Pathology (June 2017) **49(4)**, pp. 413–418

IMMUNOPATHOLOGY

Comparison of enzyme-linked immunosorbent assay and rapid chemiluminescent analyser in the detection of myeloperoxidase and proteinase 3 autoantibodies

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Summary

Antibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) are vital in the diagnosis and management of ANCA-associated vasculitis. A chemiluminescent immunoassay (CLIA; Quanta Flash) provides MPO and PR3 antibody results in 30 minutes, which is much faster than enzyme-linked immunosorbent assay (ELISA). We compared the performance of ELISA (Orgentec) and CLIA (Quanta Flash) for MPO and PR3 antibody quantitation on 303 samples, comprising 196 consecutive samples received in a single diagnostic laboratory over a 3 month period, and 107 samples collected from 42 known vasculitis patients over a 40 month period. We observed a correlation between both methods using spearman correlation coefficients (MPO, $r_s = 0.63$, $p < 0.01$; PR3, $r_s = 0.69$, $p < 0.01$). There was agreement between both methods in determining a positive or negative result. In the vasculitis cohort, CLIA performed well at clinically important stages of disease; diagnosis (eight samples all positive by both assays) and disease relapse (correlation for both MPO and PR3 antibody quantitation $r_s = 0.84$, $p = 0.03$ and $r_s = 0.78$, $p < 0.01$, respectively). Three samples were discordant at clinical relapse, testing positive by CLIA, including one high positive associated with relapse requiring a change in treatment. In summary, CLIA appears to be at least as accurate as ELISA for measurement of MPO and PR3 antibodies.

Key words: Enzyme-linked immunosorbent assay; chemiluminescent immunoassay; myeloperoxidase; proteinase 3; ANCA-associated vasculitis; granulomatosis with polyangiitis; microscopic polyangiitis.

Received 19 July 2016, revised 19 February, accepted 26 February 2017
Available online 23 May 2017

INTRODUCTION

The anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides (AAV) are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis. GPA and MPA are potentially devastating small vessel vasculitides with a predilection for the kidneys and lungs.[1] ANCAs encompass autoantibodies to proteinase 3 (PR3) and myeloperoxidase (MPO), which are both sensitive and specific diagnostic markers for GPA and MPA.[2]

ANCAs are detected by indirect immunofluorescence (IIF) using ethanol and formalin fixed neutrophils.[3] Neutrophil specific antibodies referred to as atypical ANCA patterns can also be detected in other inflammatory conditions such as infections (subacute bacterial endocarditis and tuberculosis), inflammatory bowel disease and sclerosing cholangitis;[4] therefore, testing for antibodies specific for MPO or PR3 is necessary once ANCA is detected.[2] In many laboratories, testing for anti-MPO and PR3 antibodies is performed by enzyme-linked immunosorbent assay (ELISA) using commercially available kits containing purified MPO and PR3 antigens.[5,6] Direct ELISAs were found to have variable performance characteristics, and were less sensitive than capture ELISAs, where secondary specific antibodies improve epitope exposure.[7] In third generation, or anchor ELISAs, antigen is immobilised to the plate via a bridging molecule preserving epitopes for binding. These assays have a sensitivity of >95%, and differentiate GPA from non-vasculitic disease with >95% specificity.[5]

Although third generation ELISAs are highly sensitive and specific, these assays are time consuming and require operator skill, particularly if the process is performed manually. As AAV can present with rapidly progressive glomerulonephritis or lung haemorrhage, rapid testing, available at any time, is desirable. Techniques that offer these advantages include dot-blot assays, bead-based multiplex testing and chemiluminescence technology.[5] Chemiluminescent immunoassay (CLIA) is a fully automated random access chemiluminescent analyser, which provides a quantitative result in 30 minutes across a broad dynamic range, and requires minimal operator skill. There are few reports in the literature of its use in autoantibody detection.[8]

We report a comparison of parallel indirect solid phase ELISA (Orgentec) and CLIA (Quanta Flash) tests for MPO and PR3 antibody quantitation at a diagnostic laboratory in a tertiary referral centre servicing Canberra and surrounding areas in rural New South Wales. This comparison was performed over a 3 month period from May to August 2015. We also analysed stored sera from a cohort of known vasculitis patients obtained during routine clinical follow-up at our Vasculitis Clinic between 2012 and 2015. We observed

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: http://dx.doi.org/10.1016/j.pathol.2017.02.006

concordance between results obtained with both methods (correlation, r^2 = 0.7–0.9). Although numbers were small, CLIA offered greater sensitivity for the detection of patients at clinical relapse.

MATERIALS AND METHODS

Subjects

MPO and PR3 antibody testing was performed on 303 samples using both the ELISA and CLIA methods in parallel. Samples were derived from two separate patient cohorts: 196 consecutive samples from 185 patients submitted for routine testing between May and August 2015, and 107 samples from the stored sera of a cohort of 42 known vasculitis patients enrolled at our Vasculitis Clinic between July 2012 and December 2015. Sera were stored at −80°C with temperature monitoring. This study was approved by the ACT Human Research Ethics Committee.

ELISA analysis

All serum samples were tested for MPO and PR3 antibodies using a commercial MPO and PR3 ELISA kit (Orgentec, Germany) as per the manufacturer's instruction on an Evolis EIA Analyser (Bio-Rad, USA). Standards, controls and diluted patient samples (100 µL) were added to Orgentec 96 plates containing highly purified antigen, incubated then washed. Horseradish peroxidase conjugated anti-human IgG was added, incubated then washed, followed by the addition of an enzyme substrate then a stop solution. Plates were read at 450 nm with a reference at 600–690 nm within 30 min, and results provided in units of units per millilitre (U/mL). The reference range for both MPO and PR3 antibodies was <5 U/mL.

CLIA analysis

MPO and PR3 antibodies were measured on the same samples as the ELISA testing. These were performed concurrently for the consecutive laboratory samples, and retrospectively in the stored sera from the vasculitis cohort. CLIA was performed using the Quanta Flash Vasculitis assay on the Bio-Flash rapid response chemiluminescent analyser (Inova Diagnostics, USA), as per the manufacturer's instructions. This automated process involved incubation of prediluted serum, antigen-coupled beads and buffer. The magnetised beads were sedimented and washed then isoluminol conjugated antibody was added and a further incubation performed. The beads were then sedimented and washed repeatedly. Triggers were then added resulting in oxidation of the isoluminol conjugate producing a flash of light. This was measured as relative light units, and converted to chemiluminescent units (CUs). A conversion factor was then applied to the samples (0.3 for MPO and 0.25 for PR3) to convert to U/mL as per the manufacturer's instructions, with a reference range of <6 U/mL for MPO antibodies and <5 U/mL for PR3 antibodies (Quanta Flash Vasculitis product information).

Indirect immunofluorescence

IIF ANCA testing was performed on all samples using the Euroimmun (Germany) Granulocyte Mosaic kit (Hep-2 cells, formalin fixed and ethanol fixed granulocytes), according to the manufacturer's kit insert. Patterns were reported as positive based on cytoplasmic staining in the formalin and characteristic peri-nuclear or cytoplasmic pattern in the ethanol, or negative where no or minimal staining was seen in both. An atypical ANCA pattern was reported for fluorescence patterns other than those described as C-ANCA or P-ANCA, according to the International Consensus Guidelines.[2] Weak was used to denote a cytoplasmic or perinuclear pattern of staining that was lesser in intensity than the positive control but greater than the negative control. Uninterpretable was used to describe a filmy deposit that rendered the slide unable to be read.

Statistics

Spearman correlation coefficients (rs) were calculated comparing the two assays, using GraphPad Prism version 6.00 software (GraphPad Prism, USA). Values less than one were assigned a value of 1 and included in the analysis. Bland Altman plots were used to measure agreement between the two assays and 95% confidence limits of agreement were obtained.

RESULTS

Baseline characteristics and results

A total of 303 samples were tested for MPO and PR3 antibodies using both the ELISA and Quanta Flash CLIA methods. Of these, 196 samples were obtained from consecutive samples referred to a single diagnostic laboratory during the 3 month study period. The additional 107 samples were stored sera, obtained from 42 patients attending a vasculitis clinic during a 3 year period. The median age of patients tested was 57.6 years (range 6–99 years) for consecutive samples, and 57.8 years (range 18–83 years) for the vasculitis cohort. Of the consecutive samples 131 (66.8%) were obtained from females and 22 (52.3%) in the vasculitis cohort. According to information provided on pathology request forms for the consecutive samples, 58 (30.1%) samples were received from patients with known or suspected autoimmune or inflammatory disorders, including: rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease. Thirteen (6.6%) were from patients with ANCA-associated vasculitis (GPA, n = 5; MPA, n = 2; unspecified, n = 6). Thirteen (6.6%) tests were requested as part of assessment of renal impairment. The remainder were from patients with other diagnoses, or there were no clinical details provided, or the clinical information provided was illegible. Of the 42 patients in the vasculitis cohort, 26 had GPA (61.9%), 12 had MPA (28.6%), two had EGPA (4.8%), one had propothyiouracil-induced MPO antibody positive vasculitis and one had Behçet's disease.

Of the consecutive samples, 52 were found to have ANCAs by IIF (c-ANCA, 6; p-ANCA, 11; weak c-ANCA, 3; atypical ANCA, 32), and in addition, one was uninterpretable. Amongst the 107 samples from the vasculitis cohort, 73 had positive ANCAs (c-ANCA, 49; p-ANCA, 14; weak c-ANCA, 4; atypical ANCA, 6). In 33 samples, ANCA was undetectable by immunofluorescence, and in one sample, the ANCA was uninterpretable owing to technical difficulties (Table 1).

First, we evaluated samples for MPO antibodies. Within the consecutive cohort, 16 (8.2%) samples were positive for MPO antibodies by ELISA (≥5 U/mL), while 18 (9.1%) were positive by CLIA (≥6 U/mL). Sample results ranged from <1–300 U/mL for ELISA and <1–222 U/mL for CLIA. Twelve samples were positive by both methods: two of these were from patients with MPA and four were from patients with vasculitis of an unspecified type. The remainder were accounted for by individual patients with paraneoplastic vasculitis, advanced glomerulosclerosis with no active inflammatory lesions on renal biopsy, relapsing polychondritis, systemic lupus erythematosus and seropositive rheumatoid arthritis. For one sample, no history was available. Within the vasculitis cohort, 28 samples were positive by ELISA (26.2%) and 16 (15.0%) by CLIA. The range was <1–84 U/mL and <1–111 U/mL for ELISA and CLIA, respectively.

Next, we evaluated samples for PR3 antibodies. Within the consecutive cohort, seven (3.5%) were positive by ELISA (≥5 U/mL) and nine (4.6%) were positive by CLIA (≥5 U/mL), with a range of <1–36 U/mL and <1–138 U/mL, respectively. Five samples were positive by both methods: three of these had known GPA and the remainder had renal failure presumed secondary to diabetes and hypertension without features of renal vasculitis on biopsy and the other