Biological markers in inflammatory bowel disease:
Practical consideration for clinicians

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Summary
The biomarkers are important in the Inflammatory Bowel Disease (IBD) to gain an objective measurement of disease activity and severity, as well as prognostic indicator and outcome of therapy. And they can be helpful to avoid invasive procedures. The ideal biomarker does not exist for IBD and it is likely that more than one biomarker will be needed. Biological markers potentially useful in IBD include acute-phase proteins, fecal markers, several antibodies and novel genetic determinants. The C-reactive protein (CRP) is the most studied and has been shown to be an objective marker of inflammation. CRP is a good marker of measuring disease activity in Crohn’s disease (CD) and its levels can be used to guide therapy. The fecal markers (calprotectin and lactoferrin) may be helpful in differentiating patients with IBD from those with functional disorders and to predict clinical relapse. The panel of serologic markers (anti-\textit{Saccharomyces cerevisiae} antibody, perinuclear anti-neutrophil cytoplasmic antibody, anti-OmpC and anti-I2 and antiglycan antibodies) for IBD can be used to stratify IBD patients into more homogeneous subgroups with respect to disease progression. Correlating serologic markers with genotypes and clinical phenotypes should enhance our understanding of the pathophysiology of IBD. The development of biomarkers in IBD will be very important in the future with the increasing utilization of novel methodological approaches like genomics and proteomics.

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Résumé
Le rôle des marqueurs biologiques est important dans les maladies inflammatoires chroniques intestinales (MICI), à la fois pour essayer de mesurer objectivement l’activité et de la sévérité de la maladie, comme indicateurs pronostiques, ou encore pour évaluer...

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la réponse au traitement. Ils sont aussi susceptibles d’éviter de réaliser des examens complémentaires invasifs. Dans les MICI, le biomarqueur idéal n’existe pas, et plusieurs marqueurs utilisés au même moment sont souvent nécessaires. Les marqueurs potentiellement utiles sont les protéines inflammatoires, des marqueurs fécaux, certains anticorps (sérologies), ainsi éventuellement que de nouveaux marqueurs génétiques. La protéine C-réactive (CRP) a été la plus étudiée. C’est un marqueur objectif de l’état inflammatoire, corrélé à l’activité de la maladie dans la maladie de Crohn, et aidant parfois à guider son traitement. Les marqueurs fécaux (calprotectine et lactoferrine) peuvent être utilisés pour différencier les MICI de maladies ayant un trouble fonctionnel intestinal, et sont susceptibles de prédire le risque de rechute. L’éventail des sérologies disponibles (anticorps anti-Saccharomyces cerevisiae, anti-cytoplasme des polynucléaires neutrophiles de localisation péri-nucléaire, anti-OmpC, anti-I2, et anti-glycanes) peuvent être utilisés pour essayer de mieux stratifier les MICI dans des sous-groupes plus homogènes concernant leur profil évolutif. Leur corrélation avec les caractéristiques génotypiques et le phénomène clinique des maladies devrait améliorer la compréhension de la physiopathologie des MICI. Il est enfin possible que l’utilisation croissante à l’aide d’outils de génomique et/ou de protéomique fonctionnelle de plus en plus performants, permette l’identification de nouveau biomarqueurs d’intérêt dans les MICI.

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The assessment of patients with inflammatory bowel disease (IBD) is often complex, requiring a combination of clinical, laboratory and endoscopic, histopathological, or radiologic techniques. The diagnosis of IBD — particularly Crohn’s disease (CD) — is often missed or delayed because of the non-specific nature of both the intestinal and extraintestinal symptoms at presentation. The implementation of the diagnostic strategy using noninvasive biomarkers is necessary. These biomarkers have the potential to avoid unnecessary invasive diagnostic evaluations that may result in discomfort and potential complications [1]. The ability to determine the type, severity, prognosis and response to therapy of IBD, using biomarkers has long been a goal of clinical researchers [2-4]. The main biomarkers in IBD are the acute-phase proteins, fecal and serologic markers (Table 1). The presence of active gut inflammation in IBD patients is associated with an acute phase reaction and migration of leucocytes to the gut, and this is translated into the production of several proteins, which may be detected in serum such as C-reactive protein (CRP) or stool such as fecal calprotectin [5-7]. The use of serologic markers is based on the hypothesis that the IBD results from an aberrant immune response and loss of tolerance to the normal intestinal flora. This idea is supported by the occurrence of antibodies directed against microbial antigens such as antineutrophil cytoplasmic antibodies (ANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) and by the identification for Nucleotide oligomerisation domain 2/Caspase Recruitment Domain 15 (NOD2/CARD15) as a gene conferring susceptibility to CD [8]. CARD15 plays a role in the recognition of bacterial structures and in the subsequent defense against these bacteria.

### What is a biological marker?

In 2001, an National Institute of Health (NIH) working group standardized the definition of a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” and defined types of biomarkers. Type 0 biomarker is a marker of the natural history of disease and correlates longitudinally with known clinical indices and type I biomarkers capture the effects of a therapeutic intervention in accordance with its mechanism of action [9].

A biomarker will be of clinical value only if it is accurate, it is reproducibly obtained in a standardized fashion, it is acceptable to the patient, it is easy to interpret by clinicians, it has high sensitivity and high specificity for the outcome it is expected to identify, it explains a reasonable proportion of the outcome independent of established predictors consistently in multiple studies, and there are data to suggest that knowledge of biomarker levels changes management [10].
If the ideal marker exists for IBD, it would greatly facilitate the work of the gastroenterologist or surgeon treating these patients. Unfortunately, no single marker has proven to possess all the qualities although some interesting markers have been identified. In this overview, we will first briefly discuss biomarkers studied in IBD, with special reference to acute-phase proteins, fecal and serologic markers. Thereafter, the use of these markers in IBD in diagnosis and differential diagnosis, categorizing disease severity, predicting future disease course, including recurrence and response to therapy and monitoring efficacy of therapy and screening for subclinical disease will be discussed. Finally, we will discuss how one finds new biological markers. And which biological markers should be developed in the future.

Table 1  Biological markers in Inflammatory Bowel Disease (IBD).

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<thead>
<tr>
<th>Marqueurs biologiques dans les maladies inflammatoires chroniques intestinales.</th>
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<tr>
<td>Acute-phase proteins</td>
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<td>C-reactive protein (CRP)</td>
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<td>Serum orosomucoid concentrations or glycoprotein</td>
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<td>Fibrinogen</td>
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<td>Serum amyloid A</td>
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<td>α2 globulin</td>
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<td>α1 antitrypsin</td>
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<td>Lactoferrin</td>
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<tr>
<td>Cytokines: IL-6, IL-1β, TNF-α, interferon γ, transforming growth factor TGF β,IL-8, IL-10</td>
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<tr>
<td>Faecal markers (neutrophil activation)</td>
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<td>Calprotectin</td>
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<tr>
<td>Fecal lactoferrin,</td>
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<td>Lysozyme</td>
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<tr>
<td>Elastase</td>
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<td>Myeloperoxidase</td>
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Serologic markers

pANCA (perinuclear Antineutrophil Cytoplasmic Antibody); ASCA (Anti-Saccharomyces cerevisiae antibody); Anti-OMP (Antibody to Outer Membrane Porin); Anti I2; Antibodies to P. fluorescens-associated sequence I-2; Pancreatic antibodies; Cbri1: Flagellin; ALCA, ACCA, AMCA: Anti-Carbohydrate Antibodies; Transforming Growth Factor=TGF; Interleukin=IL; Tumor Necrosis Factor=TNF.

C-reactive protein

CRP is an acute phase protein that increases during inflammation and is an important component of the innate immune system. It functions by activating the classical pathway of complement and by opsonizing apoptotic cells through Fc gamma receptors [15, 16]. 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 tumor necrosis factor α (TNF-α) [17]. CRP is synthesised in the liver, and during the onset of an inflammatory response a progressively greater number of hepatocytes are recruited to its synthesis [18]. This recruitment has been shown to be extremely rapid. Within 24-48 hours the increase may be 500 to 1,000 fold higher than under basal circumstances. The reduction in the plasma CRP concentration as the acute phase response subsides may be similarly rapid, with a fall from peak with a half time of 48 hours [18]. The biological half life of the circulating protein itself is short (19 hours) [18] that is independent of any physiological or pathophysiological circumstances or of the concentration of CRP in the serum. Therefore, the synthesis rate of CRP by the liver is the only factor determining the plasma CRP concentration. Consequently, only liver failure or therapies affecting the acute phase stimulus may decrease CRP [14]. Recent studies have suggested that polymorphisms in the CRP gene account for interindividual differences in baseline CRP production in humans [19]. The short half-life of CRP makes CRP a very valuable marker to detect and follow-up inflammation, and this in contrast to other acute phase proteins.

Acute-phase proteins

An acute-phase protein has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25 percent during inflammatory disorders [11, 12]. Although the concentrations of multiple components of the acute-phase response commonly increase together, not all of them increase uniformly in all patients with the same illness. These variations, which indicate that the components of the acute-phase response are individually regulated, may be explained in part by differences in the patterns of production of specific cytokines or their modulators in different pathophysiologic states [11]. 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 (ESR). They are potential laboratory surrogate markers (biomarkers) for disease activity and are associated with endoscopic inflammation, severely active histologic inflammation in CD and ulcerative colitis (UC) [4, 6, 13, 14]. Other biomarkers of inflammation in IBD include platelet count, leukocyte count, serum albumin, and serum orosomucoid concentrations.
Erythrocyte Sedimentation Rate (ESR)

The ESR determination is a commonly performed laboratory test and reflects the changes in the various acute phase proteins. However, the usefulness of this test has decreased as new methods of evaluating disease have been developed, although it is still widely used. The test measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity [11]. ERS varies with plasma protein concentrations and the hematocrit value, and in IBD provides a crude and rapid assessment of the plasma protein alterations of the acute phase response. Thus, in general, the ESR is an indirect measurement of plasma acute phase protein concentrations, and is greatly influenced by the size, shape, and number of erythrocytes as well as by other factors, which are detailed in Table 2 [20, 21].

Repeatedly, the ESR determinations have been shown to be satisfactory monitors of acute-phase response to disease after the first 24 hours [22]. During the first 24 hours in an inflammatory process, C-reactive protein is a better indicator of the acute phase. Compared with CRP, ESR will peak much less rapidly and may also take several days to decrease, even if the clinical condition of the patient or the inflammation is ameliorated [5]. However, CRP tests are more expensive, less widely available and more time-consuming to perform than the ESR [23].

Platelets

During the last decade it has become evident that, in addition to their primary hemostatic function, platelets also play an active role in a variety of inflammatory processes [24, 25].

The high platelet number correlates well with disease severity, and, interestingly, may persist even after bowel resection in IBD patients [26]. The reason for the increased number of platelets in the circulation of IBD patients is not well understood, but is usually considered to be a non-specific response to inflammation similar to what in other chronic inflammatory diseases. Based on these observations, platelet count has been proposed as a simple method to distinguish IBD from infectious diarrhea [24, 27].

Mean platelet volume has also been proposed as a potential marker of clinical disease activity, being inversely proportional to the levels of classical inflammatory markers such as CRP and ESR. The cause of the reduced platelet volume in clinically active IBD is unknown, but it may be a direct consequence of the thrombopoiesis disturbance often observed in the early stages of systemic inflammatory processes [28].

The importance of platelets also relates to the increased incidence of thromboembolic phenomena in CD and UC. Studies have demonstrated that spontaneous platelet aggregation is present in more than 30% of IBD patients compared to none of controls, and independent of disease severity [24, 29]. But these parameters are not widely used in clinical practice.

Other acute phase reactants include α1-acid glycoprotein or orosomucoid, fibrinogen, lactoferrin, α2 microglobulin, serum amyloid A, β2-globulin, and α1-antitrypsin. Most of these markers have not been studied widely in IBD and many have shown conflicting results [14].

Emerging phase acute reactants

The cytokines are intercellular signalins polypeptides produced by activated cells. The cytokines that are produced during and participate in inflammatory processes are the chief stimulators of the production of acute-phase proteins. These inflammation-associated cytokines include IL-6, IL-1β, TNF-α, interferon γ, transforming growth factor β, and IL-8, and possibly IL-10 [11]. The expression of proinflammatory cytokines in the intestinal mucosa from IBD patients is markedly enhanced, although not always accompanied by increased concentrations of cytokines in the circulation. Future investigations will clarify the significance of impairments of the cytokine network for the initiation and progression of IBD [30].

Fecal markers

Fecal markers include a heterogeneous group of substances that either leak from or are generated by the inflamed intestinal mucosa. The inflamed hyper-permeable mucosa of patients with IBD is associated

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<tr>
<th>Table 2. Factors That May Influence Erythrocyte Sedimentation Rate (ESR). Facteurs susceptibles d’influencer la mesure de la vitesse de sédimentation.</th>
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<tbody>
<tr>
<td>Factors that decrease ESR</td>
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<td>Old age</td>
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<td>Female</td>
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<tr>
<td>Pregnancy</td>
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<td>Anemia</td>
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<td>Red blood cell abnormalities</td>
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<td>Technical factors</td>
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<td>Dilutional problem</td>
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<td>Increased temperature of specimen</td>
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<td>Tilted ESR tube</td>
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<td>Elevated fibrinogen level</td>
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<td>Infection</td>
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<td>Inflammation</td>
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<td>Malignancy</td>
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<td>Macroglobulinemia</td>
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<td>Dysproteinemia with hy perviscosity state</td>
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NSAIDs = nonsteroidal anti-inflammatory drugs.
with increased protein loss (protein cytokines and markers of neutrophil activation) into the bowel lumen [31-33]. Studies using radio-labelled proteins have demonstrated that there is fecal protein loss in patients with active IBD and it may therefore be a useful marker of disease activity. The fecal excretion of $^{111}$In-labelled leukocytes is considered the gold standard fecal marker of inflammation since strict correlations with intestinal inflammation were evidenced at colonoscopy and histology [32, 34]. Concerns about costs, radiation, and the need for prolonged fecal collections (4 days) all worked against radio-labelled leukocytes techniques for routine use, although many remain very important for research studies [31].

During intestinal inflammation, leukocytes infiltrate the mucosa, resulting in an increase in the concentration of neutrophil derived proteins in the faeces. Fecal analysis is unpleasant but an important number of these proteins present in stool have been studied, including calprotectin, fecal lactoferrin, M2-PK, S100A12, lysozyme, elastase, myeloperoxidase, and other protein cytokines (TNF-α, IL-1β, IL-4 and IL-10) and proteins (α1-antitripsina and α2-macroglobulin).

**Fecal calprotectin**

Calprotectin is the most used fecal protein. It is a 36 kDa calcium and zinc binding protein, probably the most promising marker for various reasons. In contrast with other neutrophil markers, calprotectin represents up to 50% of cytosolic proteins in granulocytes [32, 35, 36]. A strong positive correlation between fecal calprotectin concentration and fecal excretion of $^{111}$In-labeled neutrophils has been found in IBD patients, supporting calprotectin as an accurate measure of neutrophil migration to the gastrointestinal tract [37]. Although calprotectin is a very sensitive marker for detection of inflammation in the gastrointestinal tract, it is not a specific marker and increased levels are also found in neoplasia, microscopic colitis, active celiac disease, allergic colitis [37], infections, polyps [5, 7], with use of non-steroidal anti-inflammatory drugs, and increasing age [38].

Relatively high levels of calprotectin are found in the stools of normal individuals. This suggests that in normal individuals most circulating neutrophils migrate through the mucosal membrane of the gut wall and thereby terminate their circulating life [31, 32, 39, 40]. Subsequent lyses within the gut lumen and release of cytosolic calprotectin thereby accounts for the median fecal levels of 2.0 mg/l seen in healthy controls [32, 41]. The calprotectin is resistant to colonic bacterial degradation and it is easily measured in faeces by a commercially available ELISA.

**Fecal S100A12**

Fecal S100A12 is another marker of inflammation that has been proposed recently. It is a Ca-binding protein that previously was known as calgranulin C or EN-RAGE [42]. The S100A12 activates the nuclear factor-κB signal transduction pathway and induces proinflammatory cytokine release, including TNF-α [32]. Once bacterial enteritis is ruled out, fecal S100A12 may be an excellent noninvasive marker of disease activity of IBD superior to other biomarkers including fecal calprotectin, which is also derived from monocytes and potentially from epithelial cells making it less specific for infiltrating neutrophils [43]. Fecal S100A12 correlates to inflammation and can distinguish chronic IBD [43] from non-organic disease including irritable syndrome bowel with high sensitivity (86%) and specificity (96%). Fecal S100A12 levels fell during therapy in children entering remission with normal C-reactive protein levels [44]. This neutrophil-derived protein can significantly improve our arsenal of noninvasive biomarkers of intestinal inflammation [43].

**Fecal lactoferrin**

Lactoferrin is an iron-binding glycoprotein that is a major component of the secondary granules of neutrophils and has bactericidal properties [32, 45]. Fecal lactoferrin is easily quantified using an ELISA specific for human lactoferrin [46]. Other hematopoietic cells such as monocytes and lymphocytes do not contain lactoferrin [47]. The protein is resistant to proteolysis and unaffected by multiple freeze thaws, providing a useful marker in feces as an indicator of intestinal inflammation [47].

Other fecal markers include lysozyme, elastase, myeloperoxidase, TNF-α, α1-antitrypsin and α2-macroglobulin. Most of these markers have not been studied widely in IBD and many have shown conflicting results. Furthermore, their use in IBD has not proved superior to fecal calprotectin or lactoferrin [32]. Lysozyme is a polymorphonuclear neutrophil-derived enzyme which catalyses the hydrolysis of Gram-positive bacterial cell walls. Fecal lysozyme correlates with excretion of $^{111}$In-labeled granulocytes in patients with colonic disease but not in those with small bowel disease [31], therefore it is of limited value in CD patients. Myeloperoxidase is a constituent of neutrophil azure granules. Fecal levels are elevated in active IBD compared with controls and correlate with laboratory parameters and endoscopic grade of inflammation [48]. Granulocytes elastase was shown to correlate with the CDAI in CD and an activity index in UC [49].

**Other cytokines**

Serum measurements of cytokines have not correlated well with clinical activity or as predictors of response to biologics [50]. Although mucosal biopsies seem to offer best correlation, they are the least accessible clinically. Fecal TNF-α is a proinflammatory cytokine. It has been shown to be a useful marker of disease activity in children with IBD but it needs to be further assessed in adults [31]. The results in children are equivalent to other fecal markers such as calprotectin and the need to keep the stool frozen for risk of degradation makes this technique impractical for routine application [50]. Changes in the fecal pro-inflammatory cytokine IL-1β reflects mucosal inflammation and is significantly elevated in active IBD compared with healthy controls. The level of this cytokine falls with clinical resolution [33]. Fecal levels of the anti-inflammatory cytokines IL-4 and IL-10 remain normal during flare-ups of IBD but as clinical resolution occurs their levels increase significantly [48]. Other cytokines may eventually be used as markers of inflammatory activity [33].
Serologic markers

In the last decades serological markers have been studied extensively in immunology and have been used in clinical practice to detect specific pathologies. In IBD, the presence of these antibodies can serve as surrogate markers for the aberrant host immune response. Indeed, the two best-studied pANCA and ASCA cross-react with bacterial and fungal antigens, respectively [51]. New markers directed against microbial antigens have recently emerged, offering to the clinician an actual IBD serologic panel (Table 3).

**Anti-Neutrophil Cytoplasmic Antibodies (ANCAs)**

ANCAs are classically associated with vasculitis, especially Wegener’s granulomatosis, in which measurements of serum levels of ANCA are used for diagnostic, monitoring, and prognostic purposes. In addition, ANCA are found in other chronic inflammatory disorders, most notably in rheumatoid arthritis and in UC [5]. Indirect immunofluorescence showed 3 main staining patterns: a cytoplasmic granular (CANCa), a speckled (sANCA) pattern, and a perinuclear (pANCA or atypical ANCA), which is the most found in IBD. While the seroprevalence of ANCA ranges from 2%-28% in CD patients, 20%-85% of UC patients are seropositive for ANCA (Table 3), resulting in a sensitivity of 56% and a specificity of 89% in UC patients [52, 53].

**Anti-Saccharomyces cerevisiae Antibodies (ASCA)**

ASCA are antibodies primarily directed against a 200 kDa-phosphopeptidomannan cell wall component of the common baker’s or brewers yeast Saccharomyces (S.) cerevisiae [54, 55]. ASCA reactivity could be the result of cross-reacting antibodies to antigens found in a non-yeast organism that has not yet been identified [55, 56]. Mannose is not only found in yeast but also in mycobacteria and other microorganisms [54, 56]. In contrast with pANCA, ASCA are found in 39% to 69% of CD patients but only in 5% to 15% of UC patients [14, 57] (Table 3). Interestingly, the sensitivity of ASCA IgA is lower in Japanese and Chinese CD patients when compared to Caucasian CD patients [52, 58-61] suggesting that the ASCA response may be influenced by several distinct genetic determinants and/or environmental risk factors.

**Anti-OMPC: Antibody to Outer Membrane Porin**

OmpC is a major outer-membrane protein, originally isolated from Escherichia coli, for which an excessive secretion of antibodies has been recently reported in CD (55%) [52, 58], but in children and young adults it was only reported 24% [54, 61]. The prevalence of anti-OmpC was insignificant in UC patients and in healthy subjects (5%-11% and 5%, respectively). Anti-OmpC may be of value to aid diagnosis of ASCA negative CD patients [54]. A recent study found that OmpC was required by adherent-invasive E. coli (AIEC) E. coli in order to establish a niche in the gastrointestinal tract [62]. This AIEC E. coli has been found in CD lesions.

**Anti I2: Antibodies to P. fluorescens-associated sequence I-2**

IgA seroreactivity against I2 has been reported in 30-50% in CD [56, 63], 10% in UC [56, 58], 36-42% in indeterminate colitis, 19% of patients with other inflammatory gastrointestinal diseases and 5% of healthy controls [52, 58]. The presence of anti-I2 correlates with small bowel perforating disease.

**Pancreatic antibodies**

Antibodies against exocrine pancreas have been described in patients with CD, and have been reported to be specific at a low prevalence (30%) [8,64]. But also, the antibodies have been detected by indirect in low titers, in UC patients [8,65] and in first degree relatives of IBD patients [8]. The relevance of pancreatic antibodies is unclear.

**Cbir1: Flagellin**

Cbir1 has been identified as an immunodominant and colitogenic antigen of the enteric microbial flora in mice [52]. Cbir1...
is closely related to flagellin from Butyrivibrio, Roseburia, Thermogota, and Clostridium species, and appears in the Clostridium subphylum cluster XIVa of Gram-positive bacteria [52, 66]. Immune responses to this antigen are detected in approximately 50% of CD patients, whereas minor serum reactivity to CBir1 was observed in UC patients (6%), other inflammatory gastrointestinal diseases (14%), and control subjects (8%) [66, 67]. The frequency of anti-CBir1 antibodies has been reported to be greater in patients with an increased number of antibody reactivity to ASCA, I2, and OmpC, without correlation between the level of response to CBir1 and the other antibodies [52, 68]. Serum responses to CBir1 may be of help in differentiation between atypical p-ANCA positive CD and UC patients independently of ASCA [67].

ALCA, ACCA, AMCA: Anti-Carbohydrate Antibodies

Three novel anti-glycan antibodies were recently identified and associated with CD: anti-laminaribioside carbohydrate IgG antibodies (ALCA), anti-chitobioside carbohydrate IgA antibodies (ACCA), and anti-mannobioside carbohydrate IgG antibodies (AMCA) [69]. ALCA IgG, ACCA IgA, and AMCA IgG were specific for CD; their sensitivity was poor compared to ASCA [52, 70] (Table 3), suggesting a need for further prospective studies in IBD.

Usefulness of available biological markers (Table 4)

Diagnostic and differential diagnostic value of the biologic markers in IBD

There is currently no standardized method for evaluation of a patient with suspected IBD. A number of previous studies have evaluated the value of routine laboratory testing in IBD with varied results (Table 5). CRP is a valuable index of activity of IBD. But its usefulness as a screening test has not been fully assessed. But, CRP is the most sensitive compared to other biomarkers of inflammation in adult population for detecting IBD [5] and appears to be a useful addition in screening patients with gastro-intestinal symptoms. The sensitivity of CRP ranges between 70% and 100% in discriminating CD from irritable bowel syndrome and between 50% and 60% sensitivity in UC [5]. In high prevalence paediatric populations, the sensitivity of routine testing (anemia, ESR, CRP, or platelet count), has ranged from 62% to 91% when evaluating the combination of ≥2 routine laboratory tests (anemia, ESR, CRP, or platelet count), whereas specificity ranged from 75% to 94%. These investigations are useful markers of inflammation, anemia, nutritional deficiency, or intestinal damage. If positive, these tests are used as an indicator for further radiologic, endoscopic, and histologic evaluation for the diagnosis of IBD. As a paediatric inflammatory bowel disease screening strategy for the general paediatrician or gastroenterologist, the measurement of the combination of ESR and hemoglobin has a higher positive predictive value and is more sensitive, more specific, and less costly than commercial serologic testing [71]. Levels CRP tend to be higher in active CD than in UC [72] and this difference might be used to differentiate both types of IBD. But measurements of circulating levels of CRP, ESR, platelets count are not useful at all for differentiation between CD and UC [6].

Orosomucoid does not appear to be a useful test for screening health populations, or for distinguishing between patients with inflammatory versus functional disorders [73].

Fecal marker measurement may aid gastroenterologist in the differential diagnosis of CD, UC and irritable bowel syndrome. Their use could decrease the number of invasive and radiological investigations. Fecal marker levels are significantly higher in patient populations with IBD than in patients with irritable bowel syndrome, and they seem to be correlated with clinical and endoscopic disease activity in patients with UC and, to a lesser degree, with CD [7, 46, 74, 75]. They have excellent negative predictive value in

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<tr>
<th>Biological markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>C-reactive protein (CRP)</td>
<td>100-50%</td>
<td>100-65%</td>
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<tr>
<td>Erythrocyte sedimentation rate (ERS)</td>
<td>23-85%</td>
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<tr>
<td>Calprotectin</td>
<td>76-100%</td>
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<tr>
<td>Lactoferrin</td>
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<tr>
<td>Anti-Saccharomyces cerevisiae antibody (ANCA)</td>
<td>50-70%</td>
<td>80-85%</td>
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<tr>
<td>Perinuclear antineutrophil cytoplasmic antibody (pASCA)</td>
<td>65-70%</td>
<td>80-85%</td>
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| Table 4 | Role of biomarkers in Inflammatory Bowel Disease.  
Rôle des biomarqueurs dans les maladies inflammatoires chroniques intestinales. |
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<td><strong>Biomarkers</strong></td>
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<td>None</td>
<td>Fair-good</td>
<td>Good</td>
<td>Possibly</td>
<td></td>
</tr>
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ruling out IBD in undiagnosed, symptomatic patients. Their positive predictive value in this setting is somewhat lower but still generally better than the current laboratory marker of acute phase reactants [37]. However, the clinical application of these markers as a screening tool in pediatric and adult populations has been hindered by an apparent lack of consensus with respect to normative values and units of measurement in published reports [74, 75]. Large-scale comparative trials in pediatric patients are needed to evaluate the relative utility of these biomarkers in clinical practice [75]. Fecal markers are not useful for distinguishing infection from IBD.

Accurate diagnosis of pouchitis requires endoscopy with biopsies to identify the characteristic histopathological changes of neutrophilic infiltrates, crypt abscesses, and ulceration [37]. Because of the patchy nature of the inflammation, however, the diagnosis may be unreliable [37]. The fecal markers (lactoferrin and calprotectin) have shown promise in the non-invasive diagnosis of poucit [76, 77]. Because of their high sensitivities 100% and a specificity of 85%, these biomarkers can be used as screening tests to select patients for more invasive pouch endoscopy with biopsies.

The clinical value of pANCA or ASCA testing in patients presenting with non-specific gastrointestinal symptoms is limited because of inadequate sensitivity. Thus tests are infrequently positive in individuals who do not have IBD. With the addition the latest panel of 7 antibodies has improved the positive and negative values of serologies. Using all of the serologic markers reported for CD, the sensitivity for diagnosing CD is greater than 80% and the positive predictive value is over 90% but only when the prevalence of CD is high, 38% [78]. ANCA positivity has been observed in other colitides, such as eosinophilic and collagenous colitis. The specificity of ASCA seems to be higher, but ASCA positivity has been observed in patients with Behçet’s disease, primary biliary cirrhosis, autoimmune hepatitis, and celiac disease [79]. The cost effectiveness of serologic tests in the sequential diagnostic testing of IBD in children has been shown to avoid unnecessary and costly evaluations [1, 79], but it has not been confirmed by other studies [79-81].

Serologic evaluation of ANCs and ASCAs could be of help in patients with indeterminate colitis [52, 82, 83]. In these patients, early knowledge of the exact diagnosis could be of clinical importance with regard to therapeutic decisions and prognosis [8, 83]. Patients who are pANCA positive and ASCA negative are 19 times more likely to have UC, whereas patients who are ASCA positive and pANCA negative are 16 times more likely to have CD [51, 84]. A remarkable finding is that patients who do not have antibodies, to either ASCAs or ANCs, are remaining indeterminate colitis after a mean duration of 9.9 years [8, 83]. Further refinement of serologic tests and/or the combination of serologic testing with routine laboratory and fecal tests testing and non-invasive imaging may offer efficient cost-effective screening in the future.

**Prediction of relapse in IBD**

The natural history of the IBD is characterized by alternating flares and remissions. IBD is generally viewed as an unpredictable disease. After medically induced remission, about 50% of patients not on immunosuppressive therapy will relapse in the following year [85]. The ability to predict relapses would greatly help both physicians and patients for the following reasons. First, management of patients at high risk of relapse may be improved through early treatment that could prevent relapse or at least minimize its severity. Second, the identification of patients at high risk of relapse will improve the design of clinical trials to assess the efficacy of therapeutic regimes designed to maintain patients in remission [31]. Large prospective studies are necessary to further address this hypothesis.

A number of studies [85] in CD have investigated a panel of acute phase reactants. Recently, a prospective study in CD patients [85] with medically induced remission measured laboratory markers (full blood count, CRP, ESR, α1 antitypsin, orosomucoid) every six weeks after recent weaning off steroids given for a flare-up. The best predictor of short-term relapse was a combination of CRP and ESR. Patients with CRP (>20 mg/l) and ESR (>15 mm) had an eightfold increased risk for relapse with a negative predictive value of 97%, suggesting that normal CRP and ESR could almost certainly rule out relapse in the next six weeks. In other study where the patients were followed up until relapse, the ESR, globulin and α1-glicoprotein were best at distinguishing relapers from non-relapers [86]. Other studies [5, 87, 88], have demonstrated that a one-third of patients with clinically quiescent disease have elevated serum CRP levels suggesting that abnormalities in acute phase reactants without are frequent in asymptomatic CD patients. The likelihood of relapse after two years was higher in patients with an increased CRP compared with patients with normal CRP [5, 87]. Conversely, it has been described that the up to 10% of CD patients have persistently low CRP in the presence of CD-defined active disease. This low CRP group had a strikingly lower body mass index (BMI) than the high CRP group [15]. The strong association between low CRP and low BMI is entirely consistent with an emerging literature of an inverse association between CRP and obesity [89]. Mesenteric fat is a major site of synthesis of TNF-α [25] and IL-6 [26]. CRP is produced mainly by hepatocytes in response to circulating IL-6, and TNF-α. Despite an abnormally low BMI in this low CRP group, the majority of patients undergoing intestinal resection for their disease had clear evidence of fat-wrapping (85%). This may indicate that while BMI may influence the systemic inflammatory response (i.e. CRP), it may not significantly influence the local response to inflammation [15]. Colombel et al. [90] reporting a significant correlation between serum CRP levels and increased mesenteric fat density assessed by computed tomography enterography in patients with CD [90, 91]. It is clear that alone CRP cannot predict clinical relapse in IBD. ESR, CRP, IL-1b, IL-6, and IL-15 were not found to be predictive of clinical recurrence in UC [92]. In CD, serum IL-6 and soluble IL-2 receptor have been associated with a higher risk of relapse [93].

The fecal calprotectin and lactoferrin appear to be good predictors of relapse in patients with IBD, although their value may be higher in patients with UC than in those with CD [37, 75, 94, 95]. In clinical practice, fecal markers could be measured at intervals during follow-up (every 2-months), which may allow early detection of relapse rather than just prediction of relapses.
The serological markers are not useful for prediction of relapses, but might reflect a specific natural history of the disease described below.

Association with clinical phenotypes and prognostic indicators

In IBD, very few studies have assessed the value of the acute phase reactants in predicting outcome of disease or association with clinical phenotypes. In severe UC, after three days intensive treatment (hydrocortisone and/or cyclosporine) patients with frequent stools (>8/d), or 3-8 stools/d together raised CRP (>45 mg/l) need to be identified, as most will require colectomy on that admission [5, 87]. In CD, the patients who had persistently low CRP in the presence of active disease had significantly more pure ileal disease and a higher rate of prior intestinal resection compared to the high CRP subgroup, and there was a trend for them to evolve more stricturing disease. These associations were independent from the NOD2/CARD15 variant carriage rate [15].

It is commonly accepted that the presence of ANCA in UC is not related to the duration and age of onset [57]. It has been suggested that in patients with CD, the presence of atypical pANCA in serum characterizes a UC-like clinical phenotype [8, 96]. Patients with “UC-like CD” have endoscopically and/or histopathologically documented left-sided colitis and symptoms of left-sided colonic inflammation, clinically reflected by rectal bleeding and mucus discharge, urgency, and treatment with topical agents. pANCA in CD patients has been negatively associated with small bowel disease, fibrostenosis and small bowel surgery [14]. On the other hand, ASCAs have been associated with several CD clinical phenotypes. The strongest phenotypic association of ASCA in CD with small bowel rather than colonic disease has been confirmed [8, 68, 97, 98]. ASCAs have also been associated with strictureing as well as penetrating disease behaviour [8, 68, 97, 98] and requirement for surgery [98] and ASCAs have been negatively associated with UC-like behaviour. Like ASCA, the anti-OmpC and anti-I2 also appear to be associated with an increased risk for complications in adult [99-101] and children CD patients [99]. They are an independent risk factor for the development of the stenosing form and the need for surgical interventions [99-101]. Expression of I2 antibodies, but not the ASCA, pANCA and anti-OmpC was highly associated with clinical response to fecal diversion in CD patients [102]. Recent research has shown that the anti-CBir1 antibody is associated with ileal involvement in adult CD patients independent of other serologic markers, and it predisposes to the development of both stenosing and penetrating forms [67], but not with the need for surgery in a large independent CD patient cohort.

Quantitative, but not qualitative, response to CBir1 is also significantly associated with the CD associated NOD2 variants. And it raises the possibility that other genetic alterations variants may be associated with this antibody reactivity in patients with CD [103].

Among the anti-glycan antibodies in CD, ALCA was more often positive in the penetrating form and ACCA in the stenosing one when compared to the inflammatory type; although, the differences were small. No correlation was found between the anti-glycan positivity and the need for small-bowel resections [54, 70]. In summary for the serological markers in both adult and paediatric IBD patients, high-level immune response (ASCA, anti-OmpC and anti-I2) toward microbial antigens is associated with more severe, complicated CD phenotypes, defined by a younger age at onset of disease, a higher frequency of ileal involvement, strictures, fistulas and abdominal surgery [52, 68, 70, 79]. Previous studies have failed to show an association between ASCA, anti-I2, and anti-OmpC expression and NOD2 variants [68, 79, 98, 104, 105], but finally, recent studies have demonstrated an association between NOD2 variant status and antibodies [106-109].

Recently a multinational European and Israeli study [110] has described that CD patients positive for the NOD2/CARD15 mutation Gly908Arg and ASCA showed higher health care costs. It is possible that measurement of Gly908Arg and ASCA at disease diagnosis can forecast the expensive CD patients. To evaluate the prognostic value of serological markers in IBD, a prospective study enrolling pediatric CD patients showed that the presence and magnitude of immune responses influence disease behavior [52, 99]. More interestingly, while seronegative patients remained complication free, disease of seropositive patients progressed to internal penetrating and/or strictureing disease [99].

Previous reports suggested that the presence of ASCA may predict the development of postoperative fistulas and pouchitis [79]. Therefore, the initiation of immunosuppressive drugs, such as azathioprine or 6-mercaptopurine, for the prevention of postoperative complications might be indicated in IB patients with positive preoperative ASCA response [79]. Lastly, the utility of serologic testing in predicting postoperative complications after ileal-pouch anastomosis would also need to be clarified. In patients with a high level of pANCA before surgery there was a strong association with the development of pouchitis [79, 111], compared with patients with low pANCA or no pANCA expression. This has not been confirmed in other study [112]. These observations support the hypothesis that patients who are reactive to particular microbial antigens may respond to manipulation of bacterial flora [79].

Follow-up and response to treatment in IBD

CRP level is a good predictor of remission and response to treatment. No anti-inflammatory or immunosuppressive drug has proven to affect CRP production. Therefore, modifications of the CRP response during treatment occur only as a result of the effect of the drug on the underlying inflammation or disorder [5, 14]. A consistent observation is that patients with elevations in C-reactive protein have a better response to anti-TNF-α agents, suggesting C-reactive protein may be a surrogate marker for TNF-α dependent inflammation [113-115]. It also decreases with effective therapy of CD [113, 116]. In several studies have been suggested that CRP levels may be an important factor influencing remission and response rates in the placebo arm of clinical trials. Two recent large clinical studies [115, 117] have been investigated new biological agents for the treatment of active CD (respectively, certolizumab, a TNF monoclonal antibody fragment, and natalizumab, an alpha4 integrin monoclonal antibody) reported a large number of patients (approximately 50%) with low CRP (<10 mg/l) at entry to the trials. Furthermore, the patients
with low CRP and active CD in these trials were associated with an uncharacteristically high placebo response rate. Conversely, subgroup analyses demonstrated that the patient groups with high CRP at entry to these trials for active CD had a lower placebo response rate, which is in keeping with the previous literature, and were more likely to respond to the active treatments [115, 117]. CRP data were not given in the original infliximab trial papers [118], but low CRP correlated with the poor infliximab responder group in a Belgian study [119]. Recently, Will et al [19] performed a pooled analysis of response to placebo therapy using primary data from 13 clinical trials. In both acute disease and maintenance trials, mean CRP levels at randomization were lower in placebo responders than nonresponders [120].

The fecal markers (calprotectin [37, 121] and lactoferrin [46]) have been also demonstrated a transient decrease in their concentrations in adult [121] and paediatric patients [46] with CD after treatment with infliximab. Although small groups of patients have been studied, monitoring IBD patients by their fecal markers levels during the medical treatment will allow the physician to optimize the choice of invasive diagnostic procedures and to adjust drug dosing with the aim to tailor effective medical therapy. Clearly, however, larger prospective studies with histological confirmation of disease activity both before and after treatment are needed to evaluate the role of fecal markers in this setting.

The serological markers are not useful for follow-up of disease activity. In UC the presence of atypical P-ANCA have been associated with resistance to treatment of left-sided UC, and early surgery [8, 122]. Treatment-resistant disease was defined as a failure to respond to mesalazine or topical steroids. These data suggest that pANCA-positive UC patients may require earlier intervention with immunomodulators [51]. In UC patients a pANCA+/ASCA- serotype at first IFX infusion have been associated with a suboptimal early clinical response [123]. Individuals seropositive for sANCA had a better response to infliximab in a small series of IBD patients [52]. Larger retrospective and prospective studies demonstrated that the presence of ASCA and/or pANCA does not predict response to anti-TNF-α and 5-ASA therapies [124, 125]. Although a trend toward decreased responsiveness to infliximab with pANCA positivity was also seen especially patients who were pANCA positive and ASCA negative [123, 126]. Patients expressing ASCA, anti-OmpC, or anti-I2 were more likely to respond to antibiotics than serologically negative patients [113]. Although the results of this study do not justify assessing serology before institution of antibiotics, ultimately it may be possible to identify a subgroup of patients who are particularly sensitive to long-term antimicrobial therapy.

How to find new biological markers in IBD?

The process begins with the identification of target biomarkers with the use of standardized technology platforms, followed by validation of the assays, statistical evaluation of biomarker distributions in reference samples and in those with disease, and assessment of the correlation between biomarker levels (or expression patterns of biomarkers) and clinical measurements that define disease status [127] (Figure 1).

The ideal biomarker needs to be easy to quantify and measure in the accessible tissue or biofluid, not subject to wide variation in the general population and unaffected by co-morbid factors. Measurements are reliable, quick and reproducible at a different time or in a different centre. For evaluation of therapies the biomarker needs to change linearly with disease progression and closely correlate with established clinico-pathological parameters of the disease. And the use of biomarkers must be cost-effective. It is unlikely that any one biomarker will fulfill all these characteristics, and it is likely that more than one biomarker will be needed for early diagnosis and similarly for evaluation of disease progression for therapeutic trials [128].

The development of biomarkers in IBD will be very important in the future. First, the development of molecular biology tools (microarrays, proteomics and nanotechnology) have revolutionized the field the biomarker discovery. Second the advances in bioinformatics coupled with cross-disciplinary collaborations (clinicians, chemists, physicists, etc.) have greatly enhanced our ability to retrieve, characterize, and analyze large amounts of data generated by the technological advances. Third, there is increased recognition that diseases arise out of the dynamic dysregulation of several gene regulatory networks, proteins, and metabolic
alterations, reflecting complex perturbations (genetic and environmental) of the “system” in IBD [10, 129].

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

Genetics studies

Significant advances have been made over the last decade in the understanding of the genetics of IBD with several susceptibility loci identified through genome-wide linkage studies, association mapping and candidate gene association studies, and confirmed by multiple replication studies. The relative drought that followed the identification of NOD2 in 2001 has finished in a flood with 12 confirmed novel CD susceptibility genes and loci identified in the last 12 months and more to come [130]. The current literature supports the finding that CARD15 variants are consistently more common in Caucasian patients with CD than in healthy controls, and that a gene dosage effect exists [131, 132]. In Asians, Arabs, Africans, and African-Americans the contribution of these variants and genotype risks are either reduced or entirely absent [133]. However, even within Europe there is clearly variation in the importance of the NOD2/CARD15 allelic variants in contributing towards disease susceptibility—the importance of these variants in Northern Europe (Scandinavia, Scotland, Ireland) is less than in the index studies reported from Central Europe [131, 134].

One powerful new method for the identification of complex disease genes is genome-wide association (GWA) scanning, genotyping large panels of affected individuals and appropriately matched population controls for hundreds of thousands of polymorphic markers across the genome and using appropriately stringent statistical thresholds for significance [135, 136]. Since October 2006, 3 international consortia (NIDDK from North-America, a Belgian consortium, and the Wellcome Trust Case Control Consortium [WTCCC] from UK) have published the results of GWA scans in CD [137-140], and a German group has published a nonsynonymous (ns) SNP scan [141]. All these studies have confirmed that CARD15 variants are aberrantly expressed proteins that may represent new biomarkers, analysis of secreted proteins (in cell lines and primary cultures), and direct serum protein profiling [10]. Recently, a study has been published in IBD patients [2], in that the methodology used, was a 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 Hpα2) and two of these: PF4 and Hpα2 were also detected in sera by classical methods. Their true diagnostic value needs to be determined. In the future, the application of protein interaction maps to intestinal cell models in IBD will produce a comprehensive picture of protein dynamics regulating signalling homeostasis.

Proteomics

Proteomic approaches to the identification of disease biomarkers rely principally on the comparative analysis of protein expression in normal and disease tissues to identify aberrantly expressed proteins that may represent new biomarkers, analysis of secreted proteins (in cell lines and primary cultures), and direct serum protein profiling [10].

Gene expression

The availability of rapid, high-throughput analytical platforms has facilitated molecular phenotyping of disease states by analyzing the transcriptome. The global analysis of gene expression represents a paradigm shift from the traditional single-molecule approach to the evaluation of gene regulatory networks [10, 144].

Further issues that have been addressed with the help of different techniques and that are related to CD concern the function of NOD2/CARD15 and supporting the hypothesis that CD may result from impaired innate immunity, which later triggers exaggerated adaptive immunity.

Conclusion

It is important that IBD biomarkers be measured with highly reliable and standardized methods. This is particularly relevant when dealing with biomarkers since the accuracy of the measurement predicates the quality of clinically relevant decisions based on marker results.

The diagnosis, outcome of disease and treatment of CD and UC should remain based upon our current clinical, endoscopic, histological, radiological, and/or biochemical criteria. Initial laboratory investigations should include full blood count and CRP. The CRP is standard laboratory surrogate of the acute phase response to inflammation. In CD, CRP levels correlate well with disease activity and they can be used to guide therapy, follow up and to assess a patient’s risk of relapse. CRP levels correlate better with CD rather than UC. ESR indirectly assesses inflammation and it should only be used when CRP is not possible.
The fecal markers allow non-invasive assessment of selective cellular components of the intestinal inflammatory cascade. They may be helpful in differentiating patients with IBD from those with irritable bowel syndrome and for upcoming clinical relapse. But more studies are needed to define fully role of fecal markers in IBD.

Serological tests (ANCA, ASCA and anti-OmpC) are available commercial tests that can be helpful for CD and UC discrimination. Although, they show a quite good specificity, their sensitivity is rather low. The panel of serologic markers can be used to identify predominantly the subset of patients with disease of the ileum, who may be at increased risk of surgery. The predictive value is too low and they are therefore not recommended for broad clinical practice. In the future, the addition of new serological markers will add significant beneficial. Correlating serologic markers with genotypes and clinical phenotypes should enhance our understanding of pathophysiology of IBD. Actually, the integration of serological markers and NOD2/CARD15 genotype, or other genetic markers, could not be justified on current evidence. However, with the rapidly increasing utilization of novel methodological approaches like genetics, and proteomics, it is reasonable to anticipate that the etiopathogenesis of IBD will be unveiled in the next couple of decades and more definitive, perhaps disease-modifying, approaches will be discovered and implemented. Overall, it is anticipated that all these biomarkers in the future will be implemented in an integrated molecular diagnostic and prognostic approach of our patients.

Conflict of interest:

Maria Abreu carried out clinical trials as main investigator for Procter & Gamble. She gave advisory services to Procter & Gamble, Elan, UCB, Prometheus. She attended conferences organized by Procter & Gamble, Elan, UCB, Prometheus as contributor and as audience member. Juan Mendoza has not declared any conflict of interest.

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