Methods quantify the incidence of antibody switching. Typically, PR3/MPO-ANCA are both performed as specificity screening usually includes indirect immunofluorescence (IIF) and PR3/MPO-ANCA if positive or ANA present. Both tests are performed if our centre. Their applicability in other settings (general hospitals) is pending. We are evaluating this issue currently.

Reference

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P5 Incidence of PR3- and MPO-ANCA autoantibody specificity changes in ANCA associated vasculitis
S. Holding, M. Abuzakouk
Hull Royal Infirmary, Hull, United Kingdom

Introduction.— Monitoring of ANCA associated vasculitis (AAV) in remission usually includes indirect immunofluorescence (IIF) and PR3/MPO-ANCA. Typically, PR3/MPO-ANCA are both performed as specificity sometimes changes. Published data is limited to case reports and incidence of change is not reported. The aim of this study was to quantify the incidence of antibody switching.

Methods.— We serve a population of 720,000. IIF is used with reflex PR3/MPO-ANCA if positive or ANA present. Both tests are performed if previously positive. We reviewed all ANCA results from January 2000 to August 2012. A total of 22,002 IIF screens (14,518 patients) were performed. 9,838 also had both PR3/MPO-ANCA (6439 patients). Patients that changed specificity from PR3- to MPO-ANCA and vice versa were identified and case notes reviewed.

Results.— Two hundred and fifty patients positive for PR3/MPO-ANCA were followed for a mean of 2.4 years (range < 0.1 to 12.4 years; with 177 patients followed for > 90 days). Five patients (2%) changed antibody specificity during follow up (three GPA, one MPA & one AGP). In two of these patients this was associated with relapse. The incidence of specificity change was one per 66 years (including reversion to original specificity) and one per 199 years if only events associated with a rising CRP/relapse are considered.

Discussion.— In the five cases, we observed two were associated with relapse (and raised CRP). In two further patients switching was at low level, transient and was not considered clinically significant. If only the original antibody specificity was used in monitoring patients we would have missed a clinically significant event once every 199 patient years.

Conclusion.— We have shown that antibody specificity changes in AAV are rare. We show that monitoring using only the initial antibody specificity would have resulted in missed clinical events but CRP presaged relapse in these cases. Decisions on the optimum monitoring strategy require assessment of cost and benefit.

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P6 Multi-center evaluation of a novel chemiluminescent rapid assay for the detection of PR3-ANCA

1. INOVA Diagnostics, San Diego, USA
2. University of Ljubljana, Faculty of Medicine, Institute of Pathology, Ljubljana, Slovenia
3. San Carlo Borromeo Hospital, Faculty of Medicine, Institute of Pathology, Ljubljana, Slovenia
4. Maastricht University Medical Center, Laboratory of Clinical Immunology, Maastricht, Netherlands
5. AP–HP, Pitié-Salpêtrière Hospital, Department of Immunology, Paris, France
6. Wilhelminen Hospital, Laboratory Medicine, Vienna, Austria
7. University of Lubeck and Clinic Bad Bramstedt, Department of Rheumatology, Bad Bramstedt, Germany
8. University of Calgary, Department of Medicine, Calgary, Canada
9. Jagiellonian University Medical College, Department of Medicine, Krakow, Poland
10. University Hospitals Leuven, General Internal Medicine, Leuven, Belgium
11. University Hospitals Leuven, Department of Microbiology and Immunology, Laboratory Medicine, KU Leuven, Leuven, Belgium

Introduction.— Anti-PR3 antibodies represent an established and widely applied marker for the diagnosis of small vessel vasculitis, such as granulomatosis with polyangiitis (GPA). This multi-center study was designed to critically analyze the performance of QUANTA Flash PR3, a chemiluminescent immunoassay (CIA) on a random access rapid response (30 min) auto-analyzer for the detection of anti-PR3 antibodies, by testing a large number of samples from multiple clinical sites, selected samples in comparison to EliA PR3s (Thermo Scientific).

Methods.— Sera from 292 GPA patients and other diseases (n = 1356) were collected from 11 different laboratories in multiple countries (Germany, Italy, The Netherlands, Slovenia, France, Canada, Belgium, Poland, Austria) and tested by QUANTA Flash PR3. A total of 196 samples from two sites were also tested by EliA PR3s.
Results.— Clinical sensitivity and specificity for QUANTA Flash among all patients (n = 1648) were 62.7% (95% CI 56.8–68.2%) and 98.0% (95% CI 97.1–98.7%), respectively. Among the samples run on both QUANTA Flash and ELiA (n = 196), the discriminations between GPA patients and controls were similar: 56.1% (95% CI 44.7–67.0%) and 98.2% (95% CI 93.8–99.8%) sensitivity/specificity for QUANTA Flash and 58.5% (95% CI 47.1–69.3%)/96.5% (91.3–99.0%) sensitivity/specificity for ELiA. In addition, good agreements were found between assays: 86.5% (95% CI 74.2–94.4%) positive agreement, 97.9% (95% CI 94.0–99.6%) negative agreement and 94.9% (95% CI 90.8–97.5%) overall agreement. Spearman’s rho was 0.74 (95% CI 0.67–0.80).

Discussion.— Large multi-centric studies are important to evaluate the performance of novel diagnostic assays. In our study with samples from various countries, we found good performance characteristics of QUANTA Flash CIA and good agreement with ELiA.

Conclusion.— With the availability of QUANTA-Flash PR3 CIA the detection of anti-PR3 antibodies can now be performed with high reliability in clinical settings where rapid turnaround times (30 minutes) are important.

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P7
Comparison of PR3-specific ANCA assays performance for diagnosis of granulomatosis with polyangiitis (GPA)

A. Radice1, L. Bianchi2, S. Giomanì2, B. Trezzi2, U. Maggiore3, R.A. Sinico2
1. San Carlo Borromeo Hospital, Microbiology Institute, Milan, Italy
2. San Carlo Borromeo Hospital, Nephrology and Clinical Immunology, Milan, Italy
3. Maggiore Hospital, Nephrology and Transplantation, Parma, Italy

Introduction.— PR3-ANCA are usually detected by immunometric assays, with purified PR3 directly coated onto the solid-phase. Novel methods for PR3-ANCA detection have been proposed with the goal of improving the traditional PR3-specific assays, although little is known about their diagnostic performance in real-life clinical settings. The purpose of this monocentric retrospective study was to investigate and compare the diagnostic performance of nine different commercial PR3-specific assays, representative of the 1st, 2nd, and 3rd generation tests (direct, capture and anchor assays). The third generation assay, employing both human and recombinant PR3, was also evaluated.

Methods.— The study population consisted of 55 GPA, 175 disease controls, including 52 microsopic polyangiitis and 20 healthy subjects. GPA pts were selected on the basis of the clinical diagnosis, according to the internal criteria & definitions available. Median age of GPA was 53.5 yrs (13–82), 31 were 69 yrs. The primary evaluation of test sensitivity was carried out using cut-off points, which provided adequate and identical specificity for each test.

Results.— Although sensitivity & area under the ROC curves did not differ significantly between any of the PR3-specific assays, substantial differences in sensitivity at 98% specificity were found in some instances (Supplementary data, P < 0.001).

Discussion.— Our study shows that, compared to direct PR3-ELISAs, most of the capture/anchor tests improve the PPV for GPA diagnosis. Indeed, some of the 2nd & 3rd generation assays show a better performance than the traditional direct ones when the relevant cut-point is selected to guarantee the high specificity (98%) requested for such critical investigations. In addition, most of the 2nd and 3rd generation assays make easier the discrimination between +ve and –ve samples due to the reduction of borderline ANCA values.

Conclusion.— The usefulness of the new assays in monitoring the disease activity needs to be ascertained.

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P8
PR3-ANCA: A promising biomarker for the differentiation of ulcerative colitis and Crohn’s disease

M. Mahler1, C. Bentow1, D. Blockmans2, S. Vermeire3, P. Rutgeerts4, X. Bossuyt4
1. INOVA Diagnostics, San Diego, USA
2. University Hospitals, General Internal Medicine, Leuven, Belgium
3. University Hospitals Leuven, Gastroenterology, Leuven, Belgium
4. University Hospitals Leuven, Department of Microbiology and Immunology, Laboratory Medicine, Leuven, Belgium

Introduction.— Anti-PR3 antibodies represent an established and widely applied marker for the diagnosis of small vessel vasculitis, such as granulomatosis with polyangiitis (GPA). However, the prevalence of anti-PR3 antibodies in inflammatory bowel disease (IBD) patients is poorly defined. Although anti-PR3 antibodies have also been detected in IBD patients, their impact on diagnosis is unclear. Here we used three independent automated methods (QUANTA Flash, ELiA2, BioPlex 2200) to evaluate the prevalence of anti-PR3 antibodies in IBD.

Methods.— Sera from 76 ulcerative colitis (UC) and 55 Crohn’s disease (CrD) patients were collected (University Hospitals of Leuven, Belgium). The diagnoses were made based on current standard clinical, radiological, endoscopic and histological criteria. Additionally, samples from vasculitis patients (n = 55) were collected. As controls, patients with celiac disease (Cd) and patients with systemic rheumatic diseases were tested.

Results.— Anti-PR3 antibodies were detected in all three methods, albeit at a lower level than in GPA. The antibodies were mainly found in ulcerative colitis (UC) patients (prevalence varied between 15.8 and 71.1%, depending on the method) and rarely in patients with CrD (prevalence varied between 1.8 and 30.9%, depending on the method). Using receiver operating characteristics (ROC) analysis the cut-off selection was identified as the major cause of discrepancies. The likelihood ratios (LR+/LR–) for UC vs. CrD were 15.2/0.74 for QUANTA Flash and BioPlex 2200 and 8.7/0.86 for ELiA2. Overall, 15.8% of UC, but none of the CrD patients were positive by all three methods.

Discussion.— Anti-PR3 antibodies can be detected in UC patients using three independent methods. The putative clinical association between GPA and UC has to be investigated.

Conclusion.— Anti-PR3 antibodies are found in patients with UC and colitis has to be excluded in patients with a positive test result. Anti-PR3 antibodies represent a promising tool to help differentiating UC from CrD.

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