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Serological markers are associated with disease course in ulcerative colitis. A study in an unselected population-based cohort followed for 10 years

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KEYWORDS
Disease course;
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Abstract

Objectives: Perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) have been proposed as markers for diagnosis and for subtyping of inflammatory bowel disease (IBD). The aim of this study was to investigate the association of p-

Abbreviations: p-ANCA, Perinuclear anti-neutrohphil cytoplasmic antibody; ASCA, Anti-Saccharomyces cerevecias antibody; AZA/6MP, Azathioprine and 6-mercaptopurine; 5-ASA, Sulfasalazine and 5-aminosalicylic acid; BI, Binding index; CD, Crohn`s disease; CI, Confidence interval; EC-IBD, European Collaborative Study Group of Inflammatory Bowel Disease; GCS, Glucocorticosteroids; IBD, Inflammatory bowel disease; IQR, Inter-quartile range; RR, Relative risk; UC, Ulcerative colitis.

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Serological markers;
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ANCA and ASCA with a 10-year disease outcome in terms of cumulative rate of colectomy and relapse in a population-based European inception cohort of ulcerative colitis (UC) patients.
Methods: Serum samples from 432 consenting patients were analysed for p-ANCA and ASCA. The results were compared with the cumulative colectomy rate, relapsing disease and total number of relapses. We used multiple regression analyses adjusted for age, sex, residence, disease extent at diagnosis, smoking, familial IBD and drug treatment to study the relationship between serological values and disease course.
Results: The relapse rate was higher in the p-ANCA-positive patients: 82% (95% confidence interval [CI] 75–89%) compared with 67% (CI 62–72%, $p=0.011$) in the p-ANCA-negative patients. The risk of relapsing disease course was higher by a factor of 1.4 (CI 1.1–1.8, $p=0.009$) for p-ANCA-positive patients than for p-ANCA-negative patients, and the corresponding relative risk (RR) for the total number of relapses was 1.9 (CI 1.7–2.1, $p<0.001$). In ASCA-positive patients RR for the total number of relapses was 1.8 (CI 1.5–2.1, $p<0.001$). No significant association with colectomy rate was found for the presence of either p-ANCA or ASCA.
Conclusion: UC patients positive for p-ANCA and possibly for ASCA may have a more unfavourable long-term disease outcome in terms of relapse than UC patients without these markers.
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1. Introduction

It has been proposed that serological markers such as the perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) and the anti-Saccharomyces cerevisiae antibody (ASCA) are potential diagnostic tools in inflammatory bowel disease (IBD). P-ANCA has been detected in 50–80% of patients with ulcerative colitis (UC) and in 10–30% with Crohn's disease (CD).[1] ASCA has been found in 40–70% in CD and 5–15% in UC patients. In a population-based cohort of IBD patients, the combination of a positive p-ANCA and a negative ASCA had a positive predictive value of 75% for the diagnosis of UC.[2] However, although the predictive value of these serological markers for defining sub-groups of IBD patients[1] has been investigated, there are diverging opinion with regard to their association with a long-term disease outcome.

The aims of the present study were to evaluate p-ANCA and ASCA as markers of disease outcome by determining the cumulative colectomy rate, the cumulative number of patients with first relapse after diagnosis and the total number of relapses in a population-based multicentre European–Israeli inception cohort of UC patients followed for 10 years.

2. Methods

2.1. Study population

Between October 1 1991 and September 30 1993, a total of 2201 patients with IBD at 20 treatment centres in 12 European countries and Israel were recruited at inception in a prospective population-based study conducted by the European Collaborative Study Group of Inflammatory Bowel Disease (EC-IBD).[3] UC was diagnosed in 1379 of the patients according to the criteria established by Lennard-Jones and Truelove and Witts.[4] The patients were treated and followed according to the established procedures at the individual centre. After 10 years all the treatment centres were invited to participate in a follow-up study. Thirteen of the centres were able to participate; the others dropped out for technical or logistical reasons. Four of the 13 did not meet the criterion of at least 60% patient follow-up, which was arbitrarily set in order to maintain the population-based nature of the study.

2.2. Data collection

The patients were invited to a 10-year follow-up visit, which took place between August 1 2002 and January 31 2004. Data from a patient questionnaire and a patient-per-physician follow-up form were registered in a common database, which could be accessed by all the participating centres. Supplementary information was gathered from hospital records when necessary. The study population, including the follow-up, has previously been described in more detail.[5,6]

2.3. Definitions

2.3.1. Relapse
A relapse was defined as an increase in UC-related symptoms leading to consultation with a physician and resulting in an increase in the dose of ongoing medical treatment, the introduction of new medication, or surgery. Patients without symptoms or whose symptoms were too slight to fulfill these criteria were considered to be in clinical remission.

2.3.2. Colectomy
Data on total or partial resection of the colon and/or rectum with or without additional surgical procedures were noted at the10-year interview or obtained from hospital records.

2.3.3. Geographical location
The centres in Greece, Israel, Italy and Spain were classified as southern centres and those in Denmark, the Netherlands and Norway as northern centres.

2.3.4. Education level
The highest level of completed education was noted at the 10-year interview, and the patients were divided into two

categories for comparison: primary (0–9 years) combined with lower secondary school (10–11 years), and upper secondary school (12 years or more) combined with higher education.

2.3.5. Disease extent

The extent of inflammation at diagnosis was assessed mainly on the basis of colonoscopy, but in a small number of cases distal colonoscopy was combined with double contrast barium enema. Disease activity proximal to the splenic flexure was classified as extensive colitis, colonic inflammation distal to the splenic flexure as distal colitis, and disease limited to the rectum 15 cm above the linea dentata as proctitis.

2.3.6. Smoking

At the 10-year interview the patients were asked about their smoking history. Patients who had never been daily cigarette smokers were classified as non-smokers; patients who had stopped smoking in any calendar year prior to the year they entered the study were classified as ex-smokers before study inclusion; and those who had stopped smoking in any year including the calendar year they entered the study were classified as ex-smokers after study inclusion. All patients who at the 10-year interview had an average consumption of at least one cigarette daily, were classified as current smokers regardless of when they started smoking. The cumulative cigarette consumption in pack years throughout the smoking period was assessed for the current and ex-smokers.

2.3.7. Relatives with IBD

Familial occurrence of one or more first-degree relatives with IBD was noted at the 10-year interview.

2.3.8. Medical treatment

Information on medical treatment was obtained from the medical records that had been kept up to date during follow-up. For patients who had taken medication for one or more periods, the proportion of the follow-up period during which they had used the drug (referred to as the use period) was calculated. The most commonly used systemic drugs were chosen for analysis and divided into three groups: aminosalicylates (5-ASA), including sulfasalazine and 5-aminosalicylic acid, glucocorticosteroids (GCS), and azathioprine or 6-mercaptopurine (AZA/6MP). The use of 5-ASA and GCS was closely related to relapse as defined in this study, but otherwise we had no information about whether these drugs were used as maintenance or to treat relapse. We therefore excluded them from the regression analyses. AZA/6MP was included in regression analysis because it is mainly used for maintenance of remission. The use of other drugs for the treatment of UC was considered negligible due to the small number of patients concerned, and these drugs were therefore excluded from further analysis.

2.3.9. Serological analysis

At the end of the follow-up whole blood and serum samples for serological testing were obtained at the 10-year visit from the consenting patients. The analysis of ASCA was performed by a standard ELISA method (Medipan Diagnostica, Germany)[7] and interpreted by calculating the binding index (BI). ASCA (either IgG or IgA) were considered positive at BI >1. P-ANCA IgG was determined by indirect immunofluorescence using ethanol-fixated neutrophil slides (Inova Diagnostics, San Diego, Calif).[8] All samples were frozen and shipped to the University Hospital Gasthuisberg, Leuven, Belgium, for analysis.

2.3.10. Statistical analysis

The relapse rate was calculated as the cumulative rate for the first relapse in patients with at least one relapse after diagnosis. The number of relapses was calculated as the total number of relapses per unit of time at risk, which was defined as time from diagnosis until time of the 10-year interview, death, most recent consultation or colectomy. The cumulative number of patients with a first relapse was calculated by the Kaplan-Meier method, and relapse rates between sub-groups were compared using log-rank tests. The Mann–Whitney, Kruskal–Wallis, chi-squared and Fisher's exact tests were used when appropriate to identify possible differences in sex, age, geographical residence, smoking status, drugs and extent of disease between patients with and without serological test results. Adjustments for co-factors associated with the first relapse or with colectomy were made by Cox regression using the SPSS statistical software package version 14.0 (SPSS Inc., Chicago IL). On the assumption that the number of relapses would have a Poisson distribution, we used a Poisson regression model to adjust for co-factors for the total number of relapses for each participant. The follow-up time was included as an offset term to allow for different at-risk times for each patient. The Poisson model was fitted using the R software version 2.2.0 (the R Project for Statistical Computing). The two models provide information on two related but supplementary aspects of the development of the disease. Cox regression models the time to first relapse and important factors during this initial period. Poisson regression models the total number of relapses during the follow-up period and factors associated with the total burden of disease. *P* values less than 0.05 were considered statistically significant.

2.3.11. Ethical considerations

The study protocol was approved by the local committee for medical research ethics for all the participating centres and informed consent was obtained from all patients before they entered the study.

3. Results

3.1. Patients and centres

Data for the patients included in the 10-year follow-up are presented in Table 1. Of the 20 centres in the original cohort, nine centres in seven countries met the criterion of at least 60% patient follow-up. Status of the 781 patients from these centres diagnosed with UC has been outlined in Fig. 1. Ten patients had not been followed up and were therefore excluded, while 771 had been followed up for varying time periods. Blood samples were collected from 432 of the patients who attended the 10-year visit. The median follow-up period for these patients was 123 months (range 107–133). Of the 339 patients who were not tested for serology,

Table 1 Demographic details, serological results, potential risk factors and therapeutic data for the patient cohort

		p-ANCA		ASCA		No test	Total cohort
		Negative	Positive	Negative	Positive		
Centre							
	Beer Sheva	15	3	17	1	21	39
	Copenhagen	36	15	45	6	38	89
	Cremona	16	3	19	0	22	41
	Ioannina	25	2	27	0	10	37
	Heraklion	9	3	9	3	41	53
	Oslo	94	53	141	6	122	269
	Reggio Emilia	31	7	37	1	12	50
	South Limburg	64	12	72	4	64	140
	Vigo	37	7	37	7	19	63
Sex							
	Male	181	48	213	16	180	409
	Female	146	57	191	12	169	372
Age at diagnosis							
	Below 20 years	16	7	20	3	19	42
	20–29 years	77	29	97	9	87	193
	30–39 years	88	26	111	3	60	174
	40–49 years	67	17	79	5	44	128
	50–59 years	48	11	57	2	39	98
	60–69 years	25	11	32	4	47	83
	70–79 years	6	4	8	2	42	52
	80 years and above	0	0	0	0	11	11
Disease extent at diagnosis							
	Proctitis	101	29	120	10	95	225
	Distal colitis	136	44	171	9	158	338
	Extensive colitis	78	27	98	7	85	190
	No data available	12	5	15	2	11	28
Smoking status							
	Never smoked [ref]	136	52	180	8	45	233
	Ex-smoker before inclusion	73	28	94	7	25	126
	Ex-smoker after inclusion	41	6	44	3	6	53
	Current smoker	63	8	63	8	26	97
	Unknown	14	11	23	2	247	272
First-degree relative(s)							
	None	277	84	334	27	90	451
	Yes	37	11	48	0	17	65
	No data available	13	10	22	1	242	265
Education							
	Primary and lower secondary school	156	37	180	13	50	243
	Higher secondary school and above	158	58	202	14	57	273
	No data available	13	10	22	1	242	265
Drugs							
5-ASA	Drug used	273	95	342	26	271	639
	Drug not used	45	9	53	1	58	112
	No data available	9	1	9	1	20	30
GCS	Drug used	137	61	184	14	131	329
	Drug not used	181	43	211	13	198	422
	No data available	9	1	9	1	20	30

(continued on next page)

Table 1 (*continued*)

		p-ANCA		ASCA		No test	Total cohort
		Negative	Positive	Negative	Positive		
AZA/6MP	Drug used	28	11	31	8	16	55
	Drug not used	288	92	361	19	313	693
	No data available	11	2	12	1	20	33
Colectomy							
	Yes	27	12	34	5	39	65
	No	300	93	370	23	393	716
Total		327	105	404	28	349	781

No test: patients not tested for p-ANCA or ASCA, 5-ASA: sulfasalazine and 5-aminosalicylates, GCS: glucocorticosteroids, AZA/6MP: azathioprine and 6-mercaptopurine, CI: 95% confidence interval.

73 had died, 81 were lost during follow-up, and 185 patients were not tested for other reasons.

There were no significant differences in sex, north–south residence, number of colectomies or disease extent at diagnosis between patients tested for serology ($n=432$) and non-tested ($n=339$) patients. The tested patients had a median age (inter-quartile range, IQR) of 38 years (29–49) at diagnosis, which was significantly lower than the median age of the non-tested patients, which was 42 years (28–64) ($p=0.001$). However, when the patients who died were excluded, no significant difference in age between the two groups was found. With regard to outcome variables, there were no significant differences in colectomy rates, but a significantly higher cumulative rate of relapsing disease of 0.71 (CI 0.67–0.75) versus 0.61(CI 0.55–0.67, $p=0.009$) and a significantly higher median number of relapses per year at risk of 0.19 versus 0.10 ($p=0.002$) were found for the 432 tested patients than for the 339 not tested. Among the 617 patients who attended the 10-year visit, no significant differences in relapse rate or number of relapses were found between tested and non-tested subjects.

3.2. Serological results

Of the 432 UC patients who were tested, 105 (24%) were p-ANCA-positive, with a variation between the centres of 7% to 36%. Twenty-eight (6%) of the UC patients were ASCA-positive, with a variation between the centres of 3% to 25%.

3.3. Disease outcomes

The cumulative colectomy rate, cumulative rate of first relapse and median number of relapses during disease course are presented in Table 2. The status of p-ANCA was significantly related to first relapse. In p-ANCA-positive patients the 10-year cumulative rate of first relapse was significantly higher than in p-ANCA-negative patients. The difference in relapse rates between p-ANCA-positive and p-ANCA-negative patients increased with time, as shown by the Kaplan-Meier plot in Fig. 2. No significant difference was found between ASCA-positive and ASCA-negative patients with regard to risk of first relapse. The total number of

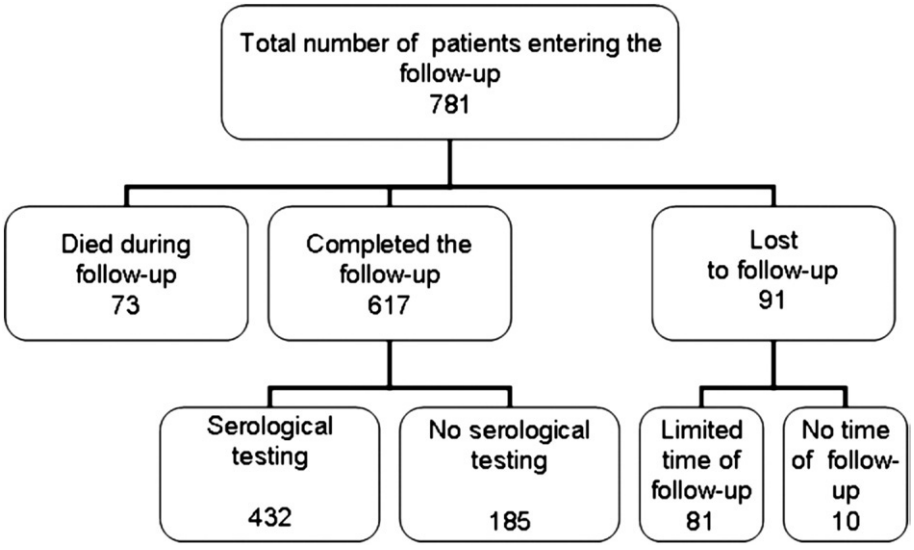

Figure 1 Flow chart demonstrating the status of the patients at the end of the 10-year follow-up study.

Table 2 Disease outcome for p-ANCA and ASCA-positive and negative UC patients

	10-year cumulative colectomy rate			10-year cumulative relapse rate			Total number of relapses per 10 years at risk		
	Cum %	CI	*P* value	Cum %	CI	*P* value	Median	IQR	P value
p-ANCA									
Positive	11.4	5.3–17.5	0.32	81.7	74.3–89.1	0.01	2.1	1.0–5.8	<0.001
Negative	5.4	5.3–11.5		67.1	62.0–72.2		1.1	0–3.2	
ASCA									
Positive	17.9	3.8–32.0	0.09	77.8	62.1–93.5	0.23	2.6	0.3–5.8	0.09
Negative	8.2	5.5–10.9		70.2	65.7–74.7		1.9	0–3.7	

Cum %: cumulative percentage, CI: 95% confidence interval, IQR: inter-quartile range. Log-rank test and Mann–Whitney *U*-test were used.

relapses during follow-up was significantly higher in p-ANCA-positive than in p-ANCA-negative patients. We found no significant relationship to total number of relapses for ASCA-positive patients compared with ASCA-negative patients with the Mann–Whitney test ($p=0.09$), but the relationship was significant with a Poisson regression model ($p<0.001$). ASCA-positive patients had a higher risk of colectomy than ASCA-negative patients, but the difference was not statistically significant ($p=0.09$). Of the 28 ASCA-positive patients, five had undergone colectomy, and of the eight patients who were positive for both ASCA and p-ANCA, three had undergone colectomy. No significant difference in colectomy rate was found between p-ANCA-positive and p-ANCA-negative patients.

3.4. Medical treatment

For all the 432 patients tested, the median drug period was compared between the p-ANCA-positive and p-ANCA-negative patients, and between the ASCA-positive and ASCA-negative patients (Table 3). A significantly higher median use percentage for 5-ASA ($p<0.001$) and GCS ($p=0.005$) was seen in the p-ANCA-positive patients. The median use percentage for AZA/6MP was higher in the ASCA-positive than the ASCA-negative patients ($p<0.001$).

3.5. Smoking

A history of cigarette smoking was obtained from 407(93%) of the 432 patients (Table 1). The median (IQR) for cigarette exposure in terms of number of pack years was 12 (5–20) for ex-smokers before study inclusion, 12 (5–31) for ex-smokers after study inclusion and 15 (6–28) for current smokers. The differences were not statistically significant ($p=0.5$). The prevalence of p-ANCA was 12% in patients who were smokers at diagnosis versus 28% in non-smokers ($p=0.001$), and related to smoking status at the end of the study the figures were 11% and 26% respectively ($p=0.009$). The prevalence of ASCA was higher among smokers than among non-smokers both at diagnosis and at the 10-year follow-up, but the difference was not statistically significant (9% versus 5% at diagnosis, $p=0.12$, and 11% versus 5% at the 10-year follow-up, $p=0.10$).The prevalence of p-ANCA and ASCA respectively among patients who quit smoking during follow-up was not significantly different from the prevalence among current smokers.

3.6. Regression analyses

We used multivariate regression analysis to study the relationships between the serological variables and disease course, accommodating any modulating effect of potential confounding variables. The potential confounding variables were age, sex, level of education, disease extent, north–south residence, familial IBD, treatment with azathioprine and smoking status. Multivariate Cox regression analysis showed an adjusted RR for first relapse of 1.4 (CI 1.1–1.8, $p=0.009$) for p-ANCA-positive patients and a non-significant difference between ASCA-positive and ASCA-negative patients. Multivariate Poisson regression analysis of the total number of relapses during follow-up showed significant differences in adjusted RR both for p-ANCA, 1.9 (CI 1.7–2.1, $p<0.001$), and for ASCA, 1.8 (CI 1.5–2.1, $p<0.001$).

4. Discussion

In this study we found a low prevalence of p-ANCA among UC patients, and a significantly higher rate of first relapse and total number of relapses in the p-ANCA-positive than in the p-ANCA-negative patients after 10 years of follow-up. A significantly higher total number of relapses among ASCA-positive patients was also shown by Poisson regression.

The reported prevalence of p-ANCA among UC patients is generally higher than what we found.[9] The reason for this

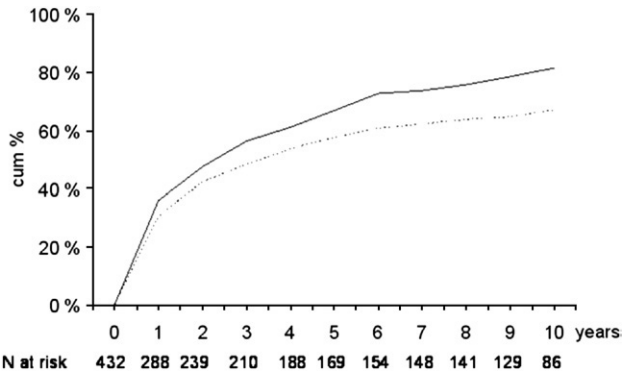

Figure 2 Ten year cumulative percentage (cum %) of p-ANCA positive (–) and negative (———) patients having at least one relapse. The difference is statistically significant by the Log-rank test ($p=0.01$).

Table 3 Use of systemic drugs during follow-up

		5-ASA				GCS				AZA/6MP			
		User *n*	Use period	Non-user *n*	No data *n*	User *n*	Use period	Non-user *n*	No data *n*	User *n*	Use period	Non-user *n*	No data *n*
p-ANCA	Negative	273	0.8	45	9	137	0.1	181	9	28	0.3	288	11
	Positive	95	1.0	9	1	61	0.1	43	1	11	0.4	92	2
ASCA	Negative	342	0.8	53	9	184	0.1	211	9	31	0.5	361	12
	Positive	26	0.8	1	1	14	0.2	13	1	8	0.1	19	1

5-ASA, sulfasalazine/5-aminosalisylic acid; GCS, glucocorticosteroids; AZA/6MP, azathioprine/6-mercaptopurine; No data, no available information on drug use; Non-user, drug not used during follow-up; User, drug used during follow-up; Use period, proportion of time on medication during follow-up, median value calculated for drug users only.

could be that most of these studies have been performed in referral populations and not in population-based cohorts, as in our study. Differences in the methods of analysis could also explain this discrepancy. The combination of IgG with IgA antibody titre could have revealed a higher prevalence of p-ANCA.[10] In a population-based cohort from Rochester, Minnesota, serum samples from 83 UC patients were tested for p-ANCA using five different assays, and the prevalence of p-ANCA varied from 0 to 63%.[2] The test used in our study was based on IgG detection, which showed a positive p-ANCA in 49.7% of 147 consecutive UC patients at a university referral centre.[8] We therefore believe that the low prevalence of p-ANCA found in our cohort is due to the population-based nature of the study and to geographical variations between the centres.

Our findings support the hypothesis that p-ANCA is associated with a more aggressive disease during follow-up. Furthermore, the larger difference in relapse rates over time in these patients is consistent with p-ANCA being a marker of a poor long-term disease outcome. The combination of p-ANCA and ASCA has been proposed as an adjunctive diagnostic tool in IBD.[11] In studies of sub-groups of UC patients, a positive p-ANCA has been associated with therapy-resistant left-sided disease,[12] more aggressive disease, a more frequent need for early surgery[1] and the development of pouchitis in ileal pouch anal anastomosis.[13,14] However, the implication of these results for long-term disease outcome in UC has not been investigated before.

A number of studies have failed to show any correlation between disease activity and the presence of p-ANCA.[15–17] However, in others a lower prevalence of p-ANCA among UC patients in remission has been reported,[18] and a positive titre of p-ANCA has been found to be associated with more aggressive UC.[19,20] However, most of these studies have been carried out on a small number of patients, using different laboratory methods. Furthermore, no uniform definitions of disease activity, study outcome or follow-up routines have been used. These factors could explain the inconsistencies in the results reported so far.

Colectomy risk was not related to p-ANCA status. Extent of disease in our cohort was shown in a previous study to be related to the risk of colectomy,[21] but not to the risk of relapse.[22] The formation of p-ANCA has been proposed to be a consequence of a mucosal immune response with high specificity for UC.[23] If so, the immune response could result in an increase in inflammatory activity and p-ANCA formation irrespective of disease extent or colectomy risk. Furthermore the decision to perform colectomy may be influenced by local treatment strategies for disease resistant to initial medical therapy or for prevention of colorectal cancer.[24] Therefore, when investigating the impact of p-ANCA status, colectomy rate may be a less reliable marker of disease activity than relapse.

Positive titre of ASCA has been associated with CD, especially stricturing and penetrating disease,[25] and with surgery[26] and higher health care costs.[27] However, there are few if any reports on the implications of positive ASCA in UC patients. Our results indicate that positive ASCA may be a marker of more aggressive disease in UC as well, as measured by the overall risk of relapse in the first 10 years of disease course and the relationship to the use of azathioprine/6MP. However, these results must be interpreted with great caution due to the low prevalence of positive ASCA among UC patients. Given the broad confidence interval in the Mann–Whitney *U*-test, the discrepancy between the results of the two statistical analyses of ASCA as a risk factor for total number of relapses could be due to a type-II statistical error.

The use period for both GCS and 5-ASA represented a significantly higher percentage in p-ANCA-positive than in negative patients. However, this should be regarded as an epiphenomenon owing to the frequent use of these drugs in the treatment of relapses that we found to be associated with positive p-ANCA. The higher levels of AZA/6MP in our ASCA-positive patients may also reflect more aggressive disease, but due to the small number of patients the higher levels could also be coincidental. The results of the multivariate regression analyses showed that the use of AZA did not contribute significantly explain the relation between disease outcome and p-ANCA status.

Cigarette smoking has been shown to protect against UC and may also alleviate the clinical course.[28] The low prevalence of p-ANCA in smokers compared with non-smokers at the time of diagnosis in our study is consistent with this reported finding. Factors responsible for the effect of smoking on the development of UC may also reduce the formation of p-ANCA as an epiphenomenon. Since the prevalence of p-ANCA did not differ between patients who stopped smoking during follow-up and those who continued smoking, it is not likely that the protective effect of smoking on UC is mediated by a change in the status of p-ANCA. Other environmental factors likely to influence disease activity, such as treatment with GCS or colectomy, may also change p-ANCA status from positive to negative.[19] This could explain

the difference in serological status, even though it was not supported by our data.

The design of cohort formation and follow-up used in our study has a strong methodological advantage. However, although we found that a number of demographic and phenotypic variables were comparable in tested and non-tested patients, this does not entirely exclude a selection bias. We have previously demonstrated an inverse relationship between age at diagnosis and risk of relapse.[22] The lower risk of relapse found in the non-tested group may have been caused by the greater age of the patients who had died. If there was a low risk of relapse among the patients who did not attend follow-up, this would also result in a selection bias. Since the risk of relapse in the non-tested patients was lower than in those tested, the association between a positive serological test and the risk of relapse for the total cohort may in fact be lower than what we found.

Despite the prospective inclusion and follow-up, the data on smoking were obtained retrospectively, and this may have resulted in recall biases. Our definition of relapse, although clinically relevant, did not include any clinical or endoscopical scoring systems, which have been used in other studies to measure disease activity.[29,30] Although some minor relapses may not have been registered, we believe that we were able to record most of the clinically important relapses during follow-up.

The tests for ASCA and p-ANCA were performed at the end of the study. We were therefore not able to analyse the possibility of a relationship between these markers and mortality as an outcome. In addition the prognostic value of these tests in our study depended on the stability of the serological status from the point of inclusion throughout follow-up. A decline in the number of seropositive patients during the follow-up could have weakened the impact of these markers on disease prognosis. However, ASCA titres have previously been shown to be consistent over time, independently of medical or surgical treatment,[31–33] which indicates that they are a stable genetic marker of disease. P-ANCA status, on the other hand has been found to change from positive to negative after treatment with steroids or colectomy.[19] This was not supported by our results, which showed neither a higher rate of colectomy nor a longer period of treatment with GCS in p-ANCA-negative than in p-ANCA-positive patients. Thus although fluctuations in p-ANCA titres over time have been reported, especially in patients with relapsing or chronic active UC, p-ANCA seems to remain stable in most patients during follow-up.[20] This is supported by a study by Israeli et al., who showed that p-ANCA was positive in 25% of UC patients even before the clinical onset of the disease.[34] These data indicate that p-ANCA remains serologically stable over time and are consistent with our finding that p-ANCA is a reliable indicator of long-term disease course. However, its serological stability means that the use of p-ANCA for monitoring disease activity and the effect of treatment during the course of disease could not be established by our data.

In conclusion, a positive p-ANCA status in a population-based cohort of UC patients followed for 10 years was a possible predictor of a relapsing disease and a larger number of relapses, information that could be useful when deciding on medical treatment. The presence of ASCA as an indicator of a more aggressive disease course in UC could not be excluded. However, the low prevalence of both markers in this population-based cohort limits their use in clinical practice, and better tests for both prediction and monitoring of disease activity in UC are still needed.[35] Further studies should be performed of both established and new serological markers during long-term follow-up.

Conflict of interest

Guarantor of the article: Björn Moum. Specific author contribution: All of the authors of this paper have contributed in planning of the study and writing of the manuscript. With the exception of Geir Aamodt, all of the authors have also participated in the collection of the data. Financial support: The study was supported by the European Commission (QLG4-CT-2000-01414) and by a research fellowship grant from the Department of Medical Research, Sörlandet Hospital, Norway. Potential competing interests: None.

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References

1. Nakamura RM, Matsutani M, Barry M. Advances in clinical laboratory tests for inflammatory bowel disease. *Clin Chim Acta* 2003;**335**(1–2):9–20.
2. Sandborn WJ, Loftus Jr EV, Colombel JF, et al. Evaluation of serologic disease markers in a population-based cohort of patients with ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2001;**7**(3):192–201.
3. Stockbrugger RW, Russel MG, van Blankenstein M, et al. EC-IBD: a European effort in inflammatory bowel disease. *Eur J Intern Med* 2000;**11**(4):187–90.
4. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol* 1989;**170:**2–6 Suppl.
5. Shivananda S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* 1996;**39**(5):690–7.
6. Wolters FL, van Zeijl G, Sijbrandij J, et al. Internet-based data inclusion in a population-based European collaborative follow-up study of inflammatory bowel disease patients: description of methods used and analysis of factors influencing response rates. *World J Gastroenterol* 2005;**11**(45):7152–8.
7. Vermeire S, Joossens S, Peeters M, et al. Comparative study of ASCA (Anti-Saccharomyces cerevisiae antibody) assays in inflammatory bowel disease. *Gastroenterology* 2001;**120**(4):827–33.

8. Peeters M, Joossens S, Vermeire S, et al. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Gastroenterol* 2001;**96**(3):730–4.
9. Roozendaal C, Kallenberg CG. Are anti-neutrophil cytoplasmic antibodies (ANCA) clinically useful in inflammatory bowel disease (IBD)? *Clin Exp Immunol* 1999;**116**(2):206–13.
10. Gigase P, De Clerck LS, Van Cotthem KA, et al. Anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease with special attention for IgA-class antibodies. *Dig Dis Sci* 1997;**42**(10):2171–4.
11. Sandborn WJ. Serologic markers in inflammatory bowel disease: state of the art. *Rev Gastroenterol Disord* 2004;**4**(4):167–74.
12. Sandborn WJ, Landers CJ, Tremaine WJ, et al. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996;**71**(5):431–6.
13. Fleshner PR, Vasiliauskas EA, Kam LY, et al. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. *Gut* 2001;**49**(5):671–7.
14. Kuisma J, Jarvinen H, Kahri A, et al. Factors associated with disease activity of pouchitis after surgery for ulcerative colitis. *Scand J Gastroenterol* 2004;**39**(6):544–8.
15. Reumaux D, Colombel JF, Masy E, et al. Anti-neutrophil cytoplasmic auto-antibodies (ANCA) in ulcerative colitis (UC): no relationship with disease activity. *Inflamm Bowel Dis* 2000;**6**(4):270–4.
16. Roozendaal C, Pogany K, Hummel EJ, et al. Titres of anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease are not related to disease activity. *QJM* 1999;**92**(11):651–8.
17. Zholudev A, Zurakowski D, Young W, et al. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. *Am J Gastroenterol* 2004;**99**(11):2235–41.
18. Lindgren S, Floren CH, Lindhagen T, et al. Low prevalence of anti-neutrophil cytoplasmic antibodies in ulcerative colitis patients with long-term remission. *Eur J Gastroenterol Hepatol* 1995;**7**(6):563–8.
19. Rump JA, Worner I, Roth M, et al. p-ANCA of undefined specificity in ulcerative colitis: correlation to disease activity and therapy. *Adv Exp Med Biol* 1993;**336:**507–13.
20. Vecchi M, Bianchi MB, Calabresi C, et al. Long-term observation of the perinuclear anti-neutrophil cytoplasmic antibody status in ulcerative colitis patients. *Scand J Gastroenterol* 1998;**33**(2):170–3.
21. Hoie O, Wolters FL, Riis L, et al. Low colectomy rates in ulcerative colitis in a European cohort followed for ten years. *Gastroenterology* 2007;**132**(2):507–15.
22. Hoie O, Wolters FL, Riis L, et al. Ulcerative Colitis: Patient characteristics may predict 10-year disease recurrence in a European-wide population-based cohort. *Am J Gastroenterol* 2007;**102:**1692–701.
23. Targan SR, Landers CJ, Cobb L, et al. Perinuclear anti-neutrophil cytoplasmic antibodies are spontaneously produced by mucosal B cells of ulcerative colitis patients. *J Immunol* 1995;**155**(6):3262–7.
24. Langholz E, Munkholm P, Davidsen M, et al. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 1994;**107**(1):3–11.
25. Vasiliauskas EA, Plevy SE, Landers CJ, et al. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996;**110**(6):1810–9.
26. Walker LJ, Aldhous MC, Drummond HE, et al. Anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn's disease are associated with disease severity but not NOD2/CARD15 mutations. *Clin Exp Immunol* 2004;**135**(3):490–6.
27. Odes S, Friger M, Vardi H, et al. Role of ASCA and the NOD2/CARD15 mutation Gly908Arg in predicting increased surgical costs in Crohn's disease patients: a Project of the European Collaborative Study Group on Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2007;**13**(7):874–81.
28. Birrenbach T, Bocker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. *Inflamm Bowel Dis* 2004;**10**(6):848–59.
29. Bresci G, Parisi G, Bertoni M, et al. Long-term maintenance treatment in ulcerative colitis: a 10-year follow-up. *Dig Liver Dis* 2002;**34**(6):419–23.
30. Edwards FC, Truelove SC. The course and prognosis of ulcerative colitis. *Gut* 1963;**41:**299–315.
31. Oudkerk PM, Ellerbroek PM, Ridwan BU, et al. Serum antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease are mainly associated with ulcerative colitis. A correlation study between perinuclear antineutrophil cytoplasmic autoantibodies and clinical parameters, medical, and surgical treatment. *Gut* 1993;**34**(1):46–50.
32. Teml A, Kratzer V, Schneider B, et al. Anti-Saccharomyces cerevisiae antibodies: a stable marker for Crohn's disease during steroid and 5-aminosalicylic acid treatment. *Am J Gastroenterol* 2003;**98**(10):2226–31.
33. Muller S, Styner M, Seibold-Schmid B, et al. Anti-Saccharomyces cerevisiae antibody titers are stable over time in Crohn's patients and are not inducible in murine models of colitis. *World J Gastroenterol* 2005;**11**(44):6988–94.
34. Israeli E, Grotto I, Gilburd B, et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005;**54**(9):1232–6.
35. Nielsen OH, Vainer B, Madsen SM, et al. Established and emerging biological activity markers of inflammatory bowel disease. *Am J Gastroenterol* 2000;**95**(2):359–67.