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Importance of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis and ulcerative colitis: prevalence, titre, and IgG subclass

D S Bansi, K A Fleming, R W Chapman

Abstract

Antineutrophil cytoplasmic antibodies (ANCA) have been reported in up to 87% of patients with primary sclerosing cholangitis with or without ulcerative colitis (PSC+/−UC) and in 68% of those with UC only. Compared with other liver and diarrhoeal diseases, ANCA have high specificity for PSC (+/−UC) and UC only. This study aimed to determine the prevalence and significance of ANCA in these two diseases and whether the ANCA titre or IgG subclass, or both, could distinguish between PSC+UC and UC only. Subjects included 63 patients with PSC, 85 with UC, 17 with coeliac disease, and 10 with dermatitis herpeteformis and 36 normal subjects. ANCA was detected using the immunoalkaline phosphatase method. The IgG subclass of ANCA was determined in 27 PSC+UC and 30 UC only patients using a panel of mouse monoclonal antibodies specific for the IgG subclasses. At a serum dilution of 1:5, ANCA had a diagnostic sensitivity of 65% for all PSC and 45% for UC only. For PSC+UC the sensitivity was 70% at 1:5 ($p=0{\cdot}004$ *v* UC only). At 1:50, the sensitivity values were 54% and 25% respectively for PSC+UC and UC only ($p=0{\cdot}0006$). In PSC, ANCA positivity was significantly associated with extensive involvement of the biliary tree but not with other clinical parameters. In UC only, the median disease duration was significantly greater in ANCA positive patients. The PSC+UC ANCA showed increased IgG3 compared with UC only ANCA ($p<0{\cdot}05$), together with increased IgG2 and IgG4 (p=NS). ANCA is a diagnostic marker in PSC and UC. While the higher titres and different IgG subclass distribution of ANCA in PSC+UC patients compared with those with UC only may reflect differences in underlying immune regulation, determination of the ANCA titre and IgG subclass is unlikely to have a role in distinguishing between PSC+UC and UC only ANCA. Future identification of the antigen(s) for ANCA should allow the development of a more sensitive and specific test for the diagnosis of these two conditions and also determine if ANCA is associated with UC or PSC.

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Keywords: primary sclerosing cholangitis, ulcerative colitis, antineutrophil cytoplasmic antibodies, IgG subclass.

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Perinuclear antineutrophil cytoplasmic antibodies (ANCA) have been detected in the serum of 26–85% of patients with primary sclerosing cholangitis (PSC) with or without ulcerative colitis (PSC+/−UC) and up to 68% with UC only. This difference in reported prevalence probably results from the different techniques used to detect the antibody, which include fixed neutrophil ELISA plus immunofluorescence,[1 2] immunofluorescence alone,[3] and immunoalkaline phosphatase determination.[4] However, it may also reflect differences between these ethnic groups. Nevertheless, ANCA is a useful marker of disease in PSC and UC.

The close association between PSC and UC[5] and the high degree of specificity of ANCA for PSC and UC compared with other liver and diarrhoeal diseases respectively[1 3 4 6] is interesting and suggests there may be an aetiological link between PSC and UC. At present it is not clear whether ANCA in a patient with PSC+UC is associated with the UC or PSC or indeed if it represents two different antibodies. Although antibodies to several constituents of neutrophils including elastase,[7] cathepsin G,[8 9] lactoferrin,[9 10] and beta-glucoronidase[11 12] have been reported in some patients, it is clear that none of these is exclusively responsible for the ANCA activity. Determination of titre may be one way of distinguishing between UC only and UC+PSC ANCA. Furthermore, in a recent report Vidrich[13] suggested that, in contrast to UC only, PSC (+/−UC) ANCA shows an increase in IgG3 subclass specificity. The IgG subclasses are known to differ in their role in immune response.[14] Thus, determination of the IgG subclass of PSC+UC and UC only ANCA may not only distinguish between these two groups and thus identify UC patients with PSC, but may give valuable information on the underlying immune mechanisms involved.

This study aimed, firstly, to determine the prevalence and significance of ANCA in PSC and UC and, secondly, to determine if PSC+UC could be distinguished from UC only by determination of the ANCA titre and IgG subclass.

Methods

PATIENTS

Outpatients with UC and PSC attending the Oxford Radcliffe Hospital were studied. Serum samples from all participants were stored at −20°C until testing. A total of 85 UC only

TABLE I *Clinical details of the ulcerative colitis patients (n=85)*

Male:female	37:48
Median age (y)	53 (range 21–89)
Ulcerative colitis extent:	
To splenic flexure	68 (80%)
Proximal to splenic flexure	17 (20%)
Treatment at time of testing:	
Nil	7 (8%)
SZP/5ASA only	59 (69%)
SZP/5ASA+prednisolone/azathioprine	18 (21%)
Azathioprine only	1 (1%)
Activity:	
Active	18 (21%)
Inactive	67 (79%)

SZP=salazopyrine, 5ASA=5 aminosalicylic acid derivative.

patients (37 men, median age 53 years, range 21–89) and 63 PSC patients (42 men, median age 62 years, range 24–86) were studied. The clinical details of UC and PSC patients are given in Tables I and II respectively. All patients with UC only had normal liver function. Thirty ANCA positive patients with UC only (12 men, median age 47) and 27 ANCA positive, PSC+UC patients (18 men, median age 63) had ANCA IgG subclass determined. Furthermore, sequential sera from four PSC+UC and one UC only patient were tested to look for variation of the IgG subclass specificity with time. These samples were drawn during subsequent routine visits to the outpatients' department over at least a two year period, and the disease was inactive on each visit. The diagnosis of UC[15] and PSC[5] was based on a combination of established histological and radiological criteria. All four patients with normal endoscopic retrograde cholangiopancreatograms (Table II) had histological evidence of PSC (that is, had 'small-duct PSC'). All seven PSC patients with no evidence of concomitant inflammatory bowel disease (Table II) had undergone complete endoscopic and histological examination of the colon. For the purpose of analysing the results, the onset of inflammatory bowel disease was defined as the time at which diagnosis was made. Disease activity was defined according to the criteria of Truelove and Witts.[15]

As a control population, we also studied 17 patients with coeliac disease (14 men, mean age 41 years, range 23–65); 10 with dermatitis herpeteformis (five men, mean age 60 years, range 45–84); seven with cytoplasmic, ANCA positive, Wegener's granulomatosis (all men, median age 51 years, range 30–62); three patients with pANCA positive systemic necrotising vasculitis (all women, median age 41 years, range 21–69) and 36 normal healthy volunteers (13 men, median age 43 years, range 18–81).

IMMUNOHISTOCHEMISTRY

The prevalence, titre, antibody class, and IgG subclass distribution of ANCA were determined by an alkaline phosphatase technique developed in this department.[4] For ANCA prevalence and titre determination, all patients' sera were initially tested at a dilution of 1:5 on alcohol fixed neutrophils. Sera positive at 1:5 dilution were then retested at 1:50 dilution on alcohol fixed neutrophils and again at 1:5 on formal acetone fixed neutrophils. (The formal acetone fixative consisted of 36 ml 39% formaldehyde BDH 28421, 180 ml 100% acetone BDH 27023, and 184 ml phosphate buffered saline.) Antinuclear antibodies were detected in the conventional way using rat liver substrate and HEP 2 cells.

For ANCA positive individuals, the class of ANCA response (that is, whether IgG, IgA, or IgM) was determined using rabbit antibody to human IgG, IgG, and IgM respectively (DAKO, High Wycombe, UK). The IgG subclass was then determined for patients with IgG ANCA. Five slides with alcohol fixed neutrophils were used for each serum sample tested. All five were incubated with patient's serum, diluted 1:5 in TBS (50 mM Tris, 145 mM NaCl, pH 7·6) for 30 minutes. After two, three-minute washes in TBS, slides 1–4 respectively were incubated for 30 minutes with specific mouse anti-human immunoglobulins directed at IgG1, IgG2, IgG3, and IgG4. The specific monoclonal antibodies used were HP 6001 for IgG1, HP 6002 for IgG2, HP 6050 for IgG3, and SK 44 for IgG4 (Sigma Immunochemicals). The monoclonal antibodies were diluted 1:100 in TBS before incubation. Slide no 5 was the internal control slide and was incubated with TBS only. After two further three-minute TBS washes, bound antibody was detected using indirect immunohistochemistry. Alkaline phosphatase conjugated rabbit antibody to mouse immunoglobulin heavy chains (DAKO, High Wycombe, UK) was used for slides 1–4. This was diluted 1:10 in TBS. Slide 5 was incubated with rabbit antibody to human immunoglobulin heavy chains (IgA/IgG/IgM) (DAKO, High Wycombe, UK). This incubation lasted a further 30 minutes. Finally, after a further two, three-minute TBS washes, fast red TR salt was used as a substrate for alkaline phosphatase.

Established negative and positive serum controls were included with each assay as a further internal control. In addition, the neutrophil donor was rotated at regular intervals to prevent any problems with the source of neutrophils. All slides were read independently by two observers (DB and KF).

Statistical analysis was by the Fisher exact and Mann-Whitney U tests. A p value of $<0·05$ was considered to be significant.

TABLE II *Clinical details of the primary sclerosing cholangitis patients (n=63)*

Male:female	42:21
Median age (y)	62 (range 24–86)
Liver histology*:	
Stage 1	12 (20%)
Stage 2	26 (43%)
Stage 3	19 (31%)
Stage 4	4 (7%)
Site on ERCP†:	
Intrahepatic only	27 (48%)
Extrahepatic only	4 (7%)
Intra/extrahepatic	21 (38%)
Normal ERCP	4 (7%)
Concomitant IBD‡:	
Ulcerative colitis	50 (79%)
Crohn's disease	6 (10%)
None	7 (11%)

*Data only available for 61 patients. †Data only available for 56 patients. ‡Data only available for 62 patients.
ERCP=endoscopic retrograde cholangiopancreatogram.
IBD=inflammatory bowel disease.

TABLE III *Antineutrophil cytoplasmic antibodies prevalence in disease groups studied. Statistical significance as indicated*

Disease	*No*	*pANCA at 1:5*	*pANCA at 1:50*
PSC+UC	50	35 (70%)*	27 (54%)†
PSC+CD	6	4 (67%)	3 (50%)
PSC only	7	2 (29%)	1 (14%)
Total PSC	63	41 (65%)*	31 (49%)†
UC	85	38 (45%)*	21 (25%)†
Coeliac disease	17	0	
Dermatitis herpeteformis	10	0	
Normal	36	0	

PSC=primary sclerosing cholangitis; UC=ulcerative colitis. *p=0·01 for total PSC *v* UC, and p=0·004 for PSC+UC *v* UC. †p=0·002 for total PSC *v* UC and p=0·0006 for PSC+UC *v* UC.

Results

ANCA PREVALENCE AND TITRE

The ANCA prevalence and titres for the different groups studied are given in Table III. In the UC group, ANCA prevalences at dilutions of 1:5 and 1:50 were 38 of 85 (45%) and 21 of 85 (25%) respectively, and exclusively of the perinuclear type (Fig 1). Taking the PSC group as a whole, ANCA was detected in 41 of 63 (65%) at a serum dilution of 1:5 and 31 of 63 (49%) at a dilution of 1:50; and again was perinuclear in type. The difference in ANCA prevalence between the two groups was statistically significant at both dilutions (p=0·01 and p=0·002 respectively, Fisher's exact test). Comparing PSC+UC and UC alone, the ANCA prevalences were 70% *v* 45% at 1:5 and 54% *v* 25% at 1:50. Again the difference was statistically significant at both dilutions (p=0·004 and p=0·0006 respectively, Fisher's exact test). Although the ANCA prevalence in the PSC+CD group was 67%, and thus similar to the PSC+UC group, the prevalence in the PSC only group was only 29% at a dilution of 1:5, dropping to 14% at 1:50.

All seven patients with Wegener's granulomatosis gave a cytoplasmic pattern of staining but, as expected, the three patients with systemic necrotising vasculitis also gave a perinuclear pattern. This pattern was indistinguishable from the perinuclear pattern in the PSC and UC groups. All other controls were negative (Fig 2).

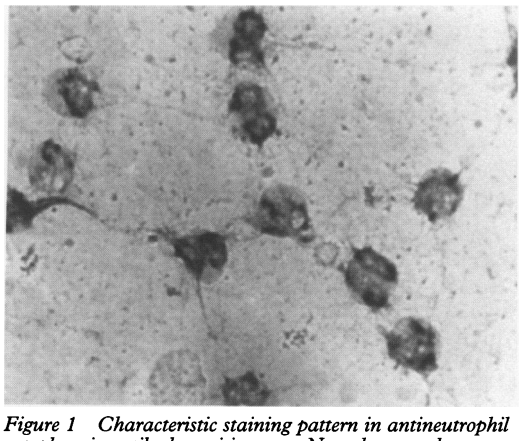

Figure 1 Characteristic staining pattern in antineutrophil cytoplasmic antibody positive sera. Note the granular cytoplasmic staining with perinuclear accentuation.

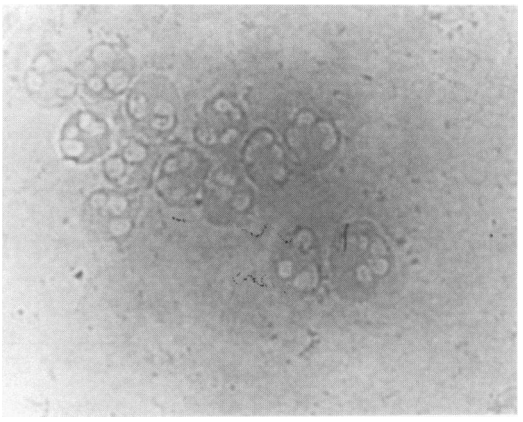

Figure 2 Antineutrophil cytoplasmic antibody negative sera. Note the 'ghostly' appearance of the neutrophils with complete absence of staining throughout the cytoplasm.

Of the 41 ANCA positive PSC patients, 34 were negative when tested on formal acetone fixed neutrophils, six gave a granular cytoplasmic pattern, and one a homogenous cytoplasmic pattern. In the UC group, 33 of the 38 patients positive on alcohol fixed neutrophils gave no staining at all on formal acetone fixed neutrophils, whereas the remaining five demonstrated a granular cytoplasmic pattern. Fifteen ANCA positive UC patients were tested for ANA on rat liver substrate and HEP 2 cells at a serum dilution of 1:5. Of these, only two had ANA present at a 1:5 serum dilution. One of these had only a weak ANA at a dilution of 1:40 but had ANCA present at a dilution of at least 1:50. In the case of the PSC ANCA positive group, six of the 18 tested had ANA at a titre of 1:40 or above.

In the case of the PSC group (Table IV), the presence of ANCA was not affected by sex, age, or liver histology. However, ANCA positivity was significantly associated with evidence of both intrahepatic and extrahepatic PSC on endoscopic retrograde cholangiography (18 of 37 or 49%) compared with ANCA negative patients (3 of 19 or 16%) (p=0·016, Fisher's exact test). The ANCA negative PSC patients were more likely to have intrahepatic PSC only (13 of 19 or 68% *v* 14 of 37 or 38%) (p=0·03). There was no significant difference in the distribution of inflammatory bowel disease between the two groups.

For the UC group (Table V), again presence of ANCA was not related to sex, age, treatment, extent, or activity of disease. However, ANCA positive patients had a significantly longer median duration of disease (median 50 months, range 2–262) compared with ANCA negative patients (median 28 months, range 1–371) (p=0·029, Mann-Whitney U test).

IgG SUBCLASS OF ANCA

Most UC and PSC ANCA belonged to the IgG class. The ANCA IgG subclass distribution for the 30 UC only and 27 PSC+UC patients tested is given in Table VI. The staining pattern was perinuclear in all cases. IgG1 was the predominant subclass in both PSC+UC and UC only ANCA and IgG3 was the second most common. Compared with UC

TABLE IV *Comparison of antinuclear cytoplasmic antibody (ANCA) positive and ANCA negative primary sclerosing cholangitis (PSC) patients. Statistical significance only where indicated*

	ANCA positive (n=41)	*ANCA negative (n=22)*
Male:female	28:13 (68% male)	14:8 (64% male)
Median age at testing (y)	63 (24–86)	49 (22–82)
Liver histology*:		
Stage 1	7/40 (17·5%)	5/21 (24%)
Stage 2	17/40 (42·5%)	9/21 (43%)
Stage 3	13/40 (32·5%)	6/21 (28·6%)
Stage 4	3/40 (7·5%)	1/21 (5%)
Site of PSC*:		
Intrahepatic only	14/37 (38%)	13/19 (68%)†
Extrahepatic only	2/37 (5%)	2/19 (11%)
Intrahepatic/extrahepatic	18/37 (49%)‡	3/19 (16%)
Normal	3/37 (8%)	1/19 (5%)
ANA >1:40*	6/18 (33%)	1/6 (17%)
Concomitant ulcerative colitis	35/41 (85%)	15/22 (68%)
Concomitant Crohn's disease	4/41 (10%)	2/22 (9%)
No colitis	3/41 (7%)	5/22 (23%)

*Data limited to patient numbers as shown. †p=0·03. ‡p=0·016. ANA=antinuclear antibody.

ANCA, PSC+UC ANCA showed a significant increase in IgG3 (26% *v* 7%, p<0·05, Fisher's exact). A trend toward higher IgG2 and IgG4 expression was also noted in PSC+UC ANCA. Most UC ANCA was IgG1 only. The combination of IgG1+IgG3 was detected in 26% of PSC+UC ANCA but only in 3% of UC only ANCA (p=0·015) (Table VII). There was no association between IgG subclass and the clinical parameters shown in Tables IV and V. Four PSC and one UC sera tested repeatedly over time showed no variation of ANCA IgG subclass distribution.

Discussion

ANCA have been reported in 26–85% of patients with PSC and up to 68% in those with UC. They have high specificity for PSC and UC compared with other liver and diarrhoeal diseases respectively. We have previously reported a highly sensitive and specific immunoalkaline phosphatase method for detecting this antibody in PSC.[4] In the present study we have shown that for adult cases of PSC, ANCA has a diagnostic sensitivity of 65% at a serum dilution of 1:5 and 49% at a dilution of 1:50. For UC only the sensitivities were 45% and 25% respectively.

As in previous studies[1 2 4] we were unable to show any relationship between ANCA and the extent of UC. Although ANCA positive UC patients in this study were more likely to have active disease and be on combination therapy with a 5 aminosalicylic acid compound and immunosuppressive drugs, this was not statistically significant compared with the ANCA negative UC patients. However, ANCA positive UC patients had a significantly longer median duration of disease than ANCA negative patients (Table V). The precise importance of this finding is unclear as this has not been shown by other groups,[2 4 6 7] and indeed in two of our 38 ANCA positive UC patients, ANCA was detected even after they had had a colectomy. This has been observed by others[2] and suggests that the diseased organ is not essential for ANCA generation once a clone of B cells has been 'programmed' for antibody production.

The only significant association in the ANCA positive PSC group (Table IV) was with the presence of both intrahepatic and extrahepatic disease, as judged by endoscopic retrograde cholangiopancreatography. This may suggest that ANCA positive PSC patients are likely to develop more extensive disease. Conversely, more extensive disease may predispose to the development of ANCA. In the present study, no association of ANCA with severity of PSC as judged by liver histology was noted, but this may simply reflect the focal nature of PSC. Indeed, whether radiologically more extensive PSC is associated with histologically more severe liver disease is also not clear. A recent report suggested that ANCA may be a prognostic marker in PSC because ANCA positive PSC patients were more likely to progress to liver transplantation.[16] However, these patients were also more likely to have biliary tract calculi and as this is associated with recurrent cholangitis and a poorer outcome it could be argued that in this study ANCA was merely a marker of the presence of calculi rather than of prognosis.

At present, the gold standard for diagnosis of PSC remains an endoscopic retrograde cholangiopancreatogram.[5] Liver histology, although characteristic, may be focal, and liver function tests are also unreliable. We have previously discussed the potential diagnostic use of ANCA as a non-invasive test to screen for PSC in childhood.[17] In the present study, patients with PSC+UC had higher ANCA titres than those with UC only (ANCA

TABLE V *Comparison of antinuclear cytoplasmic antibody (ANCA) positive and ANCA negative ulcerative colitis (UC) patients*

	ANCA positive (n=38)	*ANCA negative (n=47)*
Male:female	16:22 (42% male)	21:26 (45% male)
Median age (y)	48 (21–83)	56 (23–89)
UC extent:		
To splenic flexure	30 (79%)	38 (81%)
Proximal to splenic flexure	8 (21%)	9 (19%)
Treatment		
Nil	1 (3%)	6 (13%)
SZP/5ASA only	25 (66%)	34 (72%)
SZP/5ASA+prednisolone/azathioprine	11 (29%)	7 (15%)
Azathioprine only	1 (3%)	0
Activity:		
Active	11 (29%)	7 (15%)
Inactive	27 (71%)	40 (85%)
Median duration of disease (m)‡	50 (range 2–262)*	28 (range 1–371)†

*Data only available for 35 patients. †Data only available for 43 patients. ‡p=0·029 (Mann-Whitney U test). SZP=salazopyrine, 5ASA=5 aminosalicylic acid.

TABLE VI *IgG subclass distribution of antinuclear cytoplasmic antibody (ANCA) positive primary sclerosing cholangitis (PSC) and ulcerative colitis (UC) patients (expressed as a percentage of the total IgG ANCA). NB: Some patients have more than one isotype*

	No	*IgG1*	*IgG2*	*IgG3**	*IgG4*
UC only	30	28 (93%)	1 (3%)	2 (7%)	0
PSC+UC	27	26 (96%)	4 (15%)	7 (26%)	3 (11%)

*p<0·05 (Fisher's exact test) for UC only *v* PSC+UC.

TABLE VII *Occurrence of specific IgG isotypes in antinuclear cytoplasmic antibody (ANCA) positive ulcerative colitis (UC) only and primary sclerosing cholangitis (PSC)+UC sera*

	No	*IgG1 only*	*IgG1+IgG3*
UC only	30	27 (90%)	1 (3%)
PSC+UC	27	17 (63%)	7 (26%)
p (Fisher's exact)		0·016	0·015

diagnostic sensitivity of 54% *v* 25% at 1:50, p=0·0006). The IgG subclass also differed between PSC+UC and UC only ANCA, with a significant increase in IgG3 ANCA in PSC+UC patients. This result is in keeping with a recent report by Vidrich[13] who demonstrated increased IgG3 ANCA in PSC irrespective of the presence of concomitant UC. In the present study, higher levels of IgG2 and IgG4 were also detected in the PSC+UC group. This different ANCA titre and IgG subclass distribution may reflect differences in underlying immune mechanisms between UC only and PSC+UC patients. Typically, IgG1 and IgG3 exhibit a stronger complement activating capacity than the other IgG isotypes and bind more firmly to Fc receptors on mononuclear cells.[14] Furthermore, in Wegener's granulomatosis, patients with renal involvement have been shown to have higher levels of the IgG3 isotype of ANCA and this has been shown to correlate more closely with active disease[18] suggesting that certain isotypes may have a greater influence on disease pathogenesis and be associated with different end-organ involvement. In the current study, no association was noted between any particular ANCA IgG subclass and any of the clinical parameters shown in Tables I and II. Furthermore, serial measurements in four PSC and one UC patient failed to show any alteration in the ANCA IgG subclass distribution over time. We reviewed the case notes of the two IgG3 ANCA positive UC-only patients in this study and both had normal liver biochemistry. A larger prospective study is currently underway to investigate the significance of the IgG3 isotype in UC as a possible marker for the development of PSC and to determine if certain IgG isotypes of ANCA correlate more closely with disease activity in UC and PSC.

IgG1 and IgG3 were the predominant isotypes in both UC only and PSC+UC ANCA. This is in keeping with other autoimmune conditions such as systemic lupus erythematosus and Sjogren's syndrome.[19] In this respect they differ from the vasculitis associated ANCA which has relatively high levels of IgG1 and IgG4.[20] In particular, no IgG4 was detected in the UC group. These results are in keeping with the recent findings of Ellerbroek *et al*[9] who were also unable to detect an IgG4 response in UC ANCA, but found a predominance of IgG1 and IgG3. In contrast to this, a French group[21] determined the IgG subclass distribution in 21 patients with UC and found a 100% IgG1 response, 91% IgG3 response, and lower levels of IgG2 (5%) and IgG4 (19%).

The close association between PSC and UC[5] and the high prevalence of ANCA in PSC and UC compared with other liver and diarrhoeal diseases respectively is interesting and suggests there may be an aetiological link. At present it is not clear whether ANCA is UC or PSC associated or can exist in both conditions independently. The fact that ANCA has been previously demonstrated in patients with either PSC (without UC) or UC alone does not necessarily mean that it is independently occurring in these diseases. It is possible that patients in these studies have undiagnosed UC or PSC respectively.

The age of onset of UC typically precedes that of PSC by 10–20 years. If ANCA is UC associated and, as in this study, prevalence is associated with longer median disease duration, then it could be argued that the higher prevalence in UC+PSC may simply by a UC associated phenomenon with PSC developing as a complication of UC. In this context it is interesting to note that ANCA positive PSC patients in the present study tended to be older than ANCA negative PSC patients (Table IV) and both titre and IgG subclass of ANCA differ between UC only and PSC+UC, suggesting that an alteration in underlying immune mechanisms may play a role in the development of PSC. Similarly, it is interesting to note that in the present study only 29% of PSC only patients were ANCA positive at 1:5, dropping to 14% at 1:50. This might also suggest that ANCA in PSC+UC is UC associated. Unfortunately the duration of UC in the PSC+UC group could not be determined and compared with the UC only group. Therefore, whether the higher ANCA prevalence in the former group was due to longer duration of UC is not clear. However, patients with PSC+Crohn's disease had a similar ANCA prevalence to those with PSC+UC (67% and 70% at 1:5 respectively) as well as a similar ANCA IgG subclass distribution (unpublished observation). As the prevalence of ANCA in Crohn's disease is very low (0/32 in a previous study from our group[4]) this finding would argue against ANCA being UC associated in PSC+UC and suggests that it is due to the PSC itself. In support of this, Duerr *et al*,[1] using a combination of fixed neutrophil ELISA and immunofluorescence, detected perinuclear ANCA in 8 of 14 (58%) PSC only sera. Furthermore, the detection of ANCA in the serum of patients with autoimmune hepatitis and primary biliary cirrhosis in some studies[3 22 23] also argues against it being UC associated.

Unlike the vasculitides, the antigen(s) for ANCA in PSC and UC has not been identified. The perinuclear staining pattern is not a reliable indication of the distribution of the antigen and in Wegener's and other vasculitides is thought to be an artefact of alcohol fixation caused by the translocation of basic proteins to the negatively charged nucleus.[24] Thus, if neutrophils are fixed in formaldehyde the redistribution of antigen is prevented and the pattern becomes cytoplasmic. Abolition of the staining pattern altogether on formalin fixed neutrophils is more typical of an antinuclear antibody. In this respect our results on formalin fixed neutrophils are interesting as, while most perinuclear staining UC and PSC sera showed loss of the signal altogether, few had evidence of antinuclear antibodies when tested in the conventional way on rat liver substrate. This provides further evidence that the antigen for UC and PSC ANCA is not myeloperoxidase or other antigens conventionally responsible for a perinuclear

staining pattern on alcohol fixed neutrophils.[24] However, a predominance of the IgG1 and IgG3 isotypes for both UC only and PSC+UC ANCA is in keeping with evidence to date which suggests that the antigen is likely to be proteinaceous in nature[4] as this is the predominant response produced against protein antigens.[14] Furthermore, although some differences in IgG subclass distribution were noted between UC only and UC+PSC ANCA, suggesting possible differences in immune regulation, this does not necessarily imply that the antigen(s) for PSC ANCA differ(s) from that in UC ANCA. In Wegener's granulomatosis the same antigen (namely serine proteinase 3) is able to elicit a variable IgG subclass response and in this way influence the pathogenesis of the disease process.[18 25] The same could conceivably be true of PSC and UC ANCA antigen(s).

In conclusion, in the present study we have shown that ANCA is a diagnostic marker of disease in both UC and PSC. In UC, ANCA was associated with significantly longer median disease duration whereas in PSC, ANCA was significantly associated with the extent of involvement of the biliary tree. Further studies are needed to verify these findings. While the higher titre and different IgG subclass distribution of ANCA in PSC+UC compared with UC only suggests differences in underlying immune regulation, determination of ANCA titre and IgG subclass is unlikely to have a role in distinguishing between PSC+UC and UC only ANCA. Future identification of the antigen(s) against which ANCA is/are directed should allow the development of a more sensitive and specific test for the diagnosis of these conditions and also determine whether ANCA is associated with UC or PSC.

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