Screening and Confirmatory Anti-Neutrophil Cytoplasmic Antibodies (ANCA) Testing for Rapidly Progressive Glomerulonephritis (RPGN): A Tertiary Care Experience

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Introduction

Among ANCA associated vasculitis (AAV), 60% of the RPGN are ANCA associated. Indirect immunofluorescence performed followed by confirmation with (ELISAs). Testing for serum (ANCA) by IF and ELISA may identify patients with PR3-ANCA or MPO-ANCA despite a negative IF (IF negative MPO/PR3-positive); however, the significance of this result is not clear [1, 2]. We determine whether IF-negative MPO/PR3-positive results identified any cases of clinically meaningful systemic vasculitis at our institution. Methods: We conducted a retrospective analysis of all ANCA positive RPGN patients either by IF or ELISA identified at our institution from August 2017 - July 2018. Results: Out of 265 samples 45 were positive for both IF and ELISA, 220 were IF-negative. Among IF negative, 6 samples (2.7%), tested positive for MPO-ANCA or PR3-ANCA. Two IF-negative ELISA positive patients were previously diagnosed and treated for AAV, both with positive IF and antibody tests prior to treatment. 1 patient had SLE and 1 had inflammatory bowel disease. Mean age of patients 58±4, 56% were males and P-ANCA 67% and C-ANCA 33% only 1 pt had both C- ANCA AND P-ANCA patterns positive. Conclusion: In our study both IF and ELISA, ELISA positivity without a positive IF rarely led to a definite diagnosis of systemic vasculitis, and was more likely to occur in the context of a non-vasculitis inflammatory condition. Our results suggest that concurrent IF and MPO/PR3 testing may be important in preventing a missed diagnosis of new onset renal limited AAV. Keywords: Antineutrophil cytoplasmic antibodies (ANCA) vasculitis Rapidly Progressive Glomerulonephritis (RPGN), outcomes.

Comparisons of the utility of IF and specific antibody assays for the diagnosis of AAV have frequently, though not uniformly, suggested that IF is more sensitive than MPO-ANCA and PR3-ANCA assays [3-7]. However, because of the low specificity of P-ANCA and CANCA IF patterns for AAV, MPO/PR3-ANCA assays may provide a better positive predictive value and likelihood ratio compared to IF, while the combination of the 2 demonstrates the best results [4, 8].

The International Consensus Statement on the testing and reporting of ANCA's recommended that all samples sent for diagnostic ANCA testing be evaluated by IF, and that samples with cytoplasmic fluorescence, or nuclear fluorescence in a homogenous or peripheral

nuclear pattern, be subsequently tested for MPO-ANCA and PR3-ANCA [9]. The consensus statement also states that optimally, all serum samples should be tested for MPO-ANCA and PR3-ANCA. One common clinical approach is to screen all serum samples by IF, and then test only IF-positive samples for MPO-ANCA and PR3-ANCA. Alternatively, some practitioners utilize specific antibody tests first, followed by reflex IF testing only on MPO/PR3-ANCA-positive samples [7, 10], and in some specific cases, MPO/PR3-ANCA tests may be used alone [11].

Testing samples by IF and specific antibody tests simultaneously can identify IF-negative MPO/PR3-positive patients who would be otherwise missed if only IF-positive samples were tested for MPO/PR3-ANCA. Prior reports have noted a small number of such IF-MPO/PR3-positivesamples [4, 12]; however, the clinical significance of this result is unclear, as MPO/PR3-ANCA can also sometimes be detected in non-vasculitic conditions, including SLE and IBD [13, 14].

MATERIALS AND METHODS

This study was done at Nizams Institute of Medical sciences. Results of all ANCA tests ordered through NIMS from August 2017 - July 2018 were collected. These samples come from inpatients and outpatients at NIMS and patients seen at associated outpatient centers. Through this period, all samples sent for evaluation for serum ANCA as part of clinical care, including for the evaluation of clinically suspected systemic vasculitis, monitoring of vasculitis disease activity, or any other indication, were included. All samples were evaluated by both IF and multiplex bead assays for MPO-ANCA and PR3-ANCA as part of routine clinical laboratory practice.

IF was performed by the Nizams Institute of Medical sciences laboratory. Serum samples were diluted 1:20 and incubated on ethanol-fixed human neutrophil substrate slides as per the manufacturer’s method recommendations (Euroimmun kit, Lubeck Germany). IF patterns were reported as cytoplasmic, perinuclear, atypical, or negative. Only cytoplasmic or perinuclear patterns were considered positive in this study. Atypical results were excluded from analyses. MPO-ANCA and PR3-ANCA were measured by multiplexed bead assays, a type of solid phase immunoassay, performed at a commercial laboratory (ARUP Laboratories, Salt Lake City, UT). An antibody level >25 units/mL was considered positive. Previous analyses demonstrated very good concordance between results obtained on split samples, over a broad range of levels, analyzed with this multiplexed bead assay, with another commercial ELISA kit Euroimmun kit, Lubeck Germany )and with assays performed at Nizams Institute of Medical sciences laboratory

Statistical analysis: The data calculated from study were subjected to simple descriptive statistical analysis using percentage

RESULTS

Out of 265 samples 45 were positive for both IF and ELISA, 220 were IF-negative. Among IF negative, 6 samples (2.7%), tested positive for MPO-ANCA or PR3-ANCA. Two IF-negative ELISA positive patients were subsequently diagnosed with ANCA-associated renal limited vasculitis. Two IF-negative ELISA-positive patients were previously diagnosed and treated for AAV, both with positive IF and antibody tests prior to treatment. 1 patient had SLE and 1 had inflammatory bowel disease. Mean age of patients 58±4, 56% were males and P-ANCA 67% and C-ANCA 33% only 1 pt had both C- ANCA AND P-ANCA patterns positive

DISCUSSION

Testing for the presence of ANCA is a key component of the diagnostic evaluation for AAV; however, significant variability exists in the strategies employed to detect ANCA, including reflex testing (test by IF first, and if positive, test for MPO-ANCA and PR3-ANCA) and concurrent testing (test by IF and MPO/PR3-ANCA assays simultaneously). It is well established that isolated IF positivity, without an associated MPO-ANCA or PR3-ANCA, has a poor predictive value for AAV [4]; however, the significance of positive MPO-ANCA or PR3-ANCA in the absence of IF positivity is less clear.

In this report of over 1 year of concurrent ANCA testing by IF and MPO/PR3 multiplexed bead assays at NIMS Out of 265 samples 45 were positive for both IF and ELISA, 220 were IF-negative Among IF negative, 6 samples (2.7%), tested positive for MPO-ANCA or PR3-ANCA. Our study is unique as two IF-negative ELISA positive patients were subsequently diagnosed with ANCA-associated renal limited vasculitis. Two IF-negative ELISA-positive patients were previously diagnosed and treated for AAV, both with positive IF and antibody tests prior to treatment. 1 patient had SLE and 1 had inflammatory bowel disease. 56% were males and P-ANCA 67% and C-ANCA 33% only 1 pt had both C- ANCA AND P-ANCA patterns positive

The frequency of this result will vary with the specifics of test methods [3, 6, 16, 17], but is within the range of prior reports [4, 12, 18]. IF-negative MPO/PR3-positive results have been previously described in patients with conditions including inflammatory arthritis, IBD, connective tissue disease [7, 8, 12, 19]; however, there is little data reported on significance of this result in routine clinical testing (4, 12). Stone et al noted 5 IF-negative MPO/PR3-positive patients out of 856 consecutive patients evaluated for
AAV, but the clinical associations were not described [4]. Tsiveriotis reported 17 IF-negative MPO/PR3-positive patients out of 4786 outpatients concurrently tested, including 2 with connective tissue diseases, 4 with mixed/undefined disorders, and 11 with AAV; however, new diagnosis of AAV was not distinguished from previously treated AAV [12].

It has been suggested that MPO/PR3-ANCA testing alone may be reasonable in emergent clinical situations [11], indicating that caution must be used when interpreting positive MPO/PR3-ANCA results in the absence of concurrent IF testing. Consistent with previous reports [5, 12, 13], we identified patients with SLE, IBD, and other inflammatory disorders with IF-negative MPO/PR3-positive results. This phenomenon may be due to a number of mechanisms, including antibody cross-reactivity in the multiplex bead assay, higher sensitivity of the multiplex bead assay, specific characteristics of the antigenic epitopes targeted, or technical variability [7, 11].

In conclusion, In our study both IF and ELISA, ELISA positivity without a positive IF rarely led to a definite diagnosis of systemic vasculitis, and was more likely to occur in the context of a non-vasculitis inflammatory condition. Our results suggest that concurrent IF and MPO/PR3 testing may be important in preventing a missed diagnosis of new onset renal limited AAV.
CONCLUSION

In our study both IF and ELISA, ELISA positivity without a positive IF rarely led to a definite diagnosis of systemic vasculitis, and was more likely to occur in the context of a non-vasculitis inflammatory condition. Our results suggest that concurrent IF and MPO/PR3 testing may be important in preventing a missed diagnosis of new onset renal limited AAV.

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