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Review

ESTABLISHING THE CUT-OFFS OF LEAKY GUT SYNDROME DIAGNOSTIC: WHERE ARE WE NOW?

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Gastrointestinal mucosa forms a surface that interacts with many external factors. Beside the digestion and absorption of nutrients, it also acts as a barrier to allergens, pathogens, and toxins. Leaky gut syndrome is defined as a gut mucosal barrier dysfunction, which results in abnormally increased intestinal permeability. Research shows that leaky gut syndrome (LGS) has a pathogenetic relationship with a series of gastrointestinal and extra-intestinal disorders. This review discusses the current understanding of intestinal barrier composition and pathological contribution of LGS to various diseases. The major aim of this paper is to review different methods for diagnostics and evaluation of intestinal wall permeability, identifying their priorities and disadvantages.

Keywords: *leaky gut, intestinal barrier, gut permeability, microbiota.*

INTRODUCTION

Over the past decade, the topic of a microbiome has become increasingly relevant not only in the scientific community, but also in the context of clinical medicine. In recent years, the human microbiome has become defined as a "separate virtual organ", the detailed study of which has given rise to the hope of discovering new tailored therapy for already known diseases. The term "microbiome" is defined as a collective genome of all organisms living in a defined habitat and possessing certain physical and chemical properties (Berg *et al*., 2020). It includes bacteria, lower and higher eukaryotes, viruses and archaea, their genomes and specific environmental conditions (Marchesi and Ravel, 2015). The biotic component of a microbiome is defined as microbiota (Davenport *et al.,* 2017).

Microorganisms make up approximately 1–3 per cent of a human's body weight and have many beneficial functions, such as the production of vitamins, synthesis of anti-inflammatory factors, participation in nutrient breakdown, and aids the human immune system in pathogen recognition (Human Microbiome Project). The host immune system limits the location of the specific spectrum of the microbiota to its natural niches, such as, for example, the epithelium that covers the mucosa and the skin, and the gastrointestinal tract. The gut microbiota is important in the regulation of many physiological processes in the human body, such as weight control, blood pressure regulation, energy metabolism, immune responses, glucose homeostasis, and coagulation processes (Dominguez-Bello *et al*., 2019). Via the gut-brain axis, it regulates cognitive function, behaviour, pain, anxiety, and mood (Mohajeri *et al*., 2018). Also, it plays an important role in regulating the integrity and functionality of the gut barrier, maintaining homeostasis of the whole organism (DiTommaso *et al.*, 2021) The human gut microbiota changes throughout life. From birth to about 12 years of age, it develops, remains stable through adulthood and then declines in older age. In an adult the composition of the gut microbiome is approximately

60–70% stable (Mohajeri *et al*., 2018). Different types of pathogens, infection, changes in diet and lifestyle can lead to instability, inducing perturbations of gut microbiota (Mohajeri *et al*., 2018; DiTommaso *et al.*, 2021). The imbalance of the intestinal microbiota can lead to pathological conditions and diseases, such as leaky gut syndrome (LGS) (DiTommaso *et al.*, 2021). One of the major questions in this field so far is how to assess and evaluate intestinal wall permeability. This paper explores the essence of LGS, pointing on crucial aspects of this phenomenon.

PATHOGENESIS

Composition of the intestinal barrier. Leaky gut syndrome is defined as a gut mucosal barrier dysfunction that results in abnormally increased intestinal permeability (Kinashi and Hase, 2021). Physiological factors that ensure normal functioning of the intestinal barrier involve the epithelial surface, extracellular component (mucus), immunological factors, gut-vascular part, aforementioned microbial barrier, functional activity of the gut (peristalsis) and hepatic filter (Portincasa *et al*., 2022).

To fully understand the LGS pathogenesis, it is important to consider the essence of the gut barrier. Unlike the multilayered skin barrier, the cellular part of the gut mucosal barrier consists of a single layer of epithelial cells (Johansson *et al.*, 2016). An important role here is played by enterocytes, Goblet cells, Paneth cells, Tufts cells, and enteroendocrine cells (Portincasa *et al*., 2022).

Tight junctions. Cellular continuity and integrity of the epithelial surface, which provides a biochemical and physical barrier to allergens, pathogens and toxins, is highly dependent on apical junctional protein complex — tight junction (TJ) functionality. Intracellular TJ are located in the apical part of the lateral epithelial cell membrane (Suzuki, 2020). It is formed by a complex of numerous proteins, such as claudins, occludins, tricellulins, junctional adhesion molecule-A (JAM-A), intracellular plaque proteins — zonula occludens (ZOs) and cingulin (Krug and Schulzke, 2014; Suzuki, 2020). Certain types of claudins form selective channels for cations, anions, and water; some types are barrier-forming. Occludins and tricellulins limit the passage of macromolecules. Malfunctioning of TJ can lead to uncontrolled paracellular passage and provoke development of a variety of diseases (Krug and Schulzke, 2014).

Mucus layer. Mucus produced by enterocytes and Goblet cells provide protection against mechanical factors, and it lubricates the surface, and traps and transports bacteria and debris (Johansson *et al.*, 2016) The structure of mucus differs depending on its location in the intestinal tract; it is a single-layer barrier in the small intestine and a two-layer coating (inner and outer) in the colon (Binienda *et al*., 2020). The main components of mucus are water (90–95%), lipids (1–2%), electrolytes and proteins (Bansil and Turner, 2018). However, the most significant structural and functional elements of mucus, which give it the main protective properties, are large glycoproteins mucins (1–5%) (Johansson *et al.*, 2016; Bansil and Turner, 2018). There are two types of mucins: transmembrane mucins, produced by enterocytes, and gel-forming mucins, synthesised and secreted by Goblet cells. Gel-forming mucins are able to coat the surface, forming a defensive barrier (Paone and Cani, 2020). Transmembrane mucins cover and protect the apical surface of enterocytes, perceive change of intraluminal environment and take part in host-microbe interactions (Paone and Cani, 2020; Pelaseyed and Hansson, 2020). Notably, mucus and its components possess not only protective properties, but are also the habitat of the intestinal microbiota. Its components serve as nutrients for commensal bacteria, contributing to their adaptation and development (Sicard *et al*., 2017). In turn, metabolites of the microbiota like short chain fatty acids (SCFAs) strengthen epithelial TJ and modulate inflammatory response via regulation of inflammatory cytokine production. The stability of this ecosystem is very important (DiTommaso *et al.*, 2021).

Immunological factors. Despite the fact that mucus forms a gel-type cover, it is still permeable to bacteria and bacterial-sized beads. However due to the secretion and functioning of antibacterial peptides (AMPs), such as lysozyme C, alpha-defensins, phospholipase, regenerating islet-derived 3-gamma and C-type lectin, specific proteins and secretory IgA, they are not in direct contact with the cell surface (Takiishi *et al*., 2017; Hansson, 2020; DiTommaso *et al.*, 2021). Secretory IgA, produced by plasma cells, colonises the mucus layer, protecting the intestinal wall against pathogen adhesion and penetration. In addition, it modulates gut microbiota composition and microbiota–host interaction (Pietrzak *et al.*, 2020; DiTommaso *et al.*, 2021).

Pathological contribution of LGS to various diseases. Intestinal dysbiosis, use of numerous toxic substances and drugs, poor nutrition, sustained inflammation and infection can lead to impairment of the epithelial barrier with dysregulation of adhesion molecules and damage of TJ integrity, resulting with LGS. LGS can be associated with bacterial translocation and entry of toxins into systemic circulation, resulting with numerous gastrointestinal disorders and extra-intestinal diseases (Table 1) (Takiishi *et al*., 2017; Binienda *et al*., 2020; DiTommaso *et al.*, 2021). These include, but are not limited to: 1) inflammatory bowel disorders (IBD) (Michielan and D'Incà, 2015; Jaworska *et al*., 2019; Turpin *et al.*, 2020), 2) necrotising enterocolitis (NEC) (Jaworska *et al*., 2019), 3) irritable bowel syndrome (Shulman *et al*., 2014; Michielan and D'Incà, 2015, Jaworska *et al*., 2019), 4) gluten-related disorders, such as celiac disease, wheat-associated allergy and non-celiac gluten/wheat sensitivity (Cardoso-Silva *et al*., 2019; Jaworska *et al*., 2019), 5) non-alcoholic fatty liver disease (Michielan and D'Incà, 2015; Kessoku *et al*., 2021; Portincasa *et al*., 2022), 6) metabolic syndrome (Chakaroun *et al*., 2020), 7) type 1 (Fasano, 2020) and type 2 diabetes (Chakaroun *et al*., 2020), 8) Parkinson disease, 9) Alzheimer's disease,10) dementia, 11) autism spectrum disorders, 12) schizophrenia, 13) major depressive disorders, chronic fatigue syndrome

Table 1. Disorders pathogenetically associated with leaky gut syndrome

Disorders pathogenetically associated with leaky gut syndrome	
Gastrointestinal	Inflammatory bowel disorders
	Irritable bowel syndrome
	Necrotising enterocolitis
	Gluten-related disorders
	Non-alcoholic fatty liver disease
Metabolic	Metabolic syndrome
	Diabetes mellitus type 1 and type 2
Neurological	Parkinson disease
	Alzheimer's disease
	Multiple sclerosis
	Chronic fatigue syndrome (myalgic encephalomyelitis)
Psychiatric	Schizophrenia
	Major depressive disorders
	Autism spectrum disorders
	Dementia
Autoimmune endocrinopathies	Systemic lupus erythematosus
	Hashimoto's thyroiditis
	Graves' disease
Rheumatic diseases and musculoskeletal conditions	Ankylosing spondylitis
Allergic diseases	Allergic asthma
Oncological diseases	Hepatocellular carcinoma
	Glinoma

or myalgic encephalomyelitis, multiple sclerosis, ankylosing spondylitis, cancer (glinoma, hepatocellular carcinoma) (Obrenovich, 2018; Fasano, 2020), allergic asthma (Farshchi *et al*., 2017), autoimmune thyroid diseases (AITD) Hashimoto's thyroiditis (HT), Graves' disease (GD) (Knezevic *et al*., 2020; Zheng *et al*., 2021), and systemic lupus erythematosus (SLE) (Paray *et al*., 2020). Altered intestinal permeability has been described in some conditions, such as sepsis, systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF), acute pancreatitis, major surgery and severe trauma (Michielan and D'Incà, 2015).

It has been reported that identification of changes in gut-blood barrier can be used to predict the IBD course (Michielan and D'Incà, 2015). Elevated intestinal permeability was observed to be associated with Crohn's disease (CD) diagnosis within a few years after its identification in first-degree CD relatives (Turpin *et al.*, 2020). Thus, altered gut permeability should be considered as a potential biomarker for diagnostics and possibly a tool for assessing the severity of diseases associated with intestinal barrier dysfunction and systemic inflammation (Hollander and Kaunitz, 2020).

GUT PERMEABILITY SCORING

Currently, there are several options for measurement of intestinal permeability, among which are invasive and non-invasive diagnostic tests (Table 2) (Cardoso-Silva *et al*., 2019).

***In vivo* tests.** *In vivo* tests are based on the input of exogenous substance by oral or intravenous rout with its subsequent detection in biological material. However, the efficacy of this diagnostic test can be affected by a series of physiological factors, such as gastro-intestinal evacuation delay, changes in gut peristaltic activity, altered intestinal absorption, renal dysfunction, variable hydration status of the tested subjects and incomplete urine collection (Gerova *et al*., 2011). Among invasive tests, *in vivo* and *ex vivo* tests are distinguished. The *in vivo* test group includes the following methods: lactulose/ mannitol ratio test, iohexol test, use of polyethylene glycol (PEG), radioactive chromium complexed with ethylene diamine tetracetic acid (^{51}Cr-EDTA) and fluorescein isothiocyanate (FITC)-dextran (Cardoso-Silva *et al*., 2019).

Lactulose/mannitol ratio test. One of the most frequently used tests in contemporary clinical practice is the lactulose/mannitol ratio test (L/M test). Concentration of monosaccharides, such as mannitol, reflect the rate of small molecule absorption. Concentration of disaccharides, such as lactulose, estimate the permeability of large molecules, since its absorption occurs through the paracellular junction complex (Dastych *et al.*, 2008). Both of these sugars are passively absorbed in the gastrointestinal tract, they do not undergo extensive metabolism and are excreted in unchanged condition proportionally to the absorbed quantity (Sequeira *et al*., 2014). This method is based on oral administration of the two sugars of different molecular size and with different absorption mechanisms. Physiological aspects such as impaired gastric evacuation, altered intestinal transit, and deterioration of renal function have a lesser effect on the overall test result, since they proportionally affect the absorption of both substances. Also, there are options to measure the lactulose/creatinine ratio, and lactulose excretion in urine. These tests have very reliable and comparable values of sensitivity and specificity in assessment of intestinal permeability in groups of patients with CD and alcohol-related liver cirrhosis (Child-Turcotte-Pugh score B–C) patients (Dastych *et al.*, 2008). As an alternative, lactulose sucralose or cellobiose can be used and instead of mannitol — monosaccharide L-rhamnose can be used (Khoshbin *et al*., 2021). However, it is important to bear in mind that the lactulose/mannitol or rhamnose test is not recommended for large intestine permeability detection, as these sugars are degraded by colonic bacteria (Vancamelbeke and Vermeire, 2017).

Iohexol test. Oral administration of water-soluble contrast medium iohexol and subsequent determination of its concentration in serum and urine also can be used for intestinal permeability evaluation (Gerova *et al*., 2011). In a study with IBD patients, the iohexol test was found to be superior to the lactulose/mannitol ratio test (Halme *et al*., 2000). The iohexol test is based on the notion that high molecular weight substances reflect changes in paracellular permeability, which in turn is regulated by strong TJ compounds.

Table 2. Tests for intestinal permeability evaluation

Tests for intestinal permeability evaluation				
Assay	Probe/measurement	Material	Advantages	Disadvantages
In vivo tests:				
Administration of monosaccharides/ disaccharides	Lactulose/ mannitol ratio test (alt. sucralose, cellobiose/ L-rhamnose)	Urine	Physiological factors (gastric evacuation, intestinal transit, renal function) have lesser effect on the overall test result, since they proportionally affect the absorption of both substances	Influenced by physiological aspects, recommended only for small intestine permeability detection, only measures the paracellular permeability, time consuming, labour-intensive
Administration of water-soluble contrast medium	Iohexol test	Urine, serum	Simple to use	May be influenced by non-intestinal factors (gastric evacuation, intestinal transit, renal function and tissue distribution)
Administration of different size polyethylene glycol	PEG 400/ PEG 4000	Urine	Non-toxic (not metabolised or degraded within the human GI tract)	Non site-specific, time-consuming
Administration of ^{51}Cr-EDTA	^{51}Cr-EDTA	Urine	Recommended for both gut and colon permeability detection;	Radioactive, result can be affected by physiological non-mucosal factors, time-consuming
Administration of FITC-Dextran	FITC-Dextran	Serum	Easy to perform	Non site-specific
Ex vivo tests:				
Ussing chamber	Fluorescent probes (4kDa FITC-D, lucifer yellow), electrophysiological measurements	Biopsy, resection material	Site specific (provide information on regional differences in gut and colon permeability), differentiate between transcellular and paracellular pathways, can be used to examine cultured cells (primary cells, cell lines)	Invasive, labour-intensive, time-consuming
Non-invasive tests:				
Western blot analysis of claudins	Claudins	Urine	Non-invasive	Limited data
FABP	L-liver FABP, I-intestinal FABP, Il-ileal FABP	Urine, plasma	Non-invasive	Acute phase indicator
GSTs	α GSTs, π GSTs, μ GSTs	Urine, plasma	Non-invasive	Non-specific to intestinal tissue, acute phase indicator
Citrullin	Citrullin	Plasma	Non-invasive	
Quantitative sandwich enzyme linked immunosorbent assay (ELISA) detection of Zonulin	Zonulin	Plasma	Non-invasive, Specific for the small intestine	Could be influenced by gut bacteria (overgrown/infection)
Bacterial metabolism products	SCFA	Serum/faeces	Non-invasive	Small data

Comparison of the iohexol level in IBD (32 with CD and 26 with UC) patients and a healthy control group (n = 25) showed that its level in serum and urine was significantly higher in the IBD compared to the healthy subject group, and also reflected disease activity. Intestinal permeability disturbance was five-fold higher in a severe disease group compared with a mild/moderate disease patient subgroup (Gerova *et al*., 2011). Iohexol recovery in urine is correlated positively with IBD endoscopic activity (Halme *et al*., 2000). Investigation of intestinal permeability in liver cirrhosis (LC) patients also showed a significantly higher plasma iohexol level in comparison with healthy controls. Intestinal barrier dysfunction, detected by iohexol, was significantly more pronounced in alcoholic genesis and advanced stage liver cirrhosis patients (Gerova *et al.*, 2020).

PEG. Polyethylene glycol is a non-toxic linear polymer that is stable to bacterial enzymes and is easily excreted from the human body. Oral administration of this drug followed by identification in the urine is also used to evaluate intestinal permeability (Loret *et al.*, 2004). In most studies, it is used as the only marker with a molecular weight of 400. However, PEGs with different molecular sizes can be used, which makes it possible to determine size-dependent permeability and diagnose changes in both pore and paracellular transport (Watson *et al.*, 2001). The use of PEGs with two

different molecular masses (400 and 4000) has been proposed, as high molecular weight PEGs would more effectively identify the permeability of macromolecules (Loret *et al.*, 2004). Also the simultaneous use of two substances will reduce the influence of physiological factors on the result, since it will be proportional to the absorption and excretion of both markers. However, this method is associated with certain technical difficulties in the extraction of PEGs with different sizes from one urine sample (Loret *et al.*, 2004).

^{51}Cr-EDTA. Oral intake of radioactive chromium complexed with ethylene diamine tetracetic acid (^{51}Cr-EDTA) followed by measurement of radioactivity in urine samples has advantages and disadvantages. Among the advantages of this method, ^{51}Cr-EDTA is a marker of the permeability of both the small intestine and colon, since it is not subjected to degradation by intestinal bacteria. It also does not require prior extraction from biological fluids. The disadvantage of the method is that its result can be affected by physiological non-mucosal factors like the time of gastric emptying, intestinal transit time and the intensity of renal excretion.

Increased urinary recovery of ^{51}Cr-EDTA has been observed in Type 2 DM patients, comparing with control subjects (Horton *et al*., 2013). It was significantly elevated in different time periods (0–6 h, 6–24 h, total 24 h recovery), which indicated altered paracellular permeability, both in the gut and colon.^{51}Cr-EDTA was also significantly correlated with serum CRP, IL-6, and TNFα levels. However, the validity of this study was questioned since a high level of ^{51}Cr-EDTA was excreted in the urine in subjects of the control group (Peled *et al*.,1985). This can be explained by the fact that of 27 participants in the control group, 13 had a diagnosis of irritable bowel syndrome, which, according to modern literature, has a pathophysiological association with LGS.

FITC-Dextran**.** In FITC-Dextran test, an alternative to the previously described using radio-labelled substances, a fluorescently labelled sugar molecule is used (Schoultz and Keita, 2020). Detection of fluorescein isothiocyanate dextranFITC-D (4–6 kDa) in serum one hour after oral administration is a reliable marker for the diagnosis of LGS. In the case of an undamaged intestinal barrier 4–6 kDa, molecules cannot pass through the intestinal wall because of their large size (Vuong *et al.*, 2021). Use of the FITC-D predominantly provides information about leak pathway functionality. In addition to this test, oral administration of creatinine (6Å) and rhodamine B isothiocyanate-70 kDa dextran (rhodamine70: 120Å) may provide additional information on the permeability of the pore pathway and tight junction-independent (unrestricted) pathway (Oami and Coopersmith, 2021). However, location of gut barrier dysfunction routinely cannot be determined with this method (Woting and Blaut, 2018).

Unfortunately, due to the laboriousness, the above methods are mainly used in research and have not taken a routine place in daily clinical practice (Cardoso-Silva *et al*., 2019).

***Ex vivo* tests.** The basic *ex vivo* method for intestinal permeability determination is the analysis of a gut/colon mucosa biopsy or resection material in the Ussing chamber, using fluorescent probes and electrophysiological measurements (Larsen *et al.*, 2001; Cardoso-Silva *et al*., 2019; Thomson *et al*., 2019; Schoultz and Keita, 2020). This test is based on the identification of tissue transepithelial resistance (TER) and the potential difference (PD). Tissues are placed in the chamber such that each side of the epithelial cut is isolated and faces the specific side of the chamber. Each side of the chamber is filled with identical electrolyte solution (typically Ringers type). A PD is formed by active ion transport and is detected using electrodes. Subsequently, according to Ohm's law, TER is calculated. The short circuit current (Isc) is used to analyse the transport of epithelial ions and electrical resistance. A decrease in TER is observed with dysregulation of TJ, ZO-1, JAM-A, and certain types of claudins indicate increased permeability of epithelial cells. Paracellular streams can be determined with the use of fluorescent substances, such as 4 kDa FITC-D and lucifer yellow (Clarke, 2009; Herrmann *et al*. 2016; Vancamelbeke and Vermeire, 2017; Cardoso-Silva *et al*., 2019; Thomson *et al*., 2019). The advantage of this method is that it can provide information on regional differences in gut and colon permeability, as well as on both transcellular and paracellular transport pathways (Smith, 1996; Wuyts *et al.*, 2015). The test can also be used to study cultured cells — primary cells and cell lines (Clarke, 2009). However, it should be noted that the methodology and implementation of this test can be quite challenging, and therefore it is used mainly for scientific and experimental purposes (Thomson *et al*., 2019).

Non-invasive tests. Non-invasive tests are a promising direction in gut permeability diagnostics. Their main benefit is the absence of exogenous substances administered to patients. It reduces a series of risks and subsequent potential complications, which allows their use in sensitive groups, such as children and pregnant women. Functional non-invasive tests are based on measurement of intestinal leakage consequences by detection of luminal content in systemic circulation, urine, faeces or exhaled air (Grootjans *et al*., 2010).

Claudins. As mentioned earlier, claudins, which are transmembrane proteins, are an important structural element of TJ and regulate its selective permeability to small ions and molecules. Twenty-three types of human claudins have been identified (Findley and Koval, 2009). TJ are dynamic structures that can change depending on different conditions. In case of stress or injury, TJ proteins are relocalised and internalised or are degraded (Bergmann *et al.*, 2013). This occurs because inflammatory mediators influence transcription and endocytic trafficking of selected claudins (Garcia-Hernandez *et al*., 2017). Claudin's expression in urine can be identified by Western Blot Analysis. High expression of claudine-2 was observed in neonatal NEC patient urine and it was proposed as a potential early biomarker of this difficult-to-diagnose condition; however, this

study included a very small number of patients (Blackwood *et al*., 2015). The potential usefulness of this marker was also noted in a review based on 27 published papers, which focused on changes in claudin intestinal expression and urinary concentration in the case of NEC (Griffiths *et al.*, 2021). There is an association between intestinal tight junction loss and urinary claudin-3 level elevation in rat models, and a significant increase of claudin-3 urine level in IBD active disease patients compared with patients in remission (Thuijls *et al*., 2010).

FABP. An alternative urinary biomarker of gut damage with subsequent permeability alteration, which also can be detected in plasma, is fatty acid binding protein (FABP) (Vancamelbeke and Vermeire, 2017). FABP is a group of molecules 14–15 kDa in size, which regulate cellular uptake, intracellular reactions and metabolism of lipids (Furuhashi and Hotamisligil, 2008; March, 2017). In total, nine isoforms of FABP are distinguished: L-liver, I-intestinal, Il-ileal, A-adipocyte, H-heart, B-brain, E-epidermal, M-myelin, T-testis. However, it is important to note that not one isoform is specific to any particular cell type, since tissues can produce several types of FABP (Furuhashi and Hotamisligil, 2008). Intestinal tissues express three types of FABP: I-FABP, L-FABP and Il-FABP (Furuhashi and Hotamisligil, 2008; Vancamelbeke and Vermeire, 2017) However, the expression of these proteins occurs in different parts of the intestine: I-FABP along the entire length of the intestine, prevailing in the distal part; L-FABP in the proximal part of the gut; and Il-FABP in the distal compartment of the small intestine (Furuhashi and Hotamisligil, 2008). In the case of intestinal tissue damage, these proteins enter the systemic circulation and are subsequently excreted through the kidney (Vancamelbeke and Vermeire, 2017). A significantly higher level of urinary (I-FABPu) and plasma (I-FABPp) I-FABP was observed in neonatal NEC patients, comparing with patients with different diagnosis, with the highest I-FABP peak in the first eight hours after symptom onset (Schurink *et al*., 2015). It was concluded that serial measurement of I-FABP level can be valuable not only in disease identification, but also in complicated disease prediction (Schurink *et al*., 2015).

GSTs. Another marker that is detectable in urine and plasma, and presumably could be useful for diagnosis of intestinal damage and consequent changes in intestinal permeability, is Glutathione S-transferase (GSTs). GSTs is a group of cytosolic enzymes involved in detoxification of xenobiotic substances. There are several subtypes of GTSs (alpha-α, pi-π, mu-μ, theta-θ and microsomal enzymes) with characteristic tailored distribution to each group (Khurana *et al*., 2002). Alpha, mu and pi type GSTs are localised in the gastrointestinal epithelium, α in villous enterocytes, and π and θ mostly in the crypts. Accordingly, GSTs can be detected in serum in the case of intestinal tissue damage. However, it is important to emphasise that in addition to the gastrointestinal tract, alpha GTSs can also be found in hepatocytes, kidney, adrenal gland, and testis. The mu type can be detected in liver and lymphocytes and the pi type also in placenta, lungs, kidney, liver, pancreas, and salivary glands (Campbell *et al*., 1991; Sugimoto,1995; Khurana *et al*.; 2002). Several studies have reported that GSTs proved to be useful in the early diagnostics of intestinal ischaemia (Khurana *et al*.; 2002; Gearhart, 2003). Therefore, the question of the specificity of this marker remains open (Campbell *et al*., 1991; Khurana *et al*., 2002).

Citrullin. Citrullin is a non-proteinogenic amino acid that is produced in gut enterocytes from glutamine. The plasma citrullin level can be used for determination of the functional absorptive bowel length and enterocyte damage. With a decrease of the intestinal epithelial cell mass, changes in gut permeability occur. Subsequently, the levels of circulating citrullin in blood also decrease accordingly (Bischoff *et al*., 2014). A study with short bowel syndrome patients showed that the plasma citrullin level was significantly lower, comparing with healthy controls, and decrease of the plasma post-absorbtive citrullin level significantly correlated with bowel length and made it possible to distinguish between transient and permanent intestinal failure (Crenn *et al.*, 2000). A reduced level of postabsorptive citrullin level was found in patents with celiac and non-celiac villous atrophy without gut resection, comparing with healthy controls and anorexia nervosa severely malnourished patients, and a decrease in citrullin level was significantly correlated with extent and severity of villous atrophy (Crenn *et al*., 2003). In addition, it was shown to be effective marker of cancer treatment-induced small bowel injury in comparison with sugar tests (conducted with lactulose/L-rhamnose; D-xylose/3-*O*-methyl-D-glucose and L-rhamnose/3-*O*-methyl--D-glucose), demonstrating both high sensitivity and specificity. Maximal gut damage and gut recovery was identified earlier with citrullin level changes than with sugar tests (Lutgens *et al*., 2005).

Zonulin. Zonulin is currently the only one known human protein that reversibly affects intestinal permeability by regulating the functioning of TJ. There are two known intestinal factors that can affect the secretion and release of zonulin followed by an increase in intestinal permeability: gluten and gut exposure to bacteria. In the case of bacterial exposure, the production of zonulin promotes the segregation of the ZO-1 protein from the TJ, thus opening the paracellular transport pathway. Gliadin promotes the release of zonulin by interacting with chemokine receptor CXCR3, which is over-expressed in celiac disease patients. The zonulin level can be measured in plasma by quantitative sandwich enzyme linked immunosorbent assay (ELISA) method. An increase in plasma zonulin level has been described in patients with celiac disease, and its subsequent decrease during treatment with a gluten-free diet. In T1D animal model studies, an elevated plasma zonulin level, indicating increased intestinal permeability, was detected earlier than onset, clinical manifestation and histological confirmation of disease (Fasano, 2012a; 2012b; Sturgeon and Fasano, 2016). Zonulin is involved in the development of diseases such as celiac disease, Type-1-diabetes, inflammatory bowel disease, multiple sclerosis, obesity/insulin resis-

tance, Type-2 diabetes, polycystic ovary syndrome, acute lung injury, asthma, coronary artery disease, glioma, septicemia, HIV, irritable bowel syndrome, non-celiac gluten sensitivity, environmental, enteropathy and necrotising enterocolitis (Sturgeon and Fasano, 2016).

SCFA. Gut microbiota metabolites like SCFA might serve as a marker of intestinal barrier permeability changes (Jaworska *et al.*, 2019). This method does not involve the use exogenous substances, which is especially important working with such a sensitive group of patients as children. Systemic blood SCFAs (*C*s), faecal SCFAs (*Cf*) and *C*s/*C*f ratio of SCFAs were determined in paediatric patients with IBD. Compared to the control group, IBD patients had a significantly higher *C*s /*C*f ratio for SCFAs, including acetic, valeric, isocaproic, caproic and propionic acids. There was a statistically significant positive correlation between SCFA permeability and faecal calprotectin level. However, this study incorporated a very small number of patients (n = 6 with IBD, n = 9 controls) (Jaworska *et al.*, 2019).

CONCLUSION

To the best of our knowledge, awareness of the role of elevated intestinal permeability in the development and pathogenesis of gastrointestinal and extra-intestinal diseases has noticeably improved in recent years. Methods for diagnosing altered intestinal permeability is a relatively new scientific field that requires more research. Unfortunately, now there is no single validated diagnostic test that could be actively used in routine clinical practice. There are many potential diagnostic tests to detect and measure changes in gut barrier functionality. However, many of them have certain disadvantages and limitations, such as introduction of exogenous substances, difficulties in the execution technique, high costs, etc. In addition, for some of them, there is not yet a sufficient amount of specificity and sensitivity data and also no head-to-head comparison analyses. It follows that this area requires more research in order to identify a non-invasive, easy-to-use and cost-effective way to diagnose LGS. Hopefully, in the future, this will make it possible to identify and use targeted therapy for this pathology and, possibly, preventively reduce the incidence of diseases pathogenetically associated with excessive intestinal permeability.

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PAAUGSTINĀTĀS ZARNU CAURLAIDĪBAS SINDROMA DIAGNOSTIKAS ROBEŽVĒRTĪBU NOTEIKŠANA: KUR MĒS ESAM TAGAD?

Kuņģa–zarnu trakta gļotāda ir virsma, kas mijiedarbojas ar daudziem ārējiem faktoriem. Papildus tādām funkcijām kā barības vielu sagremošana un uzsūkšana tā darbojas arī kā alergēnu, patogēnu un toksīnu barjera. Paaugstinātas zarnu permeabilitātes jeb caurlaidības sindroms tiek definēts kā zarnu gļotādas barjeras disfunkcija, kuru izraisa neparasti palielināta zarnu caurlaidība. Mūsdienu zinātniskajā literatūrā konstatēts, ka šim sindromam ir patoģenētiska saistība ar virkni kuņģa–zarnu trakta un ārpuszarnu traucējumiem. Šajā pārskatā aplūkota pašreizējā izpratne par zarnu barjeras sastāvu un paaugstinātas zarnu caurlaidības sindroma patoloģisko iesaistīšanos dažādās slimībās. Raksta galvenais mērķis ir apskatīt dažādas metodes, kas paredzētas zarnu sieniņu caurlaidības diagnostikai un izvērtēšanai, identificējot to prioritātes un trūkumus.