The Influence of Innate and Adaptive Immunity on Crohn's Disease Severity

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Abstract

Stricturing and penetrating disease are classified as severe Crohn's disease types and are frequently associated with an increased risk for bowel surgery. Research has shown that early treatment with aggressive immunosuppression (including biological and thiopurine therapies – the so-called "top-down approach") results in a diminished risk of developing these complicated disease types. However, these therapies carry significant risks and cost. Being able to predict which patients are at an increased risk of developing severe Crohn's disease may enable us to treat patients individually, with the aggressive "top-down approach" started at diagnosis in patients with a significantly increased risk of developing complicated disease types. Defects of innate and adaptive immunity both play a role in Crohn's disease pathophysiology. Identifying whether defects of innate immunity (through gene mutations) or adaptive immunity (through antibodies to microbial antigens) are associated with stricturing/penetrating disease types may enable us to predict the course of the disease and therefore decide on who would benefit most from the "top-down approach". This review discusses the role of NOD2 and other gene polymorphisms in predicting Crohn's disease severity. It also highlights the evidence linking the role of the various antibodies involved in adaptive immunity (ASCA, OmpC, GM-CSF) and complicated Crohn's disease types.

Keywords: Innate immunity; Adaptive immunity; Crohn's disease; NOD2 polymorphisms; Stricturing disease; Penetreating disease

Introduction

The Montreal classification of inflammatory bowel disease classifies Crohn's disease (CD) into three disease types: inflammatory (non-stricturing, non-penetrating) disease, stricturing disease and penetrating disease [1]. Stricturing and penetrating disease are markers of Crohn's disease severity, being also associated with an increased risk of surgical intervention [2,3]. CD is a dynamic disease which may progress over time from the non-stricturing, non-penetrating type of disease to the more severe forms of this condition. In a study on CD patients followed up for 25 years, Louis et al. found that 46% of patients had a change in disease type in the first 10 years of follow up with 27% of patients developing stricturing disease and 29% of patients developing penetrating disease [4].

Multiple studies trying to predict which patients with CD are more prone to develop the more severe (stricturing or penetrating) disease types have been carried out. Since using potent immunosuppressants in all CD patients may expose patients with mild CD to an increased risk of side-effects from these immunomodulators and biological agents, this approach may help identify which patients would benefit from aggressive immunosuppression at an early stage. This approach is frequently called the "top-down" approach and involves starting intensive therapies, including biological agents (anti-tumor necrosis factor α agents like infliximab) early on with the aim of avoiding the development of strictures and fistulas. This approach varies from the classic "step-up" approach which refers to the progressive intensification of treatment as the disease becomes more severe. Studies have shown that the "top-down" approach is better than the current "step-up" approach both in the induction and maintenance of remission, as well as in decreasing the risk of surgery [5].

However, a number of factors still limit the use of anti-tumor necrosis factor (TNF) α therapy in all CD patients. These factors include potential side-effects associated with this therapy, including risks of sepsis secondary to immunosuppression. In addition, up to 50% of patients have mild disease which can be managed adequately with therapy which is less costly and carries less risks [6]. The significant cost of anti-tumor necrosis factor (TNF) α agents when compared with other therapies like aminosalicylates and purine analogues is another important factor limiting the indiscriminate use of these agents in all CD patients.

The current challenge in CD is to distinguish at an early stage patients with an increased likelihood of developing severe disease types who would therefore benefit from the "top-down" approach. On the other hand, the "step-up approach" should be used in patients with predicted mild (inflammatory) disease types. Defects of innate and adaptive immunity both play a role in CD pathophysiology (Figure 1) and therefore identifying whether defects of innate immunity (through gene mutations) or adaptive immunity (through antibodies to microbial antigens) are associated with stricturing/penetrating disease types may be used to predict the course of the disease. This may enable physicians to choose "tailor-made" therapy for their CD patients at the time of diagnosis.

The biology of Crohn's disease is well described and involves various processes involving altered gut permeability, dysfunctional autophagy and altered immune response. In particular, Atg16l1 and Nod2 proteins are both necessary for effective autophagy in ileal Paneth cells and polymorphisms in genes responsible for these proteins may lead to defective autophagy and intestinal inflammation. Upon microbial infection of intestinal cells, Nod proteins recruit

autophagy proteins, including Atg5, Atg12 and Atg16l1, to the plasma membrane and stimulate the phagosome to engulf the pathogen. The autophagosome then interacts with the lysosome to break down the pathogen into bacterial (or viral) peptides. Polymorphisms in the NOD2 and ATG16L1 genes result in defective autophagy with a hyper inflammatory response in the intestinal mucosa [7,8].

This review focuses on biomarkers which may be used to predict the development of delayed complicated disease types in Crohn's, thus enabling us to identify which of these patients would benefit most from early, aggressive immunosuppression.

NOD2/CARD15 Mutations

Evidence that genetics plays an important part in the development of severe disease types arises from studies in monozygotic and dizygotic twins in Norwegian inflammatory bowel disease patients. Data from these studies showed that stricturing disease among twins was significantly higher than stricturing disease in unrelated CD cases [9].

The strongest evidence linking gene polymorphisms with stricturing and penetrating disease types and risk of surgery comes from mutations in the NOD2/CARD15 pathway. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) also known as caspase recruitment domain-containing protein 15 (CARD15) is encoded by the NOD2 gene located on chromosome 16. NOD2 plays an important role in the immune system by recognizing bacterial peptidoglycans and stimulating the innate immune system [10,11]. At least 27 polymorphisms in the NOD2/CARD15 gene have been identified as Crohn's disease susceptibility genes with the commonest ones being the R702W and G908R (missense mutations) and the 3020insC and 1007fs (frameshift mutations). Around 40% of CD patients of Western European and Northern American origin carry at least one of these variants compared with 0.5-20% of healthy controls [12].

The NOD family consists of a number of receptors which contain a nucleotide binding oligomerization domain and a leucine-rich repeat. These proteins are important in innate immunity since they are able to recognize pathogen (bacterial, viral) peptides. Upon pathogen recognition, they activate a specific signal transduction cascade which leads to a transcriptional response against these pathogens. NOD proteins are also important in caspase activation and apoptosis. The leucine-rich repeat allows NOD2 proteins to recognize muramyl-dipeptide, a component of bacterial wall peptidoglycan, thus leading to the activation of the autophagy pathway. Autophagy activates an inflammatory reaction in epithelial cells and releases antimicrobial peptides from cytosolic granules which degrade bacterial peptides. Therefore defective NOD2 proteins secondary to NOD2/CARD15 polymorphisms will lead to a defective innate immunity, defective autophagy and defective handling of intestinal pathogens. These changes are believed to lead to the increased inflammatory response seen in patients with CD [13]. NOD2 proteins are found in monocytes, macrophages, T cells, B cells, Paneth cells, dendritic cells and in epithelial cells of the small and large intestine.

Mutations in the CARD15 gene have also been shown to have an effect on intestinal mucosal permeability. A genetically impaired intestinal barrier function is suspected to lead to the chronic intestinal inflammation seen in Crohn's disease. In a study analyzing the intestinal permeability and CARD15 (R702W, G908R and 3020insC) polymorphisms in 128 patients with quiescent Crohn's disease, 129

first degree relatives of Crohn's patients, 66 non-related household members and 96 healthy controls, intestinal permeability was significantly increased in Crohn's patients and their first degree relatives when compared with household members and controls. In addition, 40% of first degree relatives carrying the 3020insC CARD15 mutation and 75% of those relatives with combined 3020insC and R702W mutations had increased intestinal permeability when compared with 15% of relatives with the wild-types. These results suggest that genetic factors may be involved in intestinal barrier impairment in Crohn's disease [14].

NOD2/CARD15 mutations are associated with Blau syndrome, a rare autosomal dominant condition involving granuloma formation, and possibly also with early onset sarcoidosis. Granuloma formation occurs secondary to a host reaction to intracellular infections, which results in the activation of a T helper 1 response with subsequent production of cytokines (interleukin-12, interferon gamma and tumor necrosis factor). However, mutations in the NOD2/CARD15 pathway result in inappropriate activation of the immune system with the excessive granuloma formation typically seen in these conditions [15].

Genotype-phenotype correlations between these polymorphisms have shown an association with stricturing and penetrating disease and an increased risk of surgery in different populations [12,16-27]. Abreu et al. found that patients with NOD2 mutations have nearly a 3-fold increased risk of developing fibro-stenosing disease when compared with patients without these polymorphisms [28]. The risk appears to increase in patients carrying more than one polymorphism of the NOD2 gene with an odds ratio of sticturing or penetrating disease of 1.9 in patients with one polymorphism and an odds ratio of 3.5 in patients with two polymorphisms [29]. Patients with multiple mutations in the NOD2 gene have a younger age at diagnosis, are associated with more frequent stricturing disease type and less frequent colonic involvement [30]. Meta-analysis confirmed the increased risk of complicated CD in patients carrying 2 polymorphisms of the NOD2/CARD15 gene [31].

Patients with one NOD2 polymorphism together with at least one other risk allele (like interleukin 23 receptor gene, Drosophilia discs large homologue 5 and autophagy-related 16-like 1 genes, plasminogen activator inhibitor 1 – IL23R, DLG5, ATG16L1, PAI-1) also have more severe disease types, more risk for surgery and earlier disease onset [32-34]. In addition, the need for surgical intervention appears to be required earlier in patients with these variants [35].

Phenotypic correlation of CD severity with NOD2 mutations was also confirmed in paediatric populations in several studies [36-38]. These studies confirmed that 2 or more NOD2/CARD15 polymorphisms were strongly associated with need for surgery secondary to stricturing disease [37,38]. This association appears to be even stronger for the 3020insC mutation which was shown to carry an odds ratio of 6.6 for stricturing disease and 5.8 for risk of surgery. Surgery also appears to occur earlier among children with this mutation (hazard ratio 2.24) [39].

While NOD2 mutations are the strongest genetic factors predisposing to small intestinal stricturing CD, cigarette smoking is the best characterized environmental risk factor predisposing to this phenotype. In an experiment where intestinal epithelial cells were exposed to cigarette smoke extract, there was a delayed NOD2 mRNA expression, reduced nuclear factor kappa Beta (NF-kB) activity and decreased chemokine production. This suggests that environmental

factors may also have a direct effect on NOD2 function and NOD2-mediated responses [40].

While this data may be insufficient to recommend top-down therapy based on only one NOD2 mutation, the significant risk of complicated CD associated with two NOD2 mutations or with one NOD2 mutation and at least one other risk allele may be sufficient to recommend early-intensive therapy in these patients. Expert consensus recommendations may in the future be made available once checking for these polymorphisms becomes more easily available.

Other Genetic Markers

While mutations in the NOD2/CARD15 gene have been most extensively investigated in the risk of developing severe CD types, other gene polymorphisms have also been shown to be associated with stricturing/penetrating disease. In the innate immune system, the inflammasome plays a central role in maintaining gut homeostasis and mediating host immune defenses against pathogens (Figure 1). In Crohn's disease, dysregulation of the inflammasome contributes to chronic inflammation and disease onset [41]. Polymorphisms associated with inflammasome components have in fact been associated with an increased susceptibility to inflammatory bowel disease (Table 1). Polymorphisms in IL-18, IL-18 accessory proteins and NOD-like receptors (NLRs) (which result in impaired IL-1β and IL-18 production) have all been associated with inflammasome dysregulation and increased susceptibility to Crohn's disease [42-44]. Inflammasome formation occurs in a two-step process. In the priming step, Toll-like receptors (TLRs) induce NF-κB transcription of pro-Il-1β, pro-Il-18 and NLRs while in the second step, inflammasome oligomerization leads to cytokine cleavage and mature Il-1β and Il-18 production [45-47]. The potent proinflammatory cytokines Il-1β and Il-18 can induce T cell differentiation thus bridging innate and adaptive immune responses [48]. Il-1β also activates the release of other cytokines, like TNF-α, Il-23 and Il-6 [49]. Il-1β levels are therefore markers of ongoing inflammation in Crohn's disease. They have also been shown to predict early relapse in Crohn's disease patients with quiescent disease [50].

The Asp299Gly polymorphism of the Toll-like receptor 4 (TLR4) gene has been shown to carry a significantly increased risk of stricturing disease even in the absence of NOD2 polymorphisms while penetrating disease is more common in patients carrying NOD2 polymorphisms than those with TLR4 polymorphisms [51].
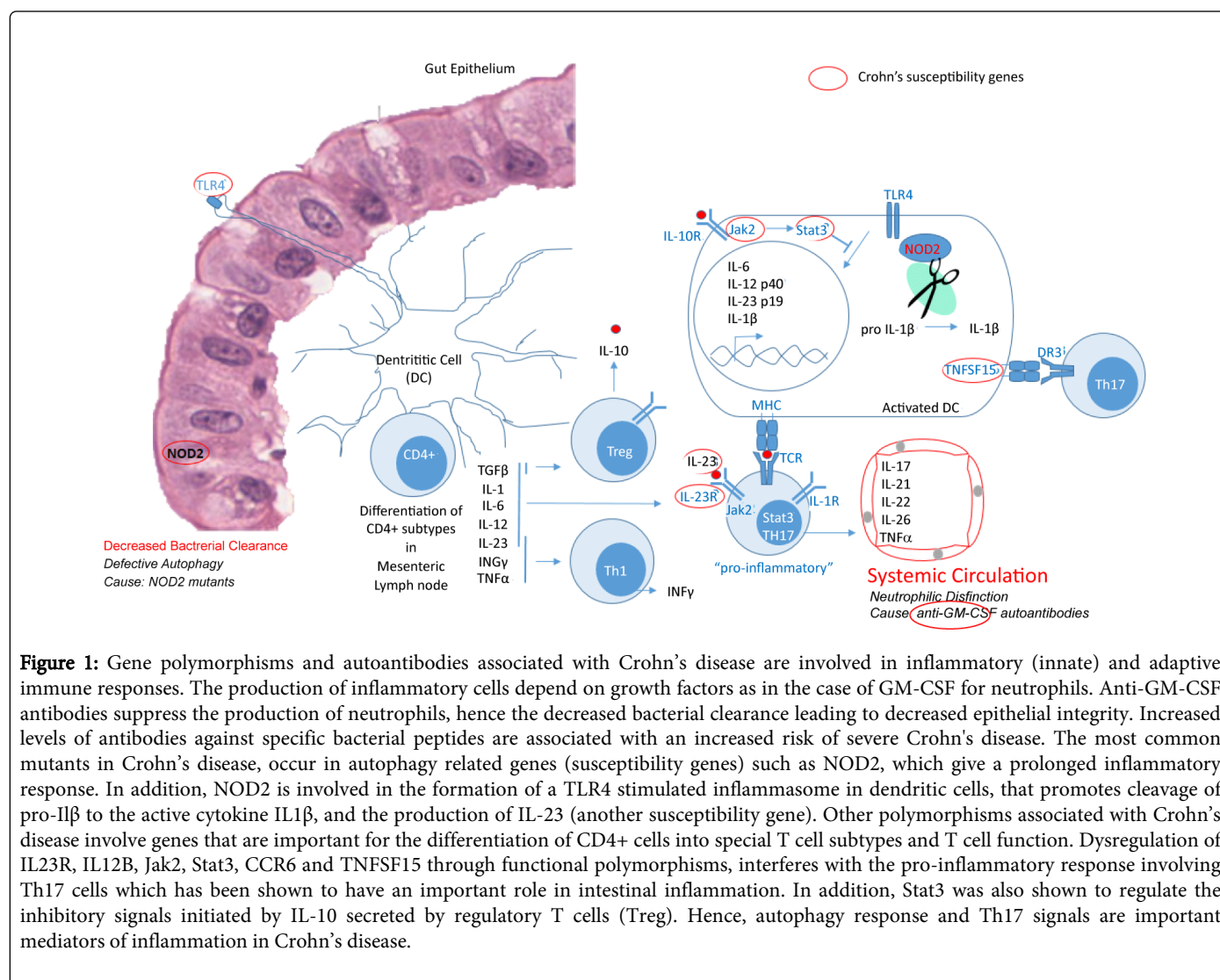

Figure 1: Gene polymorphisms and autoantibodies associated with Crohn's disease are involved in inflammatory (innate) and adaptive immune responses. The production of inflammatory cells depend on growth factors as in the case of GM-CSF for neutrophils. Anti-GM-CSF antibodies suppress the production of neutrophils, hence the decreased bacterial clearance leading to decreased epithelial integrity. Increased levels of antibodies against specific bacterial peptides are associated with an increased risk of severe Crohn's disease. The most common mutants in Crohn's disease, occur in autophagy related genes (susceptibility genes) such as NOD2, which give a prolonged inflammatory response. In addition, NOD2 is involved in the formation of a TLR4 stimulated inflammasome in dendritic cells, that promotes cleavage of pro-Ilβ to the active cytokine IL1β, and the production of IL-23 (another susceptibility gene). Other polymorphisms associated with Crohn's disease involve genes that are important for the differentiation of CD4+ cells into special T cell subtypes and T cell function. Dysregulation of IL23R, IL12B, Jak2, Stat3, CCR6 and TNFSF15 through functional polymorphisms, interferes with the pro-inflammatory response involving Th17 cells which has been shown to have an important role in intestinal inflammation. In addition, Stat3 was also shown to regulate the inhibitory signals initiated by IL-10 secreted by regulatory T cells (Treg). Hence, autophagy response and Th17 signals are important mediators of inflammation in Crohn's disease.

Polymorphisms within the promoter regions for interleukin-10 (Il-10) and tumor necrosis factor alpha (TNFα) have also been linked with an increased risk of stricturing disease type [52,53], while TNFα polymorphisms have been shown to be higher in CD patients with fistulising disease [54].

Polymorphisms in the clock gene PERIOD3 have been associated with severe forms of CD. Altered body rhythmicity and deregulated clock gene expression may lead to circadian disruption, which may cause immune dysregulation and chronic inflammatory diseases like CD. PERIOD3 polymorphisms have been associated with circadian disruption and altered levels of inflammatory cytokines. The rs2797685 polymorphism of the PERIOD3 gene has been shown to give an increased risk of stricturing and penetrating disease types (p: 0.03) [55].

Another CD susceptibility gene which has been shown to be associated with stricturing disease type is the rs7234029 polymorphism of the protein tyrosine phosphatase, non-receptor type 2 (PTPN2) gene. This gene appears to regulate autophagosome formation in intestinal epithelial cells and may therefore form an essential part of the autophagy pathway [56].

The transforming growth factor beta 1 (TGFbeta1) codon 25 has variable peptide production, predisposing to fibrosis in several organs. This codon was associated with stricturing disease (OR=2.63) and early surgical resection in CD patients [57].

Different gene polymorphisms appear to exhibit different phenotypic expressions in different populations. Genotype-phenotype

analysis showed an association between the rs1004819 and rs1495465 polymorphisms of the IL23R gene and stricturing/penetrating disease types among Korean and Finnish CD patients [58,59].

The rs10758669 polymorphism of JAK2 is a CD susceptibility gene which has been shown to strongly enhance the risk of ileocolonic disease with stricturing behavior and a significantly elevated risk of bowel resection in a population of New Zealand CD patients [60].

Other gene mutations may also act as markers of the less severe CD type. Choosing the "step-up" approach rather than the "top-down" approach may be justified by finding these polymorphisms in the absence of biomarkers which predict severe CD types. The IL12B gene is a susceptibility gene for inflammatory bowel disease which encodes the p-40 subunit of Interleukin-12. Glas et al. have shown that the rs6887695 single nucleotide polymorphism of the IL12B gene was significantly associated with the non-stricturing, non-penetrating CD type [61]. The RAGE gene (receptor for advanced glycation end products) is involved in innate immunity and the rs1800624 polymorphism of this gene has also been shown to have a protective effect against developing the stricturing disease phenotype. This protective effect probably occurs secondary to an increase in the levels of serum soluble RAGE (sRAGE) which neutralizes pro-inflammatory mediators [62].

The genetic markers which predict Crohn's disease severity are summarized in Table 1.

Biomarker	Polymorphisms	Severity of Crohn's Disease	References
NOD2	SNP8/R702W	Small bowel Involvement	Abreu et al. [28]
	SNP13/G908R	Fibrostenosing phenotype	Kugathasan et al. [39]
	SNP18/1007fs	Stricturing and penetrating disease	
	3020insC	Increased risk for surgery	
TLR4	Asp299Gly	Stricturing disease	Brand et al. [51]
	Thr399Ile		
TNFα	857C	Stricturing and fistulating disease	Fowler et al. [52]
PERIOD3	rs2797685	Stricturing and fistulating disease	Mazzoccoli et al. [55]
PTPN2	rs7234029	Stricturing disease	Glas et al. [56]
IL10	1082G	Stricturing disease	Fowler et al. [52]
IL23R	rs1004819	Stricturing and penetrating disease	Yang et al. [58]
	rs1495465		Lappalainen et al. [59]
JAK2	rs10758669	Ileocolonic disease	Ferguson et al. [60]
		Stricture formation	
IL12B	rs6887695	Non-stricturing and	Glas et al. [61]
		Non-penetrating disease	
RAGE	rs1800624	Non-stricturing disease	Däbritz et al. [62]

NOD2: Nucleotide-binding Oligomerization Domain-containing protein 2; TLR4: Toll-like Receptor 4; TNFα: Tumor Necrosis Factor alpha; PTPN2: Protein Tyrosine Phosphatase, Non-receptor type 2; IL10: Interleukin 10; IL23R: Interleukin 23 Receptor complex; JAK2: Janus Kinase 2; IL12B: Interleukin 12 beta; RAGE: Receptor for Advanced Glycation End products

Table 1: Genetic Markers of Severity in Crohn's Disease. Key markers involved in innate immune response in Crohn's disease. Mutations in these markers are associated with specific severity phenotypes thus lending a prognostic or predictive role in disease management.

Serological Markers

Research into serological markers of adaptive immunity, particularly antibodies to bacterial antigens, has also shown an association with CD phenotype. The most well recognized antibodies associated with severe CD are antibodies to granulocyte-macrophage colony-stimulating factor (GM-CSF Ab), Anti-*Saccharomyces cerevisae* antibody (ASCA), flagellin (CBir1), and *Escherichia coli* anti-outer membrane porin-C (OmpC). Neutralizing autoantibodies against GM-CSF in children with CD were significantly associated with structuring ileal CD. Stricturing prevalence on CT enterography or magnetic resonance enterography (CTE or MRE) increased from 4% in CD patients with low GM-CSF Ab levels (<1.6 µg/mL) to 19% in those with elevated levels of GM-CSF Ab (≥ 1.6 µg/mL) [63].

These findings were replicated in an independent paediatric CD cohort which confirmed that the likelihood ratio for stricturing behavior for patients with elevations in both GM-CSF Ab and GM-CSF neutralizing capacity was equal to 5. In this cohort, Jurickova et al. isolated ileal lamina propria mononuclear cells from paediatric CD patients and controls and showed that these lamina propria cells produce GM-CSF Ab in the CD patients. Neutrophil bacterial killing decreased as GM-CSF Ab levels increased confirming the role that these antibodies play in cellular immunity [64]. The association between GM-CSF Ab and CD phenotype was subsequently analyzed in 2 independent, predominantly adult CD cohorts. This analysis confirmed an association between GM-CSF Ab levels and stricturing/penetrating disease type, and showed an increased risk of intestinal resection in patients carrying high antibody levels [65].

Oligomannan (Anti-Saccharomyces cerevisae antibody - ASCA) is one of the best known biomarkers associated with complicated CD types [66-68]. In a study analyzing predictors of complicated CD in 182 CD patients in the Manitoba IBD cohort, serological and genetic markers were analyzed at enrolment and after 5 years. Only ASCA-IgG positive serology was found to be predictive of stricturing/penetrating disease behavior and surgery [69]. ASCA antibodies were also found to be predictive of ileal involvement, complicated disease behavior (stricturing/penetrating disease type) and surgery among African-American CD patients [70].

The microbial antigen CBir1 (flagellin) produces strong adaptive immune responses in mouse models of colitis. Antibodies against this antigen have been shown to be associated with small-bowel disease, penetrating disease and fibro-stenosing disease in CD patients. Investigators also showed that positivity to this antibody remained constant independently of disease activity [71] and serum ASCA levels remain stable even after curative intestinal resection [72]. This suggests that these serological markers may also be used in predicting risk of disease, progression in patients who are already in remission or who have already started treatment.

Increased levels of other serological markers, including perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), *Escherichia coli* anti-outer membrane porin-C (OmpC) and anti-flagellin have also been associated with features of complicated CD, with earlier disease onset, ileocolonic and perianal disease, penetrating and stricturing behavior, and increased risk of surgery [73,74]. Schoepfer et al. confirmed the association between anti-flagellin and ASCA antibodies with complicated CD and showed that patients with antibodies against multiple microbes have an increased risk of developing strictures or perforations and an increased risk of requiring

small bowel surgery than patients with antibodies against only one microbe [75].

Pancreatic autoantibodies are CD specific antibodies which have been shown to be associated with perforating, perianal disease and extra-intestinal manifestations in an Eastern European cohort [76]. In addition, glycoprotein 2 (GP2) has recently been discovered as a major autoantigen of CD-specific pancreatic autoantibodies (PAB). Antibodies to GP2 have been shown to be more prevalent in CD patients with stricturing behavior and perianal disease [77].

Patients having antibodies against multiple microbes have an increased immune reactivity and this may explain the increased risk of complicated CD in these patients [75,78]. Mow et al. [79], analyzed the sera from 303 CD patients for antibodies to I2 (*Pseudomonas fluorescens*-related protein), OmpC, ASCA and pANCA and compared them with disease phenotype. Patients with antibodies to I2 had a significantly increased risk of developing fibro-stenosing disease and require small intestinal surgery while patients with antibodies to OmpC had an increased likelihood of internal perforating disease and small bowel surgery. The risk of small bowel surgery increased significantly in patients who were triple positive to ASCA, I2 and OmpC (odds ratio=8.6).

Serological Marker	Severity of Crohn's Disease	References
anti-GM-CSF	Stricturing Ileal Disease	Dykes et al. [63]
	Generalized Stricturing and penetrating disease	Gathungu et al. [65]
ASCA-IgG	Stricturing and penetrating disease	Dassopoulos et al. [67]
	Risk of Surgery	Eser et al. [72]
	Ileal involvement	
anti- flagellin	Small bowel disease	Targan et al. [71]
	Penetrating and fibrostenosing disease	
Pancreatic autoantibodies	Penetrating disease	Lakatos et al. [76]
	Perianal disease	
	Extrainstinal manifestations	
anti-GP2	Stricturing and perianal disease	Bogdanos et al. [77]

GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; ASCA-IgG: Anti-Saccharomyces cerevisae Antibody Immunoglobulin G; GP2: Glycoprotein 2

Table 2: Serological Markers of Severity in Crohn's Disease. Key markers involved in adaptive mucosal immune response in Crohn's disease.

There also appears to be a synergistic effect between innate immunity (NOD2 mutations) and adaptive immunity (antibodies to microbial antigens) in increasing the risk of fibro-stenosing CD phenotype. Ippoliti et al. have shown that NOD2 variants and antibodies to oligomannan, flagellin, OmpC and I2 synergistically increase this risk [80]. This synergistic effect may occur because NOD2 mutations frequently result in an increased immunologic response to microbial antigens. Devlin et al. [81] showed that NOD2 mutations are associated with an increased adaptive immunologic response with patients having NOD2 polymorphisms having more positive antibody tests than patients not carrying these polymorphisms. Patients

carrying NOD2 mutations also had a significantly higher cumulative serologic response to ASCA, I2, OmpC and CBir1 antigens than patients not carrying the NOD2 variants.

The serological markers which predict Crohn's disease severity are summarised in Table 2.

Conclusion

Innate and adaptive immunity play a central role in the pathophysiology of Crohn's disease. Gene polymorphisms which cause defects in innate immunity and antibodies to bacterial peptides are increasingly being recognized as predictors of severe Crohn's disease types. Stricturing and penetrating disease types, with their increased risk of surgery, represent severe, complicated CD. Perhaps the most studied gene mutations which predict severe CD are the NOD2 polymorphisms, though other gene polymorphisms have also been associated with severe CD types. Research has shown that CD patients with two or more polymorphisms have a significantly higher risk of developing the severe CD phenotype.

Elevated levels of serological markers, including ASCA, anti-flagellin, OmpC and GM-CSF antibodies also appear to predict the severe CD phenotype. In addition, the presence of more than one serological marker in the same patient is associated with an exponential increase in the risk of complicated CD. Combined defects in innate and adaptive immunity give a synergistically increased risk of stricturing and penetrating disease types as shown by the increased risk of severe CD in patients having both NOD2 polymorphisms and elevated levels of serological markers.

Though the evidence linking these markers with severe CD is increasing, there are as yet no strict recommendations as to whether these patients should undergo early, aggressive immunosuppression with the "top-down" approach. Through the early use of anti-TNFα therapy, the risk of developing stricturing and penetrating disease types, and therefore also the risk of surgery, may be decreased [82]. However, further evidence from randomized controlled trials of the effect of aggressive immunosuppression in patients with these biomarkers is needed. Expert consensus opinion on which biomarkers should be analyzed upon CD diagnosis and how many biomarkers are required to justify the "top-down" therapeutic approach is required. The levels at which serological markers will predict an increased risk for severe CD must also be standardized. In addition, these biomarkers need to become more easily available before they can be recommended for routine clinical use.

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