or probable IE, and 31 (28%) had prosthetic valves. The major causative pathogens were *Staphylococcus aureus* (n = 33), *Streptococcus viridans* (n = 23), *Streptococcus bovis* (n = 10) and *Enterococci* (n = 7). C-ANCA were found in 13 patients (12%), P-ANCA in 11 (10%) and 1 case (1%) showed both patterns. ELISA revealed anti-PR3 in four cases (3%) and anti-MPO in four (3%), some with very high titters. The eight anti-PR3/anti-MPO-positive IE cases involved various pathogens and both native and prosthetic valves. Testing for ANAs (titer > 1:160), acf and RF was positive in 17 (16%), 25 (23%) and 38 (35%), respectively.

**Conclusion.–** This study suggests that ANCA, including those with anti-PR3 or anti-MPO specificities, occur in a significant subset of cases and substantiate the consideration of IE as a potential cause of ANCA positivity.

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A30

**Recurrent acquired ANCA-positive agranulocytosis after cocaine exposure: A chronic disease?**


1. Ematologia Spedali Civili, Brescia, Italy
2. Monash University, Clayton, Australia
3. University of Groningen, Groningen, Netherlands
4. Environmental Protection Agency, Research Triangle Park, USA
5. Massachusetts General Hospital, Boston, USA

**Introduction.–** Levamisole tainted cocaine may lead to skin necrotizing vasculitis with neutropenia, but also to life-threatening agranulocytosis, often occurring with ANCA positivity. While in vasculopathy-associated cases neutropenia resolves with drug withdrawal, little is known about the clinical course of isolated agranulocytosis. Levamisole is thought to cause neutropenia inducing autoimmunity. We describe three of recurrent, chronic, ANCA-positive agranulocytosis, with no vasculitic symptoms, after cocaine exposure.

**Methods.–** Anti-MPO, anti-PR3 and anti-NE ANCA were detected by direct immunofluorescence and anti-c-myel and nickel ELISAs.

**Results.–** Three pts (1 M/2 F, 36, 41, 45 y.o., respectively) without family history of neutropenia, developed recurrent episodes of agranulocytosis associated to acute tonsillitis, oral mucositis, or perianal ulcers. All admitted cocaine use. Laboratory tests showed ANCA positivity, anti-MPO in one case and anti-PR3 in the other two. Before immunosuppressive therapy was started, aycyclical drop of neutrophil count was observed in all pts, not regularly linked to further cocaine use. In one case high dose prednisone was administered for 2 months, with success. When this patient relapsed, azathioprine was given, obtaining a new remission. The other two cases achieved remission after three and two courses, respectively, of high dose Ig. Pts’ medium follow-up was 22 months.

Looking for an immune mechanism possibly responsible for an early apoptosis of neutrophil precursors, we tested pts’ sera for neutrophil elastase (NE) ANCA, i.e. the antibody directed against the same protein mutated in the congenital form. We found a significant titer of HNE ANCA in all three pts.

**Conclusion.–** Our findings support a relation between cyclic acquired neutropenia and NE ANCA, likely induced by exposure to cocaine, that would trigger an immuno-mediated process leading to a chronic disease. Drug abstinence may not be enough to prevent neutropenia, and treatment in these cases should be immunosuppressive therapy.

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A31

**ANCA epitope specificity determines pathogenicity, detectability and clinical predictive value**

A. Roth1, J. Ooi2, J. Hess1, M. Van Timmeren3, E. Berg1, C. Poulton1, J. Mcgregor4, M. Burkart5, S. Hogan5, Y. Hu4, W. Winnik5, P. Nachman1, C. Stegeman3, J. Niles5, P. Heeringa4, M. Free1, R. Kitching2, S. Holdsworth2, C. Jennette1, G. Preston1, R. Falk1

1. UNC Chapel Hill, Chapel Hill, USA
2. University of Groningen, Groningen, Netherlands
3. Environmental Protection Agency, Research Triangle Park, USA
4. Massachusetts General Hospital, Boston, USA

**Introduction.–** Anti-neutrophil cytoplasmic autoantibodies (ANCA) specific for myeloperoxidase (MPO) or proteinase 3 (PR3) are detected in > 90% of ANCA-associated vasculitis (AAV) patients. In vivo and in vitro studies demonstrate that ANCA are pathogenic, yet ANCA titers do not correlate well with disease activity. This multicenter study sought to elucidate the poor correlation between disease activity and ANCA titer, why naturally occurring anti-MPO autoantibodies exist in disease-free individuals, and the failure to detect ANCA by conventional assays in some AAV patients.

**Methods.–** An epitope excision/mass spectrometry (MS) approach entailed binding MPO-ANCA to MPO, protecting epitopes from enzymatic digestion. Bound peptides were eluted and identified by matrix-assisted laser desorption (MALDI)–MS. Antibody epitope profiles were analyzed from S2 active and 35 remission AAV patients and 10 healthy controls. Reactivity with peptide epitopes were assayed by ELISA. Pathogenic potential of ANCA were tested by ex vivo neutrophil activation and in vivo mouse model.

**Results.–** Twenty-five unique anti-MPO epitopes were detected and subcategorized: active disease-associated (12/25), persistant in remission (6/25), and natural epitopes (7/25) which reacted at very low levels with healthy control IgG. Importantly, MPO-ANCA reactive to one linear sequence were detected in 8 of 10 AAV patients who were negative by conventional assays. Autoantibodies against this epitope have pathogenic potential as demonstrated by their capacity to activate neutrophils ex vivo and were nephritogenic in mice. The confounder for clinical detection of these autoantibodies is a ceruloplasmin fragment, which masks the epitope that is readily detected with purified IgG.

**Conclusion.–** ANCA titers in clinical assays reflect both pathogenic and nonpathogenic ANCA. Pathogenic ANCA correlate better with disease activity. IgG from patients with ANCA-negative AAV reacts to a restricted pathogenic epitope, which is masked in serum by ceruloplasmin.

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A32

**Characteristics and outcomes of patients with ANCA-associated vasculitis in the Czech population**

Z. Hruskova1, E. Jancova1, V. Lanska2, J. Svojanovsky3, J. Klaboch4, P. Nemec3, K. Zamboch5, M. Kodeda6, V. Hanzal7, P. Nemeci8, V. Tesar1, On Behalf Of The Czech National Clinical Registry Of AAV

1. Department of Nephrology, General University Hospital and First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
2. Statistical Unit, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
3. 2nd Department of Medicine, St Ann’s Hospital, Brno, Czech Republic

**Introduction.–** Myeloperoxidase (MPO) and proteinase 3 (PR3) ANCA were detected in 90% of ANCA-associated vasculitis (AAV) patients. Our study aimed to evaluate clinical predictors of AAV in the Czech setting.

**Methods.–** We performed a multicenter retrospective study of 407 consecutive patients referred to the AAV reference center in the Czech Republic between 2004 and 2013. Patients were categorized in four groups: definite AAV, possible AAV, clinical AAV and “non-AAA”.

**Results.–** Of 407 patients, 280 (69%) were definite AAV, 16 (4%) possible AAV, 11 (3%) clinical AAV and 90 (22%) were non-AAA. The mean age was 57 years and 30% were men. Myeloperoxidase ANCA were detected in 96% of patients and PR3 in 4%. C-ANCA were detected in 24% of patients.

**Conclusion.–** Our findings support the hypothesis that AAV is a composite disease entity with a wide range of clinical presentations and disease courses.

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