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Non-Invasive Biomarkers of Intestinal Inflammation and Increased Gut Permeability in Inflammatory Bowel Diseases

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1. Abstract

Inflammatory bowel diseases (IBD) are chronic conditions of the intestine characterized by the inflammation of the gut's wall or mucosa. Their etiology is unknown, most probably multifactorial including genetic susceptibility, dysbiosis and environmental factors. The diagnosis is based on both clinical and endoscopic features. However, recently easy, fast, reliable, and non-invasive biological markers have been used not only in diagnosis but also in therapeutic monitoring. To date, fecal calprotectin (FCP) is widely used, and is approved by the European Crohn's and Colitis Organization (ECCO). Nonetheless, other biomarkers such as zonulin have also been investigated for IBD. These markers allow the discrimination between functional and organic bowel conditions. The aim of this study was to review currently available biomarkers of intestinal inflammation and increased gut permeability in IBD.

2. Introduction

Inflammatory bowel diseases (IBD) that comprise of Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing disorders of the intestine [1]. Their incidence is increasing globally, especially in the developed countries [1]. So far, the pathogenesis of IBD has not been fully understood but it is most probably multifactorial, and includes genetics, environmental factors, dysbiosis and impaired host immune system [1,2]. The diagnosis is based on both clinical and endoscopic features [1]. However, recently easy, fast, reliable, and non-invasive biological markers of intestinal inflammation and gut permeability have gained a lot of interest. They allow the discrimination between functional and organic bowel conditions and are used not only in diagnosis but also in therapeutic monitoring [1]. The fecal calprotectin (FCP), fecal and serum zonulin are the biomarkers that have been broadly investigated in IBD [1,2].

Calprotectin is a leukocyte protein of the S100 family belonging to the group of acute phase reactant proteins found in the granulocytes, neutrophil cytosol, in monocytes and activated macrophages [1]. When released into the extracellular space, it induces migration of neutrophils to the inflammatory lesion and stimulates their phagocytic activity. Moreover, calprotectin not only prevents the growth of bacteria within the intestinal lumen and their adhesion to the intestinal epithelium due to the ability to inhibit zinc-dependent bacterial metalloproteinases, but also induces apoptosis, both in normal and cancerous cells [1,2]. Since inflammation leads to the significant increase of calprotectin in the blood serum, other body fluids and in the stool, it has been used as a marker of inflammatory processes in the gastrointestinal tract such as IBD [1], wherein its concentration is much higher in the stool than in the blood serum [1]. There are also limited pilot studies evaluating the usefulness of immunohistochemical (IHC) detection of tissue calprotectin in bowel mucosa in children with IBD that have demonstrated its well correlation with microscopic scores. However, PCP and clinical scores seem to be better predictors of patients' outcome than tissue calprotectin [1]. Zonulin is a 47-kDa human protein which reversibly modulates the intercellular tight junctions whose proper functioning is crucial for maintaining physiologic processes in the intestine, that leads to increased permeability in the epithelial layer of the small intestine [1].

Increased serum/plasma zonulin levels have been reported in celiac disease, type 1 and type 2 diabetes or in obesity-associated insulin resistance [1-3]. However, the role of zonulin in the development of intestinal inflammation for e.g., in IBD is not clear. There are also discrepancies regarding the correlation between serum and fecal zonulin, and which of them could be more useful in the diagnostics of IBD. The aim of this manuscript is to review the role of biomarkers, calprotectin and zonulin in the diagnostics and monitoring of IBD.

3. Calprotectin

Fecal calprotecin, (FC) as a noninvasive marker of intestinal inflammation, has been used to assess and monitor disease activity, mucosal healing (MH) and disease recurrence in patients with IBD [9,10,19]. It was demonstrated that a cutoff point of 50 μg/gr discriminates patients with IBD from controls with 79.4% specificity and 91.9% sensitivity and better correlation with clinical activity than C-reactive protein (CRP) [20]. This shows better sensitivity of FCP than its specificity which is the main limitation of this marker, especially in discriminating active IBD from other intestinal inflammation, as well as CD from UC [21]. Several factors may influence FCP levels, including age, diet, exercise, use of nonsteroidal anti-inflammatory drugs, colonic cleansing, and the fecal amount of blood or mucus in stools [22-25]. As it was mentioned, FCP is also a predictor of disease relapse - Tibble et al proved that its high level could identify patients with IBD who were at risk of early relapse [26]. On the other hand, Costa et al. showed that FCP concentration higher than 150 μg/g in patients in clinical remission was correlated to a 2-fold increase in the relapse risk in CD and a 14-fold increase in UC which indicates its stronger prediction value of clinical relapse in UC than in CD [27]. Furthermore, FCP was proposed as a marker in monitoring therapy efficacy.

Wagner et al. demonstrated that FCP correlated with clinical scores after 8 and 4 weeks of conventional therapies (mesalamine, steroids or azathioprine) in patients with CD and UC respectively [28]. A significant decline in FCP levels after 4 weeks, of treatment was observed in patients with complete response to therapy but not in partial or nonresponders, which suggests its role in prediction of therapeutic response [19]. In a study by Kolho et al. evaluating pediatric patients with IBD FCP levels decreased in line with clinical improvement in children with active disease treated with steroids, but its values hardly ever declined to normal values [29]. In a prospective longitudinal cohort study in patients with IBD (24 ulcerative colitisand 71 Crohn's disease) treated with anti-tumor necrosis factor (TNF) agent FCP had a high prediction value of clinical relapse during follow-up and its levels <130 μg/g during remission correlated with persistence of clinical remission, while the concentration higher than 300 μg/g was a predictor of relapse within the next four months [30]. Moreover, some studies underline the role of FCP in predicting the risk of clinical relapse after discontinuation of biologic therapy [31]. According to Buisson et al. levels >100 μg/g, can be used to identify patients at risk of clinical relapse [32]. While it was >250 μg/g in the study by De Suray et al [33]. Many studies have demonstrated that FCP is a useful tool for assessing endoscopic disease activity and its levels correlate well with disease extension, both in UC and CD [34], Schoepfer et al. showed that FCP was the only biomarker which discriminated among mild, moderate and severe disease [35]. Moreover, it has been reported that FCP predicted histological remission in both children and adults [36] showing better correlation with short-term outcome in comparison with CRP, with a cutoff of 174 μg/g to predict MH [37]. Fabian et al. evaluated the usefulness of immunohistochemical (IHC) detection of tissue calprotectin (T-CPT) in bowel mucosa in children with UC. They focused at correlation of T-CPT with levels of F-CPT and endoscopic and microscopic disease activity at the time of diagnosis and tested whether T-CPT could serve as predictor of complicated course of the disease. The authors demonstrated that T-CPT correlated well with microscopic scores. F-CPT and PUCAI appear to be better predictors of unfavorable outcome in patients with UC [38]. Aiming for the best long-term outcome of IBD the new challenge of the coming years will be the evolution of the therapeutic target in IBD from MH to histological healing and FCP is probably the best marker for identifying this deeper remission. Aiming for the best long-term outcome of IBD the new challenge of the coming years will be the evolution of the therapeutic target in IBD from MH to histological healing and FCP is probably the best marker for identifying this deeper remission.

4. Zonulin

Zonulin is the protein that reflect the intestinal permeability, and its increased fecal levels are considered to be a marker of an impaired intestinal barrier, especially in the small intestine [39]. Increased serum/plasma zonulin concentrations have been found in different immunopathological diseases such as food allergies, infections of the gastrointestinal tract, systemic autoimmune diseases and inflammatory diseases of the intestine [40]. However, there are discrepancies on the correlation between fecal and serum zonulin levels [41]. Only few works published so far describe zonulin use in IBD, and all of them include adult patients [6,7,42]. Caviglia et al. investigated the role of zonulin in patients with IBD and the correlation between its serum and fecal levels and they demonstrated that serum concentrations were higher in IBD patients compared to control group (34.5 [26.5-43.9] ng/mL vs. 8.6 [6.5-12.0] ng/mL, P<0.001), but no correlation was observed between serum and fecal zonulin (rs=0.15, P=0.394) [6]. Chech study from 2017 that examined 40 IBD patients and 40 healthy persons for fecal and serum zonulin concentrations has shown that both of them were elevated in patients with active CD but not in UC. A very interesting outcome from this study was the observation that smokers had high zonulin levels irrespective of IBD, which may point to the significant up-regulation of gut permeability in cigarette smokers [7]. Since zonulin is considered to be the best marker of increased permeability in the small intestine [43], and CD can extent to the whole gastrointestinal tract including small

intestine its levels may be higher in CD than in UC which is restricted to the large intestine (with exception of rare backwash colitis). On the other hand, Wegh et al. who investigated which markers were most relevant to assess intestinal permeability in UC patients have demonstrated that serum not fecal zonulin was elevated in active disease and had better correlation with other inflammatory markers such as CRP [3]. It is important to mention the limitations of the current commercial ZRP ELISA assays which exercises caution in considering the measurement of serum zonulin as a marker of intestinal barrier integrity. The study by Ajamian M et al [17]. That investigated different zonulin's assays demonstrated that all of them detected different proteins, neither of which was Zzonulin. Therefore, there can be no value of circulating concentrations in assessing intestinal mucosal barrier dysfunction and permeability until the target proteins are indeed identified [44]. Commercial ELISA detection methodology may be improved with the development of specific and reliable monoclonal capture and detection antibodies to recombinant zonulin/prehaptoglobin-2 protein [45]. Taking into consideration all these results and discrepancies between them, not enough evidenced is available to draw any firm and objective conclusions on zonulin role as a potential new noninvasive biomarker of IBD activity.

5. Haptoglobin

Haptoglobin has been shown to have a protective effect against experimentally induced colitis - HP knockout mice have more weight loss and higher macroscopic and histological scores as compared with their wild-type littermates [46]. This means that HP plays an important modulatory and protective role in inflammatory colitis in experimental models. Clinical data are not unequivocal. Maza et al [47]. Found that HP11 was significantly less common in CD. However, Papp et al [47]. Discovered a higher frequency of HP11 in CD. However, a well-powered study from Marques group demonstrated that HP2 is a risk allele for IBD, with a higher frequency in both CD and UC compared with controls . Mouse model studies showed that Hp knockout mice are more susceptible to experimentally induced colitis than their wild-type littermates which would support the protective effect of the HP1 allele in IBD patients. However, more data is needed to draw any firm conclusions on HP role in IBD

6. Conclusion

To conclude, FCP is currently one of the best biomarkers in IBD, and its use will probably increase in the future, especially during biological therapies. Zonulin, as a marker of increased intestinal permeability with promising results in pilot studies is worth further research with properly designed clinical trials.

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