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Increased Immune Reactivity Predicts Aggressive Complicating Crohn's disease in Children

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Abstract

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Background and Aim—The ability to identify children with Crohn's disease who are at highest risk for rapid progression from uncomplicated to complicated phenotypes would be invaluable in guiding initial therapy. Aim: To determine whether immune responses and/or CARD15 variants are associated with complicated disease phenotypes and predict disease progression.

Methods—Sera were collected from 796 pediatric CD cases and tested for anti-Cbir1 (flagellin), anti-outer membrane protein C (anti-OmpC), anti-*Saccharomyces-cerevisiae* (ASCA) and perinuclear anti-neutrophil cytoplasmic antibody (pANCA) using ELISA. Genotyping (TaqmanMGB) was performed for 3 CARD15 variants (SNPs 8, 12, 13). Associations between immune responses (antibody sum (AS) and quartile sum score (QSS), CARD15, and clinical phenotype were evaluated.

Results—32% of patients developed at least one disease complication within a median of 32 months and 18% underwent surgery. The frequency of internal penetrating (IP), stricturing (S) and surgery significantly increased (p trend < 0.0001 for all 3 outcomes) with increasing AS and QSS. 9% of seropositive groups had IP/S vs. 2.9% in the seronegative group (p=0.01). 12% of seropositive groups underwent surgery vs. 2% in the seronegative group (p=0.0001). The highest AS group (3) and QSS group (4) demonstrated the most rapid disease progression (p < 0.0001). Increased hazard ratio was observed for AS group 3 (7.8 [2.2–28.7] p < 0.002 and QSS group 4 (11.0 [1.5,83.0] p < 0.02).

Conclusions—The rate of complicated CD increases in children as the number and magnitude of immune reactivity increases. Disease progression is significantly faster in children expressing immune reactivity.

INTRODUCTION

The currently accepted etiopathogenic hypothesis for inflammatory bowel disease (IBD) proposes the role of genetics, altered immune responses and environmental factors for disease susceptibility and development. These factors and their interactions may also be important determinants of disease phenotype and disease progression. Childhood-onset inflammatory bowel disease, Crohn's disease (CD) in particular, may be an indicator of increased genetic predisposition leading to a higher penetrance and is often described as having a more severe disease phenotype. Parallel with both the clinical and genetic heterogeneity of CD, studies have shown immune response heterogeneity exists among CD patients (1). Landers et al suggested that there are subsets of patients with differing immune responses to microbial antigens; antibodies to the E.coli outer-membrane porin C (OmpC), as well as to *Saccharomyces cerevisiae* (ASCA), and autoantigens, i.e. perinuclear anti-neutrophil antibody (pANCA). The research by Vasilias et al was the first to introduce the concept of CD phenotypes stratified by different immune responses (2,3). Subsequent studies demonstrated an association between ASCA, anti-OmpC and anti CBir1 (anti-flagellin) with complicated phenotypes (4,5) These cross sectional analyses were the first to demonstrate in adult CD cohorts that both the number of immune responses to the different microbial antigens, as well as the magnitude of these immune responses (antibody level), correlated most significantly with the presence of complicated CD phenotypes. More recent pediatric cohort studies suggest that these markers are present in patients before a complication occurs and thus predictive of disease progression from uncomplicated to complicated state (6,7). The concept of increasing immune reactivity predicting disease progression was first evaluated in an initial cohort of pediatric CD (6). Survival analysis demonstrated that reactivity to at least one microbial antigen was predictive of the development of internal penetrating and/or stricturing (IP/S) disease faster as compared to patients negative for all markers. Amre *et al* also studied a prospective cohort and demonstrated that the time to first surgery was more rapid for ASCA positive patients (7). The small number of patients in these existing studies, however, limited the number of

subgroups evaluated and a larger cohort such as the one presented in this paper provides a more robust evaluation of the true predictive nature of both number and magnitude of immune responses.

The research to date supports the hypothesis that immune reactivity in CD patients may be a risk factor or indicator of disease progression from uncomplicated to complicated disease behavior. Predicting risk for disease progression is important in the management of pediatric CD and preliminary data cited above require verification in a larger pediatric cohort. In the present study we set out to determine whether immune responses and/or variants in NOD2/CARD15 are associated with complicated disease phenotypes and predict rapid disease progression in a large multicenter prospectively recruited pediatric cohort.

MATERIALS AND METHODS

Patient population

Pediatric CD patients were enrolled from 21 participating sites of the Western Regional Pediatric IBD Research Alliance, The Pediatric IBD Collaborative Research Group and the Wisconsin Pediatric IBD Alliance (6, 8, 9)

In order to be eligible, all CD patients must have undergone complete colonoscopy with ileal intubation or complete colonoscopy and small bowel follow through. A diagnosis of CD for this study was based on standard diagnostic criteria (10). Blood was drawn at each of the participating sites and sent to The Immunobiology Institute at Cedars-Sinai Medical Center (CSMC) for all sites in the Western Regional and Wisconsin Alliance. Serological analyses were run at Prometheus laboratory (San Diego, CA) for patients drawn at sites of the Pediatric IBD Collaborative Research Group. Genotyping was performed at the Genotyping Core Facility of the Medical Genetics Institute/GCRC at CSMC for all Western Regional sites, at the Children's Hospital of Wisconsin (SK) for the Wisconsin Alliance, and at Prometheus Laboratory for all sites of The Pediatric IBD Collaborative Research Group. This study was approved by the Ethics Review Board at all participating sites.

Data collection

Demographic and clinical data were stored in a secure database. Key variables included: gender, date of diagnosis, age at diagnosis, date of last follow up and duration of disease as of last follow up, ethnicity, family history, disease location (small bowel only, large bowel only, small and large bowel, upper digestive tract and perianal location), disease behavior (non complicated [non penetrating non stricturing (NPNS)] vs. complicated [internal penetrating (IP), stricturing (S) and perianal penetrating (PP)], and surgical history.

Immune Responses

All blood samples were taken at the time of consent and enrollment. Sera were analyzed for expression of pANCA, ASCA, anti-OmpC, and anti-CBir1 antibodies in a blinded fashion by ELISA as previously described (1,4–6). Serological analyses were performed at CSMC or Prometheus Laboratories using the same technology. Antibody levels were determined and results expressed as ELISA units (EU/ml), which are relative to a Cedars-Sinai Laboratory or a Prometheus Laboratory Standard which is derived from a pool of patient sera with well-characterized disease found to have reactivity to this antigen. The following definitions were used for all analyses involving ASCA, anti-OmpC and anti-CBir1 immune responses. pANCA was analyzed separately given that pANCA has been shown to be negatively associated with the majority of disease phenotypes except large bowel disease location (4)

Antibody sum (AS): number of positive antibodies per individual: 0, or 1 or 2, or 3 positive

Antibody Quartile Score: quartile score for each antibody level (< 25% =1, 25–50% =2, 51%– < 75%=3, 75%–100%=4).

Quartile Sum Score(QSS): sum of quartiles score for all 3 antibodies (ASCA (A or G, anti-OmpC and anti-CBir1). Minimum score of 3 (all antibodies had a quartile score of 1) and maximum score of 12 (all antibodies had a quartile score of 4).

Quartile Sum Score (QSS) Group: In order to minimize the number of patient subsets i.e quartile sum score 3–12, we have regrouped patients based on a range of quartile sum scores: Quartile sum score 3–5 = group 1, 6–7 = group 2, 8–9 = group 3 and 10–12 = group 4.

Genotyping

CARD15 SNP's R675W (rs2066844, CEPH-IBD1-snp8), G881R (rs2066845, CEPH-IBD1-snp12), and 3020insC (rs2066847, CEPH-IBD1-snp13) were adapted to the TaqMan MGB genotyping platform following the manufacturer's instructions and using PrimerExpress design software (Applied Biosystems, Foster City, CA) as previously described (11–13).

Statistical Analysis

To determine the associations between disease phenotype and immune responses, univariate analyses using χ^2 tests were performed. Odds ratios (OR) and 95% confidence intervals were calculated to compare the odds of positive antibodies to CBir1, OmpC, and ASCA in the group of patients with a certain disease characteristic with the group of patients without such a characteristic. For the OR calculations the minimum antibody sum of 0, the minimum quartile sum score of 3 and the minimal quartile sum score group 1 were set as baseline, i.e. OR of 1.0. Quantitative comparison of immune response levels between groups for each antibody was performed using non-parametric Wilcoxin Rank test. Stepwise multivariable analysis using logistic regression modeling determined the primary associations among qualitative serological responses with disease phenotypes. Kaplan-Meier estimator of survival probability was calculated to construct survival curves and the log-rank test was used to test if the survival curves were significantly different between subgroups of patients (14). The hazard ratio (HR) of occurrence of complication or surgery among patients who were sera positive compared to those who were sera negative as well as who were in higher antibody sum or quartile sum group compared to those who were in baseline group were estimated from Cox's proportional hazards model and adjusted for all other covariates (14). All HRs were expressed as a point estimate with 95% confidence interval. Age at diagnosis and gender were included as covariates in all the multivariable analyses. The OR/HR for age at diagnosis was explained as the times of odds/hazards increase (e.g. OR-1) per one year older at diagnosis. All analyses were performed by using Statistical Analysis Software (Version 9.1; SAS Institute, Inc., Cary, NC).

RESULTS

Patient demographics

A total of 796 pediatric CD patients were eligible for analysis. The median age at diagnosis was 12 [0.6–18] years and the median disease duration as of last follow up was of 32 [1–235] months. The cohort was comprised of 56% males and 44% females and 87% were Caucasians and 28% were of Jewish background.

Clinical phenotypes

A total of 236 (30.3%) patients presented with (96/796 [12%]) or developed (140/796[18%]) at least one disease complication within the median follow up time of 32 months (Table 1). One hundred and forty patients (18%) underwent a CD related surgery of which 89 were small bowel resections. Of the remaining surgeries: a total of 42 were involving perianal penetrating disease; 24 patients underwent colectomy and 3 patients a limited colonic resection. Fifteen patients had more than one surgery.

Immune response and genotype frequencies

Serum was collected at the time of diagnosis or within 1 month of diagnosis in 18% (146/796) of patients and 30% (241/796) within 3 months of diagnosis. The remaining patients had serum collected greater than 3 months from time of diagnosis. The median time to sera collection for all patients was 10 [0–180] months. A total 73 % of patients were positive for at least one microbial driven immune response (ASCA, anti-OmpC or anti-Cbir1), 27% of whom were positive for a combination of any 2 of these immune responses and 8% of patients were positive for all 3 responses. ASCA, anti-OmpC, anti-CBir1 and pANCA were present in 45%, 18%, 52%, and 19% respectively. NOD2/CARD15 (any variant) was observed in 34% of patients (25% heterozygote and 9% homozygote or compound heterozygote).

Cross sectional analyses

Univariate analysis for the association between immune responses and NOD2/CARD15 genotype and disease location and disease behavior is illustrated in table 2 and table 3. Figures 1A and 1B illustrate the cumulative influence of number of immune responses (antibody sum) as well as the magnitude of the immune response (quartile sum score group) on disease behavior. The frequency of internal penetrating, stricturing disease and surgery significantly increased (p trend < 0.0001) as the number of immune responses increased (antibody sum 0–3) (Figure 1A) and magnitude of immune response (quartile sum score group 1–4) increased (Figure 1B). The odds ratios for the 3 disease behaviors and surgery associated with antibody sum and quartile sum score groups are shown in Table 4.

Multivariable analysis confirmed the association of small bowel location with ASCA (OR [95%CI]:2.3 [1.6–3.2]; p <0.0001), anti-CBir1 (OR 1.5 [1–1.2]; p =0.03), pANCA (OR: 0.6 [0.4–0.9]; p = <0.007); and NOD2/CARD15 (OR: 1.7 [1.1–2.4]; p =0.007). Large bowel location was associated with pANCA (OR: 2.8 [1.4–5.4]; p <0.004). Table 5 illustrates the results of the multivariable analysis for the independent associations with disease behavior and surgery. All individual antibodies were included in the model as well as a single unit change in antibody quartile sum score as a co-variable (e.g. increase in score of 3 to 4). There was a significant association seen with quartile sum score change and complicating disease behaviors as well as surgery, such that for each unit of quartile sum increase the OR increased by 1.3 for internal penetrating and stricturing disease and 1.2 for surgery. The difference between a score of the minimum 3 and the maximum score of 12 equates to an OR of 10.6 (= 1.3)⁹ and 5.2 (= 1.2)⁹, respectively. Quartile sum score was not independently associated with small bowel disease location as compared to the presence of the individual antibodies as noted above. Additional independent associations were found between female gender and older age at diagnosis (Table 5).

Predictors of Disease progression

For the second aim of our study, we set out to examine whether seropositive patients (1, 2, or 3 positive for ASCA, anti-OmpC and/or anti-CBir1) had a greater risk to progress to internal penetrating and/or stricturing (IP/S) disease as well as to surgery, as compared to

seronegative patients (0 such immune responses). We used a longitudinal study to answer this question which included only those patients who *did not* have IP/S or surgery at diagnosis (NPNS +/- PP) *and* continued to have uncomplicated disease status at the time the serum was collected for immune response measurement. A total of 536 patients met these inclusion criteria and the median time from diagnosis to serum draw was 10 [0–211] months. A total of 90 of 536 developed IP/S in follow up; however 59% (53 patients) were eliminated from this analysis as they had immune responses collected after the complication occurred. Among the 37 patients who developed IP/S during the follow-up after serum was drawn, the median [range] time from diagnosis to the onset of IP/S was 26 [4–108] months. Thirty two of the 363 seropositive patients (9 %) had IP/S vs. only 2.9% (5/173) in the seronegative group ($p=0.01$). Among the 61 patients who underwent surgery (any CD related surgery after serum was drawn) the median [range] time from diagnosis to surgery was 30 [1–105] months. Twelve per cent (57/464) of the seropositive (at least one positive) patients had undergone surgery vs. only 2% (4/189) in the seronegative group ($p=0.0001$). Because longer disease duration increases the chance of developing IP/S as well as surgery, and not all patients were followed for the same amount of time, we performed survival analysis to take the length of follow-up into consideration (Figure 2A–2D). The Kaplan-Meier survival analysis, followed by the log-rank test for the different antibody sum and quartile sum score group comparisons, showed that overall survival times for IP/S and CD-related surgery were significantly lower for those positive for immune responses, and this was true when both the quantity of immune responses and magnitude of those responses were assessed. Given the same length of follow up, among those patients with antibody sum greater than 1, more progressed to IP/S than those negative for all 3 or positive for *only* 1 antibody ($p < 0.0001$). In other words, those patients positive for at least 2 immune responses (antibody sum 2 or 3) progressed to IP/S faster than those negative for all or positive for only 1 antibody. The group positive for all 3 antibodies demonstrated the most rapid disease progression with a median [range] time to disease progression of 20 [4–65] months. The same rapid progression to surgery was seen among the higher antibody sum group (Figure 2B). Like antibody sum, those patients in the highest quartile sum score group (group 4 = Quartile sum score 10–12) progressed faster to IP/S and surgery and the median [range] time to IP/S and surgery was 21[4–65] months and 27 [1–93] months, respectively (Figures 2C and 2D). The survival curves were very similar when evaluating intestinal resection only ($n=48$) as compared to any CD surgery ($n=61$) (Log Rank: $p < 0.0001$ for the 4 antibody sum groups and $p = 0.001$ when comparing survival among the 4 quartile sum groups) (data not shown). The most conservative way to evaluate the predictive abilities of immune response was to limit inclusion in the survival analysis to only patients whose serum was drawn before a complication or surgery. Given the inconsistent data on whether serologies changes after a complication has occurred, we did however also perform survival analysis on all 90 patients who developed IP and/or S in follow up regardless of when serologies were drawn. For both antibody sum and quartile sum score group, the results showed a significantly higher number of patients progressing to complication faster in the face of seropositivity (Figures 3A and 3B).

The predictive ability of immune responses for rapid progression to the first IP/S or surgical event was further evaluated by fitting Cox-proportional hazards models (Table 6 and 7). Hazard Ratios for all CD related surgeries as well as for intestinal resections only were calculated controlling for both disease location and disease complication (IP, S and PP). When examining intestinal resection surgery, an increased hazard was observed for antibody sum 3 (7.8 [2.2–28.7]; $p < 0.002$ and quartile sum score group 4 (11.0 [1.5–83.0]; $p < 0.02$).

Discussion

Longitudinal analysis in our large pediatric multicenter cohort demonstrated that disease progression from uncomplicated to internal penetrating and/or stricturing disease phenotypes and CD related surgery is accelerated in the presence of anti-microbial immune reactivity. Both the number of immune responses as well as the magnitude of immune response to various microbial antigens was predictive of aggressive disease phenotypes based on the results from the subgroup of patients who had serologies drawn before a complication occurred and were evaluated in a longitudinal manner. The cross sectional analyses which evaluated all patients with a complication at any time in the course of disease also demonstrated a strong association between immune responses and the presence or absence of internal penetrating and or stricturing disease and surgery. This is the largest pediatric cohort to report on the prognostic potential of serum biomarkers in early onset Crohn's disease.

Our results confirm and extend previous observations that serum immune responses are associated with complicated disease phenotypes in both children and adults (3–7, 15). This concept was adopted by Dotan et al and applied to a large CD cohort investigating a novel panel of anti-glycan antibodies and similar associations were found with disease behavior (16). Like other studies that have preceded ours, the cross sectional analyses are impressive in demonstrating a strong association between immune responses and disease behavior (4,15). However, the potential variability in immune responses over time and the fact that a complication itself can lead to an alteration in mucosal permeability and hence possibly to variation in sero-reactivity to microbial antigens remained valid concerns that might call into question the predictive abilities of immune responses when evaluating studies that used cross sectional analyses only. The prospective component of our study was done to address these questions. We recognize the possibility that we would not have known if a sub-clinical complication had already developed at the time serum was drawn. Nevertheless, we feel that the results from the prospective component of our study strongly support the predictive abilities of immune responses. The group positive for all 3 antibodies and those patients with the highest magnitude of response (the highest quartile sum group 4) demonstrated the most rapid disease progression.

The ability to stratify patients relative to risk of disease progression from the time of diagnosis is crucial in developing a treatment plan and for explaining the risk:benefit of these treatments to patients and families. Natural history observations in children (17) have prompted the increasing use of biologic therapy that has shown efficacy in inducing as well as maintaining response and remission in children (18). The development of lymphoma, particularly a rare almost uniformly fatal hepatosplenic T cell lymphoma (19) in individuals receiving infliximab concomitantly with immunomodulators have resulted in clinicians wanting to carefully select those patients who are appropriate candidates for these therapies. Current controversy exists concerning the relative value of “step up” versus “top down” therapy. To date, the top down strategy has been defined by use of anti-TNF α therapy in patients whose disease activity necessitates corticosteroids (20), which may be better defined as an early intervention strategy. Taking this one step further, the identification of those patients at greatest risk for rapid disease progression would be of great value in stratifying patients into more or less aggressive treatment paradigms at the time of diagnosis and would not be based on disease activity or need for corticosteroids to guide therapy. Our data suggest that markers of immune reactivity can identify those children most likely to benefit from the early use of highly effective therapies with the goal of altering the natural history of disease. Risk stratification models incorporating clinical, immune, genetic and bacteriological risk markers in children with newly diagnosed Crohn's disease are needed.

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Abbreviations

ASCA	anti-Saccharomyces cerevisiae antibodies
pANCA	perinuclear anti-neutrophil cytoplasmic antibodies
OmpC	outer membrane protein C
I2	Pseudomonas fluorescens-associated sequence
OR	odds ratio
SNP	single nucleotide polymorphism
CD	Crohn's disease
CI	confidence interval
OCTN	organic cation transporter
NOD	nucleotide binding domain
IP	internal penetrating disease
S	stricturing disease
NPNS	non-penetrating, non-stricturing disease
PP	perianal penetrating

References

1. Landers CJ, Cohavy O, Misra R, et al. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology*. 2002; 123:689–699. [PubMed: 12198693]
2. Vasilias EA, Plevy SE, Landers CJ, et al. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology*. 1996; 110:1810–1819. [PubMed: 8964407]
3. Vasilias EA, Kam LY, Karp LC, et al. Marker antibody expression stratifies Crohn's disease into immunologically homogeneous subgroups with distinct clinical characteristics. *Gut*. 2000; 47:487–496. [PubMed: 10986208]
4. Mow WS, Vasilias EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology*. 2004; 126:414–424. [PubMed: 14762777]
5. Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology*. 2005; 128:2020–2028. [PubMed: 15940634]
6. Dubinsky MC, Lin YC, Dutridge D, et al. Serum immune responses predict rapid disease progression among children with Crohn's disease: immune responses predict disease progression. *Am J Gastroenterol*. 2006; 101:360–367. [PubMed: 16454844]

7. Amre DK, Lu SE, Costea F, et al. Utility of serological markers in predicting the early occurrence of complications and surgery in pediatric Crohn's disease patients. *Am J Gastroenterol*. 2006; 101:645–652. [PubMed: 16464223]
8. Kugathasan S, Maresso K, Collins N, et al. L1007Fsinc variant of CARD15/NOD2 is strongly associated with early onset and fibrostenosing behavior in pediatric Crohn's disease. *Clin Gastroenterol Hepatol*. 2004; 2:1003–1009. [PubMed: 15551253]
9. Markowitz J, Hyams J, Mack D, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn's disease. *Clin Gastroenterol Hepatol*. 2006; 4:1124–1129. [PubMed: 16861053]
10. Loftus EV, Silverstein MD, Sandborn WJ, et al. Crohn's disease in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. *Gastroenterology*. 1998; 114:1161–1168. [PubMed: 9609752]
11. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*. 2001; 411:599–603. [PubMed: 11385576]
12. Afonina I, Zivarts M, Kutuyav I, et al. Efficient priming of PCR with short oligonucleotides conjugated to a minor groove binder. *Nucleic Acids Research*. 1997; 25:2657–2660. [PubMed: 9185578]
13. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal*. 1999; 14:143–149. [PubMed: 10084106]
14. Kleinbaum, DG.; Klein, M. survival Analysis: A Self learning text. 2nd edition. New York: Springer; 2005.
15. Arnott ID, Landers CJ, Nimmo EJ, et al. Sero-reactivity to microbial components in Crohn's disease is associated with disease severity and progression, but not NOD2/CARD15 genotype. *Am J Gastroenterol*. 2004; 99:2376–2384. [PubMed: 15571586]
16. Dotan I, Fishman S, Dgani Y, et al. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology*. 2006; 131:366–378. [PubMed: 16890590]
17. Gupta N, Cohen SA, Bostrom AG, et al. Risk factors for initial surgery in pediatric patients with Crohn's disease. *Gastroenterology*. 2006; 130:1069–1077. [PubMed: 16618401]
18. Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology*. 2007; 132:863–873. [PubMed: 17324398]
19. Mackey AC, Green L, Liang LC, et al. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. *J Pediatr Gastroenterol*. 2007; 44:265–267.
20. Hommes D, Baert F, van Assche G, et al. Management of Recent Onset Crohn's Disease: A controlled randomized trial comparing Step-up and Top-down therapy. *Gastroenterology*. 2005; 129:371.

Figure 1.

Figure 1A: Frequency of non-penetrating non-stricturing disease, internal penetrating disease, stricturing disease and surgery amongst the different antibody sum groups. P trend < 0.0001

Figure 1B: Frequency of non-penetrating non-stricturing disease, internal penetrating disease, stricturing disease and surgery amongst the different quartile sum score groups. P trend < 0.0001

Figure 2.

Figure 2A: Survival estimates for internal penetrating and/or stricturing disease by antibody sum 0, vs. 1, vs. 2, vs. 3 (prospective patients only). P value < 0.0001

Figure 2B: Survival estimates for CD related surgery by antibody sum 0, vs. 1, vs. 2, vs. 3 (prospective patients only). P value < 0.0001

Figure 2C: Survival estimates for internal penetrating and/or stricturing disease by quartile sum score (QSS) group 1, vs. 2, vs. 3, vs. 4 (prospective patients only). P value < 0.0001

Figure 2D: Survival estimates for CD related surgery by quartile sum score (QSS) group 1, vs. 2, vs. 3, vs. 4 (prospective patients only). P value < 0.0001

Figure 3.

Figure 3A: Survival estimates for internal penetrating and/or stricturing disease by antibody sum 0, vs. 1, vs. 2, vs. 3 (all patients). P value < 0.0001

Figure 3B: Survival estimates for internal penetrating and/or stricturing disease by quartile sum score (QSS) group 1, vs. 2, vs. 3, vs. 4 (all patients). P value < 0.0001

Table 1

Patient Demographics

Clinical Phenotype	N (%)
Disease location (n=796)	
Small bowel only	112 (14)
Large bowel only	249 (31.2)
Small and large bowel	430 (54.0)
and /or upper tract	287 (36)
and/or anal disease	167 (21)
Disease Behavior (N= 780) *	
Non-penetrating Non-Stricturing	544 (69.7)
Stricturing only	71 (9.1)
Stricturing and internal penetrating	24 (3.0)
Stricturing and perianal penetrating	11 (1.4)
Internal Penetrating only	26 (3.3)
Internal penetrating and perianal penetrating	10 (1.3)
Perianal penetrating only	84 (11)
Stricturing and internal and perianal penetrating	10 (1.3)

* Disease Behavior as of last follow up was not available for 16 patients

Table 2

Associations of Immune Responses with Disease Location: Univariate analysis

	Small Bowel OR[95%CI]* P value	Large Bowel OR[95%CI] P value	Upper Tract OR[95%CI] P value	Perianal OR[95%CI] P value
ASCA	2.9 [21.-4.0] < 0.0001	0.67 [0.44–0.99] 0.04	NS	1.5 [1.1–2.2] <0.02
Anti-OmpC	NS	NS	NS	NS
Anti-CBir1	1.6 [1.2–2.3] 0.002	NS	NS	NS
pANCA	0.44 [0.30–0.63] < 0.0001	4.0 [1.8–8.8] <0.0001	NS	NS
NOD2/CARD15	1.9 [1.4–2.7] <0.0001	NS	NS	NS

*OR [95%CI]: Odds Ratio and 95% confidence intervals

Table3

Associations of Immune Responses with Disease Behavior and Surgery: Univariate analysis

	NPNS OR[95%CI]* P value	IP OR[95%CI] P value	S OR[95%CI] P value	PP OR[95%CI] P value	Surgery OR[95%CI] P value
ASCA	0.45 [0.3–0.6] 0.0001	2.3 [1.4–3.9] 0.002	2.4 [1.6–3.7] 0.0001	NS	2.2 [1.5–3.2] 0.0001
Anti-OmpC	0.37 [0.3–0.5] 0.001	3.7 [2.2–6.4] 0.0001	2.7 [1.7–4.3] 0.0001	1.6 [0.99–2.6] 0.05	4.0 [2.6–6.1] 0.0001
Anti-CBir1	0.56 [0.4–0.8] 0.001	2.3 [1.3–4.1] 0.003	2.0 [1.3–3.1] 0.002	NS	2.0 [1.3–3.0] 0.001
pANCA	1.8 [1.1–2.7] 0.01	NS	0.45 [0.2–0.9] 0.02	0.50 [0.3–0.95] 0.03	NS
NOD2/CARD15	NS	NS	NS	NS	NS

*OR [95%CI]: Odds Ratio and 95% confidence intervals

Table 4

Odds Ratios for disease behavior and antibody sum* and quartile sum score groups** : Univariate analysis

	Antibody Sum 1 OR [95%CI]*	Antibody Sum 2 OR [95%CI]	Antibody Sum 3 OR [95%CI]	P value	Quartile Sum Score Group 2 OR [95%CI]**	Quartile Sum Score Group 3 OR [95%CI]	Quartile Sum Score Group 4 OR [95%CI]	P value
Non penetrating Non stricturing	0.40 [0.3–0.7]	0.30 [0.2–0.4]	0.10 [0.07–0.3]	<0.0001	0.3 [0.2–0.5]	0.3 [0.1,–0.4]	0.2 [0.1–0.3]	<0.0001
Internal Penetrating	2.2 [0.9– 5.8]	5.2 [2.1–12.9]	9.5 [3.4–26.4]	<0.0001	3.2 [1.0–9.8]	4.0 [1.3–12.2]	8.5 [2.9–24.9]	<0.0001
Stricturing	1.7 [0.9–3.4]	4.2 [2.2–8.1]	6.1 [2.7–13.5]	<0.0001	6.8 [2.3–19.9]	8.6 [2.9–24.9]	12.5 [4.3–35.9]	<0.0001
Surgery	2.7 [1.4–5.2]	4.5 [2.4–8.8]	10.3 [4.8–22.1]	<0.0001	4.4 [2.0–9.7]	4.8 [2.1–10.6]	8.4 [3.8–18.5]	<0.0001

* Baseline antibody sum is 0 (OR = 1.0)

** Baseline quartile sum score group is 1 (OR = 1.0)

Table 5

Associations with disease behavior: multivariable analysis

Variables	NPNS OR [95%CI]* P value	IP OR [95%CI] P value	S OR [95%CI] P value	PP OR [95%CI] P value	Surgery OR [95%CI] P value
Female gender	NS	2.7 [1.5–4.7] 0.0006	1.6 [1.0–2.5] <0.04	0.6 [0.40–0.96] 0.03	1.7 [1.1–2.6] <0.009
Age at Diagnosis	0.9 [0.90–0.99] <0.02	1.1 [1.0–1.3] 0.004	NS	NS	NS
Anti-OmpC	NS	NS	NS	NS	2.6 [1.6–4.2] 0.0002
pANCA	2.0 [1.2–3.2] 0.005	NS	0.37 [0.2–0.8] <0.007	NS	NS
Quartile Sum score (Δ OR/Unit)	0.80 [0.7–0.9] < 0.0001	1.3 [1.2–1.5] <0.0001	1.3 [1.2–1.4] <0.0001	1.1 [1.0–1.2] 0.008	1.2 [1.1–1.3] <0.002

* OR [95%CI]: Odds Ratio and 95% confidence intervals

Table 6

Hazard Ratios for Disease Complication and Surgery and Immune Responses

	IP and/or S Disease Hazard Ratio [95% CI] p value	Any CD-Related Surgery Hazard Ratio [95%CI] p value
Antibody Sum (Baseline Risk of Antibody Sum = 0)		
ASCA	NS	3.2 [1.1,9.5] < 0.04
Anti- OmpC	2.4 [1.2,4.9] 0.01	2.2 [1.3,3.8] < 0.004
Anti-CBir1	2.5 [1.2, 5.2] < 0.02	NS
pANCA	0.16 [0.04,0.70] < 0.02	NS

Table 7

Hazard Ratios for Disease Complication and Surgery According to Antibody Sum and Quartile Sum Group

	IP and/or S Disease Hazard Ratio [95% CI] p value	Any CD-Related Surgery Hazard Ratio [95%CI] p value
Antibody Sum (Baseline Risk of Antibody Sum = 0)		
Antibody Sum 1	1.1 [0.3, 3.7] NS	3.2 [1.1,9.5] < 0.04
Antibody Sum 2	5.5 [2.0,15.2] 0.005	3.4 [1.2,10.1] < 0.03
Antibody Sum 3	6.0 [1.7, 20.5] < 0.005	6.6 [2.1,20.6] 0.001
Quartile Sum Group (Baseline Risk of Quartile Sum Score Group 1 (= Quartile sum score 3–5))		
Quartile Sum Score Group 2 (= Quartile sum score 6–7)	3.4 [0.7, 16.4] NS	4.7 [1.1, 20.5] 0.04
Quartile Sum Score Group 3 (= Quartile sum score 8–9)	5.2 [1.1, 23.7] 0.03	3.5 [0.8,15.4] NS
Quartile Sum Score Group 4 (= Quartile sum score 10–12)	10.0 [2.3, 43.4] 0.002	7.1 [1.7,30.5] 0.008