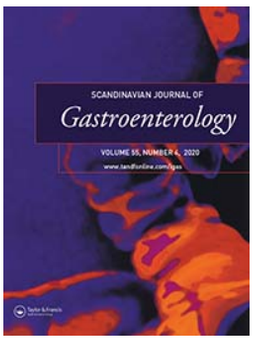

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ORIGINAL ARTICLE

Serological antibodies and surgery in a population-based inception cohort of Crohn's disease patients – the IBSEN study

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ABSTRACT

Introduction: Serological antibodies have been associated with complicated disease course in Crohn's disease (CD), including the need for surgery.

Aim: The aim of this study was to investigate if a panel of relevant antibodies could predict surgery in a prospective population-based cohort of patients with CD.

Methods: The population-based IBSEN cohort has been followed prospectively for 20 years. At the 10- and 20-year follow-up, the following panel of serological antibodies was analysed: pANCA, ASCA IgA, ASCA IgG, anti-OmpC, anti-I2, and anti-CBir1. At the 20-year follow-up or until lost to follow-up, all CD-related surgeries were registered.

Results: Serum was available from 159 patients at 10-year follow-up and 135 patients at 20-year follow-up. In 113 patients, serum was available at both time points. No significant change of antibody status (positive vs. negative) was found from 10-year to 20-year follow-up. Negative pANCA, positive ASCA IgA and positive ASCA IgG at 10-year follow-up were all individually associated with increased risk for CD-related surgery. There was no association between anti-OmpC, anti-I2 or anti-CBir1 and CD-related surgery. In a multiple regression model including disease location and behaviour, only stricturing or penetrating disease behaviour and negative pANCA remained significantly associated with higher odds for surgery.

Conclusion: Positive ASCA IgA and IgG, and negative pANCA were associated with higher odds for CD-related surgery in univariate analysis. Since disease phenotype changes during the disease course, while serological antibodies are stable, our results support the use of pANCA, ASCA IgA and ASCA IgG as prognostic markers in CD.

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Introduction

Complications of Crohn's disease (CD) are common and include intraluminal strictures, abscesses and fistulae. These complications are usually handled with surgical procedures, and more than one third of the patients require surgery within 10 years from diagnosis [1,2]. In the era of biological therapy, it is suggested that early start of treatment may reduce the need for future surgery [3]. However, increased costs and risk of side effects may be the downside of this strategy; thus, high-risk patients should be identified early in the disease course. Consequently, biomarkers predicting a complicated disease course are requested.

Seroreactivity to specific antigens, both autoantigens and microbial antigens, is seen in subgroups of patients with inflammatory bowel disease (IBD) and have attracted attention as potential non-invasive biomarkers with a diagnostic and prognostic potential. Such antibodies as anti-*Saccharomyces cerevisiae* antibody (ASCA) and atypical perinuclear antineutrophil cytoplasmic antibody (pANCA) have been widely studied [4]. However, studies have shown diverging results regarding diagnostic specificity and stability of titres over time [5–8], questioning the clinical importance of these antibodies. Studies of both short- and long-term stability of serological antibodies are therefore crucial to estimate the diagnostic significance as well as prognostic impact of such markers. Current guidelines do, so far, not recommend routine analysis of any single serological antibody due to limited diagnostic accuracy [9]. The long-term stability and predictability of such antibodies applied in population-based inception cohorts require further studies. Also, panels combining several antibodies in a combination model or a risk matrix model may improve the clinical use as predictors of disease outcome in IBD.

The purpose of the present study was therefore:

1. To assess the long-term stability of a panel of serological antibodies at 10 and 20 years after diagnosis in a population-based inception cohort of CD patients (the IBSEN cohort).

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2. To investigate associations between these serological antibodies and CD-related surgery in the IBSEN cohort that has been followed for 20 years after diagnosis.

Materials and methods

Study population

The IBSEN cohort is a population-based inception cohort of patients diagnosed with IBD in four counties of Norway in the period of 1 January 1990 to 31 December 1993. Details about the establishment of the cohort are previously described [10]. The cohort included a total of 756 patients with IBD, 519 UC patients and 237 CD patients. Prescheduled follow-up visits, including collection of clinical data, were performed 1, 5, 10 and 20 years after inclusion. If a patient missed a study visit, clinical information was collected from the patient's record. In the presented study, the CD patients were followed for 20 years or until lost to follow-up. Disease course and CD-related surgery (bowel resection, colectomy, fistula and abscess surgery) were recorded. Before the 10-year follow-up, patients were asked to provide blood samples for biobanking. This was repeated at the 20-year follow-up. Serum samples from these two time points were analysed in the present project.

Laboratory analyses

At the 10- and 20-year follow-up, serum samples were collected and stored until examination in one single batch. The following panel of serological antibodies was analysed: atypical perinuclear antineutrophil cytoplasmic antibody (pANCA), anti-*Saccharomyces cerevisiae* antibody (ASCA) IgA and IgG, anti-*Escherichia coli* outer membrane porin C (anti-OmpC) IgA, anti-Pseudomonas fluorescens-associated sequence I2 (anti-I2) and anti-flagellin expressed by Clostridial phylum (anti-CBir1) IgG. All analyses were performed at Prometheus Laboratories Inc. (San Diego, CA, USA). ASCA IgA, ASCA IgG, anti-OmpC, anti-I2 and anti-CBir1 were analysed using enzyme-linked immunoassay (ELISA) and values expressed in ELISA units (EU). Reference values (EU/ml) were provided by Prometheus Laboratories: ASCA IgA <8.5, ASCA IgG <17.8, OmpC IgA <10.9, CBir1 IgG <78.4 and I2 <368. Values above the reference range of the relevant antibody were considered positive. Analysis of pANCA was performed using indirect immunofluorescence on polymorphnuclear leukocytes and expressed as detected (positive) or not detected. The laboratory methods and reference ranges have been previously described [11,12].

Statistical analyses

Continuous data are presented as median with ranges, categorical data as counts and percentages. Possible differences between pairs of variables were assessed using the chi-square test (categorical data) and Mann–Whitney–Wilcoxon test (continuous data). Wilcoxon signed-ranks test was used to detect changes in biomarker status (positive vs. negative) from 10- to 20-year follow-up. Associations between gender, age at diagnosis, disease location, disease behaviour, serological antibodies and need of CD-related surgery were assessed using univariate logistic regression. Variables that were statistically significant in univariate regressions were entered into a multiple logistic regression model. The results are expressed as odds ratio (OR) with 95% confidence intervals (CI) or probabilities for surgery given selected levels of covariates. The risk matrix model was constructed of biomarkers that were stable and significantly associated with probability of surgery, and results are expressed as probabilities (%) with 95% CI. p values $<.05$ were considered statistically significant and all tests were two-sided. Since our study was exploratory in nature, we did not perform any correction for multiple testing. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (Armonk, NY, USA).

Ethical considerations

The study was approved by the Norwegian South East Regional Committee for Medical and Health Research Ethics. All patients (legal guardians if patient was <18 years of age) signed an informed consent before entering the study.

Results

Study population

The number of patients eligible for 10-year and 20-year follow-ups in the IBSEN cohort has previously been published. As described by Solberg et al. [2], 197 CD patients completed the 10-year follow-up, 18 were dead and 22 were lost to follow-up. As described by Ossum et al. [13], 156 CD patients completed the 20-year follow-up, 38 were dead and 43 were lost to follow-up. Serum samples for analyses of the antibody panel were available from 159 CD patients at 10-year follow-up and 135 CD patients at 20-year follow-up. For 113 CD patients, serum samples were available at both time points. Patient characteristics of the analysed patients compared to the remaining CD patients in the IBSEN cohort without available serum samples are presented in Table 1. The study population was significantly younger at diagnosis; however, no significant differences regarding gender, disease location or disease behaviour were found.

In the study population, 34 (24%) had received treatment with anti-tumour necrosis factor alpha (anti-TNF), 72 (50%) had received azathioprine and 6 (4%) had received methotrexate during the 20 years of follow-up.

Need of surgery

During 20 years of follow-up, or until lost to follow-up, 72 patients had at least one bowel resection performed, 22 patients had colectomy performed and 22 patients had at least one fistula or abscess surgery performed. Altogether, 82 patients (52%) experienced minimum one incident of CD-related surgery. A total of 46 patients (29%) had more than

Table 1. Patient characteristics of the 159 patients that provided serum samples before the 10-year follow-up compared to the 78 patients that did not provide serum samples.

	Study population ($n = 159$)	Rest of cohort ($n = 78$)	p value
Age at diagnosis – median (range)	27.8 (7.9–75.8)	31.4 (13.6–84.6)	.037
Female gender – n (%)	78 (49%)	40 (51%)	.747
Disease location at diagnosis			.222
Terminal ileum	43 (27%)	21 (27%)	
Colon	71 (45%)	44 (56%)	
Ileocolon	42 (26%)	12 (15%)	
Upper GI	3 (2%)	1 (1%)	
Disease behaviour at diagnosis			.78
Inflammatory	98 (62%)	49 (63%)	
Stricturing	42 (26%)	22 (28%)	
Penetrating	19 (12%)	7 (9%)	

Table 2. Proportions of patients testing positive for each antibody at 10- and 20-year follow-up.

Serological antibody	10-year $n = 159$	20-year $n = 135$
pANCA – n positive (%)	53 (33%)	57 (42%)
ASCA IgA – n positive (%)	61 (38%)	48 (36%)
ASCA IgG – n positive (%)	59 (37%)	46 (34%)
Anti-OmpC – n positive (%)	10 (6%)	13 (10%)
Anti-I2 – n positive (%)	129 (81%)	118 (87%)
Anti-CBir1– n positive (%)	23 (15%)	13 (10%)

one incident of surgery; 19 patients (12%) had two incidents, and 27 patients (17%) had three or more incidents.

Prevalence and stability of antibodies

Proportions of patients testing positive for each antibody at 10- and 20-year follow-up are presented in Table 2. For the 113 patients who provided serum samples at both visits, we investigated whether the presence of each antibody was stable from 10 to 20 years. Positivity at both time points (stable positivity) was found in 33%–90%, and negativity at both time points (stable negativity) was found in 47%–97% of the tested antibodies. The change of antibody status from positive to negative or opposite was not statistically significant for any of the analysed antibodies (Table 3).

Antibodies and associations with CD-related surgery

CD-related surgery during the 20 years of follow-up or until lost to follow-up was associated with disease location in ileum or ileocolon, stricturing or penetrating disease behaviour, and with negative pANCA, positive ASCA IgA and positive ASCA IgG, all at 10-year follow-up (Table 4). There was no statistically significant association between CD-related surgery and anti-OmpC, anti-I2 or anti-CBir1. Variables that were statistically significantly associated with the outcome in univariate regressions were entered into a multiple model. Only stricturing or penetrating disease behaviour and negative pANCA remained significantly associated with higher odds for surgery (Table 4).

To assess the odds for CD-related surgery based on serological antibodies alone, multiple logistic regression models including pANCA, ASCA IgA and ASCA IgG were fitted and the results were expressed as probabilities and organized in a risk matrix model (Table 5). The antibody combination associated with the lowest probability of CD-related surgery was positive pANCA, negative ASCA IgA and negative ASCA IgG. Based on our risk matrix, approximately 25% of the patients with this antibody combination would need CD-related surgery during the 20-year follow-up period. The antibody combination associated with the highest probability of CD-related surgery was negative pANCA, positive ASCA IgA and positive ASCA IgG. As many as 79.5% with this antibody combination would need CD-related surgery during the 20-year follow-up period.

Discussion

In the present project, we have investigated the selected factors associated with the need of surgery as an expression of complicated CD. Biomarkers usable for predicting disease complications must be stable over time. However, studies of serial measurements of serological antibodies in CD are scarce. A cohort of incident paediatric IBD patients has been studied regarding stability of some serological markers; however, small sample size (54 patients) and short follow-up time (median 20 months) restrict any definitive conclusions [12]. A recent study of a panel of serological antibodies in UC patients concluded with stability over time; however, median follow-up time was only 21 months [8]. Smids et al. [7] included untreated and newly diagnosed IBD patients and studied serological antibodies (ANCA, ASCA, anti-glycans) at diagnosis and follow-up. In 49 CD patients eligible for follow-up, and with a median time period between the two measurements of 30 months, they found that pANCA and ASCA positivity remained stable, but ASCA fluctuated in concentration over time [7]. To the best of our knowledge, we present the first study of the stability of the serological antibodies; ASCA IgA, ASCA IgG, pANCA, anti-OmpC, anti-I2 anti-CBir; assessed in a population-based cohort and with a 10-year follow-up period. We found no significant change in antibody status (positive vs. negative) from the 10-year follow-up to the 20-year follow-up. Fluctuation in concentration over time may be of pathophysiological interest but beyond the scope of our clinical approach. Our conclusion is therefore that our data did not reveal clinically relevant changes in antibody status.

Complicated CD with stricturing and penetrating disease behaviour often requires surgery, as confirmed in our regression model. Others have also, in line with our univariate model, found that disease location is important. Colonic CD is associated with lower risk of surgery than ileal and ileocolonic disease [14,15]. However, in a multiple model adjusted

Table 3. Stability of serological antibody status from 10-year to 20-year follow-up from the 113 Crohn's disease patients with serological samples from both follow-ups.

Serological antibody	Positive at 10 years	Stable positive, *n* (%)	Positive to negative, *n* (%)	Negative at 10-year, *n* (%)	Stable negative, *n* (%)	Negative to positive, *n* (%)	*p* value change of status
pANCA	42	32/42 (76%)	10/42 (24%)	71	56/71 (79%)	15/71 (21%)	.32
ASCA IgA	43	38/43 (88%)	5/43 (12%)	70	64/70 (91%)	6/70 (9%)	.76
ASCA IgG	40	36/40 (90%)	4/40 (10%)	73	69/73 (95%)	4/73 (6%)	1.0
Anti-OmpC	6	2/6 (33%)	4/6 (67%)	107	99/107 (93%)	8/107 (8%)	.25
Anti-I2	96	91/96 (95%)	5/96 (5%)	17	8/17 (47%)	9/17 (53%)	.29
Anti-CBir1	16	7/16 (44%)	9/16 (56%)	97	94/97 (97%)	3/97 (3%)	.08

Table 4. Univariate (crude) and multiple (adjusted) logistic regression analyses showing associations between clinical variables at the 10-year follow-up and serological antibody status at the 10-year follow-up, and Crohn's disease-related surgery in the 20 years of follow-up or until lost to follow-up as dependent variable.

	n	Surgery+* (% of *n*)	Crude OR (95% CI)	Adjusted OR (95% CI)
Clinical variables				
Gender				
Female (ref)	78	38 (48.7)	1	
Male	81	41 (50.6)	1.1 (0.6–2.0)	
Age at diagnosis				
A1 (≤16) (ref)	15	7 (46.7)	1	
A2 (17–40)	101	57 (56.4)	1.5 (0.5–4.4)	
A3 (> 40)	43	15 (34.9)	0.6 (0.2–2.0)	
Disease location				
Colitis (ref)	52	14 (26.9)	1	
Ileocolitis	70	42 (60.0)	4.1 (1.9–8.9)	1.5 (0.5–4.4)
Ileitis	30	18 (60.0)	4.1 (1.6–10.6)	0.8 (0.2–3.1)
Disease behaviour				
Inflammatory (ref)	69	10 (14.5)	1	
Stricturing	52	38 (73.1)	16.0 (6.5–39.7)	11.7 (3.9–34.9)
Penetrating	38	31 (81.6)	26.1 (9.1–75.4)	17.8 (5.8–55.0)
Serological antibodies				
pANCA negative	106	62 (58.5)	2.6 (1.3–5.2)	2.6 (1.0–6.5)
ASCA IgA positive	61	42 (68.9)	4.1 (2.1–8.2)	1.5 (0.5–4.7)
ASCA IgG positive	59	39 (66.1)	3.7 (1.9–7.4)	1.2 (0.4–3.8)
Anti-OmpC positive	10	8 (80)	4.1 (0.8–19.7)	
Anti-I2 Positive	129	68 (52.7)	1.8 (0.8–4.0)	
Anti-CBir1 positive	23	11 (47.8)	1.0 (0.4–2.5)	

*Minimum one incident of Crohn's disease-related surgery.
OR: Odds ratio; CI: confidence interval.

Table 5. Risk matrix model demonstrating probability of Crohn's disease-related surgery within different combinations of serological antibodies in a population-based inception cohort of Crohn's disease patient followed for 20 years after diagnosis.

	ASCA IgA negative		ASCA IgA positive	
	ASCA IgG		ASCA IgG	
	Negative	Positive	Negative	Positive
pANCA				
negative	44.3% (36–53)	59.7% (51–68)	67.5% (60–75)	75.9% (73–86)
positive	25.1% (18–32)	38.4% (30–47)	46.6% (38–55)	61.9% (54–70)

for available contributory factors, disease behaviour was the only phenotypic factor that remained statistically significant. Inflammatory disease behaviour in the early disease course has by us and others been shown to progress to stricturing and penetrating disease in a substantial part of the CD patients [16,17]. This will unevitably increase probability of surgery. Since the serological antibodies were stable from 10-year to 20-year follow-up, we assume a stability from diagnosis and throughout the disease course. We therefore suggested a risk matrix model based on serological antibodies, which could be used at time of diagnosis.

Previous studies of serologic biomarkers have found associations with disease phenotype, disease progression and disease duration [4]. ASCA, anti-OmpC, anti-I2 and anti-CBir have been associated with complicated CD, progression of disease and need of surgery, whereas the presence of pANCA has been associated with an inflammatory behaviour and a less severe disease course [18–25]. Our findings of associations between CD-related surgery and positive ASCA IgA, positive ASCA IgG and negative pANCA are therefore in line with previous studies. The associations may, however, be an expression of ongoing inflammation. For example, ASCA concentrations have been reported to decrease after resection for CD [26], indicating coherence with disease activity. Vermeire et al. found, however, that concentrations remained stable throughout the disease course and did not depend on disease activity [27], in line with the present findings of stable serological markers over time. The association with surgery may also represent a more severe disease course or a higher inflammatory burden. A study of paediatric IBD patients found that ASCA-positive and pANCA-negative children were more likely to receive early aggressive treatment with anti-TNF [12]. This is in line with our results, where the most favourable combination of antibodies (positive pANCA, negative ASCA IgA and negative ASCA IgG) was associated with a much lower probability for CD-related surgery than the least favourable combination (negative pANCA, positive ASCA IgA and positive ASCA IgG). This may indicate that serological antibodies may be used to identify patients in need of early intensive treatment strategies. However, the question of whether the disease course can be changed with an early aggressive treatment strategy remains unanswered. One study investigated a panel of serological biomarkers including ASCA IgA, ASCA IgG, anti-Cbir and anti-OmpC as predictors of anti-TNF response. In that study, the concentrations of antibodies were similar among responders and non-responders, and age at first anti-TNF administration, body mass index and previous surgery was the only predictors of anti-TNF response [28].

Compared to a review of the literature [4], this study showed a relatively high prevalence of anti-I2 and pANCA, and a low prevalence of anti-OmpC and anti-CBir in CD. The serological pattern may, however, be influenced by the selected cohort, and a population-based sample may not be comparable with CD cohorts recruited from referral centres where a larger proportion of patients have complicated disease. Lower prevalence of ASCAs in population-based cohorts has previously been described [29]. No statistically significant associations were found between CD-related

surgery and the presence of anti-OmpC, anti-I2 or anti-CBir in the present cohort. This may also be due to the population-based design. Moreover, anti-OmpC, anti-I2 and anti-CBir are all microbial antibodies and their presence might depend on environmental factors that differ between populations from different geographical regions [30,31].

This study demonstrated a higher prevalence of ASCA and pANCA positivity at 10-year follow-up than previous analyses of the same serum samples from this cohort performed with an assay from another manufacturer [16]. This demonstrates that choice of analytical assay may influence the estimated prevalence and the results are not directly comparable between assays.

The fact that serum samples were drawn from patients at the 10- and 20-year follow-ups, and not from time of diagnosis, is a limitation to the extrapolation of our results. There might have been changes in antibody status in the early stage of disease related to disease activity or response to treatment. However, the subsequent long follow-up period of 10 years without significant change in antibody status might imply that the presence of antibodies is indeed stable and may be unchanged from the time of diagnosis. The study population were significantly younger than the patients from the cohort not providing serum samples. However, we do not believe that older patients have less stable antibody status nor different associations between antibody status and surgery. The number of patients included restricts further subanalyses of associations with other disease complications as well as ongoing inflammation. However, the population-based design may increase the generalizability of our results.

Conclusion

In this population-based inception cohort of CD patients followed for 20 years, all investigated serological antibodies were stable from 10 to 20 years after diagnosis. Positive ASCA IgA and IgG, and negative pANCA were associated with higher odds for CD-related surgery in univariate analysis. In a multiple regression model including disease location and disease behaviour, negative pANCA was the only serological antibody significantly associated with CD-related surgery. Since disease phenotype changes during the disease course, while serological antibodies are stable, our results support the use of pANCA, ASCA IgA and ASCA IgG as prognostic markers in CD.

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Disclosure statement

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