Posters

ANCA testing

P1
The diagnostic value of distinguishing and reporting different perinuclear ANCA (P-ANCA) immunofluorescence patterns: A prospective study
S. Perel, K. Prain, R. Wilson, R. Wong, D. Gillis, P. Hogan
Pathology, Queensland, Brisbane, Australia

Introduction.— Detection of anti-neutrophil cytoplasmic antibodies (ANCA) by indirect immunofluorescence (IIF) is the most widely utilised laboratory screening test for systemic necrotising vasculitis. While markedly different clinical associations have been described for the different subtypes of “perinuclear” or P-ANCA staining patterns, the 1999 International Consensus Statement does not require laboratories to distinguish between P-ANCA pattern subtypes (instead, allowing laboratories to report any type of perinuclear or granulocyte specific nuclear fluorescence as “P-ANCA”, followed by reference to MPO-ANCA/PR3-ANCA results). As we felt this recommendation was sub-optimal, we investigated whether discrimination of the classical P-ANCA pattern from atypical P-ANCA and uninterpretable patterns improves the diagnostic utility of ANCA testing.

Methods.— Over a 4 month period, all ANCA requests (n = 3544) referred to our Immunology Laboratory (Pathology Queensland Central Laboratory) were analysed prospectively for subtypes of P-ANCA staining patterns and MPO-ANCA/PR3-ANCA results, and then correlated with clinical/laboratory/radiological evidence of necrotising small vessel vasculitis.

Results.— The classical P-ANCA pattern had a significantly stronger association with vasculitis than the atypical P-ANCA pattern (67% vs. 1.2%; p < 0.0001) or ANA/uninterpretable patterns (67% vs. 3.5%; p < 0.0001). The combination of a classical P-ANCA pattern and a positive MPO-ANCA/PR3-ANCA result was also more strongly associated with vasculitis, than a positive MPO-ANCA/PR3-ANCA result in isolation (83% vs. 48%; p = 0.003).

Conclusion.— This study clearly demonstrates that the reporting of different P-ANCA patterns (including ANA/uninterpretable patterns) provides additional diagnostic information to the results of MPO-ANCA/PR3-ANCA testing. We therefore propose that future revisions of this International Consensus Statement should include an optimal recommendation to distinguish the different subtypes of P-ANCA staining patterns.

http://dx.doi.org/10.1016/j.lpm.2013.02.072

P2
ANCA testing in routine clinical laboratory
S. Perel, K. Prain, R. Wilson, R. Wong, D. Gillis, P. Hogan
Pathology, Queensland, Brisbane, Australia

Introduction.— Most samples sent for ANCA testing to routine immunological laboratory comes from patients suspected for other diagnosis than vasculitis. Possible diagnostic procedure recommended by SLI CSAKI was published for such cases [1]. This diagnostic procedure and scheme follows on the recommendation of SLI CSAKI for ANCA from 2005 year [2] and statements presented in the article published 2 years ago [3]. The mentioned scheme assumes ANCA screening using IFA on ethanol-fixed granulocytes followed by IFA, ELISA, immunoblot or ALBIA confirmation and typing of positive samples. If the diagnosis of vasculitis is assumed, it can already be used ELISA, immunoblot or ALBIA for screening in the first step including at least anti-PR3 and anti-MPO determination. The validity of the procedure mentioned above was supported and verified on data collected during the ANCA testing in author’s laboratory. Results of this testing are shown in this presentation.

Methods.— The analysis results of 5514 samples sent for ANCA testing to the author’s laboratory during 2011 year were evaluated. ANCA testing was done using diagnostic chart recommended by SLI CSAKI [1].

Results.— The overview of ANCA test results in the authors’ laboratory in 2011 year is listed in the attached table (table I).

Discussion.— Our analysis confirmed us in the opinion that the terms C-ANCA, P-ANCA and A-ANCA should be used only for IFA. Anti-PR3

| Table I |
The characteristic and outcome of treatment of EGPA patients

<table>
<thead>
<tr>
<th>Results of ANCA testing</th>
<th># (%)</th>
<th>Total (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2961 (93.79)</td>
<td>5 (0.16)</td>
</tr>
</tbody>
</table>

from 196 in ANCA positive or marginal samples screened on EtOH-GR:
- 135 (68.8%) was typed as A-ANCA
- 30 (15.31%) was typed as P-ANCA
- 23 (11.73%) was typed as C-ANCA
- 5 (2.55%) was typed as P-ANCA together with C-ANCA
- 2 (1.02%) was typed as P-ANCA together with A-ANCA
- 1 (0.51%) was typed as NSA due to the presence of ANA in high

ANCA testing by IBD IFA screening (most of ANCA positive samples were not typed)

<table>
<thead>
<tr>
<th>Results of ANCA testing</th>
<th># (%)</th>
<th>Total (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2148 (93.51)</td>
<td>7 (0.30)</td>
</tr>
</tbody>
</table>

60 from 196 IFA positive and marginal samples were ELISA typed. 39 were found positive: 16 anti-PR3, 21 anti-MPO (2 anti-PR3 and anti-MPO)and 9 other ANCA positivities (Lf, eia, cat, bpi)

52 from 196 IFA positive and marginal samples were typed using immunoblot.

21 were found positive: 11 anti-PR3, 14 anti-MPO (4 anti-PR3 and anti-MPO)

33 in IBD screening ANCA positive samples were IFA typed, 85% as A-ANCA
antibodies are mostly associated with C-ANCA and anti-MPO antibodies with P-ANCA fluorescence patterns. It cannot be expected that any specific, precisely defined antigen for A-ANCA could be find in the future [3]. The detection of A-ANCA is not practicable using ELISA, immunoblot or ALBIA.

**Conclusion.**– It seems that it is high time to supplement the internationally recognized ANCA terminology and procedures for their diagnosis [4,5] to be effective not only in the diagnosis of vasculitis, but also other neutrophil mediated inflammatory diseases.

**References**

anca/).


http://dx.doi.org/10.1016/j.lpm.2013.02.073

**P4**

**Results of ANCA standardisation and their diagnostic usefulness in ANCA-associated vasculitides (AASV) in a respiratory referral centre in Mexico**

F. Contreras-Rodríguez1, J. Rojas-Serrano2, J.E. Figueroa-Aguirre3, L.F. Flores-Suárez1

1. Instituto Nacional de Enfermedades Respiratorias, Primary Systemic Vasculitides Clinic, Mexico City, Mexico
2. Instituto Nacional de Enfermedades Respiratorias, Department of Intestinal Lung Diseases, Mexico City, Mexico
3. Instituto Nacional de Enfermedades Respiratorias, Clinical Laboratory Department, Mexico City, Mexico

**Introduction.**– ANCA have been fundamental for recent advances in primary small-vessel vasculitides, and are a valuable diagnostic tool. However, their standardisation has been difficult and other conditions can present with positive results. Our aim was to know the diagnostic usefulness of ANCA in a nationwide respiratory referral centre.

**Methods.**– All patients with suspicion of AASV were prospectively and consecutively included. Sample size calculation was done according to Flahault et al. [1]. Intrinsic properties of the tests were evaluated, and results of IIF (cutoff ≥ 1:20) and direct ELISA (≥ 20 U/mL, as per manufacturer’s cutoff) were compared by estimation of areas under the curve (AUC). Diagnosis of an AASV was established by clinical and histopathological data. Serology was performed in duplicate without knowledge of clinical data by lab personnel according to manufacturer (Euroimmun).

**Results.**– Ninety-eight patients, 23 AASV (14 GPA, eight MPA, one EGPA), 75 disease controls. Test properties are shown in the table (table I), according to diagnostic categories. Regarding AUC of IIF and ELISA, there were no differences between P-ANCA vs MPO-ANCA or C-ANCA vs PR3-ANCA overall, nor when specific diseases were compared, except for MPA, in where the AUC for MPO-ANCA was higher than IIF (90% vs 82%, P = 0.05).

**Discussion.**– Considering interpretation of IIF done by expert personnel, automated tests feasibility and characteristics of centres in where ANCA are ordered and performed, from our results, we can advise for either test to be used diagnostically in a referral centre where patients have respiratory symptoms in a high percentage. For MPA, ELISA may be better to use initially.

http://dx.doi.org/10.1016/j.lpm.2013.02.074

**P3**

**Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis relapses? Results of a longitudinal study on 126 patients**

L.H. Thai, P. Charles, M. Resche-Rigon, L. Guillevin

AP–HP, Paris, France

**Introduction.**– Predicting granulomatosis with polyangiitis (GPA) relapses by measuring of ANCA titers remains a source of debate. Our objective was to evaluate the relevance of monitoring PR3-ANCA titers for GPA management.

**Methods.**– This retrospective study included 126 patients recruited from Cochin Hospital, all fulfilling the 1990 ACR criteria for GPA and PR3-ANCA-positive at the time of diagnosis. GPA activity was assessed with the Birmingham Vasculitis Activity Score for Wegener granulomatosis (BWAS/WG). cANCA were detected in an immuno-fluorescence assay and their PR3-ANCA specificity was determined in (Euroimmun).

**Results.**– Ninety-eight patients, 23 AASV (14 GPA, eight MPA, one EGPA), 75 disease controls. Test properties are shown in the table (table I), according to diagnostic categories. Regarding AUC of IIF and ELISA, there were no differences between P-ANCA vs MPO-ANCA or C-ANCA vs PR3-ANCA overall, nor when specific diseases were compared, except for MPA, in where the AUC for MPO-ANCA was higher than IIF (90% vs 82%, P = 0.05).

**Discussion.**– Considering interpretation of IIF done by expert personnel, automated tests feasibility and characteristics of centres in where ANCA are ordered and performed, from our results, we can advise for either test to be used diagnostically in a referral centre where patients have respiratory symptoms in a high percentage. For MPA, ELISA may be better to use initially.

Conclusion.– Our results can lead to test optimisation and recommendations in referral units in our country. Both assays performed equally in