Review

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Inflammatory bowel diseases: from pathogenesis to laboratory testing

Abstract: Inflammatory bowel diseases (IBDs), which comprise the two major clinical subtypes, Crohn’s disease and ulcerative colitis, incur high morbidity and potential mortality. The present study reviews data on the pathogenesis and diagnosis of IBDs. The pathogenesis depends on complex interactions between susceptibility genes, environmental factors, and innate and adaptive immunity, the understanding of which is crucial to discovering novel laboratory biomarkers. Traditional laboratory tests for the diagnosis, prognosis and assessment of disease activity of IBDs are reported on, and the biochemical properties, preanalytical and analytical aspects and clinical utility of the fecal markers lactoferrin and calprotectin are described. DNA testing and established (ASCA and pANCA) and emerging (ACCA, ALCA, AMCA, OmpC) serum markers are described; a further aspect to be addressed is the clinical use of pharmacogenetics for the treatment of IBDs.

Keywords: anti-saccharomyces cerevisiae antibody (ASCA); calprotectin; Crohn’s disease; inflammatory bowel diseases; lactoferrin; perinuclear antineutrophil cytoplasmic antibodies (pANCA); thiopurine methyl transferase; ulcerative colitis.

Inflammatory bowel diseases (IBDs) – epidemiology, genetics and pathogenesis

Inflammatory bowel diseases (IBDs) comprise a heterogeneous group of chronic inflammatory disorders of the intestinal tract, most cases having an onset during young adulthood, although about 20%–25% of patients are diagnosed during childhood [1–3]. Unlike adult IBDs, pediatric IBDs have a particularly severe clinical activity, and are predominant in males, the most frequent clinical presentation being ileocolonic inflammation in cases of Crohn’s disease (CD) and pan-colitis in ulcerative colitis (UC), the two major clinical subtypes. IBDs, one of the largest healthcare challenges in developed countries, affect over one million people in Europe, with a North-South incidence gradient [1]. While the incidence of IBDs in western Europe has progressively increased since the World War II, apparently plateauing and now affecting 0.5%–1.0% of the population, it is on the increase in areas with a low incidence (eastern and southern Europe, the Middle East, Asia, Latin America and Indian subcontinent). IBDs have higher morbidity and mortality rates than most other diseases, and incur an important risk of complications (in children 29% at diagnosis and 59% at follow-up; in adults 16% after diagnosis) [2]. Currently, pediatric IBDs are considered a distinct disease rather than simply a particularly severe and early presentation of a common form of IBDs evolving from pediatric to adult age. The exact cause of IBDs is still unknown as no effective genetic and/or biochemical IBDs markers are available, the diagnosis calling for colonoscopy and histology [1]. It is believed that the pathogenesis of IBDs depends on complex interactions between susceptibility genes, environmental factors, and innate and adaptive immunity [4–11].

In the last decade, genome-wide association (GWA) studies have been conducted to shed light on genetic loci potentially underlying susceptibility to IBDs. The majority of studies have been made on adult IBDs, but two large studies have been performed on pediatric IBDs [12–18]. Overall, it has been confirmed that CD and UC share a common genetic ground, but each has distinct genetic features, the IBD5 locus (5q31-33 region) being strongly associated with UC, and the NOD2 (16q12) and major histocompatibility complex (MHC) (6p21) locus with CD [19, 20]. Furthermore, adult and pediatric IBDs have some susceptibility loci in common but each also has distinct genetic markers. Imielinski et al. [14, 16], who recently conducted the largest GWA study among children, validated 31/49 known adult
loci and found five new loci associated with early-onset IBDs. Several genetic polymorphisms associated with IBDs depend on proteins entering the inflammasome multiprotein complexes formation, with the consequent activation of the cysteine protease caspase-1 and the processing and activation of pro-IL1β, pro-IL18 and pro-IL33 [21]. This complex is formed by a nucleotide binding domain leucine rich repeat containing receptors (NLRs), the adaptor molecule apoptosis associated speck-like protein (ASC) containing the caspase recruitment domain (CARD) and pro-caspase-1 (Figure 1). The specific name of inflammasome (i.e., NLRP1 inflammasome or NLRP3 inflammasome) stems from the individual NLR number, which might be one of the 22 known molecules included in the complex [21]. NOD2 may also be required to activate NLRP1 inflammasome. Three NOD2 polymorphisms [rs2066844 (R702W), rs2066845 (G908R), rs5743293 (L1007fsinsC)], leading to loss of function, may be associated with CD [6], while NLRP1 polymorphism rs12150220 (Leu155His) has been associated with resistance to steroid treatment in young IBDs patients [22]. The above data indicate that NLRP1 inflammasome is implicated in IBDs, but epidemiological and experimental data suggest that NLRP3 inflammasome is also involved [23]. Decreased NLRP3 expression and IL1β production, associated with the rs6672995 and rs4353135 risk alleles, has been linked to increased CD susceptibility [24]; as demonstrated in an experimental animal model, this decrease in NLRP3 expression enhances susceptibility to dextran sodium sulphate induced colitis [25]. In brief, a number of genetic polymorphisms associated with an increased IBDs risk are associated with reduced inflammasome complex proteins expression, and consequently with a reduced caspase-1 activity.

The gut mucosa, an extensive surface deputed to nutrient absorption, is primarily involved in maintaining a delicate balance between host defense and uncontrolled inflammation. Immune system cells, extensively present in the gut mucosa, regulate the balance between tolerance and inflammation in response to the huge bulk of environmental and intestinal microbial antigens reaching this large tissue-environmental interface. Intraepithelial lymphocytes (IELs), the primary mediators of the innate immune response to antigens in the epithelial layer of the small intestine, constitute a unique T cell population in that they differ from circulating, spleen and lymph node T cells (MHC class II restricted CD4+ αβ and MHC class I restricted CD8+ αβ). IELs are not MHC class II restricted, mainly being made up of (>80%) CD8+ T cells, which include CD8+ ααT cell (absent in blood), and 10% of CD8-CD4- T cells (double negative T cells, rare in blood) [26]. IELs are involved in the primary response to foreign antigens (e.g., food and microbial proteins), but are also autoreactive to “self-engendered” molecules deriving from infection or cell transformation; these molecules are independent of foreign epitopes [27]. The adaptive immune response taking place in the lamina propria, which has been well characterized in celiac disease with CD4+ T cells responding to antigens in a MHC class II restricted manner [28–31], is also implicated in IBDs. Innate and adaptive immune response are mediated by dendritic cells in the intestinal mucosa which, through the release of different cytokines, may either induce tolerance or evoke a cascade of inflammatory events ultimately leading to chronic inflammation. Dendritic cells in the gut mucosa may favor tolerance by releasing high amounts of the anti-inflammatory cytokine IL10 or promote inflammation through the release of IL12. The finding that IL10 and IL12 genetic polymorphisms are associated with the risk of developing IBDs supports the hypothesis that the adaptive immune response plays a primary role in the pathogenesis of IBDs [12, 14, 19, 32]; this is further supported by the observation that several of the more recently identified genetic susceptibility genes (IL23R, IL12B, IL27, IL1R1, IL18RAP, JAK2, IL10, TNFRSF6B and STAT3) are involved in innate and adaptive immunity [16].

CD-related inflammation in the gut mucosa is characterized by a marked infiltration of CD4+ T cells secreting T helper type 1 (Th1) and Th17 cytokines, whereas in UC the local immune response is less polarized, although IL5, IL13

![NLRP1 and NLRP3 inflammasomes multiprotein complexes.](image-url)
and Th17 cytokines production may be enhanced [33]. Th17 cells and related cytokines may be involved in the expansion and accumulation of myeloid-derived suppressive cells (MDSCs), which have been described in animal models of experimental colitis, and in patients with IBDs [34]. Th17 and MDSCs appear to be implicated in the pathogenesis of autoimmune diseases, and it has been suggested that autoimmunity can also contribute to the onset of IBDs [35]. This hypothesis is supported by the observation that in sera of patients with CD pancreatic autoantibodies recognizing the major zymogen granule membrane glycoprotein 2 (GP-2) are detected in about 30% of cases [36].

However, despite the numerous genetic studies conducted so far in large patient cohorts, as much as 80% of hereditability in IBDs has yet to be clarified. The belief that environmental factors play a crucial role in IBDs expression is in line with the epidemiological observations of a rise in the incidence of these diseases proportionate to improvement in socio-economic conditions, and the observed surge of the incidence of IBDs in South Asian children migrating to Canada or the UK, mirroring that of the host country [37]. One of the most important environmental factors to be suspected is eating habit, particularly the western and “westernized” diet, which may contribute to IBDs pathogenesis through several mechanisms, including the direct effect of dietary antigens, alterations in gene expression and in the composition of the enteric flora and the consequent effects on gastrointestinal permeability [37]. An increased consumption of refined carbohydrates, high total fat and protein intake have been associated with CD and/or UC, while a high intake of dietary fibers (vegetables and fruits) has been associated with a decreased risk of IBDs [8]. Animal protein consumption has been cited as potentially involved in the pathogenesis of this disease in Europe, US and Japan [8, 37]. In only a few epidemiological studies have different sources of animal protein been investigated to ascertain the risk of IBDs; Bernstein et al. [38] found a higher risk of CD and UC in pork than in chicken meat consumers, while Sakamoto et al. [39] found an increased risk of CD, not UC, in fish consumers.

### IBDs – laboratory diagnosis

#### Fecal markers

In the presence of suggestive symptoms, blood and stool laboratory tests are integral to the overall diagnostic work-up, which includes a physical exam, clinical history, endoscopy, biopsy and imaging [1]. A complete blood count, ESR and CRP, which may indicate the presence of intestinal inflammation, are, however, unreliable indexes of disease activity in patients with confirmed IBDs. Other blood parameters, including electrolytes, ferritin, calcium, magnesium, cobalamin, liver enzymes and function tests (INR, albumin, bilirubin), may indicate malabsorption and/or intestinal protein loss. Calprotectin and lactoferrin are the most reliable available fecal markers to be used as indexes of intestinal inflammation [1, 40–44]; both proteins, produced mainly by polymorphonuclear and mononuclear inflammatory cells, are shaded in the intestinal lumen in the presence of mucosal inflammation. Fecal calprotectin/lactoferrin, considered hallmarks of neutrophilic intestinal inflammation, are invariably present in IBDs, thus explaining the high sensitivity of these markers in detecting the condition. Other organic intestinal diseases (e.g., celiac disease, diverticulosis, colorectal carcinoma) are also associated with neutrophilic intestinal inflammation, and consequently with an increased fecal calprotectin/lactoferrin output (Table 1). Therefore fecal calprotectin, with a sensitivity of 64%–95% and a specificity of 79%–93%, has been suggested as an index of organic bowel disease [43]. Nonetheless, an increased fecal level of calprotectin and/or lactoferrin leads to the identification of patients most likely to have IBDs and calls for immediate endoscopy; moreover, the use of fecal calprotectin/lactoferrin for screening allows a reduction in the number of negative findings at endoscopy in both adults and young patients with suspected IBDs [45]. In adults, the overall sensitivity of these markers in discriminating between patients with IBDs and healthy subjects is 93%, and the specificity 96%; the sensitivity is also high

<table>
<thead>
<tr>
<th>Infections</th>
<th>Drugs</th>
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<tbody>
<tr>
<td><em>Giardia lamblia</em></td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>Bacterial or viral gastroenteritis</td>
<td>Proton pump inhibitors</td>
</tr>
<tr>
<td>Helicobacter pylori gastritis</td>
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<table>
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<tr>
<th>Malignancy</th>
<th>Other gastrointestinal diseases</th>
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<tbody>
<tr>
<td>Colorectal cancer</td>
<td>Untreated food allergy</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Untreated celiac disease</td>
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<tr>
<td>Intestinal lymphoma</td>
<td>Gastro-esophageal reflux disease</td>
</tr>
<tr>
<td>Colorectal adenoma</td>
<td>Diverticular disease</td>
</tr>
<tr>
<td>Juvenile polyp</td>
<td>Protein losing enteropathy</td>
</tr>
<tr>
<td></td>
<td>Cystic fibrosis</td>
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<td></td>
<td>Microscopic colitis</td>
</tr>
</tbody>
</table>
(92%) in children and teenagers, but the specificity is less satisfactory (76%) [45, 46]. The reasons for the discrepancy in specificity between adults and children probably depends on both age-related variations and the higher prevalence among children of intestinal disorders that can lead to augmented calprotectin fecal levels. Overall, fecal calprotectin and lactoferrin, which are correlated with each other, perform equally well in diagnosing IBDs and therefore should be used as an alternative, rather than an addition, to the diagnostic flow-chart.

In patients with a confirmed diagnosis of IBDs, fecal markers are also sensitive and specific markers of disease activity. Table 2 reports the sensitivity and specificity of lactoferrin, calprotectin and of CRP in discriminating between active and inactive IBDs [47–54]. High levels of calprotectin/lactoferrin are also predictive of relapse for both UC and CD [55, 56]. The calprotectin thresholds specified by different authors for predicting relapse vary, ranging from 50 to 400 μg/g (Table 3). In their recent paper, Louis et al. [60] demonstrated that a simple algorithm based on six clinical and laboratory parameters (no previous surgical resection, male gender, Hb ≤145 g/L, leucocyte count >6×10⁹/L, hsCRP ≥5 mg/L, fecal calprotectin ≥300 μg/g) allows the stratification of CD patients into four categories according to risk of relapse.

### Lactoferrin and calprotectin: biochemical properties, pre-analytical and analytical aspects

Lactoferrin, an 80 kDa protein, stored in secondary granules of neutrophils, is released upon cell activation. Calprotectin, a 36.5 kDa hetero-trimer, consisting of one S100A8 light chain and two S100A9 heavy chains belonging to the S100 calcium binding proteins family, is present in the cytoplasm of granulocytes and accounts for about 60% of soluble proteins; it is secreted extracellularly from stimulated neutrophils and monocytes or released by cell disruption or death [44]. Both lactoferrin and calprotectin have antimicrobial properties: histidine-based zinc-binding sequences (His-X-X-X-His motif) are involved in the antimicrobial activity of calprotectin, while the amino-terminal domain (lactoferricin) liberated by pepsin

### Table 2 Sensitivity and specificity of lactoferrin, calprotectin and of C reactive protein (CRP) in discriminating between active and inactive IBDs.

<table>
<thead>
<tr>
<th>Author [ref.]</th>
<th>IBDs type</th>
<th>Lactoferrin</th>
<th>Calprotectin</th>
<th>CRP</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sens/Spec</td>
<td>Sens/Spec</td>
<td>Sens/Spec</td>
</tr>
<tr>
<td>Røseth et al. [47]</td>
<td>CD and UC</td>
<td>77%/100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solem et al. [48]</td>
<td>CD</td>
<td>77%/80%</td>
<td>81%/80%</td>
<td></td>
</tr>
<tr>
<td>D’Incà et al. [49]</td>
<td>CD</td>
<td>75%/60%</td>
<td>78%/70%</td>
<td></td>
</tr>
<tr>
<td>D’Incà et al. [49]</td>
<td>UC</td>
<td>77%/100%</td>
<td>87%/100%</td>
<td></td>
</tr>
<tr>
<td>Sipponen et al. [50]</td>
<td>CD</td>
<td>86%/93%/71%/88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schoepfer et al. [51]</td>
<td>UC</td>
<td>89%/58%</td>
<td>68%/58%</td>
<td></td>
</tr>
<tr>
<td>Schoepfer et al. [52]</td>
<td>CD</td>
<td>80%/67%</td>
<td>80%/89%</td>
<td></td>
</tr>
<tr>
<td>Sipponen et al. [53]</td>
<td>CD</td>
<td>74%/90%</td>
<td>84%/38%</td>
<td></td>
</tr>
<tr>
<td>Lobatón et al. [54]</td>
<td>UC</td>
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CD, Crohn’s disease; Sens, sensitivity; Spec, specificity; UC, ulcerative colitis.

### Table 3 Fecal calprotectin thresholds for predicting IBDs relapse rate.

<table>
<thead>
<tr>
<th>Author [ref.]</th>
<th>IBDs type</th>
<th>Cut-off, μg/g</th>
<th>Relapse rate with low calprotectin, %</th>
<th>Relapse rate with high calprotectin, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibble et al. [55]</td>
<td>UC</td>
<td>50</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>50</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Sipponen et al. [53]</td>
<td>UC+CD</td>
<td>100</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>D’Incà et al. [57]</td>
<td>UC</td>
<td>130</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Costa et al. [58]</td>
<td>UC</td>
<td>150</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>150</td>
<td>57</td>
<td>87</td>
</tr>
<tr>
<td>Walkiewicz et al. [59]</td>
<td>CD</td>
<td>400</td>
<td>11</td>
<td>56</td>
</tr>
</tbody>
</table>

CD, Crohn’s disease; UC, ulcerative colitis.
clease is involved in the antimicrobial activity of lactoferrin. Both proteins are resistant to bacterial proteolysis, their integrity being maintained in stools, where they can easily be identified; since they remain stable in feces for up to 1 week when stored at room temperature, and for months when stored at −20°C [43], it may be possible to adopt low-stringency transport conditions for fecal material, although transport in refrigerated boxes is advisable for a better overall standardization of the pre-analytical phase. Laboratory processing of the samples includes: 1) sampling and dilution; and 2) analysis. In the initial pre-analytical phase, 100 mg of feces should be collected from the fecal mass and diluted into an exact w/v ratio. The time-consuming weighting of fecal samples has now been obviated by the use of devices allowing the easy and rapid obtaining of standardized w/v fecal sample dilutions. However, the devices cause an under-recovery of calprotectin with respect to the classical manual procedure, and this appears to be particularly relevant in the case of liquid stool samples [61]. We obtained similar results for lactoferrin, when manual weighting was compared with the approach using the Schebo device; under-recovery was particularly evident when levels were below the upper normal limit. These findings suggest that each laboratory should investigate the transferability of published and/or manufacturers’ cut-off values not only to its own patients’ population but also to its own pre-analytical procedures for sample preparation. The upper normal limits for both calprotectin and lactoferrin, which have mainly been established by using manual processing of the stool samples, appear to be age-dependent: while for adults and adolescents it is estimated at 50 μg/g for calprotectin and 7–11 μg/g for lactoferrin [44, 62], in children younger than 10 years, values three to four-fold the reference cut-off are suggested [63]. Likewise, a separate reference range two-fold higher than the reference cut-off is required in patients over 60 years of age [63].

The commercially available ELISAs for calprotectin have a total assay imprecision with mean % CV values of <12% [61]. By contrast the biological variability may span from low (<10%) to very high (58%) values [64], suggesting that a single stool test result should be interpreted with great caution in clinical practice.

The complex scenario of calprotectin laboratory testing includes several rapid immunochromatographic tests [point-of-care tests (FC-POCT)]. Three types of FC-POCT (qualitative, semi-quantitative and quantitative) are available – and good correlations between the latter and ELISAs have been reported [54, 65–68]. However, it has yet to be established whether FC-POCT results call for ELISA confirmation, and the way in which quality should be monitored. Since FC-POCT is not suitable for monitoring IBDs in patients likely to have high concentrations, it is reasonable to suggest that ELISA should be used to confirm positive FC-POCT findings, although this is time-consuming and incurs additional costs. Furthermore, the set of results of each ELISA plate can be monitored by quality control of the manufacturers’ and patients’ extracts [61], but this does not apply to any FC-POCT. These considerations indicate that the uncontrolled use of FC-POCT is inadvisable and that batch ELISA testing is preferable in high volumes centralized laboratories.

**Serum markers to define type of IBDs**

Although the two main IBDs clinical subtypes, UC and CD, have distinct clinical and pathological features, IBDs remain unclassified in about 6% of pediatric and 8% of adult onset cases [2]. Serum markers of clinical utility in diagnosing IBDs and aiding the differentiation between CD and UC are: 1) IgA and IgG class antibodies anti-*Saccharomyces cerevisiae* (ASCA), which can be determined by means of enzyme immunoassays; and 2) IgG class anti-neutrophil cytoplasmic antibodies (ANCA) demonstrating atypical perinuclear staining (pANCA), which can be assessed by indirect fluorescent antibody assays. While ASCA are associated with CD, positive pANCA are more frequently encountered in UC [69–71]. The antigenic target of ASCA has been identified as the mannose residue from the phosphopeptidomannan of the *S. cerevisiae* cell wall. In making a diagnosis of CD, ASCA have a high specificity (96%–100%), but a limited sensitivity (50%–63%) [72, 73]. If CD is suspected, ASCA IgA and IgG should be measured: while about two-thirds of CD patients with ASCA IgG are also positive for ASCA IgA, one-third of these patients are IgA ASCA negative, and 0%–19% have only ASCA IgA antibodies. The prevalence of ASCA is much higher in cases of sporadic CD and family members with CD alone (63%) than in those with both CD and UC (33%). Furthermore, ASCA is detected more frequently in healthy relatives of CD patients (20%–25%), and may therefore be considered a familial and hereditary quantitative trait. pANCA are spontaneously produced by the lamina propria and mesenteric node lymphocytes with the antigenic target present on the inner side of the nuclear periphery [74]. Positive pANCA antibody detects 60%–80% of patients with UC, but will also identify 6%–20% of subjects with CD. The combined analysis of pANCA and ASCA, indicated for defining the IBDs type, raises the specificity of the test to 94.3% with a sensitivity of 51.3% in adults [69]. In the pediatric population pANCA combined with ASCA raises
sensitivity to 70.3% with no significant variation in specificity (93.4%) [69]. If IBDs patients with ASCA positive/pANCA negative findings are more likely to have CD, while those with ASCA negative/pANCA positive are more likely to have UC, double negative or double positive ASCA and pANCA results do not support one diagnosis or another. A study was conducted in 2006 to clarify this aspect, a platform with 384 new antibodies anti-glycans, the predominant components of the cell wall surface of many micro-organisms such as fungi, yeast and bacteria, being tested: those directed against laminaribioside (ALCA), chitobioside (ACCA) and mannobioside (AMCA) were found more frequently and in higher levels in CD patients than in subjects with UC and healthy controls [75]. The sensitivity and specificity of these serum markers in distinguishing between CD subjects, healthy controls and UC patients are reported in Table 4 [76]. ANCA, ALCA, ACCA and AMCA levels, increased in both CD patients and in their first degree relatives, have been found to be associated with CD-associated NOD2 mutations [77-79]; this suggests that they may be the expression of an increased stimulation of the immune system in CD predisposed subjects.

Other potential serum biomarkers of CD, with respective clinical uses, sensitivity and specificity, are reported in Table 5. Although none of them is currently recommended instead of or in addition to ASCA determination, a combination of pANCA, ASCA, anti-CBir1 and anti-OmpC were shown to predict CD and UC in individuals from a low-risk population several years before any clinical sign of the disease [80]. Moreover, it has been suggested that anti-GP2 IgG and IgA serum autoantibodies combined with ASCA are of potential clinical utility in distinguishing CD from UC [36, 81], but that they may also be helpful in distinguishing between ileocolonic and colonic CD [35].

### Intestinal permeability

Increased gastrointestinal permeability, known to play a role in the pathogenesis of IBDs, has been found in a large number of familial studies on CD patients and in their asymptomatic first-degree relatives [82-84]; it can be evaluated by sugar testing, which is based on the measurement of the urinary excretion of orally administered non-metabolized sugar probe molecules (lactulose/mannitol). Normal intestinal epithelium allows the transcellular absorption of about 10% of the monosaccharide mannitol, while only traces (<1%) of the disaccharide lactulose are absorbed through the paracellular pathway. A leaky intestinal epithelium causes increased paracellular lactulose absorption and urinary excretion with respect to mannitol, thus leading to an increased lactulose/mannitol urinary excretion ratio that, in excess of 0.025, indicates the presence of increased intestinal permeability [85].

### Genetic markers

The development of IBDs depends on complex interactions between environmental factors, genetic predisposition, and innate and adaptive immune response. A series of GWA studies identified more than 70 loci for CD and 47 for UC, each locus typically spanning about 150 kb and encompassing an average of 3–4 genes. NOD2 (16q12) and MHC loci (6p21) are associated with an increased risk of CD, while the IBD5 locus (5q31-33 region) is associated with an increased risk of UC [12-19]. The CARD15/NOD2 proteins enter the formation of inflammasomes multiprotein complexes that, upon activation, lead to the activation of the cysteine protease caspase-1 and the resultant processing and activation of pro-IL1β, pro-IL18 and pro-IL33 [21]. Although multiple variants in the gene can increase CD susceptibility, the most commonly identified mutations of NOD2 are R702W, G908R and L1007fsinsC. NOD2 mutations, found in approximately 30% of CD patients, are associated with a more severe form of the disease, early age at onset, and ileal lesions [86, 87]. Heterozygosity

<table>
<thead>
<tr>
<th>Anti-glycan antibody markers</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
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<tbody>
<tr>
<td></td>
<td>Mean (min-max)</td>
<td>Mean (min-max)</td>
</tr>
<tr>
<td>CD vs. UC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCA</td>
<td>57 (52–61)</td>
<td>88 (86–90)</td>
</tr>
<tr>
<td>ALCA</td>
<td>18 (12–27)</td>
<td>92 (85–96)</td>
</tr>
<tr>
<td>AMCA</td>
<td>24 (18–31)</td>
<td>92 (88–95)</td>
</tr>
<tr>
<td>ACCA</td>
<td>16 (11–22)</td>
<td>92 (85–96)</td>
</tr>
<tr>
<td>CD vs. controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCA</td>
<td>53 (45–61)</td>
<td>70 (86–90)</td>
</tr>
<tr>
<td>ALCA</td>
<td>19 (9–31)</td>
<td>72 (22–99)</td>
</tr>
<tr>
<td>AMCA</td>
<td>26 (18–37)</td>
<td>87 (56–97)</td>
</tr>
<tr>
<td>ACCA</td>
<td>15 (9–23)</td>
<td>81 (22–99)</td>
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increases the risk two to three-fold, whereas homozygosity is associated with a 20–40-fold higher risk of developing CD. The IBD5 locus contains immunoregulatory genes, including IL4, IL5, IL13 and the interferon regulatory factor-1 [88]. Despite the numerous efforts made to identify predisposing genes, the reported percentage for IBDs heredity, as high as 80% has yet to be explained, and further prospective studies must be undertaken before any attempt is made to translate GWA study findings to clinical practice.

**IBDs – pharmacogenetics**

The drugs employed for treating IBDs include anti-inflammatory agents (5-aminosalicylic acid, sulfasalazine, corticosteroids), immune modifiers (azathioprine and 6-mercaptopurine (6-MP), calcineurin inhibitors, methotrexate), anti-TNF agents (infliximab, adalimumab, cortolizumab), antibiotics (most commonly metronidazole and ciprofloxacin) and probiotics. Experimental agents and symptomatic therapy complete the IBDs treatment panel. Before starting thiopurines AZA or 6-MP administration, thiopurine methyl transferase (TPMT) phenotyping (enzyme levels) or genotyping is recommended in order to direct dosing thus maximizing patient safety [1, 89]. TPMT plays an important role in the metabolism of AZA and 6-MP, which it converts into inactive metabolites. Genetically determined differences in the enzymatic activity of TPMT may influence the likelihood of AZA- and 6-MP-induced myelotoxicity. More than 20 single nucleotide polymorphisms can cause a reduction in TPMT production; however, the most prevalent allele among whites associated with a reduced TPMT enzyme activity is TPMT*3A (0.035), followed by TPMT*3C (0.0042) and TPMT*2 (0.0019). To diagnose TPMT deficiency, phenotype or genotype analyses may be performed [90]. Phenotype analysis has the advantage of verifying the effects of different factors (e.g., blood transfusions, medications, alcohol, food, post-myelotoxicity), which might affect the genetically-based expected TPMT enzyme activity, but must be repeated throughout treatment and has a high inter-laboratory variability mainly due to differences in assay types (HPLC followed by enzymatic assay, tandem mass spectrometry or radiochemical assay). The sensitivity of genotype analysis, which is not influenced by exogenous factors and need not be repeated, maybe limited, but exceeds 90% when this assay method is used for detecting the major alleles (i.e., wild type TPMT*1 and the most frequent mutant alleles TPMT*2, *3A and *3C), rather than for rare mutations [91]. Treatment with AZA or 6-MT is contraindicated in homozygous TPMT mutant allele carriers, since these subjects have an extremely low TPMT enzyme activity and are therefore at a high risk of myelotoxicity [1, 89, 92]. It has not yet been demonstrated in heterozygote patients, for whom a reduced enzyme activity should be expected, whether dosage adjustments might improve the outcome of treatment or reduce myelotoxicity, although a regimen starting with a 50% standard dose reduction followed by adjustment based on the degree of myelosuppression (continued for 2–4 weeks to reach steady state) is advisable in these cases. In all cases periodic monitoring of cell blood count is mandatory for the early identification of myelotoxicity, and for the patient’s safety.

In conclusion, several genetic and biochemical laboratory testing strategies are currently available for supporting a diagnosis of IBDs, differentiating between IBDs types, predicting relapse and establishing the risk of adverse events from therapy.

### Table 5. Potential new serum biomarkers for IBDs differential diagnosis.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Antigen</th>
<th>Disease indication</th>
<th>Prevalence</th>
<th>Sens, %</th>
<th>Spec, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CBir1 IgG</td>
<td>Flagellin, NCir (Clostridium subphylum)</td>
<td>CD</td>
<td>May help differentiate CD from UC in pANCA+</td>
<td>50%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Anti-I2 IgA</td>
<td>Bacterial DNA sequence derived from Pseudomonas fluorescens</td>
<td>CD</td>
<td></td>
<td>54%</td>
<td>10%</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>Outer membrane porin, originally isolated from E. coli</td>
<td>CD</td>
<td>May identify CD &lt;15% of ASCA-</td>
<td>20%–55%</td>
<td>10%</td>
</tr>
</tbody>
</table>

CD, Crohn’s disease; HC, healthy controls; Sens, sensitivity; Spec, specificity; UC, ulcerative colitis.
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53.ARRAY


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