.*, 2013). The mucous membrane cells of the small intestine are one of hierarchical tissues (HTs). HTs are characterized by a three-component cellular composition (Johson, 2012). HT cells can be divided into:

— multipotent stem cells,

— differentiating transitional cells, capable of a limited number of cell divisions, and

— fully differentiated mature cells incapable of proliferation.

Most tissues and organs of a living body contain a population of undifferentiated and immature cells, known as stem cells. They are the primary pluripotent cells and give rise to all the cells in the body. A key characteristic of those cells is the ability to self-renew and differentiate. The highly proliferative stem cells constitute approximately 1% of all mucous membrane cells and are the precursors of all other intestinal epithelial cells. Located closest to the basement membrane, these cells exhibit the highest mitogenic potential. Thus, they are the most vulnerable to damage (Schofield, 1983). Cell division is the ability to produce identical daughter cells by splitting of a parent cell. An asymmetrical division of a stem cell results in the formation of a progenitor cell, with the original stem cell retaining all its former characteristics. The newly-formed progenitor cell gains the ability to differentiate and generate functionally mature cells, with its subsequent divisions leading to more and more mature cells, until the fully mature final stage is achieved. Transitional cells are also capable of proliferation. Mature, fully differentiated cells of the mucosa will have lost its proliferative activity. They exfoliate as part of a natural life cycle. Their loss is compensated for by differentiating transitional cells (Solanas & Batlle, 2011; De Mey & Freund, 2013). The villous epithelium comprises closely packed cells making up on impermeable barrier between chyme in the intestinal lumen and the intestinal stroma. Small intestinal epithelium undergoes continuous renewal. From intestinal crypt renewal zones the cells migrate along the lateral surface of the villi and exfoliate at the top. Intestinal crypts are the regenerative zone of the intestinal mucosa. Crypt cells are the progenitor cells for all types of intestinal epithelial cells. They give rise to differentiated cells. Stem cells and transient cells of intestinal crypts constitute a proliferative pool of 50–70% of the epithelial population. Intense cell proliferation in the lower regions of the crypts results in epithelial cell migration along the crypt and the villi towards the intestinal lumen. As they migrate, the intestinal epithelial cells mature and differentiate, gaining the complete set of enzymes and carriers necessary for their digestive-resorptive function. The cells undergo exfoliation from the surface of villous tips (Goke & Podolsky, 1996; Wright, 1998). The cycle of intestinal epithelium renewal lasts approximately 3–5 days. Around 10^9 new cells (~1 g) are produced and die every 5 days (Wong *et al.,* 1999). The onset of mucosal inflammation is determined by the lifespan of mature epithelial cells (Sonis, 2004). Any condition that causes flattening of the mucosa may cause small intestinal absorptive dysfunction.

INTESTINAL MUCOSITIS

Mucositis following radiation and chemotherapy

The epithelium constitutes approximately 60% of differentiated tissue in the human body (Slack, 2000). Thus, radio- and chemotherapy-induced enteritis constitutes an important clinical problem in oncology. This kind of enteritis occurs following the patient's systemic exposure to chemotherapy or irradiation. The overall risk of developing this complication varies depending on the diagnosis, patient's age, the condition of the gastrointestinal tract, type of chemo/radiotherapy, and dosing frequency (Blijlevens, 2007). Regimen-related mucosal toxicity is extremely common following cytotoxic chemotherapy or radiotherapy (Gibson & Bowen, 2011). Mucositis following chemotherapy occurs in about 40% of patients receiving standard anti-cancer therapy and in 80% of bone marrow stem cell recipients (de Vita, 2011). Radiotherapy for abdominal and pelvic malignancies often causes severe small bowel toxicity (Onal *et al.,* 2011).

Mucositis is characterized by physiological changes in epithelial cells — ranging from erythema to ulceration. Its onset occurs very early during the course of treatment. Epithelial injury is preceded by damage to the epithelial tissue, microvasculature, and connective tissue. Anti-cancer treatments both damage and deplete of stem cells and transient cells, with the consequent inability of the body to fully compensate for the deficiency resulting from exfoliation of differentiated cells. This results in morphological changes of the mucosa in the form of inflammation. The rapid natural turnover of intestinal mucosal epithelium makes these cells particularly vulnerable to cytotoxic treatment. Mucositis has complex pathology and complex clinical presentation (Wardill & Bowen, 2013). Based on anatomical and functional differences between the segments of the gastrointestinal system, two types of mucositis have been identified: oral mucositis (OM) and gastrointestinal mucositis (GIM). The cells of the oral cavity have a fast turnover rate, with a cycle of 7–14 days (López-Galindo *et al.,* 2006), while complete epithelial renewal in the small intestine requires only 3–5 days. These differences in epithelial renewal speeds are responsible for the differences in the time to onset of inflammation (Shaw, 1979; Sonis, 2004). For reasons not entirely understood, the mucous membrane of other organs and systems does not sustain significant damage during chemotherapy or radiotherapy (Elting, 2004; Sonis *et al.,* 2004). The mucosal barrier is highly susceptible to the direct and indirect toxic effects of anti-cancer therapy. This is due to a number of factors, including the high cellular turnover rate of the mucosa, and the complex and diverse microflora of the oral cavity and the gastrointestinal tract. Normally, the mucous membrane constitutes an effective protective barrier. Its damage and inflammation increase the risk of local and systemic infection, especially in a period of neutropenia. The MBI is either a result of a direct action of the drug upon the mucosa (direct toxicity), or an indirect consequence of therapeutic drug-induced bone marrow suppression or myelosuppression (indirect toxicity).

DIRECT MECHANISM OF MUCOSITIS

Both chemo- and radiotherapy cause stem cell and transitional cell damage and depletion. The consequence is a lack of full compensation for the natural loss, resulting from exfoliation of differentiated cells. The mucous

membrane undergoes structural inflammatory changes. These changes develop after approximately 5-10 days following the administration of chemotherapy or exposure to irradiation. The exact time when the inflammatory changes develop is determined by the life-span of differentiated mucosal cells. When the cytotoxicity stops, rapid repopulation of stem cells and transitional cells ensues, resulting in a complete resolution of inflammatory changes (López-Galindo, 2006; Chaveli López, 2011). In general, the repair of mucosal barrier injury (MBI) parallels hematological reconstitution as peripheral blood counts return to normal (Nicola, 2007), with complete resolution occurring within 2–3 weeks (Blijlevens, 2007).

Indirect mechanism of mucositis is associated with the myelosuppressive effect of cytostatics and an increased risk of viral, fungal, and bacterial infections leading to mucosal barrier damage. The time of inflammation induced by the direct mechanism coincides with the peak myelosuppressive effect of cytostatics (usually 10–14 days following exposure). Due to this co-occurrence in time, the inflammation induced by the direct mechanism together with myelosuppression pose a high risk of systemic infections (López-Galindo, 2006; Blijlevens, 2007; Chaveli López, 2011).

PATHOGENESIS OF MUCOSITIS

Mucosal barrier injury is a complex and dynamic pathobiological process manifested throughout the entire digestive tract, occurring in rapid, and in some cases parallel, phases (Sonis, 2013). The pathogenesis of mucositis comprise sequential biologic events coupled with the influence of the local environment and microbiome (Sonis, 2009). The mechanisms for radiation-induced and chemotherapy-induced mucositis are believed to be similar. The majority of pathways that lead to mucositis are the same whether the initiating event is chemotherapy, radiation, or concomitant chemoradiation. Patients treated with cycled chemotherapy receive an acute challenge that is administered systemically, while radiation is considered to be administered locally. Patients undergoing radiation receive fragmented (fractionated) radiation doses which trigger a cascade of biologic events detectable systemically with resulting constitutional effects. In both cases, "bystander" events result in collateral injury (Mothersill & Seymour, 2012). Recent studies have indicated that the mechanisms involved in the pathogenesis of mucositis are much more complex than direct damage to epithelium alone (Treister & Sonis, 2007). According to the model introduced by Sonis the pathogenesis of radiotherapy-induced and chemotherapy-induced mucositis is a five-stage process (Sonis, 2004; Peterson *et al.,* 2011). This model of injury has been demonstrated in the oral mucosa but may also take place in other parts of the alimentary tract (Shaw, 1979; Goke, 1996; Wright, 1998; Sonis *et al.,* 2004).

During the first phase of inflammation — the initiation phase — epithelial cells are directly damaged by chemo- or radiotherapy with subsequent basal membrane and submucosal vessel damage. Radiation and/or chemotherapy induce cellular damage resulting in death of basal epithelial cells. The generation of reactive oxygen species (free radicals) by radiation or chemotherapy is also believed to play a role in the initiation of mucosal injury (Gate *et al*., 1999). These small highly reactive molecules are byproducts of oxygen metabolism and can cause significant cellular damage. The formation of reactive oxygen species (ROS) leads to the activation of nuclear factor kappa B (NFκB) (Sonis, 2002).

The second phase of inflammation — the upregulation/activation phase — involves activation of inflammatory cytokines (interleukin 1, TNF-alpha, IFN) and initiation of angiogenesis. In addition to causing direct cell death, free radicals activate second messengers that transmit signals from receptors on the cellular surface to the inside of the cell. This leads to upregulation of pro-inflammatory cytokines, tissue injury and cell death (Maddens, 2002). The induction of messenger molecules results in treatment-related tissue inflammation and apoptosis. The intestinal changes of this phase include enterocyte apoptosis and flattening of the villi.

The next, third phase of inflammation — signaling and amplification — involves intensified release of cytokines resulting in mucous membrane damage and loss of its integrity and continuity. Upregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), produced mainly by macrophages, causes injury to mucosal cells, and also activates molecular pathways that amplify mucosal injury. The amplification of messenger molecules in this amplification/signaling phase leads to more inflammation and apoptosis. Ulcerations of the mucosa are a hallmark of the transformation of mucositis into phase four.

The fourth, or ulcerative, phase is characterized by discontinuity of the epithelial barrier resulting from apoptosis, development of mucosal ulceration, inflammatory cell infiltrates, dysfunction of local immune response mechanisms, and microbial translocation (bacteria, viruses, fungi), all of which increase the risk of systemic infection. There is a significant inflammatory cell infiltration associated with the mucosal ulcerations, based in part on metabolic byproducts of the colonizing oral/gut microflora. Production of pro-inflammatory cytokines is also further upregulated due to this secondary infection.

The fifth phase of inflammation is characterized by epithelial proliferation as well as cellular and tissue differentiation (Dorr, 1994) restoring the integrity of the epithelium. This phase involves the spontaneous healing process: epithelial cell proliferation, differentiation, and migration to restore the mucosal integrity and continuity. The normal physiological flora is restored. New undamaged epithelium forms; however, some angiogenic processes persist (Sonis *et al.,* 2000).

All of these phases can develop simultaneously in various areas of the mucosa (Sonis, 2004; van Vliet, 2010).

Sonis presented a morphological model of mucositis divided into four successive phases (Sonis, 1998): (1) an inflammatory/vascular phase followed by (2) an epithelial phase leading to (3) an ulcerative/bacteriological phase and ultimately resolving in (4) the healing phase. This model could also be applicable to the gut as a whole even though it is a more complex organ having a dynamic epithelial border with different functions and unique interactions with the immune system and luminal microflora (Sleisenger & Fordtran 1989, Blijlevens *et al.,* 2005).

The first phase is **the inflammatory/vascular phase** characterized by the induction of pro-inflammatory cytokines IL-1, TNF-alpha and IFN gamma by cytotoxic drugs or irradiation while the epithelial cells are still intact. The effects of chemotherapy result in the release of two pro-inflammatory cytokines, IL-1 and TNF-α, by activated macrophages and monocytes. Ionizing radiation at doses not directly harmful to the mucosa also leads to the release of these cytokines by epithelial cells and

the connective tissue beneath (Sherman, 1991). TNF-α is believed to cause direct cell injury and potentially initiate or accelerate the development of mucositis; IL-1, in contrast, initiates inflammation by increasing the number of blood vessels in the subendothelial layer, which in turn leads to increased local cytostatic levels in the area (Sonis *et al.,* 1990). In the gut, macrophages and monocytes, the vast majority of total circulating lymphocytes and other members of lymphoreticular system reside in gut-associated lymphoid tissue (GALT). Released into the circulation, cytokines amplify local tissue injury (Ferrara, 1993). This results in increased vascularity and probably higher local levels of cytotoxic agents. Before total cell destruction, TNF-α, IFN-γ and IL-1 induce major changes in the functionality, permeability, brush border transport, glutamine utilization (glutamine is the main source of energy for intestinal cells), and mucosal cell integrity (Adams *et al.,* 1993; Austgen *et al.,* 1992; Marano 1998).

The second phase is **the epithelial phase** when cells cease dividing and die. This coincides with neutropenia. Proliferating cells are non-specifically irritated by anti-cancer treatment. The cytokines released as a result of the treatment continuously and directly intensify cell damage, ultimately leading to increased permeability of the intestinal epithelium. The second phase involves a halt in basement membrane cell divisions and epithelial atrophy. The resulting metabolic dysfunction of epithelial cells leads to structural changes (villous flattening, epithelial thinning, brush border atrophy, and the formation of a mucoid layer that is thick but does not provide protection). Clinically, this phase may also manifest with mucous membrane redness resulting from enhanced vascularity and reduced epithelial thickness.

The third, or **ulcerative-bacteriological**, phase is characterized by clinical manifestations. It is when necrosis and ulceration occur and the resident microbial flora and its products, e.g. endotoxins, translocate into the bloodstream. Moreover, impaired local defenses and low levels of secretory IgA may allow local infection to develop. Death of the cells from the epithelial basal layer and a lack of epithelial regeneration result in necrosis and structural defects in the tissue, erosions, and ulcerations. Those mucosal ulcerations become secondarily infected by viral, bacterial, or fungal pathogens, which is additionally facilitated by progressively more severe neutropenia. The secondary infections are responsible for increased endotoxin secretion into subepithelial tissue, which enhances the production of IL-1, TNF-α and ROS, resulting in local mucosal destruction. The causative pathogens are typically microorganisms of the physiological flora as well as microflora non-typical for the given segment of the gastrointestinal tract. The forming ulcerations can be a source of generalized infection. The events that take place in the gut are more complicated than those occurring in the oral cavity. The gastrointestinal tract is a more complex ecosystem, it possesses the specialized gut-associated lymphoid tissue (GALT) system, and its resident microflora is more numerous and varied and shares a symbiotic relationship with the host. When the gut epithelium is disrupted, bacteria translocation occurs and pro-inflammatory bacterial endotoxins readily gain access to subepithelial tissues (Ferry *et al.,* 1989). The rate of bacterial translocation is strongly associated with the degree of neutropenia (Tancrède & Andremont, 1985). Microbial translocation is exacerbated by irradiation (Guzman, 1989) and chemotherapy (Berg, 1999), as evidenced by the presence of microorganism cultures from extra-intestinal sites as well as blood (Wells, 1988).

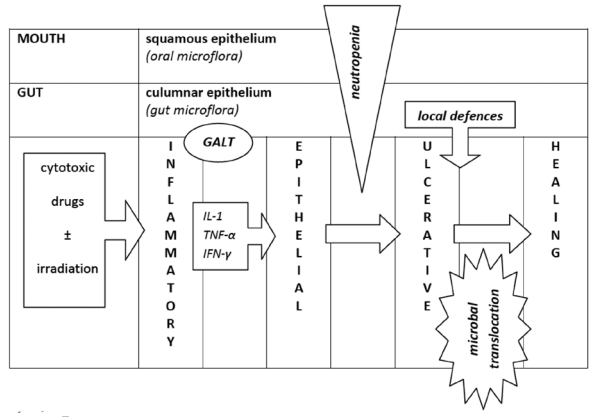

Figure 2. Morphological four-stage approach to etiopathogenesis of mucosal barrier injury

As white blood cell counts normalize, the fourth, or healing, phase occurs involving recreation of the epithelial mucous membrane structure and function. The mucous membrane returns to its physiological state. The **healing** signal derives from the extracellular matrix and initiates epithelial cell proliferation and differentiation. Also, the white blood cell count in the inflammatory area normalizes. Defense mechanisms begin to gain control over intestinal microflora. Secondary changes within the epithelium remain, which increases the risk of mucositis during the next treatment cycle (Pico *et al.,* 1998; Sonis 2004; Miller & McLeod, 2007; Bowen & Keefe, 2008).

In contrast to what is observed after resolution of oral mucositis, gut function does not return to normal after structural repair. Malabsorption and diminished enzyme activity persist for up to several weeks. The healing of mucosal damage probably occurs in two phases: the restitution of mucosal integrity and then remodeling of the mucosal architecture. The mucosal repair process depends on the severity of damage, since superficial injury can be repaired rapidly by epithelial migration without cell prolieration (Lacy, 1988).

CLINICAL PRESENTATION OF MUCOSITIS

Mucositis, also referred to as mucosal barrier injury, is one of the most debilitating side effects of radiotherapy and chemotherapy (Bellm *et al.,* 2000). It is characterized by both inflammation and cell loss in the epithelial barrier lining the gastrointestinal tract (Sonis, 2004; Blijlevens *et al.,* 2005). Clinically, mucositis is associated with bacteremia, malnutrition, the need to use total parenteral nutrition, and an increased use of intravenous analgesics (Masszi & Mank, 2012). Observations on the relationship between oral mucositis (OM) and gastrointestinal mucositis (GIM) and the incidence of fevers of unknown origin and intravenous antibiotic use in patients after high dose chemotherapy suggest that the incidence of fevers and use of antibiotics are more dependent on GIM compared to OM (Vokurka *et al.,* 2014). These complications lead to longer hospitalizations and increased health care costs. Moreover, mucositis is a frequent reason for reducing the dosages of radiotherapy and chemotherapeutics or even for postponing cancer treatment, ultimately leading to higher mortality in cancer patients (Sonis *et al,* 2001; Blijlevens *et al.,* 2005). Historically, research has focused on oral mucositis. More recently, the pathophysiology and clinical symptoms of

Table 1. Tests for the assessment of small intestinal mucosal injury — advantages and disadvantages

Test	Characteristic
Conventional endoscopy/small bowel biopsy	"gold standard" affords visual perspective therapeutic intervention possible
	invasive, painful, expensive assesses only proximal or distal regions of small intestine reflects the function of only the biopsied region additional risk for cancer patients
Sugar permeability test (Menzies *et al.,* 1979; Tooley *et al.,* 2009)	assesses barrier function = can measure gut integrity and function test measures in the permeability and absorption (loss of epithelial surface)
	requires the patient to drink fluid containing a solution of four sugars: lactulose, L-rhamnose, D-xylose and 3-*O*-methylglucose requires 2-hour blood and 5-hour urine collection not routinely used in clinical practice
Breath tests: Oro-cecal transit time Hydrogen breath test (van Wyk *et al.,* 1985; Almeida *et al.,* 2008)	non-invasive correlation with mucosal barrier injury
	dependent on the presence of hydrogen-producing bacteria in the colon, diet, the use of antibiotics and proton-pump inhibitors require specialized equipment
^{13}C-lactose breath tests (Tooley *et al.,* 2009)	non-invasive correlation with mucosal barrier injury
	precluded in lactose-intolerant patients hardly available
Diamine oxidase (DAO) (D'Agostino *et al.,* 1986; Biegański *et al.,* 1983; Bragg *et al.,* 1991; Bounous *et al.,* 1984)	plasma DAO activity is a candidate marker for ischemic small bowel injury particularly high concentration in the epithelial cells of the small intestine
	rapidly cleared by the liver
Calprotectin (Costa *et al.,* 2003)	fecal concentration has been identified as a sensitive biomarker of intestinal inflammation highly sensitive non-invasive
	low specificity does not allow for discrimination of anatomical sites of intestinal injury
Granulocyte marker protein (GMP) (Richter *et al.,* 1997)	pathophysiologically similar to calprotectin sensitive non-invasive biomarker of radiation induced injury
Cytokines (Logan *et al.,* 2008; Engel *et al.,* 1998)	markers of inflammatory response induced by various chemotherapeutic agents play key roles in the pathogenesis of mucositis biomarkers of febrile neutropenia
	non-specific
C-reactive protein (CRP)	correlates with tissue injury
	non-specific, may be influenced by other factors levels increase after the beginning of inflammation
Citrulline	sensitive and specific for small bowel epithelial cell loss simple low-cost no methodological drawbacks biomarker of small intestinal enterocyte mass
	requires repeated collection of blood samples
Thromboxane B2 Leukotriene B4 (Cole *et al.,* 1993)	eicosanoid mediators of inflammation potential biomarkers for radiotherapy-induced gut damage
	one small study only

intestinal mucositis have been drawing more attention (Blijlevens *et al.,* 2005; Lutgens *et al.,* 2005). The epithelial barrier lining the gastrointestinal tract is composed of a single layer of epithelial cells (Powell, 1981) forming a mechanical barrier separating the inside of the human body from the outside world. In patients treated with chemo- and/or radiotherapy, mucositis tends to have a rapidly aggravating course. A mucous membrane damage means a disruption of the body's natural barrier against infection. Additionally, a weakened immune system is a factor contributing to the dynamic development of infections. Inflammation and loss of mucosal integrity together with neutropenia increase the risk of local bacterial, fungal, and viral infections, which predisposes immuno-

suppressed patients to developing sepsis (Rubenstein *et al.,* 2004). **Oral mucositis** manifests with pain, edema, erythema, lesions, pseudomembrane formation, excessive mucus production and reduced of saliva, and bleeding, all of which reduce the patient's ability to eat and drink (Schubert *et al.*, 1992; Woo 1993). Intensification of an inflammatory response involves development of erosions and ulcerations covered with a fibrin coating composed of exfoliated epithelial cells. Initially, these lesions are limited but large areas become covered with confluent fibrin coating. Extreme cases result in necrosis of the mucous membrane. Oral mucositis can be accompanied by mild to severe pain and difficulty swallowing (dysphagia). The complex clinical presentation of oral mucositis also includes altered taste sensation (dysgeusia), dry mouth (xerostomia), ecchymoses, and hemorrhages, all of which cause difficulties in oral ingestion of fluids and food. The severity of oral mucositis can be assessed reliably during a physical examination (Zur, 2012; Raber-Durlacher *et al.,* 2012). By contrast, there are no reliable data on the incidence of **gastrointestinal mucositis,** although almost every transplant recipient is affected to some extent and develops manifestations that include nausea, vomiting, and watery diarrhea accompanied by macroscopic loss of blood or mucus and abdominal cramps. The exact course and severity of the bowel symptoms of gut mucositis are also difficult to ascertain as they are frequently masked either by antiemetic drugs taken by the patient as part of prevention and treatment of chemo/radiotherapy-induced nausea and vomiting, or by narcotic analgesics used for oral mucositis, which induces constipation as a result of reduced gut motility. An assessment of the mucous membrane in the distal gastrointestinal tract is considerably more difficult (Zur, 2012). Anti-cancer therapy-induced damage to the intestinal mucosa results in intestinal crypt cell apoptosis, atrophy of the villi, and reduction in enterocyte mass (Keefe *et al.,* 2000). This leads to impaired absorption of the products of digestion. Clinical consequences of mucositis include dehydration, malnutrition, potentially life-threatening infections and even increased mortality (van der Velden, 2010).

ASSESSMENT AND MONITORING OF MUCOSAL DAMAGE INDUCED BY CANCER THERAPY

Several scoring systems for oral mucositis have been proposed (Parulekar, 1998), although none are universally accepted and all lack standardization. However, there is no system for registering gut mucositis at present, although definitions for grading toxicity of individual signs and symptoms have been published (Blijlevens *et al.,* 2000). Early detection, assessment and monitoring of mucosal damage are necessary for effective management. The assessment scales for mucosal damage focus on clinical presentation. For chemotherapy complications both the World Health Organization (WHO) scale and the National Cancer Institute — Common Terminology Criteria for Adverse Event (NCI-CTC AE) scale are commonly used (Neveux, 2004). Currently, the most commonly used classification systems (scales) of radiation reactions are the Toxicity Criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) criteria (Peterson *et al.,* 2011). The toxicity scales developed for uniform reporting of side effects assess early and late complications of therapy for individual tissues and organs. The severity of individual side effects is graded from 0 to 5, where 0 means no symptoms, and 5 — death due to a side effect. The intermediate grades from 1 to 4 correspond to various severity of the reaction: 1 — mild, 2 — moderate, 3 — severe, 4 — life-threatening. Toxicity scores are considered to be the best scales for intestinal mucositis and are designed based on signs and symptoms related to gastrointestinal changes. Their reliability is subjective and subject to interobserver and intraobserver variation. The oral toxicity scale combines objective signs of mucosal damage, erythema and ulcers, with subjective and functional outcomes, pain and ability to eat.

MARKERS OF SMALL INTESTINAL MUCOSAL INJURY

The small intestine is largely inaccessible by conventional means, with endoscopy coupled with small bowel biopsy remaining the current "gold standard" technique for assessing small intestinal function. Endoscopy procedures are used regularly in clinical practice for diagnostics of gastrointestinal complaints. However, this technique is invasive, painful, expensive and only the proximal and distal parts of the small intestine can be routinely assessed. The real problem is to determine true functionality of the whole small intestine. Furthermore, cancer patients develop additional treatment-related side-effects, which makes it difficult to perform diagnostic procedures on the gastrointestinal tract. Invasive diagnostic methods often are precluded because of the high risk of infectious and bleeding complications. Currently, there is no "one-fit-all" biomarker of regimen-related mucosal toxicity. A number of biomarkers have been investigated in gastrointestinal diseases. Some of them have proved to be useful in the oncology arena.

Two significant potential biomarkers of regimen-related mucosal injury, i.e., citrulline and calprotectin, seem to be more sensitive and more specific for detecting chemo- and radiotherapy induced small intestinal mucosal damage than other markers of small intestinal function (Gibson *et al.,* 2011).

CITRULLINE ASSAYS

Amino acid composition analysis refers to analytical techniques measuring the concentration of individual amino acids in a given sample. Citrulline assays are not routinely performed in analytical laboratories because they require specialized high-resolution techniques. Various methods have been reported for quantification of citrulline. Most of them are based on chromatographic separation, which usually involves pre- or post-column derivatization of the amino acid (Stein & Moore, 1954; Efron *et al.,* 1964; Neveux *et al.,* 2004; Duranton *et al.,* 2014). Although plasma, serum and urine are commonly used as test samples, whole blood or erythrocytes are also reported as potential samples for clinical evaluation (Efron *et al.,* 1964; Rougé *et al.,* 2008). The latter, however, due to the very complex matrix require highly-selective analytical techniques such as mass spectrometry (MS). Hozyasz and coworkers (2010) proposed MS on dried blood spot specimens for screening citrullinemia as a promising candidate biomarker of abnormal embryogenesis. Dried blood spot amino acid profiling *via* tandem mass spectrometry (MS/MS) can also be used for monitoring graft function following intestinal transplantation (Yu *et al.,* 2005). Whole blood citrulline concentration may be a simple biochemical marker for diagnosis and monitoring of enterocyte loss in celiac

disease (Hozyasz *et al.,* 2006). Plasma or serum citrulline levels, measured with chromatographic separation, are ideal for small intestine function assessment. The technique shows adequate selectivity and high resolution. There is no significant difference in results or interpretation between analytical methods using serum or plasma for determination of citrulline (Crenn *et al.,* 2008).

The function of citrulline in the body and its use as a marker of small intestinal mucosal damage

Citrulline is an endogenous nonprotein amino acid, naturally occurring in the body. It has been known since 1930, when Wada first isolated it from watermelon juice (Wada, 1930). Several years later, the same scientist confirmed the absence of citrulline in protein structures (reported by Kurtz, 1937). Those events coincided with the discovery of the urea cycle by Krebs and Henseleit in 1932, of its role in the elimination of toxic ammonia from the body, and the involvement of citrulline (after Adeva *et al.,* 2012). Then, for nearly half a century, the metabolism of citrulline remained out of the spotlight of scientific interest. In the 1980s and 1990s, two subsequent discoveries were made constitut the most recent chapter in the history of citrulline. In 1981, a study in rats demonstrated that citrulline, which is necessary for the production of endogenous arginine, is synthesized in the small intestine (Windmueller, 1981). *De novo* formation of citrulline takes place in enterocytes and its concentration is currently considered a marker of intestinal function (Crenn *et al.,* 2000; Curis *et al.,* 2005; 2007). Another significant discovery involved the role of nitric oxide as a signaling factor in vascular smooth muscle relaxation and the role of citrulline in nitric oxide synthesis. In 1998, the three authors of this discovery, Furchgott, Ignarro, and Murad, received the Nobel Prize in medicine (Nobelprize. Org. Web.).

METABOLISM OF CITRULLINE

Citrulline is a rare amino acid. As a nonprotein (nonproteinogenic) amino acid it functions as an intermediate in protein (proteinogenic) amino acid metabolism and in the urea cycle. The metabolic activity of citrulline is mainly a result of its close link with arginine metabolism. Citrulline is a direct precursor of arginine as well as a metabolite of its transformations. The following are parallel metabolic transformations of citrulline:

1. The first metabolic pathway is arginine biosynthesis, which involves citrulline exchanges at the systemic level. In the gut, citrulline is synthesized from glutamine, glutamate or proline (reported by Wu *et al.,* 1997) and released into the bloodstream to be converted back to arginine (by ASS and ASL) in the kidneys. Approximately 60% of net arginine synthesis in adult mammals occurs in the kidney, where citrulline is extracted from the blood and converted to arginine (Windmueller & Spaeth, 1981). In this pathway, circulating citrulline is a masked form of arginine to avoid hepatic uptake and metabolism by arginase. The process of arginine synthesis in the adult involves the intestinal-renal axis. However, many other tissues and cell types also contain ASS and ASL for generating arginine from citrulline (Mori & Gotoh, 2004).

2. The second pathway is the arginine-citrulline-nitric oxide (NO) cycle. In most NO-producing tissues, citrulline is locally recycled to arginine by ASS to increase arginine availability for NO production (Husson *et al.,* 2003).

3. The third pathway takes place in the liver, where citrulline is synthesized by OCT from ornithine and metabolized by ASS in the urea cycle (Windmueller & Spaeth, 1981).

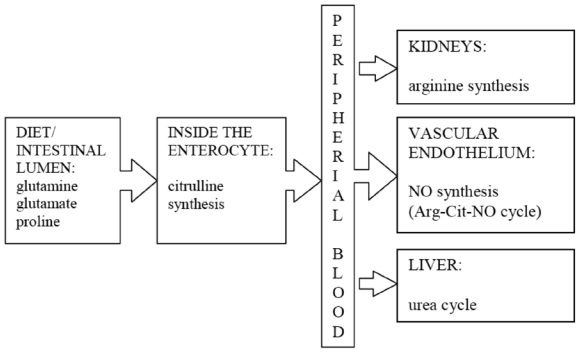

Figure 3. Metabolic transformations of citrulline

Figure 3 shows a schematic representation of citrulline metabolic pathways.

However, there are differences in citrulline metabolic pathways between mammalian adults and neonates. Young mammals including preterm infants have a particularly high requirement for arginine (Wu *et al*., 2000). In the young, arginine is an essential amino acid for optimal growth and development, and therefore must be provided in the diet. In adults, arginine is a conditionally essential amino acid. Studies in neonatal pigs (an excellent model for studying infant nutrition) showed that endogenous synthesis of arginine is crucial for maintaining arginine homeostasis (Flynn & Wu, 1996). On the basis of our current knowledge of mammalian arginine metabolism, enterocytes are responsible for the major part of net citrulline and arginine synthesis from glutamine and proline in neonates (Wu & Morris,1998). In the neonatal period, arginase level in enterocytes is low, as are the ASS and ASL levels in the proximal tubule of the kidney, where citrulline is converted to arginine in the adult. Thus, the neonatal process of arginine synthesis takes place entirely in the gut (Wu *et al.,* 2004). In neonates, whose renal ASL activity is minimal, most of the citrulline synthesized in enterocytes is converted locally into arginine by ASS and ASL. The near absence of arginase in neonatal enterocytes maximizes the intestinal release of arginine into the systemic circulation (Wu, 1997). The low activities of key enzymes limit citrulline synthesis in the enterocytes, even if the substrates are fully available.

Citrulline synthesis in the small intestine

Diet is a poor source of citrulline. Small quantities of citrulline have been found in the fruits of cucurbitaceae (watermelons and melons) (Rimando & Perkins-Veazie, 2005) and in birch sap (Ahtonen and Kallio, 1989). Endogenous synthesis is the main source of citrulline in the body (Cren *et al.,* 2000; Curis *et al.,* 2005; 2007). Small intestinal epithelial cells, enterocytes, play the key role in citrulline synthesis. The substrates for citrulline synthesis in the small intestine are amino acids provided with diet: glutamine, glutamate, proline, and also serum glutamine (Wu, 1998). Absorbed by intestinal villi, they undergo further transformations inside the enterocytes. Most of the circulating citrulline comes from glutamine conversion in enterocytes (Wu, 1998). The key enzyme

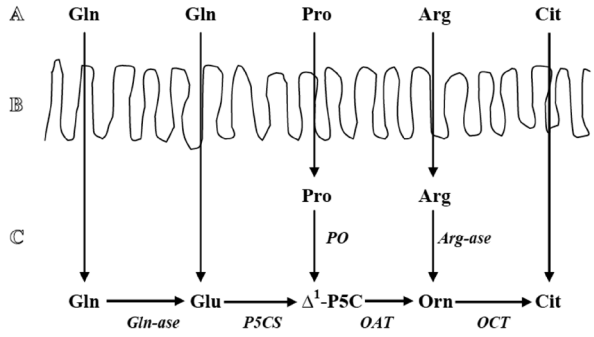

Figure 4. Citrulline synthesis in the small intestine
A — small intestine lumen, **B** — microvillis, **C** — enterocyte inside, **Cit** — citrulline, **Gln** — glutamate, **Gln-ase** — glutaminase, **Glu** — glutamine, **OCT** — ornithine carbamoyltranferase, **Orn** — ornithine, **P5CS** — pyrroline-5-carboxylate synthase, **PO** — proline oxidase, **Pro** — proline.

for glutamine and glutamate transformation to citrulline is pyrolline-5-carboxylate synthase (Crenn *et al.,* 2011), an enzyme located almost exclusively in the small intestinal mucosa. Citrulline synthesis from proline occurs by the enzyme pyrolline oxidase (Crenn *et al.,* 2008; Curis *et al.,* 2005; 2007). Figure 4 presents these transformations in small intestinal epithelial cells. Given the low levels of argininosuccinate synthetase (ASS) in enterocytes, citrulline formed in the intestine is released into the bloodstream, increasing the amount of blood-borne amino acids (Crenn *et al.,* 2008; 2011).

RENAL TRANSFORMATION OF CITRULLINE AND ARGININE SYNTHESIS

Approximately 80% of the citrulline formed in the small intestine is carried with blood to the kidneys (Moinard et al., 2008), where it is transformed to arginine by the ASS and ASL (Mori and Gotoh, 2004; Häberle *et al.,* 2012). Arginine formed in this way is subsequently released into the circulation and constitutes its main source for further metabolic transformations. Indeed, nearly 60% of endogenous arginine in humans is synthetized in the kidneys (Morris, 2002). People with renal dysfunction or post nephrectomy have elevated blood citrulline levels (Lau, 2000). This arginine-citrulline-arginine cycle can be seen as a means of protecting dietary arginine from excessive degradation in the liver, especially in situations where the intake of protein is low.

CITRULLINE — A DIRECT PRECURSOR OF ARGININE

Citrulline is a direct precursor of arginine, as well as a product of its catabolism. The biological activity of citrulline is mainly a result of its close coupling with arginine metabolism.

Circulating citrulline is used for efficient arginine synthesis by the brain, peripheral nerve cells, and vascular endothelial cells (Moncada & Higgs, 2006). Synthesized in these tissues, arginine becomes the substrate for the synthesis of nitric oxide (NO) — an important neurotransmitter and vasodilator (Luiking, 2010). The transformation of citrulline to arginine is catalyzed by two intracellular enzymes ASS and ASL (Husson, 2003). Nitric oxide is synthesized directly from arginine by nitric oxide synthase (NOS) (Husson, 2003; Luiking, 2010). Directing of arginine (derived from citrulline) for NO production optimizes NO formation (Flam, 2007; Schwedhelm, 2008). Arginine is derived from dietary sources and endogenous synthesis. In adults, arginine is synthesized endogenously. It is essential in certain settings, especially in disease and in the recovery phase following diseases. Arginine synthesis in the liver takes place only if all the necessary components of the urea cycle — especially ornithine — are present. This, as well as the high arginase activity in hepatocytes, results in only a small amount of endogenous arginine produced in the urea cycle being released into the bloodstream. The remaining fool of this amino acid comes from local intracellular sources: protein degradation and synthesis from citrulline (Curis *et al.,* 2005). The citrulline used for intracellular arginine synthesis must come from the bloodstream, since citrulline synthesized intracellularly is formed from arginine in a reaction catalyzed by nitric oxide synthase (the arginine–NO cycle). The arginine–NO cycle is responsible for arginine regeneration in various tissues (Graboń, 2006). The key enzymes involved in arginine catabolism are arginase and nitric oxide synthase. Arginase catalyzes the hydrolysis of arginine to ornithine and urea. Unlike other enzymes of the urea cycle, arginase can also be found in extra-hepatic tissues. The activity of arginase is regulated by the accessibility of its substrate arginine. Arginase competes with nitric oxide synthase for their common substrate — arginine (Wu & Morris, 1998). This competition depends on tissue type and the presence of stimuli.

FORMATION OF NITRIC OXIDE

Vascular endothelial cells function not only as a passive diffusion barrier, but also have a role in active secretion, which includes the production of nitric oxide. Nitric oxide is formed as part of arginine metabolism. This reaction is catalyzed by three izoenzyms of nitric oxide synthase (NOS) family that differ in their level of expression and their localization: nNOS is mainly present in neural cells, iNOS in macrophages and eNOS in endothelial cells (after Luiking *et al.,* 2010). All these enzymes share a common mechanism for synthesizing NO. The mechanism of nitric oxide production from arginine involves oxidation of the imino group of the guanidine residue by molecular oxygen. Arginine is first oxidized to N-hydroxyarginine, which is then further oxidized to citrulline with the formation of nitric oxide (Flam *et al.,* 2007; Schwedhelm *et al.,* 2008):

$$\text{Arginine} + O_2 \xrightarrow{\text{NOS}} \text{Citrulline} + \text{NO}$$

Citrulline is essential to the formation of arginine, which in turn is needed to produce nitric oxide. Arginine is the only substrate for nitric oxide synthesis in the human body.

THE ROLE OF CITRULLINE IN NITRIC OXIDE SYNTHESIS

In 1975, Felig showed that visceral uptake of arginine (arteriovenous difference) in humans is positive. Thus, the liver is an importer rather than an exporter of this amino acid (Felig, 1975). Recent discoveries have proven that nitric oxide synthase is limited by the availability of extracellular arginine. Therefore, it is the extracellular and not intracellular arginine that is a substrate for the production of nitric oxide in endothelial cells. Nitric oxide formation does not correlate with the levels of intracellular arginine (Dioguardi, 2011; Schwedhelm *et al.,* 2008). The direct involvement of extracellular arginine

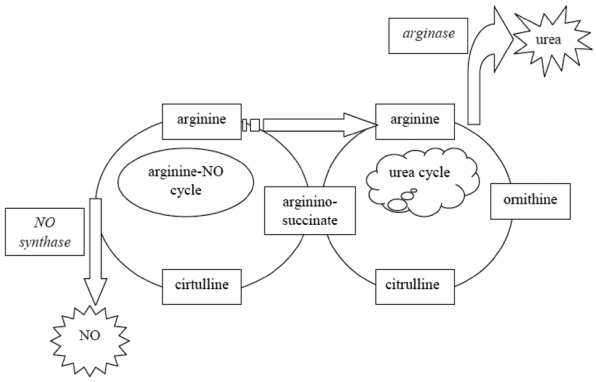

Figure 5. "Arginine paradox"

in the production of nitric oxide optimizes its formation in endothelial cells. This phenomenon is consistent with the principle that the substrate regulates the activity of the enzyme involved in its transformations. In recent years, a phenomenon called the "arginine paradox" has been discovered. It turns out that arginine supplementation in physiological setting leads only to its increased consumption in the urea cycle and its hydrolysis via arginase in the liver; and only to a small extent, if at all, does it increase the production of nitric oxide (Shin *et al.,* 2011). Conversely, citrulline supplementation increases plasma arginine levels, which offers the possibility for nitric oxide synthesis (Husson *et al.,* 2003; Asgeirsson *et al.,* 2011). This constitutes another argument for revising the medical tenet stating that supplying the body with that which is missing is always beneficial.

THE INVOLVEMENT OF CITRULLINE IN UREA CYCLE

The urea cycle (also known as the ornithine cycle or Krebs–Henseleit cycle), which occurs in mammals, is a cycle of biochemical reactions that produce urea from ammonia. It was the first metabolic cycle discovered. Nitrogen from enteral sources (dietary protein) and muscle is excreted from the body as urea *via* the urea cycle. Urea synthesis is the main pathway of the elimination of the toxic ammonia from the human body, and the only organ able to produce urea from ammonia and carbon dioxide is the liver, as it is only there that the complete set of enzymes essential for urea biosynthesis can be found (Windmueller & Spaeth, 1981). It is of note that the first two enzymes of the urea cycle, i.e., carbamoyl phosphate synthetase and ornithine carbamoyltransferase, are also present in the small intestine cells. That is why ammonia, considered to be an enterotoxin, can be transformed into citrulline and transported to the liver, where it enters the urea cycle (van de Poll, 2007). In the liver, citrulline is an intermediate metabolite of the urea cycle (Häberle, 2012). Urea formation *via* the urea cycle is a cyclic process, and therefore it does not synthesize citrulline in amounts higher than required by the stoichiometry of the cycle. The metabolism of citrulline in the liver is not connected with other pathways of its metabolism (Häberle *et al.,* 2012; van de Poll *et al.,* 2007).

ENZYMES INVOLVED IN CITRULLINE TRANSFORMATIONS

The rates of metabolic transformation of citrulline vary in individual tissues and organs, hence the various organ distribution of the enzymes involved in citrulline metabolism. Citrulline synthesis can be accomplished by three enzymes: ornithine carbamoyltransferase (OCT), proline oxidase (PO), or pyrroline-5-carboxylate synthase (P5CS) (Wu *et al.,* 1997). OCT is found in the liver and the small intestinal mucosa, PO is located mainly in the small intestine, liver, and kidneys, while P5CS is present almost exclusively in the small intestinal mucosa. The key enzyme in citrulline catabolism is argininosuccinate synthase (ASS), which catalyses the transformation of citrulline to argininosuccinic acid. In the subsequent step, argininosuccinic acid is cleaved by argininosuccinate lyase (ASL) yielding arginine and fumarate (Wakabayashi, 2004). Both of these enzymes are disseminated widely across mammalian tissues (Mori & Gotoh, 2004), hence most tissues are able to synthesize arginine from citrulline. However, the lowest levels of ASS and ASL can be found in the small intestinal mucosa (Husson *et al.,* 2003). As a result of such low enterocyte levels of ASS and ASL, citrulline formed in the small intestine does not undergo further transformations. Instead, it enters the bloodstream, increasing the level of circulating amino acids (Windmuell & Spaeth, 1981). Citrulline used for intracellular arginine synthesis must be taken up from the bloodstream, as that synthesized intracellularly is produced from arginine *via* NO synthase (the arginine-citrulline-NO cycle) (Schwedhelm, 2008). Table 2 shows relative levels of the enzymes catalyzing metabolic transformations of citrulline.

THE ROLE OF CITRULLINE IN THE ASSESSMENT AND MONITORING OF DAMAGE TO SMALL INTESTINAL MUCOSA

The nonprotein endogenous amino acid citrulline synthesized in enterocytes (cells of the small intestinal epithelium), is a marker that is independent of diet and nutritional status (Crenn *et al.,* 2008). The normal plasma citrulline level in healthy individuals ranges from 30 to 50 μmol/L (40 ± 10 μmol/L) (Crenn *et al.,* 2003). The relationship between plasma citrulline concentration and epithelial cell mass has been demonstrated previous-

Table 2. Relative levels of enzymes for citrulline transformations (Wakabayashi, 2004)

	relative enzyme levels		
	intestine	liver	kidney
OCT	high	high	low
P5CS	high	low	low
PO	high	high	high
ASS	low	low	high

ASS — argininosuccinate synthase; OCT — ornithine carbamoyltransferase; PO — proline oxidase; P5CS — pyrroline-5-carboxylate synthase

Table 3. Citrulline level in different clinical situations (based on Crenn *et al*., 2008, modificated)

Citruline level	Condition	Comments	Source
Normal 40 ± 10 μmol/L	adult Caucasian	fasting	Rabier *et al.,* 1995
	hepatocellular failure		Weber *et al.,* 1982
	malnutrition	without kwashiorkor	Crenn *et al.,* 2003
	small bowell transplantation	biomarker for rejection	Pappas *et al.,* 2002
Increased	renal failure	if creatinine clearance below 50 ml/min	Ceballos *et al.,* 1990
	ageing	can be increased due to renal function insufficiency	Pitkänen *et al.,* 2003
	urea cycle disorders (rare)	ASL deficiency ASS deficiency	Scaglia *et al.,* 2004 Brusilow *et al.,* 1996
Decreased	intestinal failure: — short bowel syndrome — chronic villous atrophy diseases — intestinal toxicity due to anti-cancer treatment		Crenn *et al.,* 2003 Crenn *et al.,* 2003 Lutgens *et al.,* 2003 Blijlevens *et al.,* 2004
	severe metabolic stress		Jeevanandam *et al.,* 1990
	urea cycle disorders (rare)	OTC deficiency	Häberle *et al.,* 2012

ly. Clinical studies involving patients with short bowel syndrome, villous atrophy-associated intestinal diseases, Crohn's disease, small intestinal transplantation have shown that citrulline levels correlate positively with overall small bowel function. In 2000, Crenn et al. demonstrated plasma citrulline levels to be significantly lower in the group of patients with short bowel syndrome than in the healthy control group. Short bowel syndrome involves conditions following surgical resection or functional exclusion of a part of or the entire small intestine. The most common cause is surgical resection due to intestinal ischemic necrosis, injury, or neoplasm. Functional exclusion is typically due to Crohn's disease, enteropathy, or chemo- and radiotherapy-induced intestinal injury. As any other organ, the small intestine with its vast absorptive surface has a large functional reserve. There is no set length of the resected intestinal segment that would determine the development of this syndrome. Usually resection of a small segment does not impair intestinal function, while if the remaining intestinal segment measures less than 100 cm, the syndrome is very likely to develop (Pappas *et al*., 2001). The manifestations of intestinal functional failure depend on the specific segment of the intestine removed and the extent of the resection. The most critical segments are the duodenum, proximal jejunum, and the area around the ileocecal valve (Pappadia *et al,* 2007; Santarpia *et al.,* 2008). The plasma citrulline level in patients with short bowel syndrome is considered a reliable marker of small intestinal mucosal function. In another study, Crenn et al. (2003) demonstrated a correlation of plasma citrulline levels and both the severity and extent of villous atrophy in patients with celiac disease and patients with non-celiac villous atrophy disease. Blood citrulline levels of <10 μmol/L were considered to be a marker of severe small intestinal mucosal injury and corresponded to total villous atrophy, levels ranging from 10 to 20 μmol/L corresponded to subtotal villous atrophy, and levels >20 μmol/L indicated partial atrophy. In patients with villous atrophy diseases, plasma citrulline level is considered to be an early and reliable biomarker of enterocyte mass. Since the publication of these two pioneer studies other researchers have assessed the usefulness of plasma citrulline level as a marker in various conditions involving small bowel mucosal dysfunction, showed by the correlation with the residual duodenum-jejunum length and enteral absorption. Moreover, there is a close correlation between the duodenal and small-intestinal length and the small-intestinal absorption capacity (Crenn, 2003; Santarpia, 2008; Piton, 2011). In small-intestinal surgery, plasma citrulline levels are used in the follow-up of graft implantation (Gondolesi *et al.,* 2002; Papas *et al.,* 2002).

Crohn's disease involves segmental inflammation of all layers of the affected intestinal wall. These segmental or skip lesions can involve any segment of the gastrointestinal system from the mouth to anus. Recent studies have demonstrated decreased plasma citrulline levels in patients with a severe form of Crohn's disease, mainly in the cases of small-intestinal involvement or extensive intestinal resection. However, the plasma citrulline level is not a marker of Crohn's disease activity (Diamanti *et al.,* 2011; Diamanti *et al.,* 2012; Elkhatib & Buchman, 2011). More studies are needed to more precisely determine the role of citrulline in this disease.

Villous atrophy-associated small bowel disease (with celiac disease being the predominant etiology in Western countries (Marsh, 1992), as well as "tropical" sprue and various forms of infectious enteritis) manifests as disturbances of intestinal digestion and absorption, and eventually — malnutrition (Hozyasz *et al.,* 2006). In patients with extensive intestinal mucosa dysfunction, citrulline concentration is low and correlates with the severity and extent of villous atrophy. In this group of patients, citrulline concentration can be used as a simple and reliable marker of reduced enterocyte mass (Crenn, 2008). Patients with untreated celiac disease have significantly deminished plasma citrulline levels (Tuchman *et al.,* 2000), which rise rapidly after the introduction of gluten-free diet (Carrit, 1977). This proves that citrulline level is a sensitive marker of the beneficial effect of diet on intestinal repair (Efron, 1964; Neveux, 2004; Duranton, 2014). There is a close correspondence between gut structure and function in HIV-infected patients (Reka & Kotler, 1998). In these patients, a citrulline concentration of <10 μmol/L was highly associated with the need for parenteral nutrition, reflecting intestinal failure due to a

reduced enterocyte mass. Patients with only mild enterocyte involvement presented with normal or only moderately lowered citrulline concentrations (Crenn *et al.,* 2009).

Blood citrulline levels of <10 μmol/L are an established marker of severe small-intestinal mucosal injury (Nagamani *et al.,* 2012). Regular citrulline level assessments help monitor the function of the small-intestinal mucosa (Häberle *et al.,* 2002; van de Poll *et al.,* 2007). Moreover, citrulline levels are closely correlated with the duodenal and small-intestinal length as well as with intestinal absorptive capacity (Hozyasz *et al.,* 2010; Rougé *et al.,* 2008; Yu *et al.,* 2005). It is equally important to underline, however, that citrulline concentration does not provide etiologic diagnosis but is only a biomarker of the expected course of intestinal disease and prognosis in severe enteropathy or intestinal failure.

PLASMA CITRULLINE LEVELS IN CANCER TREATMENT

Systemic chemotherapy and radiotherapy for abdominal or pelvic malignances often causes severe bowel toxicity, with an impaired compensation for the natural deficiency due to endothelial cell exfoliation. The small bowel epithelium regenerates every four days (Wong, 1999). The exact onset of inflammation is dictated by the lifespan of mature mucosal cells. The clinical presentation of enteritis includes nausea, vomiting, watery diarrhea with evident blood and mucus content, and abdominal cramps. A clinical examination assessing the signs and symptoms continues to be the basis for evaluating the extent of mucosal injury in routine clinical practice. Clinical symptoms are most commonly used as a surrogate endpoint during and following treatment. Endoscopic studies to evaluate the apparent mucosal changes in patients following standard chemotherapy by Keefe and coworkers (2000) have demonstrated apoptosis within the crypts of the small intestine, villous flattening, and enterocyte mass reduction. Small intestinal mucositis is associated with apoptosis in crypts that precedes hypoplastic villous atrophy and loss of enterocyte height. This results in a loss of mucosal integrity and continuity and the subsequent ulceration.

The sequelae of small-intestinal mucosal injury include:

— changes in transepithelial transport,
— changes in gut barrier function,
— motility dysfunction,

which leads to impaired absorption of the products of food digestion (Butler, 2000). These functional changes are correlated with the mass of epithelial cells capable of absorption (Juby *et al.,* 1987). A biochemical evaluation of the small intestine involves intestinal function tests that help detect absorption disturbances. The most important from the clinical point of view are sugar permeability tests (Travis & Menzies, 1992; Bjarnason *et al.,* 1995). These tests are considered to be sensitive functional gauges of the proximal part of the small intestine (Melichar *et al.,* 2001; Melichar *et al.,* 2005; Kohout *et al.,* 1999). The 2004 studies by Blijlevens et al. have demonstrated the usefulness of intestinal function testing in the form of sugar absorption evaluated in the population receiving high-dose chemotherapy and compared the results with the patients' plasma citrulline levels. High-dose chemotherapy with hematopoietic stem-cell transplantation is an established treatment modality in proliferative hematopoietic and lymphatic diseases as well as in the case of some solid tumors. This is often the only therapeutic option leading to a complete cure or improved survival by ensuring a response to treatment. The significance of high-dose chemotherapy with hematopoietic stem-cell transplantation is confirmed by an increased number of these procedures noted by international registries. Intensive studies on this treatment modality which translate to its improved efficacy and reduced side effects are going. One serious clinical issue is the toxicity of the transplantation procedure. Intestinal mucosal injury is the second most common (after pancytopenia) complication associated with cancer treatment. The incidence of intestinal mucosal reactions in patients undergoing high-dose chemotherapy exceeds 80% (De Vita, 2011; ESMO Guidelines Working Group, 2011). Mucositis can, in turn, be a source of further complications caused by the damaged natural barrier of the mucous membrane, such as increased incidence of infections, necessity to introduce nutritional support, and extended hospital stay. In the period prior to graft revitalization any invasive examinations, including endoscopy, are contraindicated in patients receiving high-dose chemotherapy, due to myelosuppression and the associated high risk of hemorrhagic and infectious complications. This approach is consistent with the generally accepted standards of management (Fallows *et al.,* 2001). The plasma citrulline levels and sugar absorption tests in the patients evaluated by Blijlevens et al. corresponded well with each other as well as with the extent of intestinal mucosal injury. Many patients from the study group were unable to drink the sugar solution due to chemotherapy complications, i.e., severe oral mucositis, nausea, and vomiting. Plasma citrulline levels proved to be an equally reliable parameter of intestinal mucosal injury as were the sugar absorption tests, with lesser burden for the patient (Blijlevens, 2004). A 2004 study by Lutgens et al. has demonstrated higher sensitivity and specificity of the plasma citrulline determination *versus* sugar absorption tests in patients undergoing chemotherapy for hematological neoplasms.

Herbers and coworkers (2010) have shown that an assessment system based on absolute plasma citrulline levels measures reliably the extent of intestinal mucosal injury for clinical purposes. However, the value of these results is compromised due to a lack of a "golden standard" in diagnostics. The knowledge of chemotherapy regimens that can induce long-term hypocitrullinemia of <10 μmol/L could help identify patients requiring adequate nutritional support. With the use of low-risk regimens without the associated long-term decrease in plasma citrulline levels, parenteral feeding may additionally exacerbate small-intestinal mucosal injury and result in further villous atrophy, at the same time increasing permeability of the mucosal barrier and the risk of bacterial translocation. Moreover, low citrulline levels have been demonstrated to be associated with bacteriemia (Herbers *et al.,* 2008). This, in turn, may indicate the necessity of additional means of daily care for patients during a transplantation procedure. The duration of hypocitrullinemia below a certain value could also be useful in classifying the severity of intestinal mucositis. Further studies are necessary in order to determine the predictive role of citrulline levels in individual patients and to identify adequate cut-off values. Low levels of citrulline have also been observed in patients with intestinal injury due to acute graft *versus* host disease (GvHD). The serum citrulline level is valuable as a suitable marker of GI involvement in acute GvHD after allogeneic stem cells transplantation in both pediatric and adult patients (Vokurka *et al.,* 2013; Merlin *et al.,* 2013). In 2009, a study conducted by van Vliet and coworkers in pediat-

ric patients treated with chemotherapy showed for the first time that plasma citrulline levels correlated well with gastrointestinal mucosal barrier injury. Plasma citrulline is a candidate marker for measuring radiation-induced epithelial small bowel damage, too. The effect of radiotherapy on citrulline concentrations was studied in a mouse model (Lutgens *et al.,* 2003) and later confirmed in humans treated for abdominal or pelvic cancer (Lutgens *et al.,* 2007). Radiotherapy plays an important role in the treatment of abdominopelvic malignances. The small bowel is one of the most radiosensitive and dose-limiting organs. Intestinal toxicity is a significant clinical problem in patients receiving radiotherapy to the abdomen or pelvis. Functional changes associated with small bowel injury have been reported in 40–50% of patients treated with conventional-dose radiotherapy (Letschert *et al.,* 1994).

Radiation-induced intestinal toxicity is clearly associated with the radiation dose to the small bowel and the volume of irradiated small bowel (Baglan *et al.,* 2002; Gunnlaugsson *et al.,* 2007; Onal *et al.,* 2009). Various attempts have been made do decrease intestinal toxicity in patients treated with radiotherapy, such as: three-dimensional (3D) conformal radiotherapy and intensity modulated radiotherapy. Intestinal toxicity remains the dose-limiting side effect for abdominopelvic radiotherapy. Early detection of mucositis as well as assessment and monitoring of mucosal damage are required for effective toxicity management. Few studies have investigated the relationship between citrulline levels and radiation-induced enteropathy. Lutgens and coworkers (2004) first found a positive correlation between citrulline concentration and intestinal dose toxicity. The study group was analyzed for the feasibility of citrulline as a marker for radiation-induced small-intestinal mucosal atrophy during and after abdominal fractionated radiotherapy. The interrelationship between plasma citrulline concentration and clinical toxicity grading on one hand and the small-bowel dose and volume parameters on the other was investigated. In patients treated with fractionated radiation therapy for abdominal or pelvic cancer sites, plasma citrulline concentration correlated better with the radiation dose and volume parameters than did clinical toxicity grading (Lutgens *et al.,* 2004). Onal and coworkers (2011) evaluated the feasibility of using plasma citrulline levels for predicting radiation-induced intestinal toxicity in patients who received pelvic radiotherapy with conventional fractionation. Citrulline levels were significantly reduced and correlated with intestinal toxicity during and after radiotherapy. The results of that study demonstrated that patients with a higher intestinal dose and Radiation Oncology Toxicity Group (RTOG) toxicity score experienced a significantly higher decline in citrulline levels, thus acute radiation enteritis induced by total body irradiation or fractioned localized irradiation can be monitored via citrulline levels, which correlate with the morphological endpoints of epithelial radiation damage, the dose received and the volume of small bowel in the radiation field.

CONCLUSIONS

Circulating citrulline concentration is emerging as an innovative and promising biomarker candidate for the assessment of intestinal function. As an amino acid excreted exclusively by enterocytes of the intestinal mucosa, citrulline is not absent from proteins or nutrition products and is a precursor for the production of arginine by the kidney. In clinical setting, plasma citrulline concentration is an established biomarker of enterocyte functional metabolic mass (trophicity) in pediatric and adult patients due to its high correlation with the residual length of the active and functional small bowel in intestinal diseases (short bowel, extensive enteropathies, chemo- and radiotherapy-induced intestinal toxicity). Independently of the nutritional status, the plasma citrulline concentration (normal range: 30–50 μmol/L) below 10 μmol/L can give an objective threshold for parenteral administration of nutrition in case of intestinal failure due to a lack or dysfunction of enterocytes. Its regular assessment allows the monitoring of intestinal function, except in the case of significant renal failure.

In summary, the present lack of objective tests to assess the degree or duration of intestinal mucosal injury resulting from cancer treatment needs to be emphasized. The intestinal mucosal injury evaluation is based on clinical presentation and is biased as a result of a lack of validation or repeatability. Moreover, the investigators in this field tend to use different scales of intestinal toxicity. In turn, functional assessments of the intestine are poorly tolerated. Invasive examinations, including endoscopy, have a high risk of complications. The plasma level of citrulline seems a good quantitative indicator of mucosal functionality. Levels that reach lowest values following chemo-/radiotherapy may indicate intestinal toxicity. Plasma citrulline level assessments are repeatable, sufficiently specific and sensitive, and meet the criteria for an assessment technique of choice for intestinal mucositis evaluation and monitoring. The simplicity of the method, its low costs and a lack of drawbacks make the citrulline assay the first choice for measuring and monitoring treatment-related gut damage. A citrulline-based assessment of chemo/radiation-induced intestinal toxicity appears to be an objective parameter for monitoring one of the most common side-effect of cancer treatment. The low levels of circulating citrulline correspond with severe intestinal damage. However, this method needs randomized studies for assessing its reliability. Plasma citrulline assays are also likely to help in the development of novel effective treatments to reduce the signs and symptoms of gastrointestinal mucositis.

Conflict of interest statement

The authors declare no conflict of interest.

REFERENCES

Adams RB, Planchon SM, Roche JK (1993) IFN-gamma modulation of epithelial barrier function. Time course, reversibility, and site of cytokine binding. *J Immunol* **150**: 2356–2363.

Adeva MM, Souto G, Blanco N, Donapetry C (2012) Ammonium metabolism in humans. *Metabolism* **61**: 1495–1511.

Ahtonen S, Kallio H (1989) Identification and seasonal variations of amino acids in birch sap used syrup production. *Food Chem* **33**: 125–132.

Almeida JA, Kim R, Stoita A, McIver CJ, Kurtovic J, Riordan SM (2008) Lactose malabsorption in the elderly: role of small intestinal bacterial overgrowth. *Scand J Gastroenterol* **43**: 146–154.

Asgeirsson T, Hang S, Nunoo R, Mascarenas C, Dujovny N, Luchtefeld M, Cavey G, Senagore A (2011) Citrulline: A potential immunomodulator in sepsis. *Surgery* **50**: 744–751.

Austgen TR, Chen MK, Dudrick PS (1992) Cytokine regulation of intestinal glutamine utilization. *Am J Surg* **163**: 174–179.

Baglan KL, Frazier RC, Yan D, Huang RR, Martinez AA, Robertson JM (2002) The dose-volume relationship of acute small bowel toxicity from concurrent 5-FU-based chemotherapy and radiation therapy for rectal cancer. *Int J Radiat Oncol Biol Phys* **52**: 176–183.

Bellm LA, Epstein JB, Rose-Ped A, Martin P, Fuchs HJ (2000) Patient reports of complications of bone marrow transplantation. *Support Care Cancer* **8**: 33–39.

Berg RD (1999) Bacterial translocation from the gastrointestinal tract. *Adv Exp Med Biol* **473**: 11–30.
Biegański T, Kusche J, Lorenz W, Hesterberg R, Stahlknecht CD, Feussner KD (1983) Distribution and properties of human intestinal diamine oxidase and its relevance for the histamine catabolism. *Biochim Biophys Acta* **756**: 196–203.
Bjarnason I, MacPherson A, Hollander D (1995) Intestinal permeability: an overview. *Gastroenterology* **108**: 1566–1581.
Blijlevens NM (2007) Cytotoxic treatment-induced gastrointestinal symptoms. *Curr Opin Support Palliat Care* **1**: 16–22.
Blijlevens NM, Donnelly JP, DePauw BE (2005) Inflammatory response to mucosal barrier injury after myeloablative therapy in allogeneic stem cell transplant recipients. *Bone Marrow Transplant* **36**: 703–707.
Blijlevens NM, Donnelly JP, DePauw BE (2000) Mucosal Barrier Injury: biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview. *Bone Marrow Transplant* **25**: 1269–1278
Blijlevens NM, Lutgens LC, Schattenberg AV, Donnelly JP (2004) Citrulline: a potentially simple quantitative marker of intestinal epithelial damage following myeloablative therapy. *Bone Marrow Transplant* **34**: 193–196.
Bounous G, Echavé V, Vobecky SJ, Navert H, Wollin A (1984) Acute necrosis of the intestinal mucosa with high serum levels of diamine oxidase. *Dig Dis Sci* **29**: 872–874.
Bowen J M, Keefe DM (2008) New pathways for alimentary mucositis. *J Oncol* **2008**: 907892.
Bragg LE, Thompson JS, West WW (1991) Intestinal diamine oxidase levels reflect ischemic injury. *J Surg Res* **50**: 228–233.
Brusilow SW, Maestri NE (1996) Urea cycle disorders: diagnosis, pathophysiology, and therapy. *Adv Pediatr* **43**: 127–170.
Butler RN (2000) Small bowel disorders. In *Bochemical tests of small intestinal function*. Ratnaiken RN, pp 222–230. Arnold, London.
Ceballos I, Chauveau P, Guerin V, Bardet J, Parvy P, Kamoun P, Jungers P (1990) Early alterations of plasma free amino acids in chronic renal failure. *Clin Chim Acta* **188**: 101–108.
Chaveli López B, Gavaldá Esteve C, Sarrión Pérez MG (2011) Dental treatment considerations in the chemotherapy patient. *J Clin Exp Dent* **3**: e31–e42.
Cole AT, Slater K, Sokal M, Hawkey CJ (1993) In vivo rectal inflammatory mediator changes with radiotherapy to the pelvis. *Gut* **34**: 1210–1214.
Costa F, Mumolo MG, Bellini M, Romano MR, Ceccarelli L, Arpe P, Sterpi C, Marchi S, Maltinti G (2003) Role of faecal calprotectin as non-invasive marker of intestinal inflammation. *Dig Liver Dis* **35**: 642–647.
Crenn P, Coudray-Lucas C, Thuillier F (2000) Postabsorptive plasma cutrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. *Gastroenterology* **119**: 1496–1505.
Crenn P, Hanachi M, Neveux N, Cynober L (2011) Circulating citrulline levels: a biomarker for intestinal functionality assesment. *Ann Biol Clin* **69**: 513–521.
Crenn P, Messing B, Cynober L (2008) Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. *Clin Nutr* **27**: 328–339.
Crenn P, Truchis P, Neveux N (2009) Plasma citrulline is a biomarker of enterocyte mass and an indicator of parenteral nutrition in HIV-infected patients. *Am J Clin Nutr* **90**: 587–594.
Crenn P, Vahedi K, Lavergne Slove A (2003) Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. *Gastroenterology* **124**: 1210–1219.
Curis E, Crenn P, Cynober L (2007) Citrulline and the gut. *Curr Opin Clin Nutr Metab Care* **10**: 620–626.
Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, Benazeth S, Cynober L (2005) Almost all about citrulline in mammals. *Amino Acids* **29**: 177–205.
D'Agostino L, Ciacci C, Capuano G, Daniele B, D'Argenio G, Barone MV, Rodinò S, Budillon G, Mazzacca G (1986) Metabolic fate of plasma diamine oxidase: evidence of isolated and perfused rat liver uptake. *Digestion* **34**: 243–250.
De Mey JR, Freund JN (2013) Understanding epithelial homeostasis in the intestine: An old battlefield of ideas, recent breakthroughs and remaining controversies. *Tissue Barriers* **2**: e24965.
Derikx J, Blijlevens N, Donnelly J (2009) Loss of enterocyte mass is accompanied by diminished turnover of enterocytes after myeloablative therapy in haematopoietic stem-cell transplant recipients. *Ann Onc* **20**: 337–342.
De Vita VT, Lawrence TS, Rosenberg SA (2011) *Cancer: Principles and Practice of Oncology*. Amazon, Filadelfia.
Diamanti A, Panetta F, Basso MS, Bracci F, Knafelz D, Papadatou B, Goffredo BM, Torre G. (2012) Plasma citrulline in Crohn's disease as a marker of inflammation or disease localization. *J Clin Gastroenterol* **46**: 622–623.
Diamanti A, Knafelz D, Panetta F, Bracci F, Gambarara M, Papadatou B, Daniele A, Goffredo BM, Pezzi S, Torre G (2011) Plasma citrulline as surrogate marker of intestinal inflammation in pediatric and adolescent with Crohn's disease: preliminary report. *Int J Colorectal Dis* **26**: 1445–1451.
Dioguardi FS (2011) To give or not to give? Lessons from the arginine paradox. *J Nutrigenet Nutrigenomics* **4**: 90–98.
Dorr W, Emmendorfer H, Haide E, Kummermehr J (1994) Proliferation equivalent of 'accelerated repopulation' in mouse oral mucosa. *Int J Radiat Biol* **66**: 157–167.
Duranton F, Lundin U, Gayrard N, Mischak H, Aparicio M, Mourad G, Daurès JP, Weinberger KM, Argilés A (2014) Plasma and urinary amino acid metabolomic profiling in patients with different levels of kidney function. *Clin J Am Soc Nephrol* **9**: 37–45.
Engel A, Mack E, Kern P, Kern WV (1998) An analysis of interleukin-8, interleukin-6 and C-reactive protein serum concentrations to predict fever, gram-negative bacteremia and complicated infection in neutropenic cancer patients. *Infection* **26**: 213–221.
Efron M, Young D, Moser HW, Maccready RA (1964) A simple chromatographic screening test for the detection of disorders of amino acid metabolism. A technic using whole blood or urine collected on filter paper. *N Engl J Med* **270**: 1378–1383.
Elkhatib I, Buchman A (2011) Plasma citrulline contentation as a marker for disease activity in patients with Crohn's disease. *J Clin Gastroenterol* **46**: 308–310.
Elting LS, Sonis ST, Keefe DM (2004) Treatment-induced gastrointestinal toxicity in patients with cancer. Educational Book *Proc Am Soc Clin Oncol* 536–541.
Fallows G, Rubinger M, Bernstein CN (2001) Does gastroenterology consultation change management of patients receiving hematopoietic stem cell transplantation? *Bone Marrow Transplant* **28**: 289–294.
Felig P (1975) Amino acid metabolism in man. *Annu Rev Biochem* **44**: 933–955.
Ferrara JL (1993) Cytokine dysregulation as a mechanism of graft versus host disease. *Curr Opin Immunol* **5**: 794–799.
Ferry DM, Butt TJ, Broom MF, Hunter J, Chadwick VS (1989) Bacterial chemotactic oligopeptides and the intestinal mucosal barrier. *Gastroenterology* **97**: 61–67.
Flam BR, Eichler DC, Solomonson LP (2007) Endothelial nitric oxide production is tightly coupled to the citrulline-NO cycle. *Nitric Oxide* **17**: 115–121.
Flynn NE, Wu G (1996) An important role for endogenous synthesis of arginine in maintaining arginine homeostasis in neonatal pigs. *Am J Physiol* **271**: R1149–R1155.
Gate L, Paul J, Ba GN, Tew KD, Tapiero H (1999) Oxidative stress induced in pathologies: the role of antioxidants. *Biomed Pharmacother* **53**: 169–180.
Gibson RJ, Bowen JM (2011) Biomarkers of regimen-related mucosal injury. *Cancer Treat Rev* **37**: 487–493.
Goke M, Podolsky DK (1996) Regulation of the mucosal epithelial barrier. *Baillieres Clin Gastroenterol* **10**: 393–394.
Gondolesi G, Fishbein T, Chehade M (2002) Serum citrulline is a potential marker for rejection of intestinal allografts. *Transplant Proc* **34**: 918–920.
Graboń W (2006) Arginine as a crucial amino acid in carcinogenesis and tumor growth. *Postepy Hig Med Dosw* **60**: 483–489.
Gray H (1918) The Small Intestine. In *Anatomy Of The Human Body Classic Illustrated Edition*, pp 1173. LEA and Febiger, Philadelphia.
Gray H (2000) The Small Intestine. In *Anatomy Of The Human Body*. Warren H Lewis, pp 168. Bartleby, New York.
Gunnlaugsson A, Kjellén E, Nilsson P, Bendahl PO, Willner J, Johnsson A (2007) Dose-volume relationships between enteritis and irradiated bowel volumes during 5-fluorouracil and oxaliplatin based chemoradiotherapy in locally advanced rectal cancer. *Acta Oncol* **46**: 937–944.
Guzman SG, Bonsack M, Liberty J, Delaney JP (1989) Abdominal radiation causes bacterial translocation. *J Surg Res* **46**: 104–107.
Häberle J, Boddaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, Karall D, Martinelli D, Crespo PS, Santer R, Servais A, Valayannopoulos V, Lindner M, Rubio V, Dionisi-Vici C (2012) Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis* **7**: 32.
Häberle J, Pauli S, Linnebank M, Kleijer WJ, Bakker HD, Wanders RJ, Harms E, Koch HG (2002) Structure of the human argininosuccinate synthetase gene and an improved system for molecular diagnostics in patients with classical and mild citrullinemia. *Hum Genet* **110**: 327–333.
Helander HF, Fändriks L (2014) Surface area of the digestive tract - revisited. *Scand J Gastroenterol* **49**: 681–689.
Herbers AH, Blijlevens NM, Donnelly JP, de Witte TJ (2008) Bacteraemia coincides with low citrulline concentrations after high-dose melphalan in autologous HSCT recipients. *Bone Marrow Transplant* **42**: 345–349.
Herbers AH, Feuth T, Donnelly JP, Blijlevens NM (2010) Citrulline-based assessment score: first choice for measuring and monitoring intestinal failure after high-dose chemotherapy. *Ann Oncol* **21**:1706–1711.
Hoffmann GF, Kölker S (2013) Defects in amino acid catabolism and the urea cycle. *Handb Clin Neurol* **113**: 1755–1773.

Hozyasz KK, Oltarzewski M, Lugowska I (2010) Whole blood citrulline concentrations in newborns with non-syndromic oral clefts--a preliminary report. *Asia Pac J Clin Nutr* **19**: 217–222.
Hozyasz KK, Szaflarska-Popławska A, Oltarzewski M, Mazur J, Muller L, Jablonska E, Milanowski A (2006) Whole blood citrulline levels in patients with coeliac disease. *Pol Merkur Lek* **20**: 173–175.
Husson A, Brasse-Lagnel C, Fairand A, Renouf S, Lavoinne A (2003) Argininosuccinate synthetase from the urea cycle to the citrulline-NO cycle. *Eur J Biochem* **270**: 1887–1899.
Huynh D, Akçora D, Malaterre J, Chan CK, Dai XM, Bertoncello I (2013) CSF-1 receptor-dependent colon development, homeostasis and inflammatory stress response. *PLoS One* **8**: e56951.
Jeevanandam M, Young DH, Ramias L, Schiller WR (1990) Effect of major trauma on plasma free amino acid concentrations in geriatric patients. *Am J Clin Nutr* **51**: 1040–1045.
Johson LR (2012) Stem cells in the gastrointestinal tract. In *Physiology of the gastrointestinal tract*. Nicolas A. Wright, pp 359–378. Academic Press. Elsevier, London, San Diego, Waltham.
Juby LD, Dixon MF, Axon AT (1987) Abnormal intestinal permeability and jejunal morphometry. *J Clin Pathol* **40**: 714–718.
Keefe DM, Brealey J, Goland GJ, Cummins AG (2000) Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. *Gut* **47**: 632–637.
Ki Y, Kim W, Nam J, Kim D, Park D, Kim D (2009) C-reactive protein levels and radiation-induced mucositis in patients with head-and-neck cancer. *Int J Radiat Oncol Biol Phys* **75**: 393–398.
Kohout P, Cerman J, Brátová M, Zadák Z (1999) Small bowel permeability in patients with cytostatic therapy. *Nutrition* **15**: 546–549.
Kurtz AC (1937) A Simple Sythesis of Citrulline. http://www.jbc.org/content/122/2/477.full.pdf
Lacy ER (1988) Epithelial restitution in the gastrointestinal tract. *J Clin Gastroenterol* **10**: S72–S77.
Lanpher BC, Gropman A, Chapman KA, Lichter-Konecki, Urea Cycle Disorders Consotrium (2011) In *Urea Cycle Disorders* Overview. GeneReviews. University of Washington, Seattle.
Lau T, Owen W, Yu YM, Noviski N, Lyons J, Zurakowski D, Tsay R, Ajami A, Young VR, Castillo L (2000) Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients. *J Clin Invest* **105**: 1217–1225.
Letschert JG, Lebesque JV, Aleman BM, Bosset JF, Horiot JC, Bartelink H, Cionini L, Hamers JP, Leer JW, van Glabbeke M (1994) The volume effect in radiation-related late small bowel complications: results of a clinical study of the EORTC Radiotherapy Cooperative Group in patients treated for rectal carcinoma. *Radiother Oncol* **32**: 116–123.
Logan RM, Stringer AM, Bowen JM, Gibson RJ, Sonis ST, Keefe DM (2008) Serum levels of NFkappaB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biol Ther* **7**: 1139–1145.
López-Galindo MP, Bagán JV, Jiménez-Soriano Y, Alpiste F, Camps C (2006) Clinical evaluation of dental and periodontal status in a group of oncological patients before chemotherapy. *Med Oral Patol Oral Cir Bucal* **11**: E17–E21.
Luiking YC, Engelen MP, Deutz NE (2010) Regulation of nitric oxide production in health and disease. *Curr Opin Clin Nutr Metab Care* **13**: 97–104.
Lutgens LC, Blijlevens NM, Deutz NE (2005) Monitoring myeloablative therapy-induced small bowel toxicity by serum citrulline concentration: a comparison with sugar permeability tests. *Cancer* **103**: 191–199.
Lutgens LC, Deutz N, Granzier-Peeters M, Beets-Tan R, De Ruysscher D, Gueulette J, Cleutjens J, Berger M, Wouters B, von Meyenfeldt M, Lambin P (2004) Plasma citrulline concentration: a surrogate end point for radiation-induced mucosal atrophy of the small bowel. A feasibility study in 23 patients. *Int J Radiat Oncol Biol Phys* **60**: 275–285.
Lutgens LC, Deutz NE, Gueulette J, Cleutjens JP, Berger MP, Wouters BG, von Meyenfeldt MF, Lambin P (2003) Citrulline: a physiologic marker enabling quantitation and monitoring of epithelial radiation-induced small bowel damage. *Int J Radiat Oncol Biol Phys* **57**: 1067–1074.
Lutgens L, Lambin P (2007) Biomarkers for radiation-induced small bowel epithelial damage: an emerging role for plasma citrulline. World J Gastroenterol **13**: 3033–3042.
Maddens S, Charruyer A, Plo I (2002) Kit signaling inhibits the sphingomyelin-ceramide pathway through PLC gamma 1: implication in stem cell factor radioprotective effect. *Blood* **100**: 1294–1301.
Peterson DE, Bensadoun RJ, Roila F; ESMO Guidelines Working Group (2011) Management of oral and gastrointestinal mucositis: ESMO Clinical Practice Guidelines. *Ann Oncol Suppl* **6**: vi78–vi84.
Marano CW, Lewis SA, Garulacan LA (1998) Tumor necrosis factor-alpha increases sodium and chloride conductance across the tight junction of CACO-2 BBE, a human intestinal epithelial cell line. *J Membr Biol* **161**: 263–274.
Marsh MN (1992) Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* **102**: 330–354.
Masszi T, Mank A (2012) Supportive care. In *The 2012 Revised Edition of the ESH-EBMT Handbook on Haemopoietic Stem Cell Transplantation*. Apperley J, Carreras E, Gluckman E, Masszi T. pp. 157–174. Forum Service Editore, Genoa.
Melichar B, Dvorák J, Hyspler R, Zadák Z (2005) Intestinal permeability in the assessment of intestinal toxicity of cytotoxic agents. *Chemotherapy* **51**: 336–338.
Melichar B, Kohout P, Brátová M, Solichová D, Králícková P, Zadák Z (2001) Intestinal permeability in patients with chemotherapy-induced stomatitis. *J Cancer Res Clin Oncol* **127**: 314–318.
Menzies IS, Laker MF, Pounder R, Bull J, Heyer S, Wheeler PG, Creamer B (1997) Abnormal intestinal permeability to sugars in villous atrophy. *Lancet* **8152**: 1107–1109.
Merlin E, Minet-Quinard R, Doré E (2010) Citrullinaemia is a helpful marker of gastro-intestinal GvHD in children. *Bone Marrow Transplant* **45** (Suppl 2): 128.
Merlin E, Minet-Quinard R, Pereira B, Rochette E, Auvrignon A, Oudot C, Sapin V, Deméocq F, Kanold J (2013) Non-invasive biological quantification of acute gastrointestinal graft-versus-host disease in children by plasma citrulline. *Pediatr Transplant* **17**: 683–687.
Miller C R, McLeod HL (2007) Pharmacogenomics of cancer chemotherapy-induced toxicity. *J Support Oncol* **5**: 9–14.
Moinard C, Nicolis I, Neveux N, Darquy S, Bénazeth S, Cynober L (2008) Dose-ranging effects of citrulline administration on plasma amino acids and hormonal patterns in healthy subjects: the Citrudose pharmacokinetic study. *Br J Nutr* **99**: 855–862.
Moncada S, Higgs EA (2006) The discovery of nitric oxide and its role in vascular biology. *Br J Pharmacol* **147**: 193–201
Mori M, Gotoh T (2004) Arginine metabolic enzymes, nitric oxide and infection. *J Nutr* **134**: 2820S–2825S.
Morris SM Jr (2002) Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* **22**: 87–105.
Mothersill C, Seymour C (2012) Are epigenetic mechanisms involved in radiation-induced bystander effects? *Front Genet* **3**: 1–6.
Nagamani SC, Erez A, Lee B (2012) Argininosuccinate lyase deficiency. *Genet Med* **14**: 501–507.
Neveux N, David P, Cynober L (2004) Measurement of amino acid concentration in biological fluids using ion exchange chromatography. In *Metabolic and therapeutic aspects of amino acids in clinical nutrition*. Cynober L, pp 17–28. Boca Raton: CRC Press.
Nicola P (2007) Mucositis in patients with hematologic malignances; on overview. *Haematologica* **92**: 222–231.
Nobelprize.org. Web. The Nobel Prize in Physiology or Medicine 1998. 16 Apr 2014.
http://www.nobelprize.org/nobel_prizes/medicine/laureates/1998/ill-pres/
Onal C, Kotek A, Unal B, Arslan G, Yavuz A, Topkan E, Yavuz M (2011) Plasma citrulline levels predict intestinal toxicity in patients treated with pelvic radiotherapy. *Acta Oncol* **50**: 1167–1174.
Onal C, Topkan E, Efe E, Yavuz M, Sonmez S, Yavuz A (2009) Comparison of rectal volume definition techniques and their influence on rectal toxicity in patients with prostate cancer treated with 3D conformal radiotherapy: a dose-volume analysis. *Radiat Oncol* **4**: 14.
Papadia C, Sherwood RA, Kalantzis C, Wallis K, Volta U, Fiorini E, Forbes A (2007) Plasma citrulline concentration: a reliable marker of small bowel absorptive capacity independent of intestinal inflammation. *Am J Gastroenterol* **102**: 1474–1482.
Pappas PA, Saudubray JM, Tzakis AG, Rabier D, Carreno MR, Gomez-Marin O, Huijing F, Gelman B, Levi DM, Nery JR, Kato T, Mittal N, Nishida S, Thompson JF, Ruiz P (2001) Serum citrulline and rejection in small bowel transplantation: a preliminary report. *Transplantation* **72**: 1212-1216.
Pappas PA, Saudubray JM, Tzakis AG (2002) Serum citrulline as a marker of acute cellular rejection for intestinal transplantation. *Transplant Proc* **34**: 915–917.
Parulekar W, Mackenzie R, Bjarnason G, Jordan RC (1998) Scoring oral mucositis. *Oral Oncol* **34**: 63–71.
Pico J L, Avila-Garavito A, Naccache P (1998) Mucositis: Its Occurrence, Consequences, and Treatment in the Oncology Setting. *The Oncologist* **3**: 446–451.
Pitkänen HT, Oja SS, Kemppainen K, Seppä JM, Mero AA (2003) Serum amino acid concentrations in aging men and women. *Amino Acids* **4**: 413–421.
Piton G, Manzon C, Cypriani B, Carbonnel F, Capellier G (2011) Acute intestinal failure in critically ill patients: is plasma citrulline the right marker? *Intensive Care Med* **37**: 911–917.
Potten CS (1998) Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos Trans R Soc Lond B Biol Sci* **1370**: 821–830.
Potten CS, Loeffler M (1987) A comprehensive model of the crypts of the small intestine of the mouse provides insight into the mechanisms of cell migration and the proliferation hierarchy. *J Theor Biol* **127**: 381–391.

Powell DW (1981) Barrier function of epithelia. *Am J Physiol* **241**: G275–G288.
Raber-Durlacher JE, Elad S, Barasch A (2010) Oral mucositis. *Oral Oncol* **46**: 452–456.
Rabier D, Kamoun P (1995) Metabolism of citrulline in man. *Amino Acids* **9**: 299–316.
Reka S, Kotler DP (1998) Correlation of intestinal structure and function in HIV infection. *Gastrointest Endosc Clin N Am* **8**: 841–856.
Richter KK, Fagerhol MK, Carr JC, Winkler JM, Sung CC, Hauer-Jensen M (1997) Association of granulocyte transmigration with structural and cellular parameters of injury in experimental radiation enteropathy. *Radiat Oncol Investig* **6**: 275–282.
Rimando AM, Perkins-Veazie PM (2005) Determination of citrulline in watermelon rind. *J Chromatogr A* **1078**: 196–200.
Rougé C, Des Robert C, Robins A, Le Bacquer O, De La Cochetière MF, Darmaun D (2008) Determination of citrulline in human plasma, red blood cells and urine by electron impact (EI) ionization gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **865**: 40–47.
Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST (2004) Mucositis Study Section of the Multinational Association for Supportive Care in Cancer; International Society for Oral Oncology. Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* **100**: 2026–2046.
Santarpia L, Catanzano F, Ruoppolo M, Alfonsi l., Vitale DF, Pecce R, Pasanisi F, Contaldo F, salvatore F (2008) Citrulline blood levels as indicators of residual intestinal absorption in patients with short bowel syndrome. *Ann Nutr Metab* **52**: 137–142.
Scaglia F, Brunetti-Pierri N, Kleppe S, Marini J, Carter S, Garlick P, Jahoor F, O'Brien W, Lee B (2004) Clinical consequences of urea cycle enzyme deficiencies and potential links to arginine and nitric oxide metabolism. *J Nutr* **134**: 2775S–2782S.
Schofield R (1983) The stem cell system. *Biomed Pharmacother* **37**: 375–380.
Schubert MM, Williams BE, Lloid ME (1992) Clinical assessment scale for the rating of oral mucosal changes associated with bone marrow transplantation. Development of an oral mucositis index. *Cancer* **69**: 2469–2477.
Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, Spicker W, Schulze F (2008) Pharmacokinetic and pharmaodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* **65**: 51–59.
Shaw MT, Spector MH, Ladman AJ (1979) Effects of cancer, radiotherapy and cytotoxic drugs on intestinal structure and function. *Cancer Treat Rev* **6**: 141–151.
Sherman ML, Datta R, Hallahan DE, Weichselbaum RR, Kufe DW (1991) Regulation of tumor necrosis factor gene expression by ionizing radiation in human myeloid leukemia cells and peripheral blood monocytes. *J Clin Invest* **87**: 1794–1797.
Sherwood L (2006) Fundamentals of physiology: a human perspective. p 768. Thomson Learning, London.
Shin S, Mohan S, Fung HL (2011) Intracellular l-arginine concentration does not determin NO production in endothelial cells: implications on the "l-arginine paradox". *Bochem Biophys Res Commun* **414**: 660–663
Slack JM (2000) Stem cells in epithelial tissues. *Science* **5457**: 1431–1433.
Sleisenger MH, Fordtran JS (1989) *Gastrointestinal disease pathofysiology, diagnosis, management*. WB Saunders Company, Philadephia.
Solanas G, Batlle E (2011) Control of cell adhesion and compartmentalization in the intestinal epithelium. *Exp Cell Res* **317**: 2695–2701.
Sonis ST (1998) Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol* **34**: 39–43.
Sonis ST (2002) The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with antineoplastic therapy. *Crit Rev Oral Biol Med* **13**: 380–389.
Sonis ST (2004) The pathobiology of mucositis. *Nat Rev Cancer* **4**: 277–284.
Sonis ST (2009) Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral Oncol* **45**: 1015–1020.
Sonis ST (2013) Oral mucositis in head and neck cancer. *Am Soc Clin Oncol Educ Book* **2013**: 236–240.
Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M, Bekele BN, Raber-Durlacher J, Donnelly JP, Rubenstein EB (2004) Mucositis study section of the multinational association for supportive care in cancer; international society for oral oncology. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients**.** *Cancer* **100** (9 Suppl): 1995–2025.
Sonis ST, Oster G, Fuchs H, Bellm L, Bradford WZ (2001) Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* **19**: 2201–2205.
Sonis ST, Peterson RL, Edwards LJ (2000) Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncol* **36**: 373–381.
Sonis ST, Tracey C, Shklar G, Jenson J, Florine D (1990) An animal model for mucositis induced by cancer chemotherapy. *Oral Surg Oral Med Oral Pathol* **69**: 437–443.
Stein WH, Moore S (1954) The free amino acids of human blood plasma. *J Biol Chem* **211**: 915–926.
Tancrède CH, Andremont AO (1985) Bacterial translocation and gram-negative bacteremia in patients with hematological malignancies. *J Infect Dis* **152**: 99–103.
Travis S, Menzies I (1992) Intestinal permeability: functional assessment and significance. *Clin Sci Lond* **82**: 471–488.
Tooley KL, Howarth GS, Butler RN (2009) Mucositis and non-invasive markers of small intestinal function. *Cancer Biol Ther* **8**: 753–758.
Treister N, Sonis S (2007) Mucositis: biology and management. *Curr Opin Otolaryngol Head Neck Surg* **15**: 123–129.
Tuchman M, McCullough BA, Yudkoff M (2000) The molecular basis of ornithine transcarbamylase deficiency. *Eur J Pediatr* **159**: S196–S198.
van de Poll MCG, Ligthart-Melis GC, Boelens PG, Deutz NEP, van Leeuwen PAM, Dejong CHC (2007) Intestinal and hepatic metabolism of glutamine and citrulline in humans. *J Physiol* **581**: 819–827.
van de Poll MC, Soeters PB, Deutz NE, Fearon KC, Dejong CH (2004) Renal metabolism of amino acids: its role in interorgan amino acid exchange. *Am J Clin Nutr* **79**: 185–197.
van der Velden WJ, Herbers AH, Feuth T (2010) Intestinal damage determines the inflammatory response and early complications in patients receiving conditioning for a stem cell transplantation. *PLoS One* **5**: e15156.
Vanuytsel T, Senger S, Fasano A, Shea-Donohue T (2013) Major signaling pathways in intestinal stem cells. *Biochim Biophys Acta* **1830**: 2410–2426.
van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ (2010) The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathog* **6**: e1000879.
van Vliet MJ, Tissing WJ, Rings EH, Koetse HA, Stellaard F, Kamps WA, de Bont ES (2009) Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. *Pediatr Blood Cancer* **53**: 1188–1194.
van Wyk M, Sommers DK, Steyn AG (1985) Evaluation of gastrointestinal motility using the hydrogen breath test. *Br J Clin Pharmacol* **20**: 479–481.
Vokurka S, Chvojkova I, Svoboda T, Brandejsova R, Jungova A, Bystricka E, Jindra P (2014) The impact of oral cryotherapy and oral and gastrointestinal mucositis after autologous stem cell transplantation. *Eur J Oncol Nurs* **18**: 228–229.
Vokurka S, Svoboda T, Rajdl D, Sedláčková T, Racek J, Koza V, Trefil L (2013) Serum citrulline levels as a marker of enterocyte function in patients after allogeneic hematopoietic stem cells transplantation - a pilot study. *Med Sci Monit* **19**: 81–85.
Wada M (1930). Über Citrullin, eine neue Aminosäure im Presssaft der Wassermelone, Citrullus vulgaris Schrad. *Biochem. Zeit* **224**: 420–429.
Wakabayashi Y (2003) The glutamate crossway. In *Metabolic and therapeutic aspects of amino acids in clinical nutrition*. Cynober L, pp 135–152. CRC Press, Boca Raton.
Wardill HR, Bowen JM (2013) Chemotherapy-induced mucosal barrier dysfunction: an updated review on the role of intestinal tight junctions. *Curr Opin Support Palliat Care* **7**: 155–161.
Weber FL Jr, Reiser BJ (1982) Relationship of plasma amino acids to nitrogen balance and portal-systemic encephalopathy in alcoholic liver disease. *Dig Dis Sci* **27**: 103–110.
Wedlake L, McGough C, Hackett C, Thomas K, Blake P, Harrington K, Tait D, Khoo V, Dearnaley D, Andreyev HJ (2008) Can biological markers act as non-invasive, sensitive indicators of radiation-induced effects in the gastrointestinal mucosa? *Aliment Pharmacol Ther* **27**: 980–987.
Wells CL, Maddaus MA, Simmons RL (1988) Proposed mechanisms for the translocation of intestinal bacteria. *Rev Infect Dis* **10**: 958-979.
Windmueller HG, Spaeth AE (1981) Source and fate of circulating citrulline. *Am J Physiol* **241**: E473–E480.
Wong MH, Stappenbeck TS, Gordon JI (1999) Living and commuting in intestinal crypts. *Gastroenterology* **116**: 208–210.
Woo SB, Sonis ST, Monopoli MM, Sonis AL (1993) A longitudinal study of oral ulcerative mucositis in bone marrow transplant recipients. *Cancer* **72**: 1612–1617.
Wright NA (1998) Aspects of the biology of regeneration and repair in the human gastrointestinal tract. *Philos Trans R Soc Lond B Biol Sci* **1370**: 925–933.
Wu G (1998) Intestinal mucosal amino acid catabolism. *J Nutr* **128**: 1249–1252.
Wu G (1997) Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. *Am J Physiol* **272**: G1382–G1390.
Wu G, Davis PK, Flynn NE, Knabe DA, Davidson JT (1997) Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. *J Nutr* **127**: 2342–2349.

Wu G, Jaeger LA, Bazer FW, Rhoads JM (2004) Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *J Nutr Biochem* **15**: 442–51.

Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. *Biochem J* **336**: 1–17.

Wu G, Meininger CJ, Knabe DA, Bazer FW, Rhoads JM (2000) Arginine nutrition in development, health and disease. *Curr Opin Clin Nutr Metab Care* **3**: 59–66.

Yu HC, Tuteja S, Moon JI, Kleiner GI, Conanan L, Gaynor JJ (2005) Utilization of dried blood spot citrulline level as a noninvasive method for monitoring graft function following intestinal transplantation. *Transplantation* **80**: 1729–1733.

Zur E (2012) Gastrointestinal mucositis: focus on the treatment of the effects of chemotherapy and radiotherapy on the rectum. *Int J Pharm Compd* **16**: 117–124.