Introduction.– It has been suggested that ear, nose, and throat (ENT) involvement in ANCA-associated vasculitis (AAV) may carry the advantage of earlier recognition of the systemic vasculitis. Alternatively, differences in histological findings between patients with MPO-ANCA and PR3-ANCA might represent different routes in the pathogenesis of vasculitic disease in these subsets of patients. This study investigates whether ENT involvement in AAV is associated with better renal function and histopathology than AAV without ENT involvement.

Methods.– Renal biopsies with ≥ 7 glomeruli were available from 152 newly diagnosed AAV patients from four international multicenter trials. Age, ENT involvement, ANCA type (PR3 or MPO), interstitial fibrosis and tubular atrophy (IFTA), tubulitis, interstitial infiltrates and the histopathologic classification of ANCA-associated glomerulonephritis (AAGN) were analyzed as candidate determinants of GFR at diagnosis (GFR0). The relation of GFR0, IFTA and the histopathological classification with ENT involvement was analyzed at the time of diagnosis.

Results.– Sixty-four patients had ENT involvement at diagnosis, 88 patients had not. Multivariate analysis revealed that in combination with ENT involvement (r = 0.25, P = 0.000), age (r = −0.34, P = 0.000), IFTA (r = 0.16, P = 0.001), tubulitis (r = 0.16, P = 0.001), interstitial infiltrates (r = 0.20, P = 0.000) and the histopathologic classification of AAGN (r = 0.411, P = 0.000) were associated with GFR at diagnosis. Patients with ENT involvement had a higher GFR0 (60 ml/min versus 44 ml/min, P = 0.000), less IFTA (P = 0.001) and a histopathologic more favourable class (P = 0.000) than patients without ENT involvement. Increasing numbers of active BVAS ENT parameters (range: 0–6) showed a high correlation with increased renal function at time of diagnosis (P = 0.000).

Conclusion.– ENT involvement in AAV with renal disease is associated with better renal function and less severe histological renal injury, probably due to diagnosis before the development of irreversible chronic lesions.

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A28 Clinical evaluation of a rapid immunofluorescence test (IIFT) for diagnosis of ANCA-associated vasculitis

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Introduction.– AAV, when untreated, generally follow a fatal progressive course so that early diagnosis is mandatory to allow timely treatment. Reliable laboratory methods for ANCA testing are essential to confirm diagnosis. Guidelines suggest combining IIFT and MPO/PR3-specific assays as the optimal strategy for ANCA detection. Aims of this monocentric, retrospective study were to evaluate:

– the diagnostic performance of a rapid ANCA kit (EuroplusTM);
– its usefulness in emergency clinical settings.

Methods.– Sera from 107 AAV selected on the basis of clinical diagnosis, 123 pathological and 20 healthy controls were tested. Materials: Granulocytes Mosaic EuroplusTM (Euroimmun); homemade ANCA-IIFT, ANCA-MPO/PR3 (Orgentec, direct ELISA). The Granulocytes Mosaic EuroplusTM system is a IIFT assay where the slide wells (“biochips”) are coated with ethanol/formaline-fixed neutrophils and purified MPO/PR3 microdots (figure 1). The system allows the contemporary evaluation of IIFT and antigen-specific assays. The test is carried out as a classical IIFT and results are available in ∼ 90’.

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A29 Prevalence of anti-neutrophil cytoplasmic antibodies in infective endocarditis: An analysis of 109 cases

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Introduction.– Sporadic reports have been published on positive ANCA tests in the context of infective endocarditis (IE) and combined with the multisystem protein presentation of IE, this situation may lead to inappropriate diagnosis and therapy. Because the frequency of ANCA in IE is unknown, we assessed the prevalence of ANCA in a relatively large number of cases with IE.

Methods.– The study was conducted in the framework of an inception cohort of consecutive cases with IE launched in 2005 in a single university hospital. Sera were stored for all patients who gave informed consent for blood sampling. All selected sera were tested for ANCA in a central laboratory using indirect immunofluorescence (IIF) assays and ELISA for anti-proteinase 3 (PR3) and anti-myeloperoxidase (MPO) specificities by use of commercially available kits. In addition, the sera were tested for antinuclear antibodies (ANA) and antcardiolipin antibodies (aCL) by use of a commercially available IIF kits and for rheumatoid factor (RF) by use of an in-house test.

Results.– Sera from 109 patients (82 [75%] men, mean age: 57.5 yrs [SD: 15.4]) were tested. All patients fulfilled Duke’s criteria for definite
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 titers. The eight anti-PR3/anti-MPO–positive IE cases involved various pathogens and both native and prosthetic valves. Testing for ANAs (titer > 1:160), aCl 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?**

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**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-neyc 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, a cyclical 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 aCl activity. In all pts, 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**

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**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 non-pathogenic 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**

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**Results.**—Among 357 patients with ANCA-associated vasculitis, 101 patients fulfilled the Chapel Hill criteria for definite AAV. In AAV patients, AAS were detected in 98 (97%), PR3 in 71 (70%), MPO in 48 (47%), PR3 and MPO in 37 (36%). In AAS patients, aCl positivity was positive in 17 (16%), anti-PR3 in 8 (8%), anti-MPO in 4 (4%), and anti-PR3 and anti-MPO in 2 (2%). AAS positivity was associated with 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.

**Conclusion.**—ANCA titers in clinical assays reflect both pathogenic and non-pathogenic 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.