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Discrepancies between two immunoassays for the determination of MPO and PR3 autoantibodies

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Abstract

Background—Testing for autoantibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) is part of anti-neutrophil cytoplasmic antibodies (ANCA) test that aids the diagnosis of a number of autoimmune diseases including small-vessel vasculitis. We characterized the differences between two automated immunoassays at three facilities for measuring MPO- and PR3-ANCA autoantibodies.

Methods—117 serum samples were analyzed for MPO and PR3 autoantibodies. The INOVA Quanta Lite® IgG assay (INOVA Diagnostics) were performed at two facilities and the Bio-Plex® 2200 Vasculitis Panel (Bio-Rad) were performed at a third reference lab. The results were compared both qualitatively (between INOVA Quanta Lite® and Bio-Plex® methods) and quantitatively (between two sites performing INOVA Quanta Lite® assays).

Results—Comparison of the INOVA QUNATA Lite® assays at two different facilities (n=36) demonstrated high concordance (97.2% for MPO and 94.4% for PR3) and quantitative correlation ($R^2=0.973$ for MPO and $R^2=0.935$ for PR3). Conversely, INOVA QUNATA Lite® and Bio-Plex® methods showed poor concordance at 70.4% for MPO (n=81; 95%CI: 59.7% to 79.2%) and at 76.5% for PR3 (n=81; 95%CI: 66.2% to 84.4%).

Conclusion—This study demonstrated low concordance between two methods for MPO-ANCA and PR3-ANCA measurements. Given the discrepancies, the performance of different autoantibody immunoassay methods should be taken into consideration when evaluating MPO-ANCA and PR3-ANCA results.

Keywords

Anti-neutrophil cytoplasmic antibodies; MPO; PR3; immunoassay

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1. Introduction

Detection of anti-neutrophil cytoplasmic antibodies (ANCA), including anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) autoantibodies is a well-established diagnostic approach for patients suspected of having ANCA-associated vasculitis (AAV), inflammatory bowel disease, as well as systemic autoimmune diseases such as Lupus [1]. An international consensus on the appropriate detection of ANCA requires that screening for MPO- and PR3-ANCA should be performed by indirect immunofluorescence (IIF) and positive results to be confirmed by enzyme-linked immunosorbent assays (ELISA) specific for MPO and PR3 [2, 3]. Although IIF still remains to be the standard method for ANCA testing, it is expensive, labor intensive and variable results can be obtained with different assay kits and laboratory operators [4, 5]. Due to the above mentioned factors along with the struggle of maintaining optimum proficiency testing, many laboratories are moving away from the IIF method [5]. ELISA and other immunoassays, in comparison, are operator independent, easily automated and offer higher specificity [4, 6]. Several multicenter studies have shown a better performance of MPO- and PR3-ANCA immunoassays as the screening tool for AAV, suggesting a potential wider clinical application of these methods [5, 7].

Over the past decade, several immunoassays have been approved by the Food and Drug Administration (FDA) for the measurement of MPO-ANCA and PR3-ANCA, including ELISA and bead-based multiplex immunoassays. These assays employed a variety of testing principles, calibration methods as well as cut-off values [8, 9]. In addition, controversial data has been published on the performance and agreement of different MPO- and PR3-ANCA immunoassays [9, 10]. In the present study we have evaluated the concordance between two widely used immunoassays, a multiplex bead assay (Bio-Plex®) and a direct ELISA method (QUANTA Lite®) in a cohort of unselected patients at three facilities for the detection of MPO and PR3 autoantibodies.

2. Materials and method

2.1 Study samples

Residual, de-identified serum samples were used for all studies. In the first part of the study, 36 samples were tested for MPO and PR3 autoantibodies by QUANTA Lite® IgG ELISA (INOVA Diagnostics, San Diego, CA, USA) at two different facilities (site 1: National Institutes of Health (NIH), Bethesda, MD; site 2: Washington University in St. Louis, St. Louis, MO). A second cohort of 81 samples were evaluated by QUANTA Lite® IgG ELISA at site 1 and Bio-Plex® 2200 Vasculitis kit (Bio-Rad, Hercules, CA) at site 3 (a global reference laboratory that provides clinical laboratory testing to health care systems and hospitals). This study was approved by the Institutional Review Board of the NIH (93-CC-0094).

2.2 Methods and Instrumentation

MPO-ANCA and PR3-ANCA were determined by QUANTA Lite® IgG ELISA and Bio-Plex® 2200 Vasculitis kit.

The INOVA QUANTA Lite® assays were performed on the DSX automated workstation (DYNEX Technologies, Chantilly, VA). In the INOVA QUANTA Lite® assay, the cut-off value for discriminating positive samples has been established at >30 U/mL, and week positive samples at 21–30 U/mL, per the manufacturers recommend specifications. The INOVA polystyrene microwell ELISA plates are coated with native antigens purified from human neutrophils for MPO or PR3 [8]. After reaction with serum sample, MPO and PR3 autoantibodies are detected using a horseradish peroxidase conjugated goat anti-human IgG antibody followed by a Tetramethylbenzidine (TMB)-based chromogenic reaction. The final results are calculated by comparing absorbance of the sample to absorbance of the low positive control provided by the manufacturer.

The Bio-Plex® assay was performed on the Bio-Plex® 2200 testing platform (Bio-Rad Laboratories, Hercules, CA). The cutoff values for MPO- and PR3-ANCA per manufacturers specifications in antibody index units (AI) are: <0.4 negative, 0.4-0.9 equivocal, and >0.9 positive. The Bio-Plex® 2200 Vasculitis assay uses multiplex bead technology (Luminex, Austin, TX) to simultaneously detect three antibodies (MPO, PR3 and GBM) in a single sample [11]. After incubation with patient serum, the coated microbeads are washed and bound patient antibodies are quantified by the binding of phycoerythrin conjugated murine monoclonal anti- human IgG. Data acquisitions are calculated as relative fluorescence intensities and then converted to a fluorescence ratio using an internal standard bead. The ratio of fluorescence is compared to a calibration curve composed of four calibrator vials to determine the analyte concentration. Assay-specific analytical characteristics are summarized in Table 1.

2.3 Statistics

All statistics were completed using EP Evaluator (Data Innovations, Burlington, VT). The results were compared both qualitatively (between INOVA QUANTA Lite® and Bio-Plex® methods) and quantitatively by Deming regression analysis (between site 1 and site 2 using INOVA QUANTA Lite® assays).

3. Results

Comparison of the INOVA QUANTA Lite® ELISA assays at two different facilities (site 1 and site 2) employing 36 patient samples demonstrated high concordance with a total agreement of 97.2% (95% CI, 85.8 to 99.5%) for MPO-ANCA and 94.4% (95% CI, 81.9 to 98.5%) for PR3-ANCA (Table 2). Quantitative method comparison by Deming regression analysis showed a slope of 0.882 (95% CI, 0.831 to 0.933) and intercept of 0.01 (95% CI, -2.11 to 2.12) with $R^2=0.9728$ for MPO (Figure 1). For PR3 measurement, Deming regression demonstrated a slope of 0.977 (95% CI, 0.889 to 1.064) and intercept of -1.98 (95% CI, -6.65 to 2.68) with $R^2=0.9350$.

Conversely, for a cohort of 81 patient serum samples, INOVA QUANTA Lite® assays at site 1 and Bio-Plex® method at site 3 showed relatively poor concordance for MPO and PR3, at 70.4% (95%CI, 59.7% to 79.2%) and 76.5% (95%CI, 66.2% to 84.4%) respectively (Table 2).

For MPO-ANCA, 24 samples showed discrepant results between INOVA QUANTA Lite® assay and Bio-Plex® assay (Table 2). In specific, five samples were equivocal by Bio-Plex® (ranging from 0.6 to 0.9 AI), but negative by INOVA (ranging from 3.5 to 12.46 U/mL). 16 samples were positive by Bio-Plex® (1.1 to 6.4 AI), but below the negative cut-off by INOVA QUANTA Lite® (3.4 to 18.1 U/mL). Three samples were positive by Bio-Plex® (3.3 to 4.7 AI), but weak positive by INOVA (21.09 to 26.48 U/mL). No sample was found to be positive on INOVA QUANTA Lite® while negative on Bio-Plex® for MPO-ANCA.

For PR3-ANCA, 19 samples revealed discordant results between INOVA QUANTA Lite® assay and Bio-Plex® assay (Table 2). In specific, six samples were equivocal by Bio-Plex® (0.4 to 0.9 AI), but negative by INOVA QUANTA Lite® (10.5 to 19.68 U/mL). Five samples were positive by Bio-Plex® (1.4 to 2.6 AI), but below the negative cut-off by INOVA QUANTA Lite® (9.7 to 16.58 U/mL). Four samples were positive by Bio-Plex® (2.1 to 6.8 AI), but weak positive by INOVA QUANTA Lite® (27.6 to 29.42 U/mL). Four samples were equivocal by Bio-Plex® (0.6 to 0.8 AI) but positive by INOVA QUANTA Lite® (33.51 to 105.5 U/mL).

4. Discussion

Detection of ANCA is essential for the diagnosis and management of a number of autoimmune diseases and especially AAV [1, 12]. There is emerging evidence that MPO- and PR3-ANCA diseases are associated with distinct genetic polymorphisms, phenotypic characteristics and the risk of disease relapse [5, 13, 14]. Therefore, a sensitive and highly specific ANCA test is important for clinicians to classify ANCA associated diseases in order to provide optimal patient care. Although dual testing with IIF and solid-phase ELISA are currently recommended for ANCA detection, ELISA and other immunoassays offer many advantages over IIF including easy automation and lower cost [7]. Among the FDA-approved immunoassays, Bio-Plex® multiplex method and INOVA QUANTA Lite® ELISA tests are utilized in many clinical laboratories, but a head-to-head comparison of these two methods is lacking. In this study, we evaluated the agreement of these two immunoassays for the detection of MPO and PR3 autoantibodies in a cohort of 81 patients. In addition, the performance of the INOVA QUANTA Lite® assays were compared at two facilities employing 36 patient samples. We showed that INOVA QUANTA Lite® assays at two different facilities demonstrated high concordance (97.2% for MPO and 94.4% for PR3), but INOVA and Bio-Plex® methods showed relatively poor concordance at 70.4% and 76.5% for MPO and PR3 respectively.

We found that there was a substantial discordance at the individual level in the detection of MPO and PR3 autoantibodies between Bio-Plex® and INOVA QUANTA Lite® ELISA assays. Indeed, Cohen's kappa coefficient was only 0.35 for MPO and 0.53 for PR3, way below the level of high agreement (0.75). The discordance between two methods could be related to the differences in test principles (Table 1). For example, whereas the Bio-Plex® multiplex bead-based assay captures ligands onto spherical beads in suspension, INOVA QUANTA Lite® ELISA relies upon flat surfaces in 96-well plates, potentially resulting in a variation in antibody binding capacity [15]. In addition, since the Bio-Plex® multiplex assay allows simultaneous measurement of three autoantibodies, it is subject to perturbations that

are caused by analyzing multiple ligands simultaneously, such as cross-reactivity [16, 17]. By contrast, INOVA QUANTA Lite® ELISA only detects one analyte at a time, and therefore the concerns resulting from multiplexing are avoided [16]. The discordance could also result from the different calibration methods employed by the assays. The Bio-Plex® method uses a calibration curve composed of four calibrators to determine analyte concentration. In contrast, no calibrator except a low positive control is provided by the INOVA QUANTA Lite® ELISA assay, making it difficult to correct for the non-linear results reported by the manufacturer.

Interestingly, among the discordant samples, Bio-Plex® method demonstrated a clear positive bias relative to the INOVA QUANTA Lite® assay (Table 2). Although further evidence is needed, this discrepancy could be caused by relatively high cut-off values of INOVA QUANTA Lite® ELISA assay (higher specificity) and relatively low cut-offs of Bio-Plex® method (higher sensitivity). Indeed, a previous study demonstrated that INOVA QUANTA Lite® PR3 ELISA had an excellent specificity but a moderate sensitivity, which could be partly explained by a relatively high cut-off value (20 U/mL compared with 10 U/mL or 15 U/mL in a few commercial ELISA kits) [8, 18, 19]. However, direct comparison of the cut-off values between these methods is complexed by the different units employed by the assays and lack of published correlation between the units.

Our results here are in contrast with a survey published by College of American Pathologist (CAP), where all MPO- and PR3-ANCA tests showed excellent (100%) concordance, including the INOVA QUANTA Lite® assay and the Bio-Plex® method [20]. The inconsistency between that survey and our study could be a result of different samples employed. It is not clear whether the results of the survey were based on real patient samples. Moreover, only samples with negative values were measured in the survey whereas we have included samples in both normal and pathological ranges.

In clinical practice a physician often assumes that the results and interpretation of different ANCA tests are comparable and reliable. Our study here showed that the results for an individual sample markedly differed between two methods, particularly for values in the pathological range. It is important to inform the caring physician and the patients that the results from different ANCA assays have to be interpreted with caution. Conclusions of diagnosis should be made based on ANCA tests as well as clinicopathological features of the patients. For instance, negative results from one assay do not exclude the disease in patients with clinical suspicion, where a different method may be used to avoid false results. Immunoassays directed against MPO and PR3 also hold special value in monitoring disease activity and predicting relapse in a number of autoimmune diseases [21–23]. However, based on our results, it is highly recommended that the same method should be used for follow-up measurements.

A limitation of the current study is the lack of patients' diagnostic information. Due to this reason, we cannot assess and compare the specificity and sensitivity of these two assays. Moreover, different lots of both commercial kits may also contribute to the variations of the results, which should be considered in the future analysis. However, despite the shortcomings, we found significant discordance between the methods, which is consistent

with a general lack of standardization of autoantibody immunoassays [10, 24]. Future studies with patient information and international standards containing MPO- and PR3-ANCA are needed for a better understanding of how MPO- and PR3-ANCA tests perform and correlate [8].

In conclusion, our data show that there is a low to moderate concordance in MPO- and PR3-ANCA measurements between the INOVA QUANTA Lite® ELISA and Bio-Plex® multiplex methods. Therefore, clinical interpretation of test results should be made depending on the specific method used. More importantly, follow-up measurements to monitor treatment efficacy and/or disease progression, it is highly recommended to use the same assay method and manufacturer to prevent discrepant results.

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Abbreviations

AAV	ANCA-associated vasculitis
ANCA	anti-neutrophil cytoplasmic antibodies
ELISA	enzyme-linked immunosorbent assays
IIF	indirect immunofluorescence
MPO	myeloperoxidase
PR3	proteinase 3
TMB	tetramethylbenzidine

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Highlights

- Differences between two MPO- and PR3-ANCA immunoassays were evaluated at three facilities
- Two methods showed poor concordance for MPO and PR3 autoantibodies detection
- Clinical interpretation should be cautious as different methods may give different results.
- The same assay should be used for follow-up measurements and monitoring of disease progress.

Figure 1.
Deming plots of (A) MPO and (B) PR3 results obtained with INOVA QUANTA Lite® IgG assay at two different facilities (n=36).

Table 1

Analytical characteristics of QUANTA Lite® MPO/PR3 IgG assays and Bio-Plex®2200 Vasculitis kit.

Assay	QUANTA Lite® MPO/PR3 IgG	Bio-Plex® 2200 Vasculitis
Manufacturer	Inova Diagnostics Inc.	Bio-Rad
Technology	ELISA	ALBIA
Assay time	90 min	45 min
Detection	Semi-quantitative	Semi-quantitative
Reportable range	N/A	0.2–8 AI
	1–20 U/ml negative	<0.4 AI negative
Cut-off range	21–30 U/ml weak positive	0.4–0.9 AI equivocal
	>30 U/ml positive	>0.9 AI positive
Solid phase	Antigen coated microwells	Fluorescently labeled microbeads
Signal detected	Colorimetric detection	Fluorescence
Detection technology	Spectrophotometry	Flow cytometry

ELISA, enzyme linked immunosorbent assay; ALBIA, addressable laser bead immunoassay; N/A, not applicable; AI, antibody index units.

Table 2

Concordance between QUANTA Lite® IgG ELISA and Bio-Plex® 2200 assays for the measurement of MPO and PR3.

		QUANTA Lite® MPO (site 1)			Concordance (95% CI)	Cohen's kappa
		Week positive				
		Negative	Week positive	Positive		
QUANTA	Negative	25	1	0	97.2% (85.8 to 99.5%)	0.93
Lite® MPO	Week positive	0	0	0		
(site 2)	Positive	0	0	10		
Bio-Plex®	Negative	48	0	0	70.4% (59.7 to 79.2%)	0.35
2200 MPO	Equivocal	5	0	0		
(site 3)	Positive	16	3	9		
		QUANTA Lite® PR3 (site 1)			Concordance (95% CI)	Cohen's kappa
		Week positive				
		Negative	Week positive	Positive		
QUANTA	Negative	22	1	0	94.4% (81.9 to 98.5%)	0.89
Lite® PR3	Week positive	0	1	1		
(site 2)	Positive	0	0	11		
Bio-Plex®	Negative	48	0	0	76.5% (66.2 to 84.4%)	0.53
2200 PR3	Equivocal	6	0	4		
(site 3)	Positive	5	4	14		