Audit of the clinical usefulness of a rapid qualitative ELISA screen for antimyeloperoxidase and antiproteinase 3 antibodies in the assessment of patients with suspected vasculitis

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Background: The need for urgent antineutrophil cytoplasmic antibody (ANCA) results when assessing patients with acute renal failure, pulmonary renal syndrome, or mononeuritis multiplex has led to the development of a rapid qualitative ELISA screening assay for antibodies to myeloperoxidase (MPO) and proteinase 3 (PR3).
Aims: To report the use of a rapid qualitative ELISA screen for PR3-ANCA and MPO-ANCA in a regional immunology laboratory and its correlation with standard indirect immunofluorescence (IIF) and quantitative ELISA for PR3-ANCA and MPO-ANCA.
Methods: Over 12 months, 103 samples requiring urgent ANCA testing were screened by a rapid qualitative ELISA and the results compared with IIF and quantitative ELISA assays for PR3-ANCA and MPO-ANCA.
Results: There was an excellent correlation between the rapid qualitative ELISA and standard ANCA IIF and a routine ELISA for MPO/PR3-ANCA, with sensitivities ranging from 82% to 100%. There were two false negatives, which gave weak to moderately positive values as determined by routine ELISA. However, the clinical relevance of these two cases is doubtful.
Conclusions: The rapid ELISA for anti-MPO and anti-PR3 correlates well with quantitative ELISA and IIF ANCA, and urgent management decisions in patients with suspected small vessel vasculitis can be based with confidence on this test.

A ntineutrophil cytoplasmic antibodies (ANCA) are well established markers of the Wegener's/microscopic polyangiitis spectrum of small vessel vasculitides.[1] Current international consensus guidelines for the performance of ANCA testing recommend the use of indirect immunofluorescence (IIF) on ethanol fixed neutrophils as the first step, followed by characterisation of any positive results on IIF by antigen specific enzyme linked immunosorbent assay (ELISA) for antimyeloperoxidase (MPO-ANCA) and antiproteinase 3 (PR3-ANCA).[2] With the increasing use of ANCA has come a need for rapid results when assessing patients with acute renal failure, the pulmonary renal syndrome, or mononeuritis multiplex. The need for urgent results has led to the use of a rapid qualitative ELISA screening assay for antibodies to PR3 and MPO.[3] Although such screening assays are widely used in the UK by diagnostic immunology laboratories, we are unaware of any studies auditing their clinical usefulness. We report our experience in a regional immunology laboratory on the use of a rapid qualitative ELISA screen for PR3-ANCA and MPO-ANCA and its correlation with standard IIF and quantitative ELISA assays for PR3-ANCA and MPO-ANCA.

METHODS

Over a 12 month period, 103 consecutive samples requiring urgent ANCA testing were screened by a rapid qualitative ELISA. The rapid qualitative ELISA assay for MPO-ANCA and PR3-ANCA was performed according to the manafacturer's instructions (Weislab AB, Lund, Sweden). Briefly, serum samples were incubated in antigen (MPO or PR3) coated wells for 10 minutes. After washing to remove unbound antibodies, goat antihuman IgG conjugated with alkaline phosphatase was added and incubated for a further 10 minutes. Substrate was added after a final wash to remove unbound conjugate and the resultant colour change was measured to give an optical density (OD). An OD ratio was calculated by dividing the OD of the sample by the OD of the negative control; the negative control was supplied by the manufacturer. A sample was determined to be positive if the OD ratio was > 4, and negative if the OD ratio was < 3. There was an additional borderline positive category for samples with OD ratios between 3 and 4. In accordance with the minimum requirements for ANCA testing all samples were re-tested by IIF at a screening dilution of 1/20.[2] Testing for MPO-ANCA and PR3-ANCA by the routine quantitative ELISA (Genesis Diagnostics Ltd, Littleport, Cambridgeshire, UK) was only performed on positive samples as determined by IIF and/or the qualitative ELISA. A cut off > 3.1 units/ml was used for the quantitative ELISA.

We were able to obtain and review the clinical records of 31 of the 34 patients who had a positive result on the rapid qualitative ELISA, the routine ELISA, or IIF. Histological data from renal biopsies were available on 22 of these patients.

RESULTS

Table 1 shows the results of the 103 samples that were screened with the rapid ELISA. Twenty nine of the samples were positive; of these, one showed dual positivity to MPO-ANCA and PR3-ANCA, 11 were positive only for MPO-ANCA, and 13 only for PR3-ANCA. The ability of the rapid ELISA to predict IIF results was expressed in terms of its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), as shown in table 2. Correlations between the rapid qualitative PR3-ANCA ELISA and the routine quantitative PR3-ANCA ELISA, and between the rapid qualitative MPO-ANCA ELISA and the routine quantitative MPO-ANCA ELISA were also analysed (tables 3 and 4).

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Table 1 Results of 103 samples tested by the rapid MPO/PR3-ANCA ELISA

	Number of samples
Positive PR3-ANCA	13
Positive MPO-ANCA	11
Positive MPO-ANCA and PR3-ANCA	1
Borderline positive	4
Negative	74
Total number of samples	103

ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; MPO, myeloperoxidase; PR3, proteinase 3.

Table 2 Correlation between the qualitative MPO/PR3-ANCA ELISA and immunofluorescence

		ANCA immunofluorescence	
		+	–
Qualitative MPO/PR3-ANCA ELISA	+	23	2
	–	5	69

Sensitivity, 82%; specificity 97%; positive predictive value, 92%; negative predictive value, 93%.
ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; MPO, myeloperoxidase; PR3, proteinase 3.

Table 3 Correlation between the qualitative PR3-ANCA ELISA and the quantitative PR3 ANCA ELISA

		Quantitative PR3-ANCA ELISA	
		+	–
Qualitative PR3-ANCA ELISA	+	13	1
	–	2	14

Sensitivity, 87%; specificity 93%; positive predictive value, 93%; negative predictive value, 88%.
ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; PR3, proteinase 3.

Table 4 Correlation between the qualitative MPO-ANCA ELISA and the quantitative MPO-ANCA ELISA

		Quantitative MPO-ANCA ELISA	
		+	–
Qualitative MPO-ANCA ELISA	+	12	0
	–	0	18

Sensitivity, 100%; specificity, 100%; positive predictive value, 100%; negative predictive value, 100%.
ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; MPO, myeloperoxidase.

The qualitative ELISA detected four borderline positive samples. One of these patients had acute renal failure secondary to sepsis, with borderline positive qualitative ELISA results to both MPO-ANCA and PR3-ANCA, a weakly positive P-ANCA, and a positive quantitative MPO-ANCA ELISA (11.9 units/ml). A renal biopsy had not been performed. The second borderline positive result involved PR3-ANCA and was subsequently found to be negative on both IIF and the qualitative ELISA. However, this patient had renal biopsy confirmed Wegener's granulomatosis and was on cyclosporin after a renal transplant. The last two borderline positive results were directed against PR3-ANCA and were subsequently found to be negative on both the routine PR3-ANCA ELISA and on IIF.

The rapid ELISA shows a good correlation with IIF, with a sensitivity of 82% and a specificity of 97% (table 2). There were five negative samples on the qualitative ANCA ELISA that were picked up by IIF. Three of these five were also negative for MPO/PR3-ANCA, as determined by the routine ELISA, indicating that the IIF pattern had resulted from antibodies with specificities directed to antigens other than MPO/PR3-ANCA.[4] Two of these patients had renal biopsies that were not consistent with small vessel vasculitis, instead showing acute tubular damage and acute interstitial nephritis. This leaves two false negatives, which were subsequently found to be positive on the quantitative PR3-ANCA ELISA (table 3). The IIF revealed a moderately positive C-ANCA of 1/160 in both, and PR3-ANCA values of 15.4 and 7.4 U/ml, as determined by the routine ELISA. One patient had a history of inflammatory bowel disease and had not had a renal biopsy, whereas patient records were unavailable for the second patient.

On two occasions, positive results on the PR3/MPO-ANCA rapid ELISA were associated with a negative IIF (table 2), resulting in a specificity and PPV of 97% and 92%, respectively. One patient had a positive qualitative PR3-ANCA ELISA and both a negative IIF and quantitative PR3-ANCA ELISA (table 3). The patient had been admitted with abdominal pain and had died from respiratory failure; a renal biopsy had not been performed. However, the second patient, also with a positive qualitative PR3-ANCA ELISA and negative IIF, had a raised PR3-ANCA titre as determined by the quantitative ELISA (8.3 U/ml). He had Wegener's granulomatosis, previously confirmed by renal biopsy.

A routine quantitative ELISA for MPO-ANCA and PR3-ANCA was performed on the 34 samples that had yielded a positive result on the rapid ELISA or were positive on IIF. Table 3 shows that for the rapid PR3-ANCA assay there was a good correlation with the routine ELISA, giving a sensitivity of 89%, NPV of 89%, a specificity of 94% and a PPV of 89%. The false positive and negative results have been described above. Thirteen of the 14 patients with both positive qualitative and quantitative PR3-ANCA ELISAs had renal biopsies consistent with small vessel vasculitis. The other patient presented with a mononeuritis and had a sural nerve biopsy consistent with small vessel vasculitis.

Table 4 outlines the correlation of the rapid MPO-ANCA ELISA with the routine ELISA. The lack of false negatives or positives resulted in a sensitivity, specificity, NPV, and PPV of 100%. Eight of the 12 patients with both positive qualitative and quantitative MPO-ANCA ELISAs had renal biopsies performed, all of which were consistent with small vessel vasculitis.

DISCUSSION

These results confirm the excellent correlation between a rapid qualitative ELISA for MPO/PR3-ANCA and standard ANCA IIF and a routine ELISA for MPO/PR3-ANCA. The sensitivities range from 82% to 100%, and the two false negatives that did occur had a specificity for PR3, with weak to moderately positive values determined by the routine ELISA. One of

the patients had inflammatory bowel disease and the moderately positive routine ELISA result is unlikely to be clinically relevant. Unfortunately we were unable to find the records of the second patient, but again the clinical relevance of a moderately positive routine ANCA is debatable and depends on coexisting clinical features.

The renal biopsy findings confirm the correlation of ANCA positivity and underlying active small vessel vasculitis in the clinical setting of acute renal failure, pulmonary renal syndrome, and mononeuritis. Indeed, all the patients who were positive for PR3-ANCA who had undergone renal biopsy and the single patient with a sural nerve biopsy had histologically confirmed vasculitis.

"The results indicate that the rapid qualitative enzyme linked immunosorbent assay is sufficiently robust to use for out of hours or weekend requests"

Two of the four patients with borderline positive qualitative ELISA ANCA results were negative on both the quantitative ELISA and IIF. However, one of the patients did have Wegener's granulomatosis, and it is probable that the negativity on the routine ELISA and IIF was a consequence of immunosuppression and disease inactivity. The final borderline positive patient had a confirmed positive P-ANCA titre of 1/40 and raised MPO-ANCA (11.9 U/ml), but the acute renal failure in this patient was thought to be secondary to sepsis, which could account for his moderately positive results, although a renal biopsy was not undertaken. Overall, the borderline positive results do not correlate with routine ANCA positivity by ELISA or IIF and are unlikely to be clinically relevant. In this situation, clinicians are advised that borderline positive results should be interpreted with caution and a definitive judgement on ANCA status will have to rely on IIF and quantitative ELISA for MPO-ANCA and PR3-ANCA.

Despite the retrospective nature of our study, the results indicate that the rapid qualitative ELISA assay is sufficiently robust to use for out of hours or weekend requests. Given that a negative anti-PR3 combined with a negative anti-MPO has a high NPV for a diagnosis of small vessel vasculitis, this assay enables the exclusion of these disorders in an emergency setting.[5] Clinicians using the rapid ELISA for anti-MPO and anti-PR3 assessments can be reassured that the results correlate reliably with quantitative ELISA results and IIF ANCA,

Take home messages

- The rapid qualitative enzyme linked immunosorbent assay (ELISA) for antineutrophil cytoplasmic antibodies (ANCA) correlated well with standard indirect immunofluorescence (IIF) and quantitative ELISA assays
- This assay is useful in an emergency setting because negative results on this test can be used to exclude a diagnosis of small vessel vasculitis
- Clinicians using the rapid ELISA can be reassured that the results correlate reliably with quantitative ELISA results and IIF ANCA, and that urgent management decisions in patients with suspected small vessel vasculitis can be based with confidence on this test

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