Letter to the Editor

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PR3-anti-neutrophil cytoplasmic antibodies (ANCA) in ulcerative colitis

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To the Editor,

Anti-neutrophil cytoplasmic antibodies (ANCA) are used in the diagnostic workup of small vessel vasculitis such as granulomatosis with polyangiitis (GPA) and microscopic polyangiitis. In a recent multinational study in which indirect immunofluorescence (IIF) was compared to antigen-specific assays for ANCA detection for the diagnosis of

vasculitis, it was concluded that IIF is not of added value if high-quality assays that detect antibodies directed against proteinase 3 (PR3) or myeloperoxidase (MPO) are used [1]. It can be foreseen that PR3-ANCA and MPO-ANCA will increasingly be used as screening assays for the diagnosis of small vessel vasculitis (SVV).

It has been reported that PR3-ANCA measured by a chemiluminescent immunoassay (CIA) is not only found in SVV (in particular GPA) but also in ulcerative colitis (UC) and that PR3-ANCA can be useful to differentiate UC from Crohn's disease (CD) and to identify patients with extended form of the disease [2, 3]. This observation prompted us to investigate whether other PR3-ANCA methods also detect PR3-ANCA in inflammatory bowel disease (IBD). This is important because on the one side, it can affect specificity of PR3-ANCA for SVV and on the other side can offer clinical utility to differentiate UC from CD. At present, the only serological biomarker for UC is atypical perinuclear (p)ANCA detected by IIF (reviewed in [4]), a technique that is prone to significant variation [5]. Of note, a rare association between vasculitis and IBD has been described and the diagnosis of IBD most often antedates the diagnosis of SVV [6, 7]. As patients with IBD were excluded as controls in the above-mentioned large multicenter study on PR3-ANCA and MPO-ANCA in SVV [1], we studied PR3-ANCA in IBD and compared the results with results reported in the international study.

Sera from 98 UC patients [median (min-max) age: 42 (21–74) years; 38 females/60 males] and 94 CD patients [median (min-max) age: 42 (20–80) years; 52 females/42 males] were collected (University Hospitals of Leuven, Belgium). Patients were diagnosed based on current standard clinical, radiological, endoscopic and histological criteria [8].

PR3-ANCA were determined by three independent automated methods: QUANTA Flash PR3 CIA (cutoff 20 CU), EliA PR3^s fluorescence enzyme immunoassay (cutoff 2 IU/mL) and BioPlex 2200 multiplex bead PR3 (cutoff 1 AI).

The results for PR3-ANCA in IBD, determined by three methods, are shown in Figure 1 and performance

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Figure 1: Comparative antibody titer distribution of PR3-ANCA among patients with Crohn's disease (CD, n=94) and ulcerative colitis (UC, n=98) by QUANTA Flash (left), ELiA (middle), and BioPlex 2200 (right). The cutoff is represented by a dashed line.

Table 1: Overview of PR3-ANCA in UC, CD, GPA and controls determined by QUANTA Flash, ELiA and BioPlex 2200.

PR3-ANCA	QUANTA Flash (CU)	ELiA (IU)	BioPlex (AI)
Cutoff	20	3	1
Assay range			
UC (n=98)			
1st quartile	<2.3	0.20	0.30
Median	4.50	0.20	0.35
3rd quartile	14.40	0.20	0.51
CD (n=94)			
1st quartile	<2.3	0.20	0.20
Median	<2.3	0.20	0.30
3rd quartile	<2.3	0.20	0.40
Controls (n=924)			
1st quartile	2.30	0.30	0.20
Median	2.30	0.50	0.20
3rd quartile	2.76	0.70	0.20
GPA (n=186)			
1st quartile	37.82	3.10	1.67
Median	198.78	20.00	8.00
3rd quartile	517.31	50.08	8.00
Sensitivity, %			
UC	18.4 (11.9–27.2)	1.0 (0.2–5.6)	8.2 (4.2–15.3)
CD	6.4 (3.0–13)	0.0 (0.0–3.9)	3.2 (1.1–9.0)
GPA	80.8 (74.4–85.7)	75.8 (69.2–84.4)	77.4 (70.9–82.8)
Specificity, %			
Controls	98.3 (97.2–98.9)	98.6 (97.6–99.2)	98.6 (97.6–99.2)
UC vs. controls			
LR(+)	10.6 (5.6–19.8)	0.7 (0.1–4.2)	5.8 (2.5–13.1)
LR(–)	0.8 (0.7–0.9)	1.0 (0.96–1.0)	0.9 (0.9–1.0)
OR	12.8 (6.3–25.7)	0.7 (0.1–4.4)	6.2 (2.6–15.1)
UC vs. CD			
LR(+)	2.9 (1.2–6.8)	+∞ (0.2–+∞)	2.6 (0.8–8.7)
LR(–)	0.9 (0.8–1.0)	0.99 (0.9–1.1)	0.95 (0.9–1.0)
OR	3.3 (1.3–8.5)	N/A	2.7 (0.7–9.7)
GPA vs. controls			
LR(+)	46.6 (28.7–75.8)	53.9 (31.5–92.5)	55.0 (32.2–94.4)
LR(–)	0.2 (0.1–2.6)	0.2 (0.2–0.3)	0.2 (0.17–0.3)
OR	236.5 (128.3–435.2)	219.6 (116.1–414.8)	240.3 (126.4–454.6)

The median 1st and 3rd quartiles are given, as well as specificity and sensitivity (with 95% confidence intervals) for UC, CD and GPA. LR(+), LR(–) and OR are shown as well.

statistics are summarized in Table 1. Using the cutoff proposed by the manufacturer, the prevalence of PR3-ANCA was significantly higher in UC vs. CD determined by QUANTA Flash (18% vs. 6%) ($p = 0.015$, Fisher exact), but not by EliA ($p = 0.49$) (1% vs. 0%) or BioPlex 2200 ($p = 0.21$) (8% vs. 3%). The likelihood ratio (LR) for a positive test result to distinguish UC from CD was 2.9 for QUANTA Flash indicating a small difference in pre-test to post-test probability. The LR for a negative test result was 0.9, indicating that a negative test result has no clinical value to distinguish UC from CD. The sensitivity of EliA for UC was too low to be useful. PR3-ANCA was also determined by CIA in 75 gastrointestinal controls: 28 samples from patients with elevated levels of anti-tissue transglutaminase (median age, 48 years; range, 17–81 years; 23 females) and 47 patients with irritable bowel syndrome (median age, 28 years; range, 15–63 years; 29 female). PR3-ANCA was low (median, <2.3 CU; 3th quartile, 2.75 CU) and weak positivity was found in two samples: 26 CU in a patient with elevated anti-tissue transglutaminase and 20.7 CU in a patient with irritable bowel syndrome. The prevalence of PR3-ANCA was higher in UC than in gastrointestinal controls ($p = 0.001$, Fisher exact test).

Next, we compared PR3-ANCA in UC to PR3-ANCA in 924 diseased controls and in 186 GPA patients included in the above-mentioned multicenter study [1]. A summary of the results is given in Table 1. PR3-ANCA levels were higher in UC than in the diseased controls with QUANTA Flash and BioPlex 2200 ($p < 0.0001$; Kruskal-Wallis), but not with EliA (for which values in UC were lower than in controls; $p < 0.0001$; Kruskal-Wallis). The LR(+) for UC vs. controls was 11 and 5.8 for QUANTA Flash and BioPlex, respectively. The LR(–) was 0.8 and 0.9, respectively, indicating that a negative result does not exclude UC.

PR3-ANCA levels were clearly lower in UC than in GPA (median, 4.5 CU vs. 199 CU with QUANTA Flash ($p < 0.0001$, Kruskal-Wallis) and with BioPlex (median, 0.35 AI vs. 8 AI) ($p < 0.0001$, Kruskal-Wallis). Figure 2 shows the results for PR3-ANCA in UC, CD, GPA and controls obtained with QUANTA Flash.

Taken together, we confirmed that PR3-ANCA reactivity is found in a fraction of UC patients when quantified by a sensitive CIA [odds ratio (OR) for UC vs. controls, 12.8]. Such reactivity was also found with a multiplexed bead assay (OR for UC vs. controls, 6.2), albeit to a lower extent, but not with fluoroenzyme immunoassay. The LR(+) to distinguish UC from CD was low (2.6/2.9). Of note, PR3-ANCA has also been described in UC patients by CytoBead, a multiplex microbead-based fluorescence assay [9]. Our findings suggest a possible breakthrough of

Figure 2: Comparative antibody titer distribution of PR3-ANCA by QUANTA Flash.

This shows the distribution of PR3-ANCA among patients with Crohn's disease (CD, $n = 94$), ulcerative colitis (UC, $n = 98$), and diseased controls ($n = 924$) and GPA ($n = 186$) from reference [1].

tolerance in UC, as antibodies to several myeloid granular proteins, including PR3, BPI, lactoferrin, lysosyme, have been found in UC (reviewed in [4]).

Considering the potential change in the ANCA test algorithm (in which PR3-ANCA and MPO-ANCA will be used to screen for ANCA-associated vasculitis) (1), laboratories should be conscious of the fact that some assays will detect PR3-ANCA in UC. Therefore, laboratories should (i) evaluate whether the test they use is sensitive for PR3-ANCA in IBD and (ii) differentiate ANCA requests in the context of SVV from requests in the context of IBD (e.g. by distinguishing these two clinical settings on the request form). Low titers of PR3-ANCA by CIA might indicate the presence of UC.

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