Pathology (October 2019) **51(6)**, pp. 634–639

IMMUNOPATHOLOGY

The significance of ANCA positivity in patients with inflammatory bowel disease

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Summary

Traditionally anti-neutrophil cytoplasmic antibodies (ANCA) are used to subtype patients with inflammatory bowel disease (IBD) and to predict primary sclerosing cholangitis (PSC). The clinical utility of this testing in the Australian context is not known. Our retrospective, cross-sectional study looked at the results of ANCA testing performed during routine clinical review and aimed to retrospectively review (1) the distribution of different ANCA subtypes for IBD patients, (2) the temporal change of ANCA status, and (3) the predictive value of ANCA for PSC. Sixty-four IBD patients attending our hospital gastroenterology clinic between 2012 and 2016 had at least one ANCA test requested. Surprisingly, >80% of the IBD patients in our cohort who underwent ANCA testing had a positive ANCA result and a significant proportion had positive PR3 antibodies. However, no specific ANCA pattern predicted a specific IBD subtype or clinical course. Pairing ANCA and anti-*Saccharomyces cerevisiae* (ASCA) did not add value in subtyping IBD for these patients. Our study suggests that there is little value in ordering an ANCA for patients with IBD.

Key words: ANCA; MPO; PR3; inflammatory bowel disease.

Received 21 November 2018, revised 11 July, accepted 18 July 2019
Available online 26 August 2019

INTRODUCTION

Anti-neutrophil cytoplasmic antibodies (ANCA) were initially discovered as a useful marker for systemic vasculitides such as granulomatous with polyangiitis (GPA) or microscopic polyangiitis (MPA) in the 1980s.[1,2] In these diseases, the primary antigens that are recognised are proteinase 3 (PR3) and myeloperoxidase (MPO).[3] The International Consensus Statement on Testing and Reporting of ANCA published in 1999 recommended that indirect immunofluorescence (IIF) be performed on all ANCA requests, with further testing for those with typical or atypical fluorescent patterns with enzyme-linked immunosorbent assays (ELISAs) that detect PR3 or MPO antibodies.[3]

In the 1990s it was noted that a significant proportion of patients with inflammatory bowel disease (IBD) had positive ANCA on IIF. This was more prevalent in patients with ulcerative colitis (UC) than patients with Crohn's disease (CD) as reviewed by Bossuyt.[4] Furthermore, anti-*Saccharomyces cerevisiae* (ASCA) was found to be highly specific for CD.[5,6] This led to the conventional teaching that paired ANCA and ASCA might be helpful in subtyping UC from CD.[4,7] As a significant proportion of patients with both inflammatory bowel disease and primary sclerosing cholangitis (PSC) are ANCA positive, ANCA status had been said to be of predictive value for PSC.[6,8] As can be seen from Table 1, the prevalence of ANCA and MPO/PR3 in primary studies in inflammatory bowel disease varies significantly across different studies (also reviewed in Bossuyt).[4] This may be secondary to differences in study population age, ethnicity, inter-assay and inter-laboratory differences. Furthermore, the distinction between atypical and typical perinuclear ANCA (p-ANCA) was not routinely made until the 2000s.[9] As a result, studies in the 1990s reported p-ANCA as the dominant ANCA pattern in IBD patients with ulcerative colitis, whereas studies in the 2000s identified atypical p-ANCA as the dominant ANCA pattern in these patients.[4,8,10–12]

While it is generally acknowledged that patients with IBD may have a positive ANCA by immunofluorescence, a significant number have also been found to be MPO and PR3 positive (Table 1); however, little is known about the significance of these observations. Do these patients have a concurrent vasculitis or do they have a different disease course for their IBD from those who are MPO and PR3 negative? As the association between IBD and MPO and PR3 positivity has not been reviewed from the Australian context, the aim of our study was to look at the characteristics of patients with both IBD and a positive ANCA, in particular the rate of MPO/PR3 positivity amongst the ANCA positive patients, to see whether there appeared to be any clinical correlations in this subgroup.

MATERIAL AND METHODS

Patients who attended the IBD clinic at the Canberra Hospital between 2012 and 2016 and had ANCA ordered during the same time frame were included. Demographic information, and clinical characteristics [including type of IBD, all ANCA testing (not just in the 4 year interval), ASCA testing, extra-intestinal manifestations, medication regime, response to treatment, and surgical history] were retrieved retrospectively from the medical records for these patients. This retrospective analysis was approved by the ACT Health Low Risk Ethics Committee (ETHLR.17.124).

The patient's previous ANCA testing was retrieved from the laboratory information system. In the testing laboratory, IIF is performed using the

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2019.07.002

Table 1 Summary of literature regarding prevalence of ANCA in IBD patients

	Hertervig[8] 1994	Broekroelofs[11] 1994	Kaneko[25] 1995	Sugi[26] 1999	Saibeni[6] 2003	Papp[12] 2009	Bouzid[10] 2011
Study population	Adult	Adult	Children	Adult	Adult	Adult	Adult
Ethnic group	Scand	Scand	Japanese	Japanese	Italian	Hungarian	Tunisian
ANCA+ in UC	50.3%	50.7%	35.0%	76.9%	–	38.0–54.4%	59.3%
p-ANCA+ in UC	43.2%	49%	–	63.5%	39.6%	Atyp p, 6.3–43%; p-ANCA, 5.1–38%	Atyp p, 44.1%; p-ANCA, 3.4%
ANCA+ in CD	24.3%	40.0%	0.0%	74.4%	–	11.1–23.2%	27%
p-ANCA+ in CD	15.6%	40.0%	–	72.1%	7.5%	Atyp p, 8.1–16.2%; p-ANCA, 1.0–10.1%	Atyp p, 8.1%; p-ANCA, 5.4%
MPO+ in IBD	1.8%	2.9%	–	54.7%	–	2.5%	0%
PR3+ in IBD	0.0%	2.9%	–	–	–	9.8%	0%
Most common ANCA pattern for IBD	p-ANCA	p-ANCA	p-ANCA	p-ANCA	–	Atyp p	Atyp p

ANCA, anti-neutrophil cytoplasmic antibody; Atyp p, atypical p-ANCA; CD, Crohn's disease; IBD, inflammatory bowel disease; Scand, Scandinavian; UC, ulcerative colitis.

Euroimmun IIFT Granulocyte Mosaic slides (Euroimmun, Germany) with both ethanol- and formalin-fixed granulocyte field, and a HEp2 with granulocyte field to identify patients with a positive ANA. The patterns reported are p-ANCA, atypical p-ANCA, c-ANCA, and atypical ANCA. MPO and PR3 antibodies were determined using enzyme-linked immunosorbent assay (ELISA; Orgentec Diagnostika, Germany) prior to May 2015 and chemiluminescence immunoassay (CIA; Quanta Flash, Inova Diagnostics, USA) after October 2015. In the intervening period the samples were run in parallel on both assays as part of a standard assay change protocol, however no IBD patients in our cohort had ANCA ordered during this time. We have published previously on the correlation between the two assays.[13] MPO or PR3 titre >5 was deemed positive for ELISA. MPO >5 or PR3 >6 was deemed positive for CIA. ASCA antibodies were analysed by the Royal Prince Alfred Hospital Immunopathology department (Sydney South West Pathology Service) using the Quanta Lite kit (Inova) on the DSX ELISA platform (Dynex Technologies, USA) prior to October 2015 (with ASCA IgA or ASCA IgG >25 U/mL considered positive), and EliA ASCA assay on the Phadia 250 (Thermo Scientific, USA) from October 2015 (<7 negative, 7–10 equivocal, >10 positive).

RESULTS

Samples and baseline characteristics

At total of 2550 patients attended the IBD clinic at the Canberra Hospital between 2012 and 2016. Of these, 64 patients (2.5%) had an ANCA test ordered within the same timeframe. Of these, 40 patients had CD, 21 patients had UC, and three patients had indeterminate colitis. There were 41 female patients and 23 male. The median age of onset of symptoms was 26 years (range 7–73). Median time delay from onset of symptom to diagnosis of IBD was 5.5 months (range 1–132 months). Forty-two patients had been on biological agents at some stage.

Review of the medical records for these patients found that arthritis (with or without a formal diagnosis of seronegative arthropathy) was the most commonly reported extra-intestinal manifestation, occurring in 26 (40.6%) patients. This was followed by hepatobiliary complications [including primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), and autoimmune hepatitis], occurring in 11 (17.2%) patients. Mucocutaneous manifestations, including Sweet's syndrome, pyoderma gangrenosum, erythema nodosum and mouth ulcers were common, each occurring in nine (14.1%) of patients.

A total of 121 ANCA orders were identified from our historical record for these 64 patients from as early as 2004. Twenty-seven (22%) patients had ANCA ordered more than once; of them, three patients who were found to be PR3 positive were tested a total of 24 times. Of the 64 patients, 55 (86%) had at least one positive ANCA, 34 (53%) were positive by IIF, while 21 (33%) had a positive MPO or PR3. Of the 121 encounters, 83 were ordered for CD patients, 31 were ordered for UC patients, and seven for patients with indeterminant colitis. The majority of encounters with negative IIF (14/20) did not have MPO and PR3 performed; all encounters with positive IIF had MPO and PR3 performed. Of the 72 encounters of MPO and PR3 performed for CD patients, ELISA was carried out for 63 encounters and CIA for nine encounters; 38 ELISA and one CIA were positive. Of the 28 encounters of MPO and PR3 performed for UC patients, ELISA was carried out for 22 encounters and CIA for six encounters; seven ELISA and two CIA were positive. Of the seven encounters of MPO and PR3 performed for patients with indeterminant colitis, all were done by ELISA; three were positive.

For subsequent analysis, if the MPO or PR3 was positive this was considered the most important finding and their IIF status disregarded to avoid confusion. Possible ANCA results in this study were therefore negative, PR3 positive, MPO positive, p-ANCA, c-ANCA, atypical p-ANCA and atypical ANCA.

The clinical reasoning for the ANCA order is difficult to determine retrospectively. The pathology request form did not contain clinical information or did not contain legible clinical information for 34 test requests (28%). For 33 requests (27%) the clinical notes listed 'IBD', 'UC' or 'CD', for two (1.7%) the clinical notes were '?IBD', and for another two (1.7%) 'IBD subtyping'. The other major indications commonly provided include arthritis/arthralgia (8/121, 6.6%), deranged liver function test/liver screen (11/121, 9.1%), rash (4/121, 3.3%), differentiating between IBD vs Behcet's disease (4/121, 3.3%), fever (3/121, 2.4%), acute kidney injury (2/121, 1.7%), and autoimmune screen (2/121, 1.7%). Importantly, 'previous positive ANCA' (2/121, 1.7%), and '?vasculitis' (2/121, 1.7%) were also provided as indication for some request encounters.

ANCA positivity and type of inflammatory bowel disease

We then looked at the rate of ANCA positivity in relation to the underlying diagnosis. Of the 40 patients with Crohn's disease, 33 (83%) were positive by either IIF or for MPO/PR3

on at least one occasion. Of the 21 patients with ulcerative colitis, 19 (90%) were positive by IIF or for MPO/PR3. The distribution of ANCA status for each IBD subtype is presented in Fig. 1. Of note, for the purpose of Fig. 1 and Table 2, if a patient had multiple ANCA encounters ordered, then only the highest ranked ANCA was considered. Hierarchy of the ANCA from highest to the lowest was as follows: PR3 or MPO > p-ANCA or c-ANCA > atypical p-ANCA > atypical > negative. The reason for counting total patient numbers as opposed to counting total ANCA encounters for Fig. 1 and Table 2 was to minimise influence of the skewed distribution by small number of patients with repeated ANCA orders as described above.

For patients with Crohn's disease, all seven ANCA results were represented. PR3 positive ANCA appeared most commonly for Crohn's disease (11 patients, 28%) and was more prevalent than atypical p-ANCA (8 patients, 20%). For patients with ulcerative colitis, PR3 positive ANCA (7 patients, 33%), and atypical p-ANCA (7 patients, 33%) were most commonly identified. Of the three patients with indeterminate colitis, two had PR3 positive ANCA and one was c-ANCA positive.

The relationship between ANCA status and surgery within 12 months of ANCA order is presented in Table 2. ANCA status did not clearly predict what surgery (if any) IBD patients would undergo within 12 months of ANCA being requested.

Change in ANCA status over time

Of the 27 patients with ANCA ordered more than once, 10 patients had an ANCA status that changed over time. Three patients had their ANCA status change more than once (see Table 3). The relationship between a change in ANCA status and whether there was a corresponding change in luminal disease activity or treatment is also shown in Table 3. Bowel resection or changes in treatment were implicated in eight of 13 (61.5%) encounter changes, but did not result in a consistent directional change in ANCA status (e.g., ANCA positive to not detected).

Is there a clinical correlation with positive PR3 ANCA in IBD?

Of the 64 IBD patients in our study, 20 patients were PR3 positive at some stage, one patient was MPO positive, and 43 patients were negative for both MPO and PR3 [double negative group (this group contained patients who were ANCA positive by indirect immunofluorescence)]. Due to this distribution, only the PR3 positive and double negative groups could be compared. Of the PR3 positive IBD patients, 11 (55%) had CD, seven (35%) had UC, and two (10%) had indeterminate colitis. This was similar to the distribution of IBD subtypes amongst double negative patients: 65.1% had CD, 32.6% had UC, and 2.3% had indeterminate colitis. The median age of onset of symptoms between the two groups were similar: 26 years for the PR3 positive group, and 25.5 years for the double negative group. The rate of patients who had undergone treatment with a biological agent (65% vs 69.8%) or bowel resection (25% vs 30.2%) at any stage of their disease was also similar between the PR3 positive and double negative group.

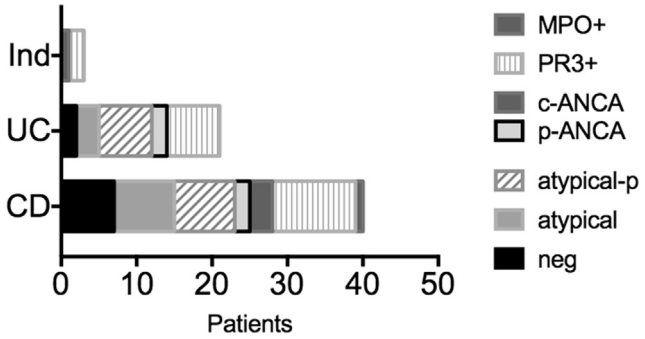

Fig. 1 ANCA status for IBD patients. Distribution of ANCA results for each of the types of IBD. CD, Crohn's disease; UC, ulcerative colitis; Ind, indeterminate colitis. ANCA results shown cumulatively from left to right: CD (40 patients): neg (7), atypical (8), atypical-p (8), p-ANCA (2), c-ANCA (3), PR3+ (11), MPO+ (1). UC (21 patients): neg (2), atypical (3), atypical-p (7), p-ANCA (2), c-ANCA (0), PR3+ (7), MPO+ (0). Ind (3 patients): neg (0), atypical (0), atypical-p (0), p-ANCA (0), c-ANCA (1), PR3+ (2), MPO+ (0).

The PR3 positive group had a lower rate of arthritis [4/20 (20%) vs 27/43 (62.8%)] but higher rate of PSC [6/20 (30%) vs 3/43 (7%)] compared to the double negative group. Of the 64 patients in our cohort, six patients with positive ANCA had been evaluated by an immunologist for assessment of vasculitis (3 of them had PR3 positive ANCA, 2 had atypical p-ANCA, 1 had c-ANCA). None were deemed to have systemic vasculitis.

Of the 121 ANCA encounters, 99 (82%) were performed at the time that our confirmatory test for MPO and PR3 was carried out by ELISA and 22 (18%) after the method change to CIA. Of the 11 patients with Crohn's disease and a positive PR3, 10 were determined using ELISA and one by CIA. The single patient with Crohn's disease and MPO positive had this determined by CIA. For patients with ulcerative colitis and positive PR3, five were tested by ELISA and two by CIA.

Due to the difficulties of retrospective analysis, we were only able to find disease activity recorded within 3 months of the ANCA order for nine patients with CD and three patients with UC. Therefore, we were not able to determine if there was a correlation between having a positive PR3 ANCA and disease activity.

Paired ANCA and ASCA results and IBD subtypes

Twenty-five IBD patients had paired ASCA and ANCA requested. The results for the 30 paired ANCA and ASCA specimens for these 25 patients are presented in Table 4. Two patients had two paired samples sent over time, for one the ASCA results changed between episodes and for the other the results remained consistent. Another patient had four paired samples sent over time, and the result of the ANCA changed. These are listed in Table 4 as independent samples.

In our study, patients with CD were found to have multiple combinations of patterns of ASCA and ANCA (see Table 4). Positive ASCA and negative atypical p-ANCA (traditionally thought to favour a diagnosis of Crohn's disease) was not the dominant disease pattern for patients with CD in our study (accounting for 5/18 encounters for patients with CD). Neither was a positive atypical p-ANCA and negative ASCA the dominant disease pattern for patients with UC (accounting for 2/9 encounters for patients with UC).

Association with PSC

Lastly, we looked at whether ANCA positivity or subtype was associated with PSC. Twenty IBD patients had a PR3 positive ANCA at some stage during the course of their

Table 2 ANCA status and surgery within 12 months of ANCA testing

ANCA status (no. patients)	Bowel resection	Perianal abscess drainage or Seton insertion	Isolated stoma creation	No previously mentioned surgeries
Negative (*n*=9)	0	1	0	8
Atypical (*n*=11)	1	1	0	9
Atypical p-ANCA (*n*=15)	1	1	0	13
p-ANCA (*n*=4)	0	1	0	3
c-ANCA (*n*=4)	1	0	0	3
PR3 (*n*=20)	2	0	2	16
MPO (*n*=1)	0	0	0	1

One patient had both bowel resection and stoma creation at the same time and was counted in the 'bowel resection category'.
One patient had both colectomy and abscess drainage at different time points and was counted in the 'bowel resection category'.
One patient only had isolated appendicectomy and was counted in the 'no previously mentioned surgery' category.
If a patient had more than one positive ANCA, they were assigned to the category of their most specific result (MPO/PR3 > p-ANCA, c-ANCA > atypical p-ANCA > atypical).

Table 3 Change in ANCA status and corresponding change in luminal disease or treatment

Patient	IBD subtypes	Pattern change	Change in treatment or luminal disease activity
1	UC	NEG → PR3 (ELISA)	Commenced biological agent
2	UC	NEG → PR3 (ELISA)	Nil
3	CD	NEG → PR3 (ELISA)	Bowel resection
3	CD	PR3 (ELISA) → NEG	Considered increasing frequency of infliximab due to increased breakthrough symptom
4	CD	NEG → Atypical p-ANCA	Nil
5	UC	NEG → Atypical p-ANCA	Nil
6	CD	Atypical → NEG	Bowel resection Commenced biological agent
7	Ind	c-ANCA → Atypical	2 episodes of luminal disease flare leading to 2 episodes of hospitalisation
8	CD	p-ANCA → PR3 (ELISA)	Changed to different biological agent
9	CD	p-ANCA → Atypical	Nil
9	CD	Atypical → PR3 (ELISA)	Commenced biological agent
10	UC	p-ANCA → Atypical	Commenced biological agent Bowel resection
10	UC	Atypical p-ANCA → Atypical	Nil

ANCA, anti-neutrophil cytoplasmic antibody; CD, Crohn's disease; CIA, chemiluminescent assay; ELISA, enzyme linked immunoassay; Ind, indeterminant colitis; NEG, negative by IIF and MPO/PR3; UC, ulcerative colitis.

Table 4 Paired ANCA and ASCA encounter results

	CD (*n*=19)	UC (*n*=9)	Ind (*n*=2)
ASCA positive			
Positive atypical p-ANCA	1	1	0
Negative atypical p-ANCA			
ANCA positive (other 5 subtypes)[a]	3	1	1
ANCA negative	2	0	0
ASCA negative			
Positive atypical p-ANCA	4	2	0
Negative atypical p-ANCA			
ANCA positive (other 5 subtypes)[a]	8	4	1
ANCA negative	1	1	0

n, number of paired sample encounters.
ANCA, anti-neutrophil cytoplasmic antibody; ASCA, anti-*Saccharomyces cerevisiae*; CD, Crohn's disease; Ind, indeterminant colitis; UC, ulcerative colitis.
[a] Other subtypes: p-ANCA; c-ANCA; atypical; PR3; MPO.

disease, seven (35%) of them had PSC. Of the 20 patients with atypical p-ANCA at one stage of their disease, only two (10%) had PSC. The nine IBD patients who had PSC had a total of 28 encounters of ANCA ordered historically, and 21 (75%) of the ANCA encounters were PR3 positive; only one (3.6%) of the encounters was atypical p-ANCA positive. PR3 positive ANCA accounted for 25 (26.9%) of the ANCA encounters ordered for IBD patients without PSC (55 patients with 93 ANCA encounters in total); atypical p-ANCA accounted for 16 (17.2%).

DISCUSSION

Our retrospective cross-sectional study is the first Australian study to look at ANCA results in patients with IBD ordered in routine clinical practice. A previous study published in 2013 presented the prevalence of p-ANCA amongst IBD patients, and focused on comparing serological differences between different ethnic groups.[14] By comparison, our study presents the distribution of different ANCA subtypes for IBD patients, delves into the temporal change of ANCA status, and assesses the relationship between ANCA and IBD disease activity, and extra-intestinal manifestations. Overall ANCA positivity prevalence amongst our cohort was much higher than reported in other studies;[8,10–12] more than 80% of patients with UC and CD in our cohort had positive ANCA (Fig. 1).

The most striking finding of our study was the high rate of positivity for PR3 ANCA. For clinicians ordering an ANCA to help with subtyping of IBD, the finding of a positive PR3 ANCA may cause concern for both the clinician and patient due to the association with systemic vasculitis. Identifying MPO or PR3 antibodies in patients with IBD may lead to a battery of investigations in an effort to exclude systemic vasculitis because ANCA vasculitis and IBD can co-exist on very rare occasions.[15] Reassuringly none of the patients in our study had diagnosed concurrent systemic vasculitis, and our study suggests that in IBD a positive PR3 result is unlikely to indicate systemic vasculitis.

There is emerging literature about the rate of PR3 positivity as a marker of ulcerative colitis.[16–19] A recent study found that chemiluminescent assays for PR3 in particular had a high positivity rate for ulcerative colitis, with 18% of ulcerative colitis patients in this study having a positive PR3 antibody on the chemiluminescent assay in comparison to 8% on a multiplex bead assay and 1% on a fluorescence enzyme immunoassay.[16] These findings have also been seen in previous analyses comparing ELISA and CIA.[17,18] In comparison, the majority of our positive PR3 results were by ELISA rather than CIA, and none of the patients who became PR3 positive over time did so after the introduction of the CIA, so this does not account for the high rate of PR3 results which we saw. None of our patients had testing with both assays for PR3 and so no comparison could be made in this respect.

Contrary to the literature cited above, PR3 positivity did not predict a specific IBD subtype or clinical course in our cohort. This may be due to the small proportion tested by CIA. PR3 positive IBD patients had similar age of onset, and similar rate of requirement for biological agents and bowel resection as IBD patients in our cohort, with neither PR3 nor MPO antibodies. There was a trend for PR3 positive IBD patients to exhibit higher rate of PSC and lower rate of arthritis as an extra-intestinal manifestation. The association between PR3 positivity and PSC has been reported previously.[20]

In our cohort, a full spectrum of ANCA results was seen and we could not replicate the use of atypical p-ANCA and ASCA to predict disease subtype. This is contrary to the traditional belief that UC patients are more likely to be atypical p-ANCA positive, and CD patients are more likely to be ANCA negative.[4] Furthermore, pairing ANCA and ASCA did not appear to be helpful in subtyping IBD in our cohort, as the phenotype of positive ANCA and negative ASCA was common in both CD patients and UC patients. The low numbers of patients analysed may be responsible for this discrepancy, but it may also indicate that this association does not hold in a general, non-selected IBD population in routine clinical practice, or that the assays have changed since this association was first noted.

The recently revised international consensus on testing of ANCA 2017 recommends for MPO and PR3 testing to be used as a primary screening method instead of IIF for patients suspected of having systemic vasculitis.[21] This recommendation came following the multi-centre European case series that revealed significant discordance between IIF methods, and superior performance of antigen-specific immunoassay in determination for ANCA subtype.[22] The revised international consensus 2017 specifies that the recommendation does not apply to patients presenting with gastrointestinal disorders.[21] Therefore, the question remains about whether laboratories should continue to provide indirect immunofluorescence testing for patients with IBD. Our study would suggest that ANCA testing does not help to subtype IBD. Our study would also suggest that should these samples come with inadequate clinical information and be wrongly assumed to be from a patient with clinical signs of vasculitis, a high rate of positivity for PR3 will result with consequent anxiety and likely further investigations.

The observation that ANCA status changes over time in patients with IBD had also been made in other studies, with some concluding that ANCA can change from positive to negative following treatment with steroids or colectomy.[23,24] While bowel resection or changed treatment occurred in the 61.5% of patients whose ANCA status changed, the direction of ANCA status change could not be predicted. This may be because the time interval between ANCA testing for each patient varies in our study from a few months to many years. As such multiple interventions may have occurred, limiting our ability to attribute ANCA status change to one particular intervention.

Our study has a number of limitations. We analysed data generated by routine clinical practice which has an inherent bias as the patients tested were not done so at random. We were also looking at clinical data from medical records rather than standardised clinical data collected in real time. However, despite this our data indicate that in a tertiary hospital IBD clinic there is little clinical value in ordering an ANCA test to evaluate for subtypes of IBD. It also suggests that patients who are PR3 ANCA positive without evidence of systemic vasculitis may have IBD.

Acknowledgements: We acknowledge Euan McNaughton's help in extracting data from the Laboratory Information System and Kathryn Nicholls' help in extracting data from the Hospital Clinical Data System. We also acknowledge the immunopathology staff at ACT pathology for performing ANCA, as well as the immunopathology staff at Royal Prince Alfred Hospital (Sydney South West Pathology Service) for performing ASCA.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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