Audit of the clinical usefulness of a rapid qualitative ELISA screen for antmyeloperoxidase 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.

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 PR3-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 ELISA, and between the rapid qualitative MPO-ANCA ELISA and the routine quantitative MPO-ANCA ELISA were also analysed (tables 3 and 4).

Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; IIF, indirect immunofluorescence; MPO, myeloperoxidase; OD, optical density; PR3, proteinase 3
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 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. 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, and that urgent management decisions in patients with suspected small vessel vasculitis can be based with confidence on this test.

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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