Review

New Metabolic and Genetic Biomarkers for Diagnosis of Ulcerative Colitis

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Abstract: Ulcerative colitis (UC) is one of the two disorders known as inflammatory bowel diseases (IBD) along with Crohn's disease (CD), with complex pathogenesis, requiring costly invasive investigations. Objective: to examine the most recent biomarkers proposed for UC diagnosis; to establish the strategy used to make the differential diagnosis between UC and CD relying on these biomarkers, also adding the benefit of finding new non-invasive tools in managing this condition. The search was performed in a single database (Web of Science) using the specific keywords „ulcerative colitis", „biomarkers" and „diagnosis" for the last five years. Study eligibility criteria: clinical trials on adults and pediatric patients with ulcerative colitis compared with Crohn's disease. Results: We selected 57 studies, randomized controlled trials (RCTs) and clinical case series (CCS), summarizing the latest most specific biomarkers in diagnosis of UC. Limitations: we considered RCTs and CCS from one database, limited to the search topics. Our findings indicate a important number of potential biomarkers with diagnostic value, which bring the advantage of a non-invasive method to approach this challenging disorder.

Keywords: ulcerative colitis; biomarkers; diagnosis; inflammatory bowel disease; Crohn's disease

1. Introduction

Inflammatory bowel diseases (IBD) consist of two major clinical phenotypes: ulcerative colitis (UC) and Crohn's disease (CD) [1]. Europe has the highest incidence of IBD, with 2.5-3 million people affected [2], while in North America there are 1.5 million people with IBD [3].

UC is an inflammatory bowel disease, a chronic condition, associated with modified gut microbiota, with unexplained causes [4,5]. It presents alternative periods of remission and relapse [6,7]. In this condition, the most affected is the mucosa of colon and rectum [8]. The most frequent symptom is bloody diarrhea, but this pathology is also manifested by other disorders, such as vomiting, abdominal pain, weight loss and fever, which contribute to an improper quality of life [9]. The first symptoms of nearly 20-30% of individuals with IBD appear before the age of 18 years [10]. During last years, the global prevalence has enhanced promptly, with a higher incidence at women. UC is the result of a complex of external, genetic, immunological and inflammatory factors [11], but it has been found that the major inflammatory compound in active UC are the neutrophils from the gut mucosa [12]. Many patients with UC have also other autoimmune diseases, e.g. rheumatoid arthritis, rheumatoid spondylitis, nodular erythema or sclerosis cholangitis. Thus, an early diagnosis of UC has a huge impact on patient's life, efficient treatments can be administrated to mitigate the patient suffering, to improve the quality of life [13], and to prevent the major complication of UC, colorectal cancer [14].

The prevalence of UC in children is between 0.5 and 4.3 per 100,000 individuals [15] and the symptoms are more aggressive than adults [16].

Both diseases are characterized by similar symptoms, but different treatment procedures; therefore, an accurate diagnosis is mandatory. UC is characterized by continuous inflammatory changes within the mucous membrane and affects only the large intestine [17]. The incidence of IBD is increasing in newly industrialized nations, being associated with consumption of processed food and low fibers intake, but the clear etiology of IBD still remains unknown. The first incriminated cause is the role of heredity pathogenesis; secondly, the abnormal interactions between the host immune system and gut flora and, as well as, dysregulation of cellular responses, such as autophagy and endoplasmic reticulum stress, resulting in intestinal inflammation. Also a possible reason is represented by the influence of other genetic, especially epigenetic changes, including microRNA-mediated regulation of gene expression [18].

The purpose of this review was to identify the newest biomarkers for diagnosis of UC, and also for differentiation between UC and CD in the patients with IBD, the major aim being the premature diagnosis of these diseases to prevent subsequent complications and to improve the quality of the patient life.

2. Materials and Methods

In the present paper, we performed a systematic qualitative review according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines searching original published papers on new potential biomarkers for diagnosis of ulcerative colitis; other criteria included: published in a scholarly peer-reviewed journal; written in English and from the last five years.

The review methods of the search were established in advance. The search was achieved in one major database, Web of Science Core Collection. The report had no significant deviations from the initial study plan.

Data sources: we searched the Web of Science database for ulcerative colitis, biomarkers, diagnosis and other related keywords. Search: we did a search in one database (filters applied: clinical trial, randomized controlled trial in the last five years) with the keywords: "ulcerative colitis" AND "biomarkers" AND "diagnosis"; we restricted searching for articles written in English; the last updated search was done on 12 July 2021.

Study selection: inclusion criteria were: patients with a diagnosis of IBD or UC or with an inflammatory status of gut microbiota in randomized clinical trials (RCTs) and clinical case series (CCS) published in the last five years.

Data extraction: the following data were selected: author(s), year of publication, country and aim of the study, study design, and main results. We selected RCTs and CCS as presented below for all the data searched and retrieved from the database sources. Firstly, the authors screened articles by title and abstract, and then by full text. Duplicates and articles not fulfilling the search criteria were excluded.

Data analysis was performed by three authors (E.E.T., M.A.C., and M.A.S.). 163 studies with biomarkers in ulcerative colitis or with biomarkers for diagnosis of IBD or with biomarkers for differentiation between UC and CD were identified and screened for eligibility by the three examiners. We completed the data collection in July 2021. The quality of the studies selected for review was evaluated.

Fifty-seven papers were included in the present study, centered on the three main topics included in the search.

3. Results

3.1. Study Selection

We searched for: TITLE: (Ulcerative colitis diagnosis) Refined by TITLE: (biomarkers) AND DOCUMENT TYPES:(ARTICLE). Timespan: 2017–2021 (last five years). Results: 163 articles.

From the databases, we selected 57 studies for full-text reading, after initial retrieving of 163 articles, screened by title and abstract or in extenso to match the search criteria, and exclusion of duplicates; the screened research was centered on twelve main topics in the UC biomarkers published research: (a) fecal analysis biomarkers, (b) different stages of disease biomarkers, and (c) organs biomarkers in UC, (d) CRP/albumin ratio, (e) microRNAs, (f) galectins, (g) gut microbiota biomarkers in UC, (h) autoantibodies (i) volatile organisc compounds, (j) protein linked carbohydrates, (h) serum fetuin, (i) transferrin and (j) other potential biomarkers . The selection process is presented in the PRISMA flow diagram (**Figure 1**).
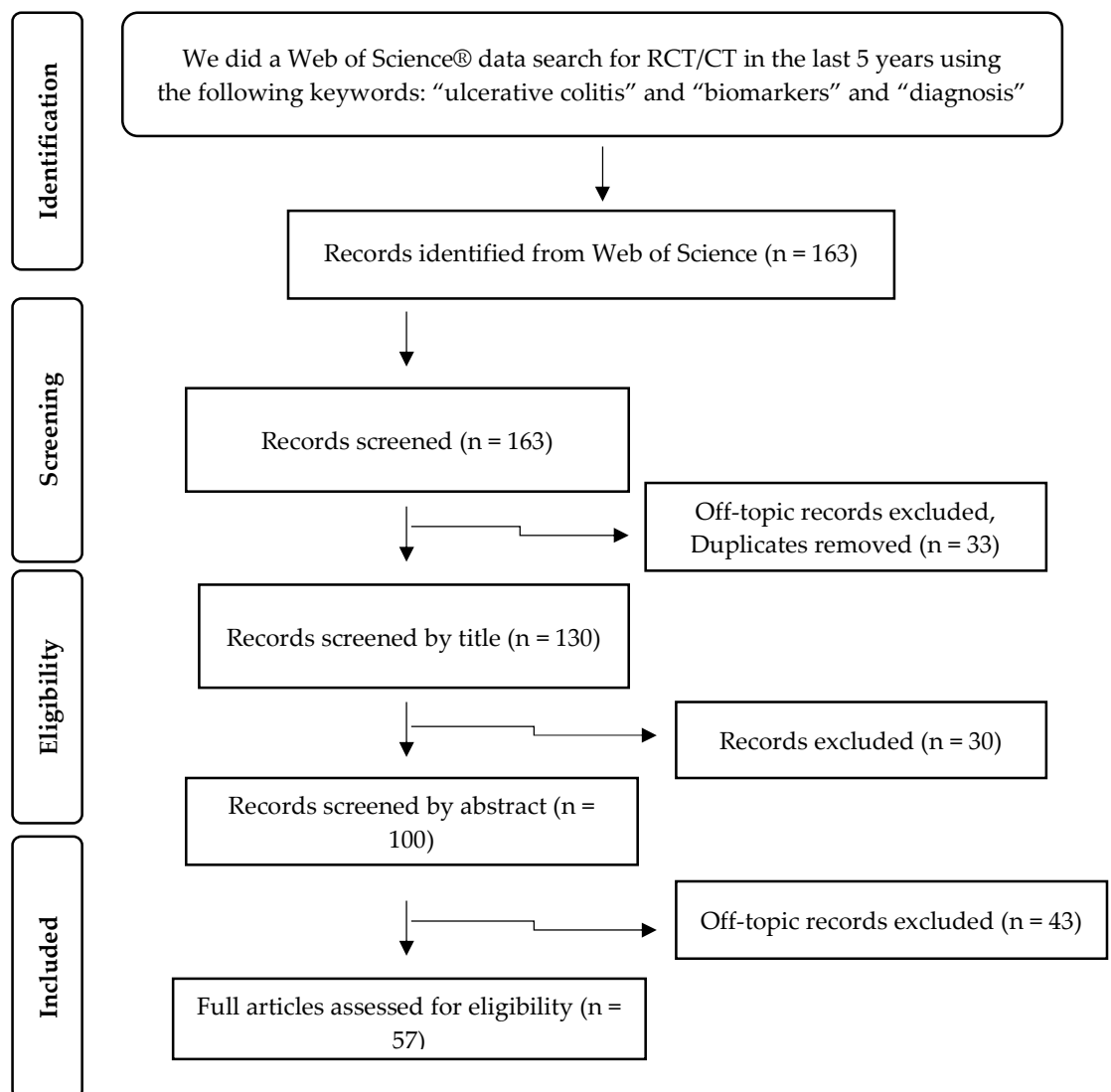
Figure 1. Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) diagram describing a systematic search and study selection process.

3.2. Biomarkers for diagnosis of UC

One of the biggest challenges is to discover new non-invasive biomarkers with a higher sensitivity and specificity to detect as early as possible the inflammation of the gut mucosa to prevent the appearance of clinical symptoms and, thus, to prevent the progressive impairment of gastrointestinal mucosa [19].

Because of its unexplained etiology, UC evaluation requires a variety of medical tests and procedures, including laboratory analysis, histopathological investigation, imaging examination and genetic tests. Regarding laboratory analysis, inflammatory biomarkers are essential for the assessment of acute phase of UC, because they are commonly used, they are cheap, they have a non-invasive character, and they are non-traumatic for the patient. In this category, we found CRP, ESR, platelets, fibrinogen, ferritin, and albumin, indicators that are investigated in the patient plasma. The major drawbacks of these markers are the low sensitivity and specificity [20].

If we talk about fecal markers, the main parameter used to evaluate the UC activity, different stages of its evolution, the mucosal healing, the response to treatment or the necessity of a surgery is fecal calprotectin (FC) [21]. It is able to differentiate between IBD and irritable bowel syndrome, but not for the differentiation of UC from CD. There are more other promising fecal parameters that are intensely studied, such as neutrophil gelatinase associated lipocalin, cathelicidins, beta-glucuronidase and osteoprotegerin [22,23].

3.2.1. Fecal analysis

A prospective cohort study led by Galipeau et al. showed that a heightened level of fecal proteolytic activity could be an early non-invasive biomarker for gut inflammation, which could be monitored in the predisposed population to ulcerative colitis (UC), before the clinical diagnosis for this pathology. During this study, there were applied several types of analysis, such as shotgun metagenomic and 16S rRNA gene sequencing, gnotobiotic colonization, and in vitro functional examination to describe the microbial composition in fecal samples, collected from pre-UC, post-UC, and healthy individuals. The study illustrated different changes in the composition of gut microbiota for pre-UC subjects, which showed higher levels for pathogens (*Actinobacillus*) and lower levels for favorable bacteria (*Adlercreutzia*, with anti-inflammatory properties and *Bifidobacillus*). The pre-UC population presents a large production of proteases and peptidases by intestinal bacteria (*Bacteroides* vulgates and *Parabacteroides*) and an augmented metabolism of proteins. Moreover, the study illustrated an increased level of elastase activity in *Bacteroides*, isolated from fecal samples. Consequently, the study opened different pathways for early investigation of predisposed people to UC and also for possible treatments by reduction of high proteolytic activity [24].

Calprotectin, a 36-kDa glycoprotein and a Ca- and Zn-binding protein, from lipocalin family, is found in the cytoplasm of inflammatory cells such as neutrophils, macrophages, and activated monocytes. The concentration of calprotectin in stool is proportional to the number of neutrophils in the intestinal lumen, making this marker more specific and sensitive for detecting inflammation in the digestive tract than other serological markers. Also, calprotectin offers the advantage of detecting inflammation at an early stage, before other non-specific markers such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and orosomucoid can be detected [25].

A new perspective for this marker is the prediction of endoscopic activity in inflammatory bowel disease. With this notion comes the „mucosal healing", an important therapeutic goal in Crohn's Disease and Ulcerative Colitis (UC). This can be defined as the "complete absence of all inflammatory and ulcerative lesions in all segments of gut" at endoscopy. As repeating colonoscopy is not feasible owing to the costs and potential adverse effects and clinical signs frequently do not correlate with

endoscopic indices or real inflammatory activity, finding a marker to predict the presence of endoscopic lesions is a key in inflammatory bowel disease diagnosis [26].

Jusue et al. showed the value of calprotectin as a predictor of endoscopic activity in IBD and that it is more accurate in ulcerative colitis than in Crohn's disease. The greater accuracy of the marker in UC was based on the fact that calprotectin was more sensitive for detecting colonic lesions than ileal lesions. Also higher levels of fecal calprotectin in patients with no evidence of endoscopic activity suggested that endoscopy may underestimate the disease activity and the need of another route of monitoring. The study also reported that the fecal calprotectin concentration in UC was closely associated with the severity of endoscopic lesions [26].

Fecal calprotectin (f-Cp) has high sensitivity and specificity for discriminating between IBD and functional bowel disorders in adults and children. Moreover, f-Cp is more sensitive and specific for IBD than commonly used inflammatory markers, and its levels link with the degree of neutrophilic infiltration. F-Cp may also be a helpful tool for monitoring disease progression and response to therapy and predicting relapse and risk of bowel resection [27].

Whitehead et al. conducted a study that has as main aim to evaluate whether between-assay variability of different fecal calprotectin (f-Cp) assays influences diagnostic accuracy in a cohort of patients and brought up the fact that this inter-assay variability can be due to the absence of standardization. They report a prospective clinical evaluation in which four commercially available f-Cp assays were compared to assess whether inter-assay variability influences clinical decision-making, and the diagnostic performance of the different f-Cp assays was similar despite inter-assay variability in same sample absolute f-Cp values. The conclusion of the study was that there is a necessity for assay standardization, but in its absence they suggest that assay-specific cut-off values may optimize their diagnostic performance and second that clinicians be made aware that f-Cp results from different assays are not interchangeable [27].

Another study which evaluated the performance of combinations of fecal and blood markers on the detection of endoscopically active disease showed that combining FCT and fecal immunochemical test improved the specificity to 95%, but the sensitivity decreased to 65.4% [28].

3.2.2. Markers for different stages of UC

During last years, many researchers have been working to discover other biomarkers for diagnosis of UC, using many analysis techniques, from blood and fecal analysis (C-reactive protein, erythrocyte sedimentation rate and fecal calprotectin – the most common analysis for UC diagnosis), to genomic and metabolomic analysis [29].

Another method of examination is peptidomics, which defines the qualitative and quantitative analysis of peptides with low molecular weight (LMW) in different biological samples. For example, Miao et al. managed such a study with 78 people, including 26 with active UC, 26 with remitting UC and 26 healthy people. Researchers applied a nano-liquid chromatography coupled with hybrid linear ion trap Orbitrap (Nano LC-LTQ-Orbitrap) mass spectrometry to evaluate the serum peptidome of all 78 subjects and there were identified 40 peptides, but only 6 biomarker peptides presented adequate features for curve analysis. These 6 peptides could be important biomarkers to differentiate the subjects with active UC from subjects with remitting UC and healthy subjects; thus, serum peptidomics represents a new method to discover new markers for UC diagnosis and for the differentiation of different phases of UC [29].

3.2.3. Biomarkers from different organs

In a study led by Hong et al., which demonstrated the effect of Compound Sophorae Decoction in UC, there were identified 36 potential biomarkers, using mass spectrometry. These metabolites were found in serum, urine, colon, kidney and liver, with various levels in UC, higher or lower [13], all this information being reviewed in Table 1.

Table 1. Potential biomarkers and their level in ulcerative colitis [13].

Matrix	Metabolite	Level in UC
Serum	8,11,14-Icosatrienoic acid	Higher
	Citric acid	
	Pyroglutamic acid	
	Arachidonic acid	
	Cytidine	
	Adenosine monophosphate	
	Thiamine	
	Carnosine	
	1-Methylhistidine	
	3b-Hydroxy-5-cholenoic acid	Lower
	Docosahexaenoic acid	
	Taurocholic acid	
	Tetranor 12-HETE	
Urine	Diaminopimelic acid	Higher
	Leucyl-phenylalanine	
	Xanthurenic acid	
	Proline betaine	
	α,β-dihydroxyisobutyric acid	
	Orotic acid	
	Niacinamide	
	Monoethylhexyl phthalic acid	
	5-Methoxytryptamine	
	Oleic acid	
	Mono-benzyl phthalate	Lower
	Methyl bisnorbiotinyl ketone	
	Deoxycortisol	
Colon	Lactic acid	Higher
	Aspartic acid	Higher
Kidney	Lysine	Higher
	Homocystine	Higher
	Phenylalanine	Lower
Liver	Glutathione	Higher
	Trihydroxycoprostanoic acid	Lower
	Tyrosine	
	Thymidine	
	Dimethylglycine	

3.2.4. CRP/Albumin ratio (CAR)

CAR or CRP to albumin ratio is an anti-inflammatory biomarker, which has been studied extensively during last years and studies showed that this is a useful indicator to

diagnose many types of cancer, acute pancreatitis or sepsis. An enhanced activity of inflammation is associated with a high level of CAR.

Although colonoscopy is the main and the conclusive medical procedure for UC diagnosis, it is important to discover and to identify other parameters that can be used more easily than a colonoscopy, a complex procedure for patient and doctor. Regarding biomarkers from blood and feces, the most used for UC investigation are CRP and fecal calprotectin [30].

CRP is a protein that characterizes the acute stage of an anti-inflammatory disease and it is the response to pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF alpha (TNF-α), but it is a nonspecific parameter, especially if UC is in a moderate stage of symptoms. Nevertheless, CRP is a superior marker to procalcitonin, erythrocyte sedimentation rate and albumin for UC diagnosis. Albumin is the opposite indicator of CRP and it is also related to the nutritional status of the patient. Therefore, an anti-inflammatory response is the outcome of an increased level of CRP and a decreased level of albumin. The combination between CRP and albumin is called CAR and it indicates the mixture of systemic swelling and nutritional situation. A recent study realized on patients with active UC illustrated that CAR had a higher specificity and AUC value comparative to CRP and albumin. It is a simple, low-cost and quickly determined indicator, so it can be a useful biomarker due to this characteristics in the diagnosis of severe UC [30].

3.2.5. MicroRNAs

Circulating microRNAa (miRNAs) is a hopeful non-invasive biomarker for IBD diagnosis. It is a small noncoding RNAs, consisting of 18-25 nucleotides with an essential role in different biological processes and it is found in serum, saliva, urine and plasma. Since 2008, miRNAs is extremely studied asa a potential biomarker for many conditions, including IBD. From all miRNAs genes, a specific role in down-regulation of patients with UC have miR-143 and miR-145, identified in the colon biopsies of this subjects and miR-7, found in the CD subjects mucosa [31].

MicroRNAs are important post-transcriptional players that regulate the expression of genes during the inflammation and thus could be appropriate biomarkers for differentiation between UC and CD.

Guz et al. confirmed in a study that the lower expression of E-cadherin (CDH1) in patients with CD, but not in patients with UC, could evidence the involvement of this protein in CD pathogenesis. Also downregulated CDH1 was negatively correlated with miR-155-5p expression in both the inflamed mucosa and adjacent mucosa of such patients, which can assume that this expression pattern may differentiate CD from UC. By comparing UC tissue to adjacent normal tissue but alo to normal mucosa from healthy controls, all analyzed miRNAs in this study were significantly overexpressed in UC tissue, with some limitations in differentiating UC and CD [3]. In addition, Mohammadi et al. identified a panel of ten miRNAs differentially expressed in CD patients in endoscopic remission compared to healthy control subjects, miR-874-3p making the difference between the UC and CD cases. Further studies are necessary for developing the method of using these miRNAs in obtaining the differentiated diagnosis, but they are still a useful tool for monitoring the disease progress [32].

Ye et al. based on the fact that circular RNAs (circRNAs) participate in the pathogenesis of a variety of diseases and are considered ideal biomarkers of human disease, conducted a study in which was examined the expression of circRNA_103516 in inflammatory bowel diseases and its associations with clinical phenotypes and inflammatory cytokines. Circular RNAs (circRNAs) are covalently closed continuous single-stranded RNA molecules that have advantageous properties because their circular structure enables rolling circle RNA replication and producing multiple

genomic copies once a single initiation event occurs. However, the characteristics of circRNAs are much less clear than those of microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). Nonetheless, research on their functions has recently emerged, and increasing evidence demonstrates that circRNAs can modulate gene expression at the transcriptional or post-transcriptional level by sponging miRNAs or by interacting with other molecules. Furthermore, circRNAs are evolutionally conserved, and their expression is relatively stable in the cytoplasm; these features indicate that circRNAs may be ideal biomarkers in human disease. CircRNAs are ideal diagnostic and therapeutic candidates due to their stability and conservation. In the study were involved 90 Crohn's disease (CD) patients and 90 ulcerative colitis (UC) patients, 80 HCs (healthy controls), and 35 PCs (patient controls). CircRNA_103516 was upregulated in CD and UC patients compared with HCs and PCs. In addition, circRNA_103516 levels were increased in active CD and UC compared with remittent groups. The conclusion of the study was that CircRNA_103516 levels in Peripheral blood mononuclear cells can be considered an ideal candidate biomarker for diagnosing IBD. Dysregulation of circRNA_103516 may participate in the molecular mechanism of IBD through hsa-miR-19b-1-5p sponging [33].

3.2.6. Galectins

Galectins are a family of galactoside-binding proteins commonly altered in the circulation of disease conditions such as cancer and inflammation. Serum levels of galectins such as galectins-2,-3, -4 and -8 are significantly higher in colon cancer patients than in healthy people and promote the dissemination of circulating haematogenous tumour cell in metastasis [34].

Yu et al. analyzed levels of galectins-1, -2, -3, -4, -7 and -8 in 208 samples from ambulant IBD patients (97 CD, 71 UC) and 40 from healthy people. Serum levels of galectins-1 and -3, but not galectins-2, -4, -7 and -8, are significantly elevated in both UC and CD patients in comparison to healthy people. The difference between the values of these galectins were not statistically significant to be able to separate active and inactive UC and CD, but they have the potential to be developed as biomarkers for general IBD determination [34].

3.2.7. Biomarkers from gut microbiota

The study of Zhou et al. aimed to identify non-invasive biomarkers for distinguishing UC from CD, based on the gut microbiom. UC was characterized by the presence of Actinobacteria, Proteobacteria, Bacilli, Bifidobacteriaceae, Bifidobacteriales, Coriobacteriales, Enterococcus, Enterococcaceae, Streptococcus, Steptococcaceae, Lactobacillales, Enterobacteriaceae, Enterobacteriales, and Pseudomonadales while healthy controls (HC) were predominantly enriched in Prevotella, Prevotellaceae, Bacteroidales, Roseburia, Lachnospiraceae, Ruminococcaceae and Clostridiales [35].

Fusobacterium nucleatum was reported to contribute to colorectal carcinoma by promoting a favourable microenvironment for carcinoma progression in UC; it has also been suggested as a biomarker for diagnosis. Also *Gardnerella vaginalis* was significantly more enriched in fecal samples from patients with UC, showing a potential use of the gut microbiome to aid in UC diagnosis being of significant clinical value in the management of IBD; study mentioned the limitation offered by the difference of gut microbiota in pediatric or elderly patients who cannot be included in the pattern [35].

As an increasing number of children are affected by inflammatory bowel disease (IBD) worldwide, the need for a diagnosis method less invasive than repeated endoscopic test emerged. Furthermore, it is challenging to distinguish Crohn's disease from ulcerative colitis endoscopically.

Tatsuki et al. conducted a study that aimed to determine a putative biomarker for distinguishing CD from UC in pediatric patients by scanning serological cytokine profile. They showed that an (interleukin) IL-1 receptor antagonist, IL-6, and macrophage inflammatory protein-1β were associated with pediatric UC activity index (PUCAI) compared with CD where the granulocyte–macrophage colony-stimulating factor was incriminated. Also growth-regulated oncogene and TNF-α were higher in UC than in controls. This study observed clear differences in cytokine profiles between CD and UC, with IL-7 alone having the highest diagnostic accuracy [36].

3.2.8. Autoantibodies

Hamanaka et al. identified 3 novel serum antibodies through Alpha LISA technique. They are anti-PARG (poly ADP-ribose glycohydrolase), anti-TCEAL 1 (transcription elongation factor A protein-like 1) and anti-PRR13 (proline-rich 13), which have been found in a large amount in UC patients comparative to CD patients. From all 3 antibodies, TCEAL 1 has been associated for the first time with UC. PARG antibody is a biomarker used for the evaluation of mucosa impairment by inflammation at patients with UC. An augmented level of PRR13 antibody is UC patients is related to an anti-inflammatory process from angiogenesis through suppression of TSP-1 (thrombospondin-1). In this study has been also studied other markers: first is pANCA, which is a common antibody found in IBD, in a percentage of 60-70% at patients with UC and it is the result of a cross-reaction between it and intestinal bacterial antigens and the other one is CUZD 1 antibody, a unique marker for IBD patients [37].

Ulcerative colitis alongside with Crohn's disease are chronic and relapsing gastrointestinal disorders with an unclear etiology. In order for a better diagnosis for these conditions, a large range of serological antibodies were studied. The most important and widely used antibodies are ASCA (anti-Saccharomyces cerevisiae antibody) and ANCA (anti-neutrophil cytoplasmic antibody). Nevertheless new biomarkers were developed and can be included in this category the following antibodies: *Escherichia coli outer membrane porin C* antibody (anti-OmpC), exocrine pancreatic antibody (PAB), intestinal goblet cells antibody (GAB), anti-glycan antibody, bacterial flagellin CBir1 antibody (anti-CBir1), and *Pseudomonas fluorescens*-associated sequence I2 antibody (antiI2). In prospective study whose purpose was to was to assess the clinical value of multiple antibodies, 130 Crohn's disease (CD) and 120 ulcerative colitis (UC) patients, compared with 80 healthy subjects, were investigated and the results showed that are serviceable biomarkers for IBD and they can lead to a better clinical decision. However, the serological positive antibodies were more inclined to be diagnosed as CD rather than UC. Positivity of ANCA was associated with disease activity of UC and showed a sensitivity of 46.7%. GAB (IgG) positivity implied extensive location in UC. A combination of ASCA/anti-OmpC IgA/GP2 (IgG) showed the best specificity in distinguishing CD from UC [38].

3.2.9. Volatile organic compounds (VOC)

VOC are gaseous molecules produced during bacterial fermentation in the gut and they give information about the gut microbiota status of the host and the interaction between the two. These compounds showed diagnosis utility in other conditions such as cancer and diabetes and IBD patients are proposed to be candidates for analyzing the benefits brought by them in establishing the diagnosis. VOC are able biomarkers to distinguish between active UC from remission UC and to evaluate the inflammation status in this disease [39].

Hassani et al. aimed to assess the potential of urinary and fecal VOC as biomarkers in both pediatric UC and CD by comparing with the endoscopic and histopathologic outcome. There is a significant difference between IBD and controls for both fecal and

urinary VOC profiles (p-value, area under the curve; 0.038, 0.73 *vs.* 0.028, 0.78), making the analysis of these compounds, potential noninvasive markers in detecting ulcerative colitis. Also, as the urine collecting method can be faster and more easily accepted by the patients, this type of establishing a preliminary diagnosis can accelerate the process. The distinction between the two methods is that analyses of fecal VOCs is believed to reflect the gut microbiota composition and local inflammatory processes, on the contrary, urinary VOCs associated with IBD are more likely to be a combination of compounds that diffused from the intestine into the bloodstream and VOCs that are associated with general inflammation [40]. Bosch et al. previously used field asymmetric ion mobility spectrometry (FAIMS) to analyze fecal VOC composition as a potential tool in differentiating two gastro-intestinal conditions relying on the accessibility of the process in pediatric patients, achieving similar diagnostic accuracies [41].

Determination of volatile organic compounds from exhaled breath is a non-invasive method for the diagnosis of IBD. The first objective of the most studies was to determine whether exhaled VOCs can be used to distinguish IBD from healthy controls and CD from UC. The volatile organic compounds from exhaled breath are a combination of bi-products of normal metabolic activity and there is an estimate of over 3000 VOCs in human breath. Ammonia, sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) were the substances described in one study and it has been showed that they contributed the most to the separation between diagnostic groups. Important changes were observed in ammonia by both UC and CD patients. Changes in NO_2 were greater in CD over UC and controls, while UC is associated with increased sulphur dioxide. Ethylene oxide in UC and controls showed small differences between groups. In other studies were identified 6 VOCs (hydrogen cyanide, ammonia, dimethyl sulphide, hydrogen sulphide, butanal, and nonanal) which significantly showed differences in concentrations, between diagnostic groups. Other analysis used VOCs (1-octene, 1-decene, (E)-2-nonene) as relevant for predicting the presence of IBD, but did not identify any significant difference in VOCs between CD and UC [42].

3.2.10. Protein-linked carbohydrates

Protein-linked carbohydrates can be used as biomarkers for UC diagnosis since now it is a need for noninvasive test for diagnose of inflammatory bowel diseases as UC and Crohn's disease. In a study was analyzed the profile of total plasma N-glycomes of 2635 patients with inflammatory bowel diseases. The plasma samples were analyzed by mass spectrometry with a linkage-specific sialic acid derivatization technique and were compared the profiles of the patients with Crohn's disease or UC and the profiles of the control group. The results showed a bigger number for large-size glycans in the samples from the patients with UC and Crohn's disease compared with control samples. In addition, it was observed a low number of hybrid and high-mannose structures, lower fucosylation, lower galactosylation, and higher sialylation (a 2,3- and a2,6-linked). A difference between patients with CD and patients with UC can be done based on the higher bisection, lower galactosylation, and higher sialylation (a 2,3-linked). Also, in this study it is showed that glycosylation models were associated with disease location and progression [43].

3.1.11. Serum Fetuin A

Fetuin A (α2-heremans-schmid glycoprotein (AHSG)) is a multifunctional plasma glycoprotein with a molecular weight of approximately 60 kDa and is a member of the cystatin superfamily. Fetuin A has been studied as an inhibitor of ectopic calcium deposition, an important promoter of insulin resistance, and a negative acute phase reactant. Acting as a negative acute phase protein, fetuin A binds macrophage deactivating cations and the resulting fetuin-cation complex also transmits an anti-

inflammatory signal. Fetuin A is synthesized by hepatocytes and its concentration in the serum falls by about 10-fold after birth. In humans, fetuin A is a negative acute phase protein, as serum concentrations are significantly reduced after major surgical procedures, trauma, burns, and severe inflammation. In one study which involved a total of 90 patients and 41 healthy individuals was found that fetuin A levels in both the UC and CD groups were significantly lower than in HC; however, there was no difference between the UC and CD groups, this means that fetuin, this means that fetuin A test can be used to differentiate UC and CD patients from HC but can't be used to distinguish UC patients from CD patients [44].

3.2.12. Transferrin

Transferrin is a glycoprotein capable of reversible iron binding, which serves as its transporter between iron donating enterocytes and macrophages and accepting erythroblasts. Systemic levels of transferrin differently respond to anemia depending on its cause. Its utility as a diagnostic marker in IBD-associated anemia is under the mark sign since transferrin concentration varies also in response to inflammation and malnutrition. Matusiewicz et al. wanted to evaluate transferrin levels in a cohort of IBD patients and relate it to indices of inflammation, malnutrition, anemia, and oxidative stress. They begin from the hypothesis that a lack of specificity caused by its relation to those indices IBD-accompanying conditions might be advantageous and transferrin may better reflect the overall disease severity. In this study were examined 234 individuals: 97 individuals as healthy controls and 137 IBD patients: 63 with Crohn's disease (36 with active and 27 with inactive disease) and 74 with ulcerative colitis (28 with active and 46 with inactive disease). It was observed a significant decrease in transferrin levels in patients with active CD and UC, without differences between these two forms. Transferrin in IBD patients negatively correlated with inflammatory indices and the strongest correlations was with IL-6 and hsCRP in CD and IL-6, WBC, and TNF-α in UC, although the latter association was found exclusively in patients with active disease. It was also examined transferrin association with indices of nutritional status and anemia and it was found out that transferrin positively correlate with all examined indices (albumin, cholesterol, hemoglobin, hematocrit, RBC, and iron) in UC patients but only with albumin and iron in CD patients. Additional it was examined the associations with antioxidants: serum paraoxonase-1 and erythrocyte catalase, glutathione peroxidase-1, and superoxide dismutase-1. Transferrin positively correlated with all of these parameters in CD patients but in case of UC patients exclusively with paraoxonase-1 [45].

3.2.13. Other studies regarding potential biomarkers for diagnosis of UC

During last five years, there have been studied many potential parameters for diagnosis or treatment of UC, but some of them illustrated several markers that could be used to diagnose UC or to differentiate between UC and CD. All these studies are reviewed in **Table 2**.

Table 2. Recent studies regarding the identification of new potential biomarkers in UC.

Subjects	Analysis technique	Main results	References
21 UC subjects 22 CD subjects 21 non-IBD controls	qRT-PCR	Identification of 2 new genes in subjects with UC: CCL11 and MMP10 Identification of 2 new genes in patients with CD: C4BPB and IL1RN These 4 new genes are potential candidate biomarkers to differentiate UC from CD, which could direct the researchers for future therapeutic targets.	[46]
80 UC subjects	ELISA	An increased level of fecal concentration of sST2 to the UC patients with higher endoscopic, clinical and total Mayo score. sST2 could be a future biomarker for the evaluation of UC severity and could open promising perspectives as a therapeutic aim.	[47]
106 UC subjects 21 control subjects	WGCNA	Identification a new potential tissue biomarkers for diagnosis or treatment of UC: DUOXA2, SAA1, SAA2, TNIP3, CXCL1, SLC6A14 and CD55; DUOXA2 and SLC6A14 could be new targets for gene therapy in UC; The pathways DUOXA2/DUOX2 and CXCL1/CXCR2 could have an essential role in the evolution of UC through the inflammatory responses.	[48]
Female C57BL/6JJ mice	Serum-associated modulation of fluorescent P450 inhibition assay	It has been found that CYP219 and CYP2E1 from the serum of UC model mice could be used as potential biomarkers for assessment of UC. These simple fluorescent P450 inhibition tests present the capacity to act like a new procedure to diagnose UC in mouse, also with promising results at human subjects.	[49]
19 pediatric and 87 adult UC subjects	Gene ontology	Identification of new potential biomarkers for UC diagnosis and treatment and also their fundamental roles in the pathological differences between UC at children and adults: CDC42, POLR2A, RAC1, PIK3R1, MAPK1 and SRC.	[50]
48 UC subjects 96 healthy controls	ELISA	There have been analyzed 2 parameters to differentiate the patients with UC from healthy subjects: NLR and PLR. They also can be used as biomarkers to diagnose UC and to evaluate the status of gut mucosa, especially in patients where colonoscopy is not possible.	[51]
42 IBD subjects 18 healthy subjects	ELISA	There have been discovered two biomarkers for UC diagnosis: LTA4H and CAT, identified from MLI (mucosal-luminal interface) proteome.	[52]
140 UC subjects 128 CD subjects 224 subjects with intestinal associated diseases as controls	ELISA Immunofluorescent assay	It has been evaluated four biomarkers to diagnose UC and CD: pANCA, PAB, ASCA and GAB. From all these markers, pANCA and PAB showed a higher level in UC and CD, while ASCA and GAB presented insignificant levels in IBD.	[53]

33 UC subjects 27 CD subjects 20 healthy controls	ELISA	Four biomarkers have been assessed as a potential indicators used for the diagnosis and evaluation of UC and CD clinical activity: NEU, HLE-α1AT, lactoferrin and calprotectin. It have been resulted higher ratios of HLE- α1AT/NEU and lactoferrin/NEU in UC subjects than with CD and higher levels of plasma calprotectin and lactoferrin in CD subjects than with UC.	[54]
158 UC subjects	-	It has been studied the NeuPla ratio as a potential biomarker for diagnosis of UC, with better results than serum biomarkers (CRP, ESR and albumin).	[55]

Abbreviations:

ASCA	antibodies to saccharomyces cerevisiae
C4BPB	complement component 4 binding protein beta
CAT	catalase
CCL11	C-C motif chemokine ligand 11
CD55	complement decay-accelerating factor (CD antigen CD55)
CDC42	cell division control protein 42
CRP	C-reactive protein
CXCL1	C-X-C motif chemokine
CXCR2	C-X-C chemokine receptor type 2
DUOXA2	dual oxidase maturation factor 2
ESR	erythrocyte sedimentation rate
GAB	antibodies against intestinal goblet cells
HLE-αIAT	leukocytes elastase-alfa 1 antytripsine complex
IL1RN	interleukin 1 receptor antagonist
LTA4H	leukotriene A-4 hydrolase
MAPK1	mitogen-activated proyein konase 1
MMP10	matrix metallopeptidase 10
NEU	neutrophils
NeuPla ratio	Neutrophil-Platelet ratio
NLR	neutrophil to lymphocyte ratio
PAB	pancreatic antibodies
pANCA	antibodies to perinuclear anti-neutrophil cytoplasmic
PIK3R1	phosphatidylinositol 3-kinase regulatory subunit alpha
PLR –	platelet to lymphocyte ratio
POLR2A	NDA-directed RNA polymerase II subunit RPB1
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RAC1	Ras-related C3 botulinum toxin substrate 1
SAA1 and SAA2	serum amyloid A 1 and 2
SLC6A14	solute carrier family 6 member 14
SRC	proto-oncogene tyrosine-protein kinase Src
sST2	soluble protein ST2, ELISA – enzyme-linked immunosorbent assay
TNIP3 –	TNFAIP3-interacting protein 3
WGCNA	weighted gene coexpression network analysis

4. Conclusions

Ulcerative colitis is an inflammatory bowel disease that causes inflammation and ulcers in the digestive tract, and sometimes can lead to complications. Since treatment can reduce signs and symptoms of the disease, it is very important to make an accurate diagnosis at an early stage. Despite the highly prevalence rate, the pathogenesis of UC is not fully elucidated and as a result, treatment does not include a wide range of medicines, the need for precision in choosing the suitable medicine being mandatory. Also the most used and accurate pathway for diagnosis is endoscopy, which is an invasive, time-consuming, expensive and painful method [56].

Biomarkers from stool and serum are non-invasive markers used as new targets of diagnosis, which have the advantages to be reproducible, accurate, standardized and to have sensitivity and specificity. The capacity to determine the type of UC, to make difference between UC and Crohn's disease, to determinate the severity and the response to therapy using markers are the major purpose of studies regarding biomarkers used in diagnosis of UC.

Studies that were analyzed in this review showed suitable non-invasive biomarkers for diagnosis and some of them indicate also highly sensitivity and specificity. However, for a better characterization of these biomarkers it is mandatory to increase the number of studies in order to demonstrate their real utility [57].

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