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The association of IgM ANCA with alveolar hemorrhage revisited

J. Clain 1, U. Specks 2, J. Stone 3, X 31
1. Mayo Clinic, Rochester, Minnesota, USA
2. Massachusetts General Hospital, Boston, Massachusetts, USA
3. Various 1 For The Rave-Itn Investigator Group.

**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 type 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

N. Hochstrate 1, C. Krause 1, K. Ens 1, M. Danckwardt 1, C. Feirer 1, K. Affeldt 1, M. Rateike 1, M. Morrin 1, W. Wuttig 1, J. Voigt 1, K. Fechner 1, E. Barth 1, T. Martinetz 2, W. Stöcker 1
1. Institute for Experimental Immunology, Euroimmun AG, Lübeck, Germany
2. Institute for Neuro- and Bioinformatics, University of Lübeck, Lübeck, Germany

**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 IF assay (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 (AVV).

**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 microdots 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. IIF 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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