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Genetic and Serological Markers in Identifying Unclassified Colitis

Valerie A. Fenech, John Schembri, Pierre Ellul, Godfrey Grech and Neville Azzopardi

Abstract

In 5–15% of the patients with inflammatory bowel disease (IBD) limited to the colon, it is difficult to distinguish histologically between ulcerative and Crohn’s colitis. This is described as unclassified colitis. Distinguishing between the two is important in terms of prognosis, since patients with Crohn's disease (CD) have a higher risk of strictures and fistulae, which may predict a more severe disease course, as well as an increased risk for surgery. In addition, colectomy may be curative in ulcerative colitis patients not responding to medical therapy, while Crohn’s patients undergoing colectomy can have relapses in other areas of the bowel and, therefore, need to be followed-up. In inflammatory bowel disease, intestinal inflammation is believed to occur secondary to an altered immune response in a genetically susceptible host. Genetic and serological markers (antibodies) may have a role in identifying unclassified colitis. Anti-\textit{Saccharomyces cerevisiae} antibody (ASCA) and anti-neutrophil cytoplasmic antibodies (pANCA) have the highest sensitivity in distinguishing ulcerative from Crohn’s colitis. Nucleotide oligomerization domain 2 (NOD2) and autophagy-related 16-like 1 (ATG16L1) polymorphisms are strongly associated with Crohn’s disease, while epithelial barrier genes are significantly associated with ulcerative colitis. This chapter describes which gene polymorphisms and serological markers may be used to distinguish between ulcerative colitis and Crohn’s disease in patients with histologically unclassified colitis.

Keywords: Crohn’s disease, ulcerative colitis, unclassified colitis, serological markers, gene polymorphisms
1. Introduction

While inflammatory bowel disease (IBD) is broadly divided into ulcerative colitis (UC) and Crohn's disease (CD), there is significant overlap in clinical presentation, endoscopic appearance, and histological findings between these two disorders. In about 5–15% of the patients with inflammation limited to the colon, it is difficult to distinguish between UC and Crohn's colitis [1]. These cases of overlap, previously referred to as indeterminate colitis, are now called unclassified IBD [2].

Distinguishing between ulcerative and Crohn's colitis is important in terms of management and prognosis, since patients with CD have a higher risk of stricture and fistula formation that may predict a more severe disease course, as well as an increased risk of surgical intervention [3]. Surgery (colectomy) may be curative in UC patients not responding to medical therapy, while CD patients undergoing surgical resection of the colon can develop delayed inflammation in other areas of the bowel, therefore, needing closer follow-up.

The exact etiology of IBD is unknown and is probably multifactorial. One of the proposed mechanisms that leads to intestinal inflammation in IBD refers to an aberrant immune response in a genetically susceptible host [2]. This mechanism involves a complex interaction between environmental and microbial factors at the intestinal epithelium of patients with susceptibility genes that might lead to an altered innate and adaptive immune response. Intestinal homeostasis relies on the interactions between environmental factors, the epithelium, and the host immune system [4]. Breakdown of any of these components will disrupt the mucosal immune tolerance and promote inflammation.

The immune defense mechanism includes a huge armamentarium of complex signaling pathways which are involved in microbial recognition and antimicrobial function. These include pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), antimicrobial peptides produced by Paneth cells, mucus production from goblet cells, and secretory immunoglobulin A [4]. Genome-wide association studies (GWAS) have identified susceptibility genes that affect the intracellular processing of bacterial components, such as autophagy. Some of these are shared in common in CD and UC; however, other genes are unique to either of these diseases, helping us distinguish between the two. Nucleotide oligomerization domain 2 (Nod2) polymorphisms are one of the most studied genetic variants in CD. Nod2 plays an important role in immune defense and tolerance in that it is expressed in different cells of the immune system. For example, Nod2 stimulation in dendritic cells activates the nuclear factor-kB (NF-kB) pathway. Nod2 in T cells plays a role in T cell function including cytokine production [5]. Polymorphisms in the genes responsible for autophagy, mainly Nod2 and ATG16L1 genes, inhibit the recruitment of autophagy proteins that are responsible for phagocytosis of the pathogen [6].

Defects in the tight junctions of the intestinal epithelial cells and changes in the paracellular permeability will inhibit the epithelium from acting as an effective mucosal barrier against luminal pathogens and contribute to inflammation. Defects which have been described in IBD include T junction abnormalities, possibly mediated by tumor necrosis factor-alpha...
(TNF-alpha), alterations in the composition of the mucous layer secreted by goblet cells, and decreased defensin production by Paneth cells, especially in ileal CD [7].

Research has also focused on the identification of environmental factors as drivers of dysregulated immunity [8]. Alterations in the gut microbiota, leading to an imbalance of the pathogenic and nonpathogenic bacteria play another important role in immune tolerance. For example, a decrease in the diversity of Firmicutes is found in patients with IBD. It is still controversial whether it is a cause or a consequence of inflammation. There have been attempts to use gut microbiota as biomarkers; however, no microbial constituents were found to be specific to CD or UC [9].

Over the past decade, IBD research has focused on the role of genetic and serological markers in the phenotypic presentation of UC and CD. However, since UC and CD have different genetic and serological markers, such markers may also be used in identifying unclassified colitis. This review looks at the evidence behind these biomarkers and their role in different IBD subtypes.

2. Serological markers in differentiating CD from UC

There is no single marker that will determine the subtype of IBD; however, the combination of different serological markers may increase our ability in distinguishing between these subtypes. In patients whom it is difficult to differentiate between UC and CD using the traditional clinical, endoscopic, and histological criteria, serological markers are becoming increasingly helpful [10]. This area of research is rapidly expanding as new antibodies to different microbial antigens and autoantibodies are being discovered [2]. Antibodies to microbial agents include the anti-glycan antibodies, while antibodies to self-antigens include anti-neutrophil cytoplasmic antibodies (pANCA) and antibodies against exocrine pancreas (PAB).

Research has produced significant data on anti-Saccharomyces cerevisiae antibody (ASCA) and pANCA. These are the best available biomarkers in distinguishing between ulcerative and Crohn's colitis (ASCA has been linked to CD, while pANCA is associated with UC) [2]. ASCA positivity is found in 29–71% of the CD patients, while only 0–29% of the UC patients are ASCA positive. A positive ASCA result in isolation has a sensitivity ranging from 37 to 72% and a specificity from 82 to 100% for the diagnosis of CD. Combining a positive ASCA and a negative pANCA profile increases specificity to 92–99% [2].

However, more recently new serum biomarkers are being discovered. These biomarkers classify the type of colitis and also play an important role in predicting disease course, risk of complications, and response to treatment. Anti-glycan antibodies are strongly associated with CD, and their presence is also linked to a more severe disease type and risk of disease progression as well as an increased risk for IBD-related surgery [10]. The anti-glycan carbohydrate antibodies include anti-chitobioside IgA (ACCA), anti-laminaribioside IgG antibodies (ALCA), antimannobioside IgG antibodies (AMCA), and more recently, anti-laminarin (anti-L) and anti-chitin (anti-C).
The overall sensitivity of the newer anti-glycan antibodies in CD is low [1]; however, combining different serum biomarkers increases the predictive value for differentiating CD from UC. Being ASCA positive and pANCA negative increases the specificity and positive predictive value for diagnosis of CD compared to ASCA positivity alone [11]. In a study published in 2009 by Seow et al. evaluating more than 800 patients with IBD, it was found that 73% of the CD patients were positive for 1 or more anti-glycan antibodies [12]. In addition, all anti-glycan antibodies were specific for CD and more prevalent in CD rather than in UC.

These findings were confirmed in 2010 by Rieder et al. who also showed a higher prevalence of serum antibodies in CD than in UC [13]. gASCA, or the combination of gASCA/pANCA were found to be most accurate for the diagnosis of CD, but using a combination of antibodies improved the differentiation between CD and UC [13].

Other antibodies that target microbial antigens include anti-outer membrane porin C (anti-OmpC), anti-CbIr1 flagellin, and anti-I2 antibody [2]. CD patients have an excessive secretion of IgA antibodies against OmpC, an outer membrane porin found in Escherichia coli [2]. CD patients are also more likely to have antibody expression against I2 which is produced by the microorganism Pseudomonas fluorescens [2]. However, adding anti-I2 and anti-OmpC to ASCA and panCA only marginally improved the predictive capacity of distinguishing CD from UC [14].

Flagellin is an antigen present on most motile bacteria in the gut and is highly antigenic [2]. Flagellin CbIr1 has been identified as a colitogenic antigen. Anti-CbIr1 is more commonly found in CD rather than in UC patients (50–56% prevalence in CD versus less than 6% in UC) [2].

Antibodies targeting the exocrine pancreas (PAB) are also highly specific for CD. The autoantigen of PAB in CD is the membrane glycoprotein (GP2) on pancreatic acinar cells [2].

A number of antibodies have been identified in IBD, pANCA being most typically associated with UC. Despite this association, the use of antibodies has been limited in clinical use in UC. The combination of ASCA-/pANCA+ results in better diagnostic accuracy for differentiating CD from UC than either test alone. Additionally, CD patients who are pANCA positive may have colonic disease resembling UC-like disease. After resection, UC patients remain pANCA positive unlike CD patients in whom ASCA titres return back to normal [2].

The pANCA staining pattern is not specific to UC and is found in a number of autoimmune diseases and in up to 2.5% of healthy controls. Loss of antigenic response after DNase digestion of neutrophils, however, seems to be a dominant characteristic of UC-specific pANCA and is termed DNase-sensitive pANCA [15].

Serum biomarkers may, therefore, be used to help distinguish IBD from other diseases of the gut to identify the type of IBD in those patients who are difficult to classify using the classical clinical, endoscopic, histological, and radiological criteria, and to predict disease severity. The latter is related to both the number of positive serological antibodies present and their levels. There is no role in repeated testing for these biomarkers to assess disease activity [2]. Nor is it useful to predict response to treatment in CD.
In IBD patients with unclassified colitis, about half of these patients will eventually manifest as CD or UC. Usually, these are the patients who have a positive biomarker (ASCA or pANCA). The other half will remain with a diagnosis of unclassified colitis. Most of these patients are negative for both ASCA and pANCA [16]. This is where newer biomarkers might help to further classify the subtype of IBD.

2.1. Prediction of disease stratification and severity in CD

Both anti-laminarin (anti-L) and anti-chitin (anti-C) are associated with more aggressive CD phenotypes. In particular, anti-C has been associated with penetrating and perianal disease [12]. Anti-L appears to be associated with more steroid-dependency but is also useful in improving the differentiation between ulcerative and Crohn's colitis when used with ASCA and ANCA antibody status [17].

Besides differentiating between ulcerative and Crohn's colitis, serological biomarkers may also help in predicting the clinical course and behavior of the disease. Elevated titres of antimicrobial antibodies makes it more likely for a patient with CD to develop more severe and complicated disease, with an increased risk of requiring IBD-related surgery. The chance of having more severe disease increases with the number of serum biomarkers present and also with increasing titres of these antibodies [2]. Seow et al. have shown that an increasing number of positive antibodies is associated with early CD onset, fistulating and perianal disease, and increased risk for surgery [12]. These findings were confirmed by Rieder et al. who showed that a higher number of anti-glycan antibodies predicts a faster progression toward more severe disease [18]. Anti-GM-CSF antibody also correlates with disease activity and an increased risk of relapses [10].

Serological markers such as anti-GP2 and anti-CBir1 may contribute to better stratification of pouchitis in patients with UC undergoing ileal pouch-anal anastomosis (IPAA). A retrospective study published in 2012 by Coukos et al. analyzed ASCA IgG and anti-CBir1 antibodies in patients with UC who underwent IPAA. Both ASCA IgG and anti-CBir1 titres were significantly associated with postoperative IPAA complications. A positive anti-CBir1 test was found to be associated with CD of the pouch and/or fistula formation (p < 0.001) [19]. Identifying patients with these positive serological markers can help the clinician predict the risk of pouchitis in patients undergoing IPAA, and therefore, choosing more aggressive treatment to prevent pouch failure [19]. A similar association between GP2 antibodies and increased risk of pouchitis has also been demonstrated, especially when the inflammation exhibits CD-like complications. Elevated anti-GP2 was also associated with more frequent bowel movements per day and presence of at least one anti-glycan antibody. Therefore, the presence of these CD-specific pancreatic auto-antibodies (PAB) could be used as a predictor of pouchitis [20].

Notwithstanding their role in distinguishing IBD subtypes and predicting IBD phenotypes, serum biomarkers are frequently not useful in diagnosing clinical remission. Their levels remain elevated even in patients who are in endoscopic and histological remission. For example, the level of CBir1 antibody in the serum does not correlate with disease severity and stable CBir1 expression has been found in the serum of CD patients during both active disease and also when in endoscopically proven remission [21].
Serum biomarkers are not specific to CD but may also be found to a lesser extent in patients with other disorders of the gastrointestinal tract or in healthy individuals. For example, ASCA and PAB, which are highly specific antibodies in CD, can also be found in patients with coeliac disease, especially prior to commencing a gluten-free diet. Therefore, these serum antibodies are not to be used on their own to diagnose IBD, but are meant to be an extra tool in the accurate diagnosis and management of IBD [2].

3. Genetic markers

3.1. Genetic markers in Crohn’s disease

Gut inflammation in IBD is believed to occur secondary to an interaction between the altered immune system of a genetically susceptible individual and environmental factors such as antimicrobial agents. IBD is regarded as a polygenic disorder with multiple susceptibility loci contributing to the overall risk of developing the disease [22]. There are frequently different genetic loci, which are involved in the pathogenesis of UC and CD, and this may be used in distinguishing between Crohn’s and ulcerative colitis.

It may occasionally be difficult to differentiate functional from organic bowel diseases based on the clinical presentation and even more difficult to distinguish CD from UC. In a study published in 2008, von Stein et al. tried to identify genes from mucosal biopsy specimens which could help discriminate between functional disease and IBD and also genes which could distinguish between CD and UC [23]. The group identified seven IBD-specific genes that could help determine IBD type in patients with colitis. These genes were solute carrier (SLC)6A14, SLC26A2, small protein associated with PDZ domain-containing protein 1 (SPAP), regenerating protein IV (RegIV), Vanin-1, matrix metalloproteinase 7 (MMP-7), and growth-related oncogene alpha (GRO-alpha). These genes have been shown to play a role in IBD, be it in inflammation, tissue injury, or carcinogenesis. For example, GRO-alpha is a chemokine that recruits and activates neutrophils at the site of inflammation [24]. Using these seven biomarkers, one could correctly classify UC or CD patients in more than 92% of cases [25].

Wu et al. also tried to identify genes that are associated with CD, UC, and non-IBD colitis [26]. Genes differentially expressed in the CD patients were related to IFN gamma-inducible TH1 processes (IFITM1, IFITM3, STAT1, and STAT3) and antigen presentation (TAP1, PSME2, and PSMB8).

The role of several genes involved in epithelial defense has been studied as a possible contributing factor to the development of CD [27]. Some of the genes studied confirm a link between defects in the immune response and the role of intracellular bacteria in patients with CD.

3.1.1. Bacterial recognition

Nucleotide-binding oligomerization domain containing 2 (NOD2) gene is found on chromosome 16 and is one of the earlier genes to be linked to CD. NOD2 variants that alter the structure of the leucine-rich repeat domain of the protein can overactivate nuclear factor
NF-kB in monocytes, thereby altering the response of the immune system to microbial pathogens [28]. Polymorphisms in the NOD2 gene have been associated with more complicated disease in CD [29].

NOD2 gene polymorphisms may also have implications on drug treatment. Gutierrez et al. studied phagocytic and bactericidal activities in neutrophils of patients with CD. Patients with a NOD2-variant had less phagocytic and bactericidal activities and increased TNFα levels in response to the presence of bacterial DNA [30]. This might have an effect on the management of these patients as they may require more aggressive treatment, with an increased requirement for anti-TNFα agents.

3.1.2. Autophagy

Hampe et al. were among the first to show a potential role of autophagy in the pathogenesis of CD. The group studied the autophagy-related 16-like 1 (ATG16L1) gene, which encodes a protein that processes intracellular bacteria. A variant of this gene was found in CD but not in UC patients [31]. It supports the role of bacteria in the pathogenesis of CD as the processing of intracellular bacteria would be altered in patients having a variant of the gene.

Another gene involved in autophagy of intracellular pathogens is the immunity-related GTPase family M (IRGM) gene. IRGM is a CD susceptibility gene [32] that does not increase the risk of developing UC [33].

The protein tyrosine phosphatase nonreceptor type 2 (PTPN2) gene is also involved in the autophagy pathway and PTPN2 polymorphisms have also been associated with IBD. The PTPN2 polymorphism rs2542151 appears to be associated with both CD and UC, while polymorphisms rs1893217 and rs7234029 are associated with CD only [34].

3.1.3. Prostaglandin system

Variants of the prostaglandin receptor 4 (PTGER4) have been implicated to have a role in CD. In a German cohort of CD patients, Prager et al. found that the variant rs7720838 increased susceptibility to CD but not to UC. Patients with this gene variant were more likely to develop strictureing disease with the risk of stricture formation increasing further if the patient also had NOD2 mutations [35].

3.1.4. TH-17/IL-23 cytokine responses

Interleukin (IL)-23R mutations and their role in CD have been studied extensively. The IL-23 cascade contributes to the differentiation of the T helper (Th) 17 cells, which play an important role in immunoregulation in the gut [25]. Dysregulated Th17 differentiation may occur in CD patients [36, 37].

The rs11209026 SNP in IL23R was found to have a protective effect for IBD. Both CD and UC were associated with IL23R in a study conducted in over 500 Dutch patients with IBD [38]. In another study involving Korean patients with CD, two other polymorphisms (rs1004819 and rs1495465) were significantly associated with CD, and when the genotype was correlated to
the disease phenotype, it was found that it was associated with both stricturing and penetrating disease [39].

3.2. Genetic markers in UC

CD and UC may share some gene susceptibility loci but significantly differ at others. While genetically determined defects in the handling of intracellular bacteria (NOD2 and the autophagy genes ATG16L1 and IRGM) are specific to CD, multiple components in the Th17 pathway (IL23R, IL12B, JAK2, STAT3) are associated with both CD and UC [40, 41].

New potential pathogenic pathways for both CD and UC have been demonstrated, initially by genome-wide association studies (GWAS) and more recently via imputation and meta-analyses to combine the power of multiple individual GWAS. Meta-analyses of GWAS have shown that CD and UC share the majority of the 163 known genetic risk factors for IBD, although to varying extents, for example, IL23 pathway is more commonly linked to CD rather than to UC despite being strongly associated with both [42].

The major histocompatibility complex (MHC) seems to be one of the notable exceptions to this. In a large cohort of patients with IBD (18,405 Crohn’s disease patients, 14,308 ulcerative colitis patients, and 34,241 controls), it was shown that the MHC region is a significant contributor to disease risk in IBD. However, while the majority of non-MHC susceptibility loci are shared between both UC and CD, most associated HLA alleles have a predominant role in either one or the other, with very few conferring shared IBD risk. Furthermore, whereas both class I and class II HLA variants contribute to disease risk in CD, class II variation seems to have a more important role in UC [43].

Altered epithelial barrier function may play a role in the development of UC but not in CD. A number of epithelial barrier genes are in fact specifically associated with ulcerative colitis (OCTN2, ECM1, CDH1, HNF4A, LAMB1, and GNA12). The role of epithelial barrier genes HNF4A, E-cadherin, and laminin was also first discovered by GWAS of a relatively small sample of 2361 white patients of European descent as part of the Wellcome Trust Case Control Consortium 2 [44]. The exact mechanisms by which such defects play a bigger role in the pathogenesis of UC than in CD are still poorly understood.

4. PSC-IBD phenotype

Primary sclerosing cholangitis (PSC) has long been associated with IBD, especially with UC. Up to 5% of patients with UC have PSC, while up to 3.6% of patients with CD have PSC, mostly those patients with extensive disease [45].

However, the phenotype of IBD in the context of a patient with both IBD and PSC is different from the IBD phenotype of a patient without PSC. De Vries et al. have recently published a systematical review of the literature to identify the distinctive features of IBD in patients with concomitant PSC [46]. The characteristics and clinical course of IBD in patients with PSC are different, making it distinct from the conventional IBD phenotypes.
The prevalence of IBD in PSC is high, ranging from 46.5% [47] to 98.7% [48]. More than 75% of these patients have UC, followed by CD and unclassified IBD.

Although the disease course of IBD in PSC is found to be quiescent, pancolitis is observed more frequently, with rates varying from 35 to 95% [49, 50]. Another two characteristics which are more commonly reported in IBD patients with PSC than in conventional IBD are backwash ileitis and rectal sparing [51]. Although the disease activity of Crohn’s is similar in patients with and without PSC, the reported rates of complications like stricturing and penetrating disease are lower in patients with concomitant PSC [52]. On the other hand, patients with IBD and PSC who undergo proctocolectomy with ileal pouch—anal anastomosis (IPAA) have a higher risk of developing pouchitis than IBD patients without PSC [46].

The risk of dysplasia or development of colorectal carcinoma is increased in PSC-IBD. For this reason, guidelines on colorectal cancer surveillance classify patients with IBD-PSC in the high risk category, recommending increased frequency of surveillance colonoscopies. The cumulative 10-year risk varies between studies, but is significantly higher than in IBD patients without PSC. In addition to this, the predominant site of dysplasia or malignancy is different in IBD patients with PSC. It tends to occur in the proximal colon, as opposed to conventional IBD, where right-sided localization is less common [46].

PSC-IBD patients have been observed to develop dysplasia or colorectal carcinoma earlier than IBD patients without PSC, where the mean interval between the diagnosis of IBD and the development of dysplasia or colorectal carcinoma is longer [53].

Patients who undergo orthotopic liver transplantation for PSC still have high rates of IBD exacerbation despite being on immunosuppressant medication. Up to 51.5% of these patients experience an exacerbation [54].

The etiology of PSC is not clear. It has been shown in genetic studies that it shares risk loci with IBD. It is genetically more similar to UC, which might explain why UC is the predominant IBD phenotype associated with PSC. However, there are also several genetic loci, which are associated with PSC but not with IBD [46]. These differences, together with the characteristic features described above, suggest that PSC-IBD is a phenotype, which is distinct from UC or CD.

5. Conclusion

Serological and genetic markers may play an important role in identifying IBD type in patients with unclassified colitis. Figure 1 summarizes some of the genetic loci and serological markers which can help us classify the type of colitis, and the degree of overlap which exists between CD and UC. These markers may have an important role in patients with unclassified colitis and may be used in these patients to create a risk score for IBD type. Thus, a patient with unclassified colitis who is ASCA+/pANCA− and carries the NOD2 and ATG16L1 polymorphisms has a significantly higher chance of having Crohn’s colitis while patients who are ASCA−/pANCA+ and have polymorphisms in epithelial barrier genes are more likely to have UC.
While there is significant evidence to link these markers to specific disease types, more evidence is needed. Since different gene polymorphisms are commoner in different geographical areas, population-based studies are needed to identify genes in specific populations. We also feel confident that with more research, other different disease-specific antibodies will be discovered. Long-term follow-up of patients with unclassified colitis may also help better characterize this disease and help us create a score based on serum biomarkers, which can predict the disease type in this group of patients.

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