**P9**

**The association of IgM ANCA with alveolar hemorrhage revisited**

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*Introduction.*—Little is known about what factors predispose patients with antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitides (AAV) to particular disease phenotypes. Case reports have suggested a potential link between the ANCA IgM isotype and AAV manifesting with alveolar hemorrhage, albeit inconsistently. The possibility of such an association has not previously been examined systematically.

*Methods.*—The study population consisted of the subset of patients who enrolled in the Rituximab in AAV (RAVE) trial [1] who had IgG proteinase 3 (PR3)-ANCA. Serum samples drawn at the baseline study visit were assessed for the presence of IgM PR3-ANCA by means of a capture enzyme-linked immunosorbent assay (ELISA). Comparisons of binary measures were analyzed by Fisher’s exact test.

*Results.*—A total of 129 patients with severe AAV were studied, and 53 (41.1%) tested positive for IgM PR3-ANCA. Alveolar hemorrhage occurred approximately three times more frequently among those who tested positive for IgM PR3-ANCA as compared to those who tested negative (45.3% versus 15.8%; P < 0.001). The frequency of IgM PR3-ANCA was similar whether AAV was newly diagnosed or recurrent, both across the entire cohort (39.6% versus 42.0%; P = 0.85) and among the subgroup of patients with alveolar hemorrhage (57.1% versus 72.7%; P = 0.47).

*Conclusion.*—In a population of patients with PR3-ANCA and severe manifestations of AAV, the PR3-ANCA IgM isotype was associated with an increased rate of alveolar hemorrhage. As IgM PR3-ANCA was not detected more frequently among those patients with newly diagnosed disease, it is unlikely that the association with alveolar hemorrhage is merely a byproduct of the acuity of the underlying AAV. These findings suggest the possibility of an association of PR3-ANCA properties with specific disease manifestations.

*Reference*


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**P10**

**Computer-aided immunofluorescence microscopy (CAIFM) for ANCA diagnostics**

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*Introduction.*—Screening by indirect immunofluorescence tests (IIFT) is the gold standard to detect anti-neutrophil cytoplasmic antibodies (ANCA). EUROPattern is a CAIFM system of microscope and software for evaluation and data management to standardize the reporting for IFT (IFA) tests. Beyond recommended diagnostics with ethanol-fixed neutrophils, we provide a test system with 5 additional substrates for more precise diagnostics of ANCA-associated vasculitis (AAV).

*Methods.*—In one reaction field, three BIOCHIPs with cell substrates and three with purified antigen spots are combined: ethanol-fixed neutrophils, formalin-fixed neutrophils, an ethanol-fixed mixture of neutrophils and HEP-2 cells, and microparticles of proteinase 3 (PR3), myeloperoxidase (MPO) and glomerular basement membrane (GBM). A magazine holds up to 50 or 100 slides with 500 or 1000 reaction fields, and a camera takes photos of the reactions. Triggering, focusing and image recording is performed automatically or interactively using EUROLabOffice.

*Results.*—EUROLabOffice connects with the Laboratory Information Management System (LIMS), further liquid handling devices and manages data and test results. IIFT images can be adjusted via a graphical user interface. Results are pre-classified as positive and negative. The user can zoom images, revise classifications, assign patterns, edit titers and results and access the patient history.

*Discussion.*—EUROPattern supports experts by simplifying and accelerating the laboratory workflow: data are processed consistently and traceable, automatic image recording enables fast archiving, the graphical user interface simplifies operators’ work and allows evaluating results with clean hands in an office instead of a dark room. The automatic pre-classification is in good correlation with manual reading.

The 6-BIOCHIP mosaic achieves a broad screening with high sensitivity and specificity, even in the presence of interfering antibodies.

*Conclusion.*—The presented CAIFM system establishes a standardized process for ANCA evaluation.

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**P11**

**Patients positive for both MPO-ANCA and PR3-ANCA do not present idiopathic systemic necrotizing vasculitis**

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*Introduction.*—Antineutrophil cytoplasmic antibodies (ANCA) are detected by indirect immunofluorescence (IIF) assays on human fixed neutrophils, with three different patterns: a cytoplasmic (cANCA), a perinuclear (pANCA), and an atypical pattern (aANCA). ANCA specificity is determined by solid phase assays (ELISA, immunodot and addressable laser bead immunoassay [ALBIA or LumineX® assay). Two relevant clinical targets are described (proteinase 3 [PR3] or myeloperoxidase [MPO]). ANCA with high titres and defined specificities (PR3 or MPO) are good serological markers of active primary systemic vasculitis (SV): c/PR3-ANCA for granulomatous with polyangiitis and p/MPO-ANCA for microscopic polyangiitis. Usually ANCA are monospecific, but some multispecific ANCA are described, of which some specific for both PR3 and MPO.

*Objective.*—Herein we describe clinical data of such a cohort in a multicenter retrospective study.
Methods.— Five thousand sera of 3095 patients from three French hospitals were found IIF-ANCA positive from 2004 to 2012. PR3 and MPO specificities were assessed by ELISA, immunodot or ALBIA.

Results.— Twenty-eight IIF-ANCA positive patients had ANCA specific for both PR3 and MPO by at least one of the solid phase test (0.7% of serum, 0.9% of patients). None of the 23 patients with an available file had a necrotizing systemic vasculitis. No relevant clinical association was noticed.

Discussion.— We described 23 documented patients with both PR3 and MPO ANCA. None of them had a SV, neither granulomatosis with polyangiitis nor microscopic polyangiitis nor Churg and Strauss syndrome.

To the best of our knowledge, no cohort studies focused on such PR3 and MPO-ANCA. Only case reports or incidentally reported cases in serological studies appear in the literature [1,2,3].

Conclusion.— This cohort study demonstrates that, unlike the mono-specific PR3- or MPO-ANCA, ANCA with both anti-PR3 and anti-MPO activity, are not associated with SV, unlike few reports of sparse MPO and PR3-ANCA cases ascertained as SV with old laboratory tests.

References

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P12 Aklides® – a highly versatile imaging platform for detection of ANCA
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Introduction.— Here we describe a highly versatile microscopy platform – Aklides® – (A) for the automated pattern analysis of ANCA with neutrophils combined with (B) multiplex microbead-based detection of Proteinase 3 (PR3), Myeloperoxidase (MPO), and glomerular basement membrane (GBM) autoantibodies (aAb). The manual interpretation of ANCA patterns is very subjective and prone to high variability. EIA results are objective but EIA have their limit in multiplex detection of specific ANCA such as PR3 and MPO.

Methods.— (A) ANCA were automatically analysed with ethanol (ethN) or formalin (formN) fixed neutrophils. Sera from 342 patients with AAV and other systemic rheumatic and infectious diseases were tested for ANCA patterns with Aklides® and results were compared to those of conventional fluorescence microscopy. (B) Overall, n = 214 patient sera with prefindings for PR3 aAb, n = 222 for MPO aAb, and n = 65 for GBM aAb were analyzed with microbead-based immunoassay (Aklides® ANCA BA) and results were compared to those of conventional routine ELISA.

Results.— (A) An interpretation software employing pattern recognition algorithms was developed enabling positive/negative discrimination and classification of cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA) pattern. Comparison of visual reading with automated interpretation revealed Cohen’s kappa (κ) values of 0.955 on ethN and 0.929 on formN for positive/negative discrimination. Analysis of the set with regard to the pattern discrimination showed a high agreement for ethN (κ = 0.746) and formN (κ = 0.847). There was no significant difference between visual and automated interpretation (P > 0.05).

(B) For Aklides® ANCA BA the following relative sensitivities and specificities were determined: anti-PR3 (98%; 95%), anti-MPO (97%; 99%), and anti-GBM (90%; 100%).

Conclusion.— Aklides® is suitable for automated ANCA pattern analysis and detection of PR3, MPO, and GBM aAb and shows a high concordance to results of conventional fluorescence microscopy and routine ELISA.

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Animal models

P13 Synergistic effect of GCSF and LPS in ANCA Vasculitis
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Introduction.— Granulocyte colony stimulating factor (GCSF) is a cytokine that is important in mobilizing neutrophils from the bone marrow and has proinflammatory effects. We have previously shown that serum GCSF is raised in patients with ANCA vasculitis and exacerbates disease in an established murine model [1]. LPS was given to all mice in the previous study, though it was not known if this was required with GCSF. We set out to investigate the relative role of GCSF and LPS in this model.

Methods.— Purified murine MPO was used to immunise MPO knockout mice to generate anti-MPO antibody. Four groups of wild type C57BL/6 mice were used (n = 5–6/group). They were given GCSF or control subcutaneously starting 8 days before the disease induction with anti-MPO (day 0). LPS or control was administered intraperitoneally on day 0 and 3. Serum, urine and histology was assessed on day 7.

Results.— The group which received both LPS and GCSF had significantly higher serum creatinine levels compared to mice with administration of neither LPS nor GCSF, administration of LPS alone or GCSF alone (12.3 ± 0.8 compared to 9.0 ± 0.5, 8.3 ± 0.6, 9.5 ± 0.4 μmol/L respectively, P < 0.001). This group also had significantly more albuminuria compared to the other three groups (106 ± 18.24 compared to 15.47 ± 1.43, 27.21 ± 4.33, 21.75 ± 2.75 mgc in 24 hrs respectively, P < 0.0001). Furthermore, this group had significantly more glomerular crescents compared to the other three groups (31.6 ± 5.2 compared to 0 ± 0, 0.3 ± 0.3, 0.2 ± 0.2 per 100 glomeruli respectively, P < 0.0001) and glomerular macrophages (15.3 ± 1.3 compared to 0.5 ± 0.1, 2.2 ± 0.3, 4.1 ± 0.8 cells per glomerulus respectively, P < 0.0001).

Discussion.— This study shows that both LPS and GCSF are required to obtain robust disease in this model, with a strong synergistic effect on both histological and biochemical disease parameters.

Conclusion.— These findings have implications for investigators using this model and for our understanding of disease pathogenesis. They suggest that endogenous GCSF may be a therapeutic target.