Long-term outcome of low dose cyclophosphamide (CYC) and plasma exchange (PLEX) induction in ANCA-associated vasculitis (AAV)

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Introduction – Adverse events to the treatment of AAV are the greatest threat for 1 year’s outcome. The use of PLEX in AAV is not commonly accepted in patients (pts.) with creatinine < 500 μmol/L. We combined low dose of CYC and PLEX in order to minimize the infection/sepsis rate, which mostly is due to the immunosuppression.

Patient – A prospective cohort study of all AAV pts. referred between 2000–2010 was performed. All pts. had AAV based on positive ANCA and a compatible clinical syndrome. The use of PLEX was decided on severity of renal biopsy and ANCA titres. Immunosuppression consisted of prednisolone 1 mg/kg/day and a low dose of daily oral CYC (100 mg/day in pts. < 65 years and 50 mg/day in pts. > 65). AZA/MMF was given for maintenance of remission.

Results – One hundred and thirty-two pts. were admitted and 118 followed for a mean of 5.7 years (range 0.2–12.3; 676 pts. years). Fifty-seven percent were male, 47% were MPO-ANCA positive. Thirty-six percent had high creatinine > 500. Forty percent were aged > 65. One hundred and five pts. received PLEX [mean 7 (5–11)]. Only five pts. (4%) died during CYC treatment (two sepsis, two AMI, one lung haemorrhage, all on HD). In total 11 pts. (9%) died within 1 year and 14 more pts. died < 5 years. The total number of deaths was 33 (28%) with an expected 12 years patient survival of 62% (Kaplan Meier). Twenty-seven pts. (23%) developed ESRD, with an expected 12 years kidney survival of 75% (K.M.). Forty-three pts. (36%) died or developed ESRD during the study, resulting in an expected 12 years dialysis free patient survival of 62% (K.M.). Pts. aged < 65 or < 500 had significantly better dialysis free survival. 29 pts. (23%) had infection < first 4 months, 15 (13%) being leucopenic. Forty-one (34%) relapses occurred during the 12 years of follow up.

Conclusion – The use of low dose CYC and PLEX for induction resulted in a high survival rate and good preservation of renal function together with low rate of complications. As the septicaemia and mortality was low, the combination of PLEX and low CYC seems to be less toxic, than the conventional AAV induction treatment regimen.

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Low %CD5+ B cells in patients with ANCA vasculitis portends a shorter time to relapse after rituximab

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Introduction – We recently reported that CD5+ B cells, as a surrogate marker of B regulatory cells, are decreased in active ANCA-small vessel vasculitis (ANCA-SVV) and normalize during disease remission [1]. After B cell depletion with rituximab, we found that patients who repopulated with a low or decreasing %CD5+ B cells and were on low maintenance immunosuppression had a shorter time to relapse than patients on similar levels of immunosuppression but normalized %CD5+ B cells or patients with similarly low %CD5+ B cells but on full dose maintenance immunosuppression with MMF. To avoid infections and adverse events from therapy, clinicians require improved markers of disease activity and impending relapse to guide immunosuppression strategies post-rituximab. In this study, we test our hypothesis that CD5+ B cells may serve this purpose in a larger cohort.

Methods – We examined B cell phenotype in patients with ANCA-SVV after rituximab therapy by flow cytometry. Whole blood or PBMCs were stained with antibodies to CD19, CD20, CD45 and CD5. Data from research samples was supplemented with data acquired from clinical Rituximab panels.

Results – Without consideration of immunosuppression dose, in 31 patients, those who had < 30% CD5+ B cells at the time of B cell repopulation (≥ 1% CD19+/CD20+ lymphocytes) relapsed sooner (14 ± 4 m) than patients who repopulated with ≥ 30% CD5+ B cells (26 ± 12 m; P = 0.03) after rituximab.

Discussion – We present data that confirm the %CD5+ B cells, as a component of the human B regulatory cell phenotype, is an indicator of future relapse. Moreover, a low %CD5+ B cells correlates with a shorter time to relapse regardless of immunosuppression dose. Monitoring whether CD5+ B regulatory cells contribute to B cell repopulation...
following rituximab therapy may help guide remission maintenance therapy in these patients.

**Conclusion.**—CD5+ B cells may be a novel immunological biomarker to follow induction of remission and impending flare in patients with ANCA vasculitis before and after treatment with rituximab.

**Reference**


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**P172**

**Monocyte chemoattractant protein-1 (MCP-1) in antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis: Biomarker potential and association with polymorphisms in the MCP-1 and the CC chemokine receptor-2 (CCR2) gene**

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**Introduction.**—ANCA-associated small vessel vasculitis (AAV) morbidity and mortality have decreased over the last four decades, but there is still a need of better biomarkers for distinguishing which patients will have a more severe outcome, in order to optimize the treatment. The aim of this study was to confirm our previous results of urinary MCP-1 (uMCP-1) as a prognostic marker, and to explore its potential as a marker of disease activity. Another part of the study was to explore the association of AAV and polymorphism in the MCP-1 gene (-2518A/G) and the CCR2 gene (V64I).

**Patients.**—One hundred and fourteen patients with AAV were followed regularly between 2002 and 2009 at Skane University Hospital. Urine samples, blood samples and clinical status were registered. uMCP-1 was analyzed by an in house ELISA. PCR-RFLP was used for the polymorphism analyses.

**Results.**—Patients with severe prognosis had significantly higher levels of uMCP-1 compared to patients with non severe prognosis and healthy controls. Patients with renal damage had higher levels compared to patients without renal damage. There was a tendency of higher uMCP-1 levels in active disease compared to remission. AA in the -2518 position of the MCP-1 gene was associated with a more severe outcome compared to the A/G or G/G genotype. The A/A genotype was associated with higher levels of uMCP-1. No significant associations were seen for the CCR2-V64I.

**Discussion.**—This study confirms the association of high uMCP-1 levels with poor prognosis of AAV. It also suggests an association of the A/A genotype at position -2518 in the MCP-1 gene with poor prognosis.

**Conclusion.**—MCP-1 is clearly a candidate biomarker of potential clinical value. The A/A genotype association needs further evaluation.

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**P173**

**Abundant neutrophil extracellular traps in thrombus of patient with microscopic polyangiitis**

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**Introduction.**—Patients with MPO-AAV have an increased risk of deep vein thrombosis (DVT). However, the mechanism remains elusive. Recently, it has been reported that aberrant formation and disordered regulation of NETs could be implicated in the development of MPO-AAV. On the other hand, NETs interact with coagulant factors and can induce thrombosis. In this study, we carried out autopsy on an MPO-AAV patient complicated with DVT, and then the association of NETs and DVT was examined.

**Methods.**—NETs in the glomeruli and thrombus were examined by immunofluorescent staining. To quantify the NETs volume in the thrombus, immunohistochemistry was performed using anti-citrullinated histone 3 antibody. The area of NETs was quantified by Image J software and then standardized by the numbers of neutrophils counted in the serial sections. The amount of NETs in the thrombus was compared to other thrombi derived from a patient who died of bacterial sepsis and from one who died of post-operative pulmonary embolism.

**Results.**—NETs were detected in the glomerular crescents despite the absence of microbes in the MPO-AAV patient. Interestingly, abundant NETs were detected in the thrombus complicated with MPO-AAV, and the amount of NETs was significantly greater compared to other thrombi unrelated to MPO-AAV.

**Discussion.**—It is reported that MPO-ANCA can induce NETs, and that NETs can induce thrombosis. This study suggests the possibility that the pathogenesis of thrombosis in MPO-AAV could be critically associated with the mechanism via NETs.

**Conclusion.**—This study suggests the possibility that the pathogenesis of DVT in MPO-AAV could be critically associated with the mechanism via MPO-ANCA and NETs.

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**Characterization of regulatory B cells in ANCA-associated vasculitis (AAV)**

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**Introduction.**—Phenotypic characterization of the regulatory B cell (BREG) subset in humans has been proposed based on expression of either CD19+CD24hiCD38hi [1] or CD19+CD24hiCD27+[2]. Functionally BREGs exert their suppressive role via production of IL-10. Recently, numerical and/or functional disturbances in BREGs have been associated with autoimmunity. This study aimed to phenotypically and functionally characterize BREG cells in AAV patients.

**Patients.**—Sixty AAV patients (14 active, 46 remission) and 41 healthy controls (HC) were included in the study. B cell subsets were determined in peripheral blood by flow cytometry. BREGs were defined within the CD19+ population as CD24hiCD38hi or CD24hiCD27+. PBMC were stimulated in vitro with Cpg-ODN to induce IL-10 production, and the percentage of IL-10+ B cells was analysed by flow cytometry. To examine the suppressive capacity of B cells, sorted CD19+ B cells were co-cultured with monocytes, to evaluate their capacity to inhibit TNF-α production upon LPS activation.

**Results.**—The percentage of circulating CD19+CD24hiCD38hi cells was different in AAV patients in remission compared to HC, but was