REVIEW

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Celiac Disease: Promising Biomarkers for Follow-Up

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ABSTRACT

Celiac disease is a common gastroenterological illness. Current diagnostics of the disease are based on serological markers and histology of duodenal biopsies. Hitherto, a strict gluten-free diet is the only effective treatment and is necessary for good control of the disease. Serological tests in current use have very high specificity and sensitivity for diagnostics, but in follow-up they have some limitations. Their levels do not accurately reflect mucosal healing, and they are unable to detect minimal transgressions in the diet. This problem is significant in patients with IgA deficiency, and there exist no robust follow-up tools for monitoring these patients' adherence to treatment. For their follow-up, we currently use IgG-based tests, and these antibodies persist for a long time even when a patient has stopped consuming gluten. More accurate and specific biomarkers are definitely needed. Adherence to a gluten-free diet is essential not only for intestinal mucosa healing and alleviation of symptoms but also for preventing complications associated with celiac disease. Here, we summarize current evidence regarding noninvasive biomarkers potentially useful for follow-up not only of patients with IgA deficiency but for all patients with celiac disease. We describe several very promising biomarkers with potential to be part of clinical practice in the near future.

Key words: celiac disease – IgA deficiency – novel biomarkers – microRNAs – gluten immunogenic peptide – citrulline.

Abbreviations: CD: celiac disease; ESPGHAN: European Society of Pediatric Gastroenterology, Hepatology, and Nutrition; FABP: Fatty acid-binding proteins; FCP: fecal calprotectin; GFD: gluten-free diet; GIP: gluten immunogenic peptide; HMBG1: high mobility group box 1; IgA: immunoglobulin A; IL-2: interleukin-2; miR: microRNAs; NO: nitric oxide; SBB: small bowel biopsies; TGA: transglutaminase.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated illness affecting the small intestine. It is caused by exposure to gluten in genetically predisposed individuals. The incidence of CD is around 1% across the global population [1]. Some data suggest that CD occurs more frequently in females [2]. Higher incidence of the disease can be expected in first-line relatives of those with CD, as well as in patients with type 1 diabetes mellitus, immunoglobulin A (IgA) deficiency, Down syndrome, autoimmune thyroiditis, and other associated diseases [3].

Celiac disease diagnostics comprise a combination of clinical symptoms, serological tests, and small bowel biopsy (SBB). In several pediatric cases, according to current recommendations of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), we can set up diagnostics based solely upon serological tests [4]. For initial screening, a combination of total IgA and anti-type 2 (tissue) transglutaminase (TGA) IgA antibodies is recommended. From another blood sample, we confirm the findings and test for the IgA antibodies against endomysium (EMA). In defined cases, it is necessary to perform esophagogastroduodenoscopy with a SBB and histological assessment using the Marsh–Oberhuber classification to evaluate histological lesions [5]. A special situation exists in patients with IgA deficiency, which is the most common primary immunodeficiency [6]. These patients have 10- to 20-fold greater risk of developing CD [7, 8]. Small bowel biopsy is mandatory in these patients [4], and it is essential also in every adult patient [7]. Therapy is then based on a lifelong gluten-free diet (GFD). Despite the existence of

several other promising approaches, these are still a long way from being relevant for clinical use [9].

Maintaining long-term adherence to GFD is a key point for good disease control and preventing complications associated with CD [10-12]. We presently use the same tests for follow-up and diagnostics (TGA IgA and EMA IgA) [7, 8] in combination with a special questionnaire (e.g., Biagi score) [13]. Nevertheless, these procedures have several limitations. A major problem is poor sensitivity of TGA IgA and EMA IgA assays in detecting persistent villous atrophy [14]. This could be especially problematic in patients with high sensitivity to gluten intake. A safe threshold for the majority of patients is less than 50 mg of gluten per day [15], but in sensitive patients even 10 mg of daily gluten intake already can lead to mucosal changes [16]. Moreover, follow-up of patients with IgA deficiency is a huge challenge. Elevated levels of IgG antibodies (TGA, EMA) persist for a long time on GFD and do not correlate with histological activity of CD [17]. Especially in this group of patients, new biomarkers are greatly needed.

The aim of our manuscript is to provide a comprehensive overview of the available follow-up biomarkers concerning which robust clinical evidence exists. Some of the discussed biomarkers have potential to improve current clinical practice and help physicians in better controlling celiac disease, particularly in selected groups of patients. Using Boolean operators, we made a comprehensive search of the PubMed database for keywords "biomarkers" AND "celiac disease" between the years 2002 and 2022. We got 1,219 results. We next reduced the number of publications to be analyzed according to inclusion and exclusion criteria. Inclusion criteria were defined as: 1) peer-reviewed article, and 2) biomarkers appearing in more than one paper. Exclusion criteria were: 1) review and systematic review (225 articles), 2) meta-analysis (17 articles), 3) absence of full text (74 articles), 4) article in language other than English (83 articles). This resulted in 49 publications, which were further evaluated (Table I).

BIOMARKERS

Citrulline

Citrulline is an amino acid produced in enterocytes. The level of citrulline in plasma depends on the ability of enterocytes to synthesize and reflects their eventual destruction [18]. A Finnish study from 2011 evaluated fasting plasma levels of citrulline in patients with CD before GFD, in patients with CD 2 years on GFD, in children with gastrointestinal problems but negative SBB, and in healthy controls [16]. Plasma citrulline levels were significantly lower in patients with CD before the initiation of GFD than in the other groups, and these increased after GFD induction. Similar results were presented by Lomash et al. [19] from a more recent study, which also captured a significant reduction in citrulline plasma levels in 558 pediatric patients with newly diagnosed CD compared to healthy controls. An increasing trend in citrulline levels was observed during follow-up on GFD and had a negative correlation with TGA IgA levels. Similar data has been published also from other pediatric and adult cohorts [20-23].

Fatty Acid-Binding Proteins

Fatty acid-binding proteins (FABP) are small cytoplasmic proteins that are involved in lipid homeostasis. Nine types of these proteins are known. One of them is the intestinal type (intestinal FABP), which is expressed throughout the gut and especially at the tip of the villi [24]. The use of this biomarker in noninvasive diagnosis and follow-up of CD [25-29] is based upon findings that increased levels of intestinal FAPB occur in the blood of individuals with damaged enterocytes, for example in cases of intestinal ischemia [30] or neonates with necrotizing enterocolitis [31].

Adriaanse et al. [32] presented a group of 90 pediatric patients with elevated TGA IgA and HLA DQ2 or DQ8 positivity within whom the level of intestinal FABP was determined before the introduction of GFD. The control group included 80 children without TGA IgA elevation. Elevated intestinal FABP (cutoff value 450 pg/ml) was found in 68% of patients with suspected CD. In all these patients, the diagnosis of CD was subsequently confirmed (following current guidelines). Sensitivity of the marker was about 85%. Measurement of intestinal FABP continued after gluten elimination. After 26 weeks of GFD, plasma levels of intestinal FABP reached the same level as in the control group.

Similar data were published by Vreugdenhil et al. [33] in a cohort of 49 pediatric CD patients. The median value of intestinal FABP in the study group was 458 pg/ml. In the control group of 19 patients, by comparison, the median value was 20 pg/ml. Level of intestinal FABP correlates with severity of villous atrophy. The authors describe quick decline in intestinal FABP levels after GFD introduction. Normal levels were reached by 80% of patients after 12 weeks of GFD.

A study by Singh et al. [34], which included 131 therapeutically naive adult patients with CD, aimed to determine an optimal intestinal FABP cutoff value that would correlate with intestinal atrophy Marsh type 2 or higher. A level higher than 1,100 pg/ml appears to be optimal. Also in this study, a decrease in the mentioned parameter was observed after the introduction of GFD.

Fecal Calprotectin

Fecal calprotectin (FCP) is a calcium- and zinc-binding protein complex mainly secreted by neutrophils. Synthesis of FCP is increasing during gut inflammation [35]. Data on the usefulness of FCP in the follow-up and diagnosis of patients with CD were presented by a Turkish team [36]. In newly diagnosed pediatric patients with CD, the mean FCP value was significantly higher (117.2 μg/g) compared to that in healthy controls (9.6 μg/g). There would also be a difference between a patient with gastrointestinal symptoms and one with extraintestinal manifestations (142.8 vs. 79.7 μg/g). A post-GFD FCP examination as part of the follow-up was performed at the time of EMA IgA normalization. A statistically significant decrease of this parameter to a mean value of 4.2 μg/g was observed.

Similar results were presented by Ertekin et al. [37]. The baseline FCP level in newly diagnosed pediatric patients with CD was 13.4 mg/l. One year after the introduction of GFD, this had decreased to 4.6 mg/l, which corresponded to the values in healthy controls (4.3 mg/l).

Table I. Actual and potential noninvasive follow-up biomarkers for celiac disease

Biomarker	References	A/P	Number of patients	Compared groups
Citrulline	Ioannou et al. [22]	P	43 + 30	CD BT, CD on GFD vs. HC
	Lomash et al. [19]	P	558 + 1565	CD vs. FDRs
	Singh et al. [34]	A	131 + 349	CD BT vs. HC
	Miceli et al. [20]	A	27 + 50	CD BT, CD refractory vs. HC
	Basso et al. [21]	P	53 + 48 + 42	CD BT, CD on GFD vs. HC
	Crenn et al. [76]	A/P	42 + 10 + 51 + 10	CD BT, non-celiac villous atrophy vs. HC, anorexia patients
	Rahmani et al. [23]	P	118	CD
Fatty acid binding proteins (FAPB)	Adriaanse et al. [25]	A	96 + 69 + 141	CD BT, CD on GFD vs. HC
	Adriaanse et al. [26]	A	20 + 43	CD on GFD vs. HC
	Adriaanse et al. [32]	P	90 + 80	CD BT vs. HC
	Derikx et al. [27]	A/P	13 + 26	CD BT vs. HC
	Singh et al. [34]	A	131 + 349	CD BT vs. HC
	Vreugdenhil et al. [33]	P	49 + 19	CD BT vs. positive CD screening, histologically not proven
	Logan et al. [28]	P	12 + 40 + 28 + 47 + 11	CD BT, CD on GFD vs. HC, Crohn's disease, ulcerative colitis
	Gandini et al. [29]	P	12 + 13 + 13	CD vs. type I diabetes mellitus vs. HC
Fecal calprotectin (FCP)	Balamtekın et al. [36]	P	31 + 33 + 34	CD BT, CD on GFD vs HC
	Ertekin et al. [37]	P	29 + 10	CD BT vs. HC
	Szaflarska-Popławska et al. [38]	P	55 + 17	CD BT vs. CD on GFD
	Capone et al. [39]	A	50 + 50	CD BT vs. HC
Gluten immunogenic peptide (GIP)	Comino et al. [40]	A/P	7 + 46 + 26	CD BT, CD on GFD vs. HC
	Comino et al. [41]	A/P	188 + 84	CD on GFD vs. HC
	Gerasimidis et al. [42]	P	19 + 44	CD BT vs. CD on GFD
	Roca et al. [43]	P	18 + 43	CD BT vs. CD on GFD
	Laserna-Mendieta et al. [44]	A/P	97	CD on GFD
	Ruiz-Carnicer et al. [46]	A	22 + 77 + 13	CD BT, CD on GFD vs. HC
	Stefanolo et al. [47]	A	53	CD on GFD
	Porcelli et al. [48]	A/P	55	CD on GFD
	Porcelli et al. [49]	A	25	CD on GFD
	Skodje et al. [50]	A	70	CD on GFD
	Monachesi et al. [51]	A	25 + 12	Healthy adult on GFD vs. healthy adult on gluten contamination elimination diet
	Moreno et al. [52]	A	4	CD on GFD
	Moreno et al. [45]	A/P	58 + 76	CD on GFD vs. HC
	Costa et al. [76]	A	44	CD on GFD
	Silvester et al. [54]	A	18 + 3	CD on GFD vs. HC
	Silvester et al. [55]	A	18	CD on GFD
	Fernández Miaja et al. [56]	P	80	CD on GFD
	Fernández-Bañares et al. [57]	A	76	CD BT
HMBG1	Manti et al. [59]	P	49 + 44	CD BT vs HC
	Palone et al. [60]	P	39	CD BT
Interleukin-2	Tye-Din et al. [61]	A	25 + 25	CD on GFD vs. HC
	Leonard et al. [62]	A	14	CD on GFD
miRNAs	Tan et al. [65]	P	33 + 20	CD BT vs. HC
	Amr et al. [63]	P	25 + 25 + 20	CD BT, CD on GFD vs. HC
	Bascuñán et al. [64]	A	10 + 10 + 10	CD BT, CD on GFD vs. HC
	Felli et al. [66]	P	40 + 40 + 40	CD BT, CD on GFD vs. HC

Table I (continued)

	Vaira et al. [67]	A	22 + 28 + 12	CD BT, CD on GFD vs. HC
	Comincini et al. [68]	P	23 + 33	CD BT vs. HC
Plasma/serum nitric oxide	Murray et al. [69]	A	12 + 9 + 45	CD BT, CD on GFD vs. HC
	Ertekin et al. [70]	P	41 + 14	CD BT vs. HC
	Piątek-Guziewicz et al. [72]	A	53 + 92 + 52	CD BT, CD on GFD vs. HC
Reg1α protein	Planas et al. [71]	A	40 + 35 + 23 + 15	CD BT vs. HC vs. type I diabetes mellitus vs. pernicious anemia
	Singh et al. [34]	A	131 + 349	CD BT vs. HC

A: adult; BT: before treatment; CD: celiac disease; FDRs: first degree relatives; GFD: gluten-free diet; HC: healthy controls; HMBG1: high mobility group box 1; P: pediatric.

In contrast to these results is a study from a Polish team that sought unsuccessfully to find in pediatric patients a correlation between FCP levels, the clinical form of CD (classical, non-classical, and asymptomatic), and histological changes in the small intestine [38]. As no statistically significant relationship was found, those authors did not consider FCP to be a promising marker for diagnosing and monitoring patients with CD. This statement is supported by data from an Italian adult cohort [39]. Further research might nevertheless bring new light into this area.

Gluten Immunogenic Peptide

Gluten immunogenic peptide (GIP) consists in fragments of gluten that are excreted undigested in the feces and urine. In 2012, Comino et al. [40] published findings from a study wherein they were investigating gluten and gliadin 33-mer equivalent peptidic epitopes (33EPs) in human feces. Gluten immunogenic peptides were detectable in the stool up to 4 days after consumption and their level correlated with the amount of gluten ingested. In another work, the same authors decided to use GIP as a marker of adherence to GFD [41]. The study included 188 patients with CD on GFD, both pediatric and adult, and 84 healthy controls. In addition to stool GIP detection, patients underwent a dietary questionnaire as well as TGA IgA and anti-deamidated gliadin peptide (anti-DGP) IgA antibodies measurement. Gluten immunogenic peptide was detected in almost 30% of patients with CD; its' positivity in patients with CD increased with age and was higher in males.

Similar work was published by Gerasimidis et al. in 2018 [42]. They investigated GIP in the stools of children with newly diagnosed CD and in children with CD already on GFD, then correlated all with commonly used methods for monitoring GFD compliance (TGA IgA levels, Biagi score, and clinical evaluation). Gluten immunogenic peptides were detected in 16% of patients on GFD. Newly diagnosed patients consuming gluten had 95% GIP positivity in the stool. Following the introduction of GFD, the presence of GIP in the feces decreased to 17% at 6 months and was 27% at 12 months.

In a similar pediatric trial, Roca et al. [43] observed some interindividual variability in stool GIP levels. Therefore, the influence of yet unknown gastrointestinal factors on their fecal excretion cannot be ruled out. Further research will certainly be needed in this area.

Another Spanish study examined correlation between presence of GIP in the stool and duodenal mucosal damage in patients with CD who followed GFD for at least a year [44]. Gluten immunogenic peptide sensitivity for duodenal mucosal damage was only 33% and specificity 81%. A limitation of this concept, however, remains the short elimination half-life of GIP in the feces and the need for longer exposure to gluten before more significant damage of the duodenal mucosa develops.

Gluten immunogenic peptide can also be measured in urine. It is detectable as early as 4–6 hours after gluten ingestion and its positivity persists for 24–48 hours [45]. Ruiz-Carnicer et al. [46] determined GIP levels in urine samples from patients newly diagnosed with CD and from patients already treated [46]. Simultaneously, serum TGA IgA levels were examined, SBB was performed, there was clinical examination, and responses to a GFD adherence questionnaire were evaluated. Twenty-four percent of patients with CD on GFD had histological findings of Marsh 2–3 while 94% of them showed GIP in their urine. Patients who had negative histology (Marsh 0) also had negative urinary GIPs in 97% of cases. Sensitivity of the examination was determined to be 94%, and the negative predictive value of the marker corresponded to 97%. GIPs have been studied and discussed also in several other publications [47-57].

High Mobility Group Box 1

High mobility group box 1 (HMBG1) protein is a marker of inflammation that is among damage-associated molecular pattern (DAMP) molecules, which play a role in immune response in inflammatory processes [58]. Manti et al. [59] measured serum levels of HMBG1 in 49 pediatric patients with CD and 44 healthy controls; HMBG1 was significantly higher in CD patients than in control individuals. They also found a significant difference between HMBG1 levels in patients with a typical form of CD and both the atypical and silent forms. Another Italian team investigated HMBG1 in the stools of 39 children at diagnosis and follow-up [60]. Levels of HMBG1 in stool at follow-up had decreased significantly. At 12 months after GFD introduction, HMBG1 was detected in only 6 patients, but the majority of them voluntarily admitted to minimal transgressions in the diet. We found no similar studies for the adult population.

Interleukin-2

Two teams investigated interleukin-2 (IL-2) serum levels in patients with CD after gluten exposure. Tye-Din et al. [61] exposed 25 celiac patients and 25 healthy controls to 6 grams

of gluten and measured IL-2 levels after 2, 4, and 6 h [41]. In 92% of patients with CD, IL-2 was elevated to >0.5 pg/ml at 4 h and was undetectable in healthy controls. A similar result was shown by Leonard et al. [62] in their randomized, double-blind trial with 14 biopsy-proven adult patients with CD. Interleukin-2 levels after gluten exposure were in correlation with symptom severity in both studies. Interleukin-2 appears to be a sensitive early biomarker of GFD nonadherence.

MicroRNAs

MicroRNAs (miRs) currently encompass one of the most progressive concepts in the field of novel diagnostics and follow-up biomarkers. They are non-coding ribonucleic acids (RNAs) of 19–24 nucleotides playing a role in post-transcriptional gene regulation. They can be detected in various body fluids and organ biopsies. Amr et al. [63] focused on the expression of miR-21 and miR-31 in the serum of 25 untreated pediatric patients with CD, 25 treated patients (on GFD), and 20 controls. Significant increases in miR-21 expression and, conversely, decreases in miR-31 expression were observed in untreated patients compared to the other two groups. The effect of GFD on the expression of these markers was not assessed in the study.

An adult study conducted by Bascuñán et al. [64] included 30 patients, 10 of whom had active CD, 10 had CD and were on GFD, and 10 were healthy controls. The expression of various miRNAs in peripheral blood mononuclear cells, monocytes, and plasma was examined in these patients. In plasma, both groups of patients with CD were detected with significant expression of miR-155, miR-21, and miR-125b. ROC analysis showed that of these parameters miR-155 had the highest sensitivity (94%) and specificity (87%) for the presence of CD.

Tan et al. [65] found 53 miRNAs that can play roles in the development of CD within a combined cohort of pediatric and adult patients. Six of these (miR-150-5p, miR-150-3p, miR-1246, miR-342-3p, miR-375-3p, and let-7a-5p) were decreased at the time of CD diagnosis and significantly increased after gluten elimination.

Very promising, too, are the results from a recent study by Felli et al. [66]. Those authors found, among other things, another three miRs (miR-192-5p, miR-215-5p, and miR-125b-5p) which can discriminate patients who are adherent to GFD from those who break the diet. A combination of these three miRs has good predictive power (sensitivity of 74%, specificity of 97%) in this situation. MicroRNAs in celiac disease follow-up have been analyzed also in two other Italian trials [67, 68].

Nitric Oxide

Nitric oxide (NO) is a radical gas produced by nitric oxide synthase. Production of NO is elevated during inflammation. Sixty-six patients were enrolled in a study by Murray et al. [69]. They underwent upper gastrointestinal endoscopy for various gastrointestinal disorders. Twenty-one of them had CD and nine were on GFD at the time of investigation. Plasma levels of NO were higher in untreated patients with CD than in those who were on GFD (mean 117.5 μM vs. 71.2 μM). Ertekin et al. [70] studied NO levels in 41 newly diagnosed pediatric patients with CD. Serum NO levels were measured at the time of diagnosis and after 1 year of GFD. NO levels after 1 year on GFD decreased to levels very similar to those in healthy controls. They describe also a statistically significant correlation between serum NO level and the degree of histologic changes in patients with CD.

REG 1α Protein (Reg1α)

The regenerating gene is part of a larger group of genes that are involved in cell regeneration. Reg1α protein (Reg1α) is expressed by small intestinal and pancreatic cells [71,72]. Its elevated serum levels can be expected in cases of intestinal cell damage and the cells' subsequent regeneration, for example in CD or inflammatory bowel disease [73]. As a potential biomarker of CD, it was investigated in a study by Planas et al. [71]. They detected elevated levels in 40 adult patients with active CD compared to healthy controls and subsequently detected a significant decrease in this parameter after GFD induction. Statistically significant differences in serum Reg1α levels between newly diagnosed patients with CD and healthy controls were described, too, in the repeatedly cited study of Singh et al. [34]. After introducing GFD, there was decline in Reg1α levels, but those levels did not correlate with the degree of villous atrophy.

Fig. 1 summarizes all those noninvasive sources of actual and potential follow-up biomarkers for CD discussed above.

DISCUSSION

A number of biomarkers are available for monitoring GFD adherence. The follow-up tools currently in use (serological tests, questionnaires, etc.) are not reliable and are not completely sensitive for detecting minor transgressions in GFD [14]. Therefore, it would be helpful to enrich the current approach with additional parameters. These parameters can help to improve disease control in all patients with CD, and especially in those patients with IgA deficiency. This group of patients represents about 2% of all individuals with CD [74]. The latest guidelines for follow-up both in pediatric and adult medicine do not offer a clear procedure for how to manage CD in IgA-deficiency patients [7,8]. The only reliable possibility remains SBB.

Especially in pediatrics, however, there is a strong need for biomarkers that would be detectable in stools or urine, because only these biomarkers are truly noninvasive. Reducing the painful experience associated with gastroenterology check-ups could improve cooperation with patients and their adherence to GFD [75]. This might well be appreciated also by adult patients.

From this perspective, GIPs comprise one of the most promising biomarkers. They satisfy the condition of noninvasiveness. Their sensitivity is by far the best from among the aforementioned biomarkers. Their detection in body fluids is dependent on gluten consumption, and potential application of GIPs in actual practice is supported by the largest number of clinical trials and commercially available diagnostic kits. Gluten immunogenic peptide's short elimination half-life from stool or urine may be limiting for detecting long-term irregular transgressions in the diet [41, 45].

Significantly limiting the usefulness of citrulline, FABP, HMBG1, and Reg1α are their abnormal levels detected also in other diseases. Citrulline is well correlated with severity of

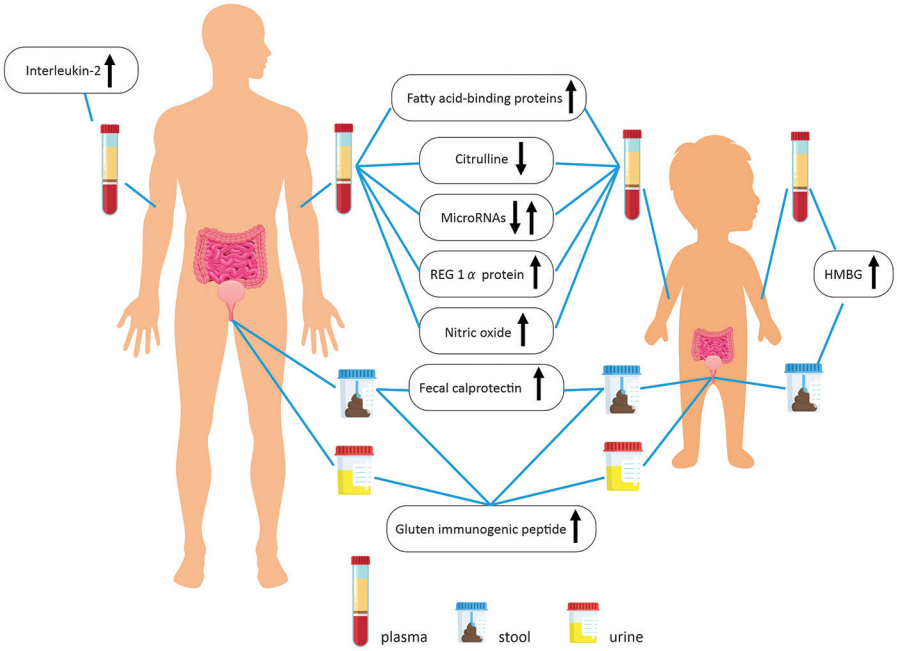

Fig. 1. Noninvasive sources of actual and potential follow-up biomarkers for celiac disease.

duodenal atrophy at time of diagnosis [19]. The lowest levels are apparent in patients with Marsh 3c duodenal lesion. In this group of patients after GFD introduction the authors describe the most significant rise in citrulline [19]. This biomarker's sensitivity is lower in patients with less severe duodenal atrophy. The main limitation appears to be low specificity of citrulline measurement. Low levels can be observed also in other small bowel diseases associated with villous atrophy [76]. Fatty acid-binding protein responds well to GFD, but its levels may interact with other causes of enterocyte damage [31]. Another problem seems to be that FABP levels depend on both the severity of villous atrophy and the extent of disease in the small intestine [33]. Therefore, false negative results can be expected in situations of patchy villous atrophy. High mobility group box 1 protein is a general marker of intestinal inflammation. Palone et al. [77] reported elevated levels of HMBG1 protein in patients with active IBD. Nitric oxide also plays a role in inflammatory reactions. Increased levels of NO have been described in patients with attention-deficit hyperactivity disorder and autism spectrum disorders [75]. Authors report some intersexual differences in levels of NO in patients with CD on GFD, with women having significantly higher levels [69]. We must not forget the crucial role of renal functions on NO clearance [79, 80]. Elevated REG1α levels can be detected also in cases of cystic fibrosis [81] or cancer of the digestive tract [82]. The diagnostic accuracy of this biomarker based on current knowledge is low, at just 52.7% [34].

Experience in other fields (e.g., oncology) certainly suggests that it will be interesting to watch how the use of miRNAs for CD follow-up will develop [74, 83, 84]. The first trials are showing promising results, albeit for now without possible application in actual clinical practice. Their limitations include current weak clinical evidence and lower availability of the biomarkers [63-65].

Despite promising results from initial studies, FCP does not seem a good biomarker for both diagnostics and follow-up of CD. Newer data do not support previous results. Significant interindividual variability of this biomarker is a major limitation [85]. Particularly in children under 4 years of age, for which the exact upper limit of normal is not specified, FCP is wholly unusable [85]. Based on current evidence, we do not expect the use of IL-2 for long-term follow-up of patients with CD. Interleukin-2 is more suited for early detection of dietary non-adherence during, for example, gluten challenge [61, 62].

CONCLUSIONS

Precise follow-up of CD is more than necessary. Good control of the disease goes hand in hand with good quality of life and lower risk of such CD complications as associated autoimmune diseases or cancer. There are some gaps in the application of those tools currently in use (serological tests, questionnaires, etc.) for monitoring specific groups of patients with CD. Their extension by additional reliable parameters would certainly be desirable. This could bring greater certainty that a patient's diet has been well adjusted. All biomarkers definitely need further validation in prospective studies on a larger group of patients.

Conflicts of interest: None to declare.

Authors' contributions: M.H. and P.J. conceive the study and the methodology. T.P., J.P., M.S., L.J. searched the literature. M.H, P.J. drafted the manuscript. L.J., M.S., T.P., J.P. revised and edited the manuscript; P.D., L.K. supervised the study. All authors have read and agreed to the published version of the manuscript.

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