*MINIREVIEWS*

# Laboratory markers in ulcerative colitis: Current insights and future advances

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## Abstract

Ulcerative colitis (UC) and Crohn's disease (CD) are the major forms of inflammatory bowel diseases (IBD) in man. Despite some common features, these forms can be distinguished by different genetic predisposition, risk factors and clinical, endoscopic and histological characteristics. The aetiology of both CD and UC remains unknown, but several evidences suggest that CD and perhaps UC are due to an excessive immune response directed against normal constituents of the intestinal bacterial flora. Tests sometimes invasive are routine for the diagnosis and care of patients with IBD. Diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. The employment of non-invasive biomarkers is needed. These biomarkers have the potential to avoid invasive diagnostic tests that may result in discomfort and potential complications. The ability to determine the type, severity, prognosis and response to therapy of UC, using biomarkers has long been a goal of clinical researchers. We describe the biomarkers assessed in UC, with special reference to acute-phase proteins and serologic markers and thereafter, we describe the new biological markers and the biological markers could be developed in the future: (1) serum markers of acute phase response: The laboratory tests most used to measure the acute-phase proteins in clinical practice are the serum concentration of C-reactive protein and the erythrocyte sedimentation rate. Other biomarkers of inflammation in UC include platelet count, leukocyte count, and serum albumin and serum orosomucoid concentrations; (2) serologic markers/antibodies: In the last decades serological and immunologic biomarkers have been studied extensively in immunology and have been used in clinical practice to detect specific pathologies. In UC, the presence of these antibodies can aid as surrogate markers for the aberrant host immune response; and (3) future biomarkers: The development of biomarkers in UC will be very important in the future. The progress of molecular biology tools (microarrays, proteomics and nanotechnology) have revolutionised the field of the biomarker discovery. The advances in bioinformatics coupled with cross-disciplinary collaborations have greatly enhanced our ability to retrieve, characterize and analyse large amounts of data generated by the technological advances. The techniques available for biomarkers development are genomics (single nucleotide polymorphism genotyping, pharmacogenetics and gene expression analyses) and proteomics. In the future, the addition

of new serological markers will add significant benefit. Correlating serologic markers with genotypes and clinical phenotypes should enhance our understanding of pathophysiology of UC.

**Key words:** Inflammatory bowel diseases; Ulcerative colitis; Crohn's disease; Serologic markers; Acute phase response

**Core tip:** Ulcerative colitis (UC) and Crohn's disease are the major forms of inflammatory bowel diseases (IBD). Tests sometimes invasive are routine for the diagnosis and care of patients with IBD. The employment of non-invasive biomarkers is needed. We describe biomarkers assessed in UC, with special reference to acute-phase proteins and serologic markers and thereafter, we describe the new biological markers. The progress of molecular biology tools have revolutionised the field of the biomarker. The techniques available for biomarkers development are genomics and proteomics. Correlating serologic markers with genotypes and clinical phenotypes should enhance our understanding of pathophysiology of UC.

## INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic, relapsing inflammatory diseases of the intestine. UC and CD can be differentiated by different genetic predisposition, risk factors, clinical, endoscopic and histological characteristics.

In UC pathogenesis are involved numerous mechanisms. The chronic inflammation of colonic mucosal in UC results from: (1) damage to the epithelial barrier; (2) equilibrium between tolerance to commensal microflora, dietary antigens and suitable sensitivity to enteric pathogens maintained by intestinal immune system; (3) dysregulation of immunological responses; and (4) genetic factors[1].

The inflammation in UC is limited to the mucosal surface. The illness starts in the rectum and generally extends proximally through the whole colon. However, some patients with proctitis or left-sided colitis might have a caecal patch of inflammation[2].

Although the precise cause of IBD is unknown, there seems to be a genetic predisposition. The risk is mainly high in first-degree relatives: from 5.7% to 15.5% of patients with UC has a first-degree relative with the same disease[3,4]. In addition, Ashkenazi Jews have a frequency of UC 3-5 times higher than other ethnic groups.

The frequency of UC is higher in developed countries and in urban vs rural areas. Numerous environmental factors act as predisposing or protective factors for UC, such as cigarette smoke. A meta-analysis showed that cigarettes smoking is protective against UC compared with non-smoking[5]. The smoking UC patients have a milder course of the disease than non-smokers, and disease activity is frequently improved in patients who have stopped smoking[6].

Previous gastrointestinal infections (e.g., *Salmonella* spp, *Shigella* spp and *Campylobacter* spp) double the risk of developing UC. This suggests that the acute intestinal infection could lead to changes in the intestinal flora triggering the onset of a chronic inflammatory process in genetically predisposed subjects[7,8].

Appendicectomy is protective against UC and a meta-analysis reported that reduces about 69% the risk of developing UC[9,10].

There are no data supporting psychological stress can promote the onset or relapse of UC[11]. The use of oral contraceptives is moderately associated with disease onset[12]. Breastfeeding is protective against later development of UC, but only when the duration of breastfeeding is more than 3 mo[13].

UC is more common than CD. In United States and in Northern Europe, the incidence of UC ranging from 9-20 cases per 100000 persons/years and prevalence rates from 156-291 cases per 100000 people. The UC has the main peak of onset between 15 and 30 years old[14] and second peak in patients aged 50-70 years. Previous studies have shown no preference for sex[14], if not a slight prevalence in men[15].

The goals of drug treatment for UC are treating the symptoms and inducing clinical remission. The ileal-pouch anal anastomosis (IPAA) is the elective surgery treatment in about 20%-30% of UC patients that eventually underwent surgery.

## DIAGNOSIS

The diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. Employment of non-invasive biomarkers is needed. Non-invasive biomarkers have the potential to avoid invasive diagnostic tests and inhibit potential complications[16].

The ability to determine the type of UC, severity, prognosis and response to therapy, using biomarkers has long been the aim of clinical researchers[17,18]. A working group of the National Institute of Health, in 2001, defined biomarker as "a characteristic that is objectively measured and evaluated as an

**Table 1  Main biological markers in ulcerative colitis**

| Serum markers of acute phase response | | Response |
| --- | --- | --- |
| C-reactive protein | | Increased |
| Erythrocyte sedimentation rate | | Increased |
| Platelet count | | Increased |
| White blood cell count | | Increased |
| Alpha1-acid glycoprotein (Oromucoid) | | Increased |
| B2-microglobulin | | Increased |
| Sialic acid | | Increased |
| Serum amyloid A | | Increased |
| Ferritin | | Increased |
| Serum albumin | Complement system | Decreased |
| Trasferrin | Complement system | Decreased |
| C1s, C2, C3, C4, B | Complement system | Increased |
| Haptoglogin | Transport proteins | Increased |
| Haemopexin | Transport proteins | Increased |
| Caeruloplasmin | Transport proteins | Increased |
| Alpha1 Antitrypsin | Proteinase inhibitors | Increased |
| Alpha 1 antichymotrypsin | Proteinase inhibitors | Increased |
| Fibrinogen | Coagulation and fibrinolytic proteins | Increased |
| Prothrombin | Coagulation and fibrinolytic proteins | Increased |
| Plasminogen | Coagulation and fibrinolytic proteins | Increased |
| Factor XII | Coagulation and fibrinolytic proteins | Decreased |
| IL-6, IL-1 β- TNF-α, IL-8, IL-10, Interferon-β | Cytokines | Increased |
| Serologic markers/antibodies | | Positive rate |
| ANCAs | Anti-neutrophil cytoplasmic antibodies (cANCA, sANCA, pANCA) | 2%-28% CD 20%-85% UC |
| ASCA | Anti-saccharomyces cerevisiae antibodies | 39%-69% CD 5%-15% UC |
| Anti-OmpC | Antibodies to outer membrane porin | 24%-50% CD 5%-11% UC |
| Anti-Cbir1 | Flagellin related antigen | 50% CD 5%-11% UC |
| Anti-I2 | Pseudomonas flourescens-associated sequence I-2 | 30%-50% CD 2%-10% UC |
| Flagellin A4-Fla2 and Fla-X antibodies | Newly identified | About 57% CD |
| Antilaminaribioside carbohydrate IgG (ALCA) | Antiglycan antibody | 17%-28% CD 4%-7% UC |
| Antichitobioside carbohydrate IgA (ACCA) | Antiglycan antibody | 20%-25% CD 5%-15% UC |
| Anti-synthetic mannoside antibodies (ASMA or AMCA) | Antiglycan antibody | 28% CD 18% UC |
| Pancreatic antibodies | Pancreatic secretion | 30%-40% CD 2%-6% UC |
| Serum p53 antibodies | | 9.3% UC |

CD: Crohn's disease; UC: Ulcerative colitis; IL: Interleukin.

indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[19]. A good biomarker must be accurate, reproducible, standardized, easy to be interpreted by clinicians and with a high diagnostic sensitivity and specificity. Unfortunately, no single marker has all these features.

First we consider biomarkers assessed in UC, with special reference to acute-phase proteins and serologic markers. Thereafter, we consider new biomarkers and which biological markers should be developed in the future. The main biomarkers in UC are the acute-phase proteins and serologic markers (Table 1).

## SERUM MARKERS OF ACUTE PHASE RESPONSE

The laboratory tests most used to measure the acute-phase proteins in clinical practice are the serum concentration of C-reactive protein (CRP) and the erythrocyte sedimentation rate. Other biomarkers of acute phase response in UC include platelet and leukocyte count, serum albumin, and orosomucoid concentrations.

### CRP
CRP is an acute phase protein produced by the liver in response to various acute and chronic inflammatory

conditions. CRP is produced mainly by hepatocytes in response to circulating interleukin-6 (IL-6), and to a lesser extent in response to IL-1β and TNF-α[20].

CRP levels range from 5 to 200 mg/L. During the onset of inflammatory response an increasingly number of hepatocytes are recruited to its synthesis. This recruitment is extremely rapid. The decrease of CRP concentration may be similarly rapid, with a decrease from peak with a half time of 48 h[21].

In literature are reported significant differences in the CRP response between CD and UC. In CD patients has been described a clear increase in CRP, whereas in UC the response is slight or absent[22,23]. There is no satisfactory explanation for these differences. Nevertheless, in literature is reported that serum IL-6 concentrations were significantly increased in patients with CD compared with UC and healthy controls[24]. Another interpretation could be that the inflammation in UC is confined to the mucosa while in CD the inflammation is transmural, but not enough to explain all the differences.

### Erythrocyte sedimentation rate

The Erythrocyte sedimentation rate (ESR) determination reflects the changes in the various acute phase proteins. Although the usefulness of this test has decreased, it is still widely used. The test measures the distance that erythrocytes have fallen after one hour in a vertical column of non-coagulated blood under the influence of gravity[25].

ESR varies with plasma protein concentrations and haematocrit value, and in IBD provides a simple and rapid assessment of the plasma protein alterations of the acute phase response.

Thus, the ESR is greatly influenced by the size, shape, and number of erythrocytes as well as by other factors, including age, gender, anaemia, blood dyscrasias and pregnancy[26].

The ESR determination monitors satisfactorily the acute-phase response of disease after the first 24 h. In contrast, during the first 24 h the CRP is a better indicator of the acute phase.

The ESR, compared with CRP, reaches the highest point less quickly, decreases more slowly and has a lesser degree of change.

### Platelets

The platelets also play an active role in several inflammatory processes[27]. The high platelet number correlates well with disease severity, and, interestingly, may persist even after bowel resection in IBD patients. Mean platelet volume has been proposed as a potential marker of clinical disease activity, being inversely proportional to the levels of CRP and ESR. The cause of the reduction in platelet volume in clinically active UC is unknown, but it may be a direct result of the thrombopoiesis disorder often observed in the early phases of systemic inflammatory

progression[28]. The platelets also relates to the increased incidence of thromboembolic phenomena in CD and UC. Some studies reported that spontaneous platelet aggregation is observed in more than 30% of IBD patients[29].

### Other serum laboratory markers

The number of white blood cells increases during the acute phase response and is also influenced by the drugs utilized in IBD, such as glucocorticoids (increased) or azathioprine and 6-mercaptopurine (decreased).

Albumin is a negative acute phase marker and decreased levels may be found during inflammation. Alpha 1-acid glycoprotein or orosomucoid is another hepatocyte derived acute phase protein related with IBD activity[30], but the long half-life (5 d) reduced its usefulness.

Other acute phase markers include sialic acid, fibrinogen, lactoferrin, β2-microglobulin, serum amyloid A, alpha 2-globulin, and alpha 1-antitrypsin. Most of these markers have not been extensively studied in IBD and the authors describe opposing results.

### Cytokines

The cytokines are intercellular signalling polypeptides produced by activated cells. The cytokines produced during inflammatory processes are the chief stimulators of the production of acute-phase proteins. The inflammation-associated cytokines include IL-6, IL-1β, TNF-α, IFN-β, TGF-β, IL-8 and possibly IL-10[25].

For UC, the cellular events are less clear, but natural killer T cells may play an important role as initiating cells. The proinflammatory cytokines include TNF-α, IL-12, IL-23, perhaps IL-17 and IFN-β[31].

In the intestinal mucosa from UC patients the expression of proinflammatory cytokines was significantly increased. Future investigations will clarify the significance of impairments of cytokine network for the beginning and UC progression. Serum cytokines assessment has not correlated well with clinical activity.

## SEROLOGIC MARKERS/ANTIBODIES

In previous years, serological and immunologic markers have been used in clinical practice. In UC, the presence of these antibodies can aid as alternate markers for the aberrant host immune response. New markers directed against microbial antigens have recently emerged (Table 1).

### Anti-neutrophil cytoplasmic antibodies

Serologic anti-neutrophil cytoplasmic antibodies (ANCAs) are assessed by indirect immunofluorescence (IIF) and are detectable showed three main staining patterns: the cytoplasmic (cANCA), the speckled

(sANCA) and the perinuclear (pANCA).

ANCAs are classically associated with vasculitis, in which ANCA serum levels are used for diagnostic, monitoring, and prognostic aims. In addition, ANCAs are found in other chronic inflammatory disorders, such as rheumatoid arthritis and in UC[32]. While ANCA values ranges from 2%-28% in CD patients, 20%-85% of UC patients are positive for ANCA, resulting a sensitivity of 56% and a specificity of 89% in UC patients[33,34].

### Anti-Saccharomyces cerevisiae antibodies

Anti-Saccharomyces cerevisiae antibodies (ASCA) are found in 39%-69% of CD patients and in 5%-15% of UC patients[35]. It is interesting to note that ASCA IgA sensitivity is lower in Japanese and Chinese CD patients than Caucasian CD patients[33], suggesting that the ASCA response may be influenced by several distinct genetic determinants and/or environmental risk factors.

### Antibodies to outer membrane porin, Flagellin, Pseudomonas flourescens-associated sequence I-2 and antibodies to Flagellin A4-Fla2 and Fla-X

Antibodies to outer membrane porin (Anti-OmpC) are a major outer-membrane protein isolated first from Escherichia coli. The positivity of anti-OmpC was very low in UC patients and in healthy subjects (5%-11% and 5%, respectively). Anti-OmpC may aid diagnosis of ASCA negative CD patients[36].

Antibodies to Cbir1 Flagellin (Anti-Cbir1) are positive in about 50% of CD patients, in contrast lower positivity was observed in UC patients (5%-11%), other inflammatory gastrointestinal diseases (14%) and control subjects (8%). The positivity of anti-CBir1 antibodies is greater in patients with increased antibody reactivity to ASCA, I2 and OmpC, without correlation between the level of response to CBir1 and the other antibodies[33]. Serum responses to CBir1 aid the differentiation between atypical p-ANCA positive CD and UC patients independently of ASCA[37].

Pseudomonas fluotescens-associated sequence I-2 (Anti-I2) has been studied and appears to be associated with CD[38]. IgA positivity against I2 has been reported in 30%-50% in CD, 2%-10% in UC[39], 36%-42% in indeterminate colitis, 19% of patients with other inflammatory gastrointestinal diseases and 4%-8% of healthy controls.

Flagellins A4-Fla2 and Fla-X have recently been found in CD patients. Flagellins A4-Fla2 and Fla-X positivity is more prevalent in subjects with post-infectious irritable bowel disease.

### Anti-carbohydrate antibodies: Antilaminaribioside carbohydrate IgG, antichitobioside carbohydrate IgA, anti-synthetic mannoside antibodies

Antilaminaribioside carbohydrate IgG (ALCA), anti-chitobioside carbohydrate IgA (ACCA), and anti-synthetic mannoside antibodies (ASMA or AMCA) are new antiglycan antibodies. ALCA and ACCA are found respectively in 17%-28% and 20%-25% of CD patients. These antibodies may improve the sensitivity of diagnostic test since they are positive in 34%-44% of ASCA-negative patients[40,41].

ASMA are antibodies against two major oligo-mannose epitopes that were positive in 28% of ASCA-negative CD patients. Anti-C, anti-chitin carbohydrate, anti-L, and anti-laminarin carbohydrate antibodies have a low sensitivity but moderately high specificity in CD patients, related to UC patients[42,43].

### Pancreatic antibodies

Antibodies directed against exocrine pancreas (PAbs) have been reported in patients affected with CD with a low prevalence (30%-40%)[44,45]. Anti-pancreatic antibodies have been detected by IIF in UC patients (2%-6%) and in healthy subjects (0%-2%)[36,46].

### Serum p53 antibodies

During the tumor progression, the genetic alterations are a key feature of malignant cells. The *p53* gene is frequently mutated in human cancers. Cellular accumulation of mutated p53 protein can initiate an immune response with generation of circulating anti-p53 antibodies. Patients with UC have an increased risk of developing colorectal cancer, among the different genes involved in carcinogenesis, *p53* may play a key role. Serum p53Ab were detectable in 9.3% of patients with UC. Serum p53Abs assessment could be used as a complementary test to improve surveillance program performance[47].

## USEFULNESS OF SERUM MARKERS AND ANTIBODIES IN UC

### Diagnostic and differential diagnostic value

Previous studies have valued the usefulness of routine laboratory testing in UC. CRP is a helpful index of UC activity, but its utility, as a screening test has not been totally evaluated.

CRP is the most sensitive compared to other serologic markers of inflammation in adult population for detecting IBD. The sensitivity of CRP ranges from 70%-100% in the differential diagnosis between CD versus irritable bowel syndrome and from 50%-60% in UC[32]. In high percentage of paediatric patients, the sensitivity of routine testing (anemia, ESR, CRP or platelet count), varies from 62%-91% when evaluating the combination of $\geqslant$ 2 routine laboratory tests, whereas specificity ranged from 75%-94%.

Levels of CRP are higher in active CD than in UC and this difference might be used to differentiate between CD and UC. The measurements of circulating levels of CRP, ESR, platelets count are not useful at all for differentiation between both types of IBD[48].

Orosomucoid is not useful test for screening healthy populations or differentiating patients with inflammatory *vs* functional disorders.

The clinical usefulness of pANCA or ASCA testing in patients with non-specific gastrointestinal symptoms is limited, because of the low sensitivity. Assaying all the serum markers available for CD, the sensitivity for the diagnosis of CD is greater than 80% and the positive predictive value is over 90% but only when the prevalence of CD is > 38%[49].

Serum evaluation of ANCAs and ASCAs could help patients with indeterminate colitis. In these patients, early diagnosis could positively influence treatment decisions and prognosis[50]. Patients pANCA-positive and ASCA-negative have a 19 times greater likelihood of developing UC, while patients ASCA-positive and pANCA-negative are 16 times more likely to suffer CD[51].

### Prediction of relapse

IBD is characterized by acute episodes followed by remissions. The management of patients at high risk of relapse may be improved with early treatment. Previous studies about CD have investigated a panel of acute phase markers. Recently, a prospective study measured every six weeks, after recent weaning of steroids, some laboratory parameters (full blood count, CRP, ESR, a1 antitrypsin, orosomucoid) in CD patients[52]. The best predictor of short-term relapse is the combination of CRP and ESR.

Patients with CRP > 20 mg/L and ESR > 15 mm had an eightfold increased risk of relapse with a negative predictive value of 97%, suggesting that normal CRP and ESR could almost exclude relapse in the next six weeks. In another study in which the patients were followed until relapse, the ESR, globulin and alpha-1-glycoprotein have been described like the best markers for discriminate relapse from non-relapse.

It is clear that alone CRP cannot predict clinical relapse in IBD. ESR, CRP, IL-1b, IL-6 and IL-15 are not predictive of clinical recurrence in UC[53]. In CD, serum IL-6 and soluble IL-2 receptor have been associated with a higher risk of relapse.

### Association with clinical phenotypes and prognostic indicators

In UC, very few studies have assessed acute phase markers in predicting outcome of disease or association with clinical phenotypes. In severe UC, after three days of intensive treatment (hydrocortisone and/or cyclosporine) patients with frequent stools (> 8/d), or 3-8 stools/d and CRP > 45 mg/L should be identified, as most of them will need to undergo colectomy. It is commonly accepted that the presence of ANCA in UC is not related to the duration and age of onset[54]. Previous studies agree that in patients affected with CD, the presence of atypical pANCAs in serum characterizes an UC-like clinical phenotype[44].

### Follow-up and response to treatment

CRP level is a good predictor of remission and response to treatment. Anti-inflammatory or immunosuppressive drugs do not affect CRP production. Therefore, changes of CRP concentrations during treatment occur only as a result of the effect of the drug on the inflammation or disorder.

The serological markers are not useful for follow-up of disease activity. In UC the presence of atypical pANCAs have been associated with resistance to treatment of left sided UC and early surgery. These data suggest that pANCA-positive UC patients may require earlier intervention with immunomodulators. In patients affected with UC pANCA-positive and ASCA-negative at first infliximab infusion have been associated with a suboptimal early clinical response[55]. In patients ASCA, anti-OmpC and anti-I2 positive was observed a better response to antibiotic therapy, compared with negative patients[56].

## FUTURE BIOMARKERS

The development of biomarkers in UC will be very important in the future. The progress of molecular biology tools (microarrays, proteomics and nano-technology) has revolutionized the field of biomarker discovery[57]. The advances in bioinformatics associated with interdisciplinary collaborations have greatly improved the ability to collect, characterize and analyse large amounts of data.

The techniques available for biomarkers development are genomics [Single nucleotide polymorphism (SNP) genotyping, pharmacogenetics and gene expression analyses] and proteomics.

### Metabolome biomarkers

The search for metabolic biomarkers in CD as evidence of microbial functions in the gut is a new and interesting diagnostic approach[58].

Recent studies described the role of Enzyme indoleamine 2,3 dioxygenase (IDO1) in gut inflammation and IBD. IDO1 is an enzyme that degrades the essential amino acid tryptophan. The concept that cells expressing IDO can suppress T-cell responses and promote tolerance is a relatively new paradigm in immunology[59]. IDO1 is significantly increased in the intestinal inflammation[60]. IDO induction might therefore contribute to a T-cell-mediated negative feedback-loop by inhibition of their further activation. Thus, some papers hypothesized a potential anti-inflammatory role of IDO in IBD, especially in CD, which is characterized by an exaggerated Th1-cell response[61].

L-arginine (L-Arg) serum levels are increased in patients with severe UC and *L*-Arg serum levels were highly correlated with disease activity index[62]. Metabolomics array studies have also identified high levels of *L*-Arg in colonic mucosal specimens of UC patients[63,64].

### Gene expression profiling

In the recent years there have been significant advances in understanding the genetics of UC. Gene expression profiling is considered a predictive marker in IBD.

Several studies explore the associations of UC with gene polymorphisms (*i.e.,* vitamin D receptor, interleukin, interleukin receptor gene and OCT-1). Genetic polymorphisms of *VDR* are significantly correlated with UC. Mutation of *VDR* is a protective factor for UC. Moreover, mutant genotype (TC/CC) of *VDR* and vitamin D deficiency may exert synergistic effects on the susceptibility to UC[65].

The cytokine network is highly complex with interactive cascades of gene activation and suppression. Not only the *IL* and *ILR* gene polymorphisms are in relation with UC pathogenesis but also the downstream signalling components of several ILs (*i.e.*, JAKs, STATs), which could be potential targets of novel treatment strategies[66].

Several SNPs in the TNF-$\alpha$ promoter region are known to affect the level of gene expression. The G → A polymorphism at position -238 in the *TNF* gene is associated with lower production of TNF-$\alpha$ in UC patients. In contrast, the -308A polymorphism is associated with enhanced TNF-$\alpha$ production in cells *in vitro* and in CD patients in *vivo*[67].

The -857 C → T SNP, located in promoter region of TNF-$\alpha$, is functional through binding to the transcription factor octamer transcription factor-1 (OCT-1). Carriers of the 857C allele show higher levels of circulating TNF-$\alpha$ and was suggested that the TNF-857C/T SNP increased the susceptibility to IBD in English population through it's effects on the interaction between the *OCT-1* gene and the NF-κB transcription factor[68].

In literature was reported in Australian subjects a possible association of TNF-$\alpha$-857 variant with an increased CD risk. In another study was described an association of TNF-857C with IBD overall and with sub-phenotypes in either UC or CD, only in patients not carrying other common mutations[69].

Genetic markers are associated with disease phenotype and long-term evolution, but their value in clinical practice is limited.

### Proteomics

The current advances in proteomic array profiling technology have sparked interest in using this technique for diagnosis of IBD.

Proteomic approaches for the identification of disease biomarkers are mainly based on the comparative analysis of protein expression in healthy and diseased tissues to identify aberrantly expressed proteins, analysis of secreted proteins (in cell lines and primary cultures) and direct serum protein profiling.

Recently has been published a study in patients with IBD, using the methodology of Surface Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometer (SELDI-TOF-MS). Four proteins of acute phase inflammation biomarkers were identified (PF4, MRP8, FIBA and Hp-$\alpha$2). PF4 and Hp-$\alpha$2 were also detected in serum by classical methods and their true diagnostic value should be confirmed. In the future, the application of protein interaction maps to intestinal cell models in IBD will produce a detailed photograph of protein dynamics regulating signalling homeostasis[17].

Furthermore, in inflammatory bowel diseases, proteomic arrays have shown promise for identify active disease, to differentiate between CD and UC and to study the pathogenesis of the diseases[17,70].

## CONCLUSION

Several reliable serum biomarkers are currently used to aid in the diagnosis of IBD, to differentiate between CD and UC, to assess disease activity and to predict relapse. However, the available serological markers are limited in their capacity as predict longer-range disease course.

Advances in genomic, proteomic, and metabolomics array are easing biomarker finding in UC.

In the future, the addition of new serological markers will add significant benefit. In the last years have been identified 163 risk loci, which include a variety of immunologic functions. IBD is a highly heterogeneous disease for the onset, course and progression of the illness. Significant differences were also reported in the response to therapies and susceptibility to therapy-related. Therefore, it is important identify predictors of the disease course, complications, probability of response to therapy and any adverse events, in order to enable a targeted therapeutic process. The genotype of an individual is constant and unchangeable, and thus could potentially play the role of important predictors of these outcomes.

Currently, the integration of serological markers with genetic markers may not be justified. However, with the increasing use of innovative methodological approaches such as genetics and proteomics, it is reasonable to expect that the aetiology of IBD could be clarified in the near future. In the future it is expected that all these biomarkers will be implemented in an integrated molecular diagnostic and prognostic approach of patients.

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