Pathogenesis—Immune Predisposition and Infectious Etiology of Systemic Vasculitis

26-019

SHORTENING OF TELOMERES: EVIDENCE FOR REPLICATIVE SENESCENCE OF T-CELLS DERIVED FROM PATIENTS WITH WEGENER'S GRANULOMATOSIS

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Background: Replicative senescence describes the fact that somatic cells undergo a finite and predictable number of cell divisions before entering an irreversible state of growth arrest. Thus, replicative senescence is a reliable indicator of preceding and persistent activation of the cellular immune response because it is invariably associated with clonal expansion of B- or T-lymphocytes. Since repeated cell division results in a progressive shortening of telomeres, determination of telomere length by Southern blotting is a useful tool to analyze replicative senescence.

Results: Based on these considerations, we analyzed DNA derived from T-cells of patients suffering from Wegener's granulomatosis. Shortened telomeres, in addition to telomeres of normal length, were detected in patients with disease for five years or more (n=9), but not in patients with newly diagnosed disease. Because T-cells in culture undergoing replicative senescence become negative for CD28, the major T-cell co-stimulatory receptor, its expression on T-cells of patients with Wegener's granulomatosis was tested. Reduced expression of CD28 was noted, particularly in patients with disease for more than five years and shortened telomeres. In conclusion, our data provide evidence that a portion of T-cells had undergone replicative senescence, which in turn indicates clonal expansion of T-cells as consequence of activation.

27-047

PRESENCE OF AUTOANTIBODIES IN RELATIVES OF PATIENTS WITH PRIMARY SYSTEMIC VASCULITIDES (PSV)

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The presence of autoantibodies in relatives of patients with PSV has been occasionally reported.

Objective: To transversally evaluate the presence of different autoantibodies in relatives of a PSV cohort.

Methodology: Three groups were studied: Group I: 188 first-degree relatives (mother-25, father-18, sister-66, brother-45, son-9, daughter-25) of 77 PSV patients. Group II: 77 PSV patients (Wegener's granulomatosis-32, Behçet's disease-17, Takayasu arteritis-8, microscopic polyangiitis-7, polyarteritis nodosa-7, Henoch-Schönlein purpura-4, giant cell arteritis-1, cutaneous PAN-1). Group III: 65 healthy sex- and age- to case-matched controls. The presence of the following autoantibodies was evaluated: antinuclear and anticytoplasmic, rheumatoid factor, PR3-ANCA, MPO-ANCA, anti-dsDNA, anticardiolipin (IgG, IgM, IgA), anti-β2-GPI, anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase. Statistical analysis: χ^2 with Yates correction or two-tailed exact Fisher test. The probability of having PSV according to the presence of each autoantibody was calculated as odd ratio (OR).

Results: No differences regarding sex and age distribution was seen between groups. Differences in antibody prevalence between patients and their first-degree relatives were as follows (patients vs. their relatives, respectively): **RF:** 16/75 (21.3%) vs 12/188 (6.4%), OR 3.97 (CI 95% 1.78-8.9), $p < 0.002$. **PR3-ANCA:** 13/74 (17.6%) vs 4/187 (2.1%), OR 9.8 (CI 95% 3.06-31), $p < 0.0009$. **MPO-ANCA:** 5/75 (6.7%) vs 2/187 (1.1%), OR 6.6 (CI 95% 1.25-34.8), $p < 0.023$. **Anti-β2-GPI:** 6/74 (8.1%) vs 42/187 (22.5%), OR 0.3 (CI 95% 0.12-0.75), $p < 0.008$. For the rest of the antibodies tested there were no differences between PSV patients and their first-degree relatives. When compared to healthy subjects, anti-β2-GPI and anti-Tg were the only antibodies increased in relatives (22.5% vs 0%; $p < 0.009$ and 17% vs 6.3%; $p < 0.039$, respectively).

Conclusions: The prevalence of anti-β2-GPI autoantibodies was higher in relatives of patients with PSV than that seen in patients and healthy non-related individuals. The low OR suggests a lesser probability to develop PSV. In contrast, for RF, PR3-ANCA and MPO-ANCA a high OR may indicate an increased probability for the development of PSV in relatives in whom these autoantibodies are present. The significance of the presence of these autoantibodies in relatives or patients with PSV is unknown. Follow-up of these subjects is being performed.

28-064

POLYMORPHISMS OF CANDIDATE GENES IN ANCA POSITIVE VASCULITIS

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Genetic factors have often been suspected in ANCA-associated small vessel vasculitis, but only few have proven to be of importance. Deficiency of alpha-1-antitrypsin, the main inhibitor of proteinase 3 (PR3), and an increased frequency of the C3F allele have been found to correlate with PR3-ANCA positive vasculitis.

We have searched for associations between ANCA-associated small vessel vasculitis and polymorphisms in the genes for three candidate molecules: IL-1Ra, Fcγ-RIIa and CTLA-4. IL1RN*2, an allele of a polymorphism in intron 2 of IL-1Ra, has been detected in increased frequency in various inflammatory and renal diseases. In CTLA-4, a microsatellite in exon 3 has been associated to autoimmune disease, for instance Wegener's granulomatosis. The R/H131 polymorphism in Fcγ-RIIa has been connected to autoimmune and infectious disease. Patients at our departments with positive ANCA tests during the period March 1991 and December 1998 were iden-

tified, and blood samples were collected after informed consent. Patients were categorized according to ANCA serology using ELISA and to disease phenotype using the "Chapel Hill" nomenclature. Of the 109 patients, 51 had Wegener's granulomatosis (WG) and 58 had microscopic polyangiitis (MPA), 61 had PR3-ANCA and 46 had MPO-ANCA. Genotypes were determined using PCR technique. Fisher's exact test was used for statistic calculations.

	MPA	WG	MPO	PR3	Total	Contr.
IL1RN*2	0.198	0.304	0.206	0.287	0.248	0.255
Fcγ-RIIa H	0.474	0.480	0.435	0.525	0.477	0.482
CTLA-4 long	0.647	0.657	0.641	0.656	0.651†	0.558

† = p<0.05.

This study confirms and extends earlier observations concerning CTLA-4. An increase was found for the long alleles, which are considered to be linked to T-lymphocyte activation. The increase was moderate and reached statistical significance only in the whole study population, but frequencies were similar in both serology and phenotype subgroups. No significant increases were found for the IL1-Ra and Fcγ-RIIa polymorphisms.

29-072

IMMUNOFLUORESCENCE FINDINGS IN KIDNEYS OF SCG/KJ MICE: A MODEL OF PAUCI-IMMUNE CRESCENTIC NEPHRITIS?

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SCG/Kj mice are a recombinant inbred strain of mice spontaneously developing crescentic GN, systemic vasculitis and MPO-ANCA, and have therefore been suggested as an animal model for ANCA associated pauci-immune crescentic GN. We evaluated the development of renal lesions in 24 SCG/Kj mice at 8, 10, 12, 14, 16, 24 and 40 weeks by light and immunofluorescence (IgG, IgM, IgA, C3) microscopy. MRL/lpr mice served as controls. In all animals the typical picture of a diffuse immune complex GN was found, demonstrating initially significant mesangial deposition of IgG, IgM and C3 as early as 8 weeks. Intensity increased with age and became strongly positive for all three Ig and C3 in the mesangium and along peripheral capillary loops. Interestingly, IgA was more dominant in the SCG/Kj strain compared to observations in MRL/lpr mice. Crescent formation also began early at week 10 and was affecting about 90% of the specimens from week 12 to 40 correlating with the amount of proteinuria. Significant Ig deposition was already present early in the course and only a weak correlation was found between glomerular Ig deposition and proteinuria. In conclusion, the SCG/Kj strain of mice provides an animal model for spontaneous ANCA positive crescentic GN. The massive presence of mesangial or glomerular immunoglobulin deposits, however, differs from the usually pauci-immune pattern in man. Thus, SCG/Kj mice seem not to be a representative model for human ANCA positive crescentic nephritis.

30-079

ANTI-MYELOPEROXIDASE ASSOCIATED PAUCI-IMMUNE FOCAL SEGMENTAL GLOMERULONEPHRITIS IN RATS

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The presence of MPO-ANCA in humans is associated with a pauci-immune focal segmental glomerulonephritis. We have developed a novel model of this disease in rats.

Wistar Kyoto rats were immunized with purified human MPO (50 micrograms im). Over 2 to 4 weeks all rats developed anti-myeloperoxidase antibodies as confirmed by ANCA IIF (on rat and human neutrophils) and ELISA (titers 60 - 100%). Hematuria (dipstick 1+ to 4+) was detected in 95% by week 5, accompanied by mild proteinuria, mean 6.22 mg/day (0.08-34.3) vs controls 0.17 mg/day (0.001-0.67). Kidney sections taken from rats killed at 8 weeks showed glomeruli with segmental inflammation (83% of rats) and occasional fibrinoid deposits (20%), tubular red cell casts (100%) and tubulo-interstitial inflammation (100%). Lung sections showed evidence of fresh hemorrhage and hemosiderin deposition (83%). Immunofluorescence microscopy revealed no deposits of IgG and only scanty tubular deposits of C3.

To investigate the effect of a local renal immune stimulus one group of rats was immunized with MPO as previously described, followed by a sub-nephritogenic dose of rabbit anti-rat glomerular basement membrane antibody at 5 weeks. After 1 week these rats developed macroscopic hematuria and marked proteinuria, mean 40.0 mg/day (0.81-106.1) vs control rats given anti-rat GBM alone, mean 3.1 mg/day (0.45-6.28). Kidney histology revealed segmental inflammation in 100%, fibrinoid necrosis in 80%, and crescents in 80% of rats.

This novel rat model of ANCA associated focal segmental glomerulonephritis should facilitate the further study of human small vessel vasculitides.

31-112

SEROLOGIC AND MOLECULAR PARVOVIRUS B19 (B19) ANALYSES IN ANCA-ASSOCIATED VASCULITIDES: A CASE-CONTROL STUDY

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Objectives: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS) are ANCA-associated vasculitides of unknown etiology. Because B19 has been associated with various vasculitides and with ANCA, we examined its potential role in ANCA-associated vasculitides.

Methods: We tested the sera from 13 selected patients with

newly diagnosed ANCA-positive WG, MPA or CSS. Every case was matched to 3 healthy controls according to age (±3 yr) and gender. All sera were tested for specific IgG and IgM antibodies (Ab) to B19 (3rd-generation ELISA, Biotrin, France) and DNA was analyzed by polymerase chain reaction (PCR). Cases and controls were compared with respect to the presence of B19-specific AB (IgG and/or IgM) and DNA detection by PCR.

Results: The 13 patients (mean age: 50.1 ± 11.1 yr, M/F sex ratio: 1.6) comprised 6 WG, 6 MPA and 1 CSS. ANCA were distributed as follow: cytoplasmic labeling and/or PR3-ANCA, n = 7, and perinuclear labeling and/or MPO-ANCA, n = 6. IgG Ab to B19 were equally detected in the sera of cases (77%) and controls (79%) (OR = 0.84, p = 0.84). All 13 cases and 39 controls were negative for IgM Ab and B19 DNA.

Conclusion: These results suggest that neither acute nor persistent B19 infection is an etiological factor of ANCA-associated vasculitides. However, a potential pathogenic role of B19 in individual cases cannot be excluded.

32-121

FREQUENCY OF FUNCTIONAL IL-10 AND TGF-β GENE-POLYMORPHISMS IN ANCA-ASSOCIATED VASCULITIS (AAV)

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Rationale: Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are anti-neutrophil cytoplasmic (ANCA) associated primary systemic vasculitides (AAV) of unknown origin. Since the immunopathogenesis of MPA suggests a strong Th2 response giving rise to the hypothesis that genotypes suppressing Th1 responses or augmenting Th2 responses are more frequent in MPA than in WG, TGF-β_1 and IL-10 genes are suspected to modify the course of AAV. The purpose of this study was to identify any association between genotype frequencies of functional polymorphisms (PMs) of the genes of these two cytokines and AAV.

Methods: 161 patients with AAV and 153 healthy blood donors were genotyped for a biallelic PM in codon 25 of the TGF-β_1 gene using PCR, and for the biallelic PM at position -1082 of the IL-10 gene using the amplification refractory mutation system - PCR methodology.

Results: For TGF-β_1 codon 25 PM no significant difference was found between control and any of the patient groups. For IL-10 (-1082) PM we found a significant shift towards the homozygous AA genotype in WG and in MPA. This significance was significantly more impressive in MPA. Moreover, we found a gender-associated significant difference in MPA for IL-10 (-1082) PM. In this group the AA homozygous genotype was more frequent in females compared to males.

Conclusion: On the basis of the analyzed cohorts a significant contribution of the named TGF-β_1 codon 25 PM to the susceptibility defining genetic backgrounds of AAV appears unlikely. However, the significant differences suggest a role of the enhanced IL-10 (-1082) PM in WG and MPA with a newly described gender difference in MPA.

Pathogenesis—Patterns of Injury: Implications for Pathogenesis

33-038

T CELL RECEPTOR V-BETA REPERTOIRES IN SYSTEMIC VASCULITIDES OF CHILDHOOD

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Introduction: Despite conflicting evidence and much debate, superantigenic stimulation of the immune system in Kawasaki disease (KD) remains an attractive hypothesis since there is considerable overlap between the clinical and immunological phenotypes of KD and classical superantigen-mediated diseases such as the toxic shock syndrome. Moreover, although there are limited data in adults suggesting that SAgs may be involved in the initiation of other primary systemic vasculitides, no such data exist for children.

Methods: To investigate the possible etiological role of SAgs, this study examined peripheral blood TCR Vβ repertoires in children with KD (n=6), polyarteritis nodosa (PAN, n=23), Wegener's granulomatosis (WG, n=1), and microscopic polyangiitis (MPA, n=1). 20 normal children and 30 children with non-vasculitic inflammatory disease or recipients of renal allografts served as controls and disease controls, respectively. 3 color FACS analysis of peripheral blood mononuclear cells stained with conjugated monoclonal antibodies to CD3, CD4, CD8, and 17 different Vβ families was performed.

Results: The mean % of CD4+ T cells bearing Vβ2 was significantly increased in the KD group versus controls and disease controls (p=0.03 and p=0.01, respectively). Individual KD patients were also noted to have CD4+ T cell Vβ expansions other than Vβ2 (Vβ5.1 n=2; Vβ12 n=1). 60% of the primary systemic vasculitis patients had one or more TCR Vβ expansions in the CD4+ lymphocyte population, compared with 30% of the controls (p=0.02-0.05), and 36% of the disease controls (p=0.05-0.1). Unlike KD, however, the pattern of Vβ families expanded in individual patients was more diverse, perhaps indicative of the involvement of several different SAgs. Follow-up studies of 7 primary systemic vasculitis patients demonstrated a normalization of the CD4+ T cell Vβ repertoire following induction of remission of vasculitis.

Conclusion: Our preliminary data provide indirect evidence for an etio-pathogenetic role for SAgs in KD and primary systemic vasculitides affecting children.

34-053

PERIPHERAL BLOOD- AND GRANULOMA CD4+CD28– T-CELLS DISPLAY CYTOKINE PRODUCTION RESTRICTED TO IFN-γ AND TNF-α IN WEGENER'S GRANULOMATOSIS

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Objective: Expansion of T-cells lacking CD28 expression has been reported in Wegener's granulomatosis (WG). We addressed the question, whether the fraction of peripheral blood and granuloma CD28– T-cells within the CD4+ T-cell population is a source of Th1 like cytokine production in WG.

Methods: 12 patients with active, generalized, biopsy-proven WG were analyzed. We assessed surface antigens and intracytoplasmic cytokine expression of peripheral blood fractions of CD28– and CD28+ T-cells within the CD4+ T-cell population by flow-cytometry (FACS). Cytokine secretion was additionally confirmed by an enzyme-linked immunosorbent assay (ELISA). Immunohistologic studies were performed on biopsies from the respiratory tract.

Results: The fraction of CD28– T-cells within the CD4+ T-cell population was significantly expanded compared with healthy controls (mean 14.4 vs. 2.1%, P<0.01). CD57 (differentiation marker) and CD18 (β_2 integrin) were upregulated on CD4+CD28– T-cells and generally missing on CD4+CD28+ T-cells. CD25 (IL-2Rα) was missing on the CD4+CD28– subset but found on their CD4+CD28+ counterparts. CD4+CD28– T-cells displayed a cytokine expression restricted to IFN-γ- and TNF-α, whereas CD4+CD28+ T-cells displayed a broader cytokine expression including IL-2. Immunohistologic analysis using serial sections demonstrated that the majority of CD4+ T-cells lacked CD28 and expressed IFN-γ- and TNF-α within granulomatous lesions.

Conclusion: CD4+CD28– T-cells appeared highly differentiated, displayed a Th1-like cytokine production restricted to IFN-γ- and TNF-α. β2 integrins, i.e. CD18, may promote recruitment of CD4+CD28– T-cells into granulomatous lesions, where they support granuloma formation by their restricted cytokine production. Moreover, IFN-γ- and TNF-α producing T-cells within the expanded peripheral blood and within the granuloma CD4+CD28– population may represent an important target of anti-TNF-α directed therapies.

35-055

A HUMAN IN VITRO GRANULOMA MODEL FOR WEGENER'S GRANULOMATOSIS (WG)

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Objective: Chronic granuloma formation occurring in one or several organs and mostly accompanied by systemic vasculitis are hallmarks of Wegener's granulomatosis. Although animal models mimicking the vasculitic pathogenesis exist, currently no model is available to investigate granuloma formation in WG. The aim of our study was to develop an in vitro granuloma model that is suitable for examining mechanisms of granuloma formation in WG.

Methods: PBMC and PMN were isolated from healthy controls and WG patients (n=5). 6 x 10^5 cells PBMC + PMN in a ratio of 10/1 were added onto a layer of human umbilical vein endothelial cells (HUVEC) in a transwell over agarose-coated wells (12 wells/assay), stimulated (superantigen + PR3-ANCA-IgG) and incubated at 37°C for four days. Viability of the granuloma-like spheroids was characterized using fluoresceindiacetate and propidium iodide staining. Phenotype (CD3, CD20, CD26, CD28, WGM2, CD164) and functional features (Osteopontin, TNFα) were determined using immunohistochemistry and ELISA.

Results: Our human in vitro granuloma model for WG proved to be stable and reproducible. Viability of the granuloma-like spheroids was higher than 95%, except for the PMN. The mean number for granuloma-like three-dimensional spheroids was 12/12 for the WG cases, but only 5/12 for healthy controls (n=4; p<0.05). At the two-dimensional level only 1/5 healthy controls exhibited granulomatous structures, whereas 5/5 WG cases displayed such structures. Further, the more active the disease, the more differentiated monocytes (CD164+) and CD26+ T cells were found. On the other hand, in vitro granulomas from inactive disease displayed mainly CD3+ and CD28+ cells, but only few if any CD164+ or CD26+ cells.

Conclusion: Employing a combination of transendothelial migration and spheroid in vitro assay, WG-specific three-dimensional granuloma-like spheroids were formed, which can be used as a tool to better analyze the nature and/or cause of granulomas in WG.

36-068

CIRCULATING ANTI-INFLAMMATORY CYTOKINES (IL-10, IL-13 AND TGF-β IN GIANT CELL ARTERITIS (GCA)

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Background: GCA is characterized by an intense systemic inflammatory response although remarkable differences may be observed among patients. We have previously reported a correlation between the intensity of the acute phase response and circulating levels of TNF and IL-6 in GCA patients (Hernández-Rodríguez et al, *Arthritis Rheum* (AC&R) 2002, in press). IL-10, IL-13 and TGFβ have been considered to have anti-inflammatory properties because they inhibit the synthesis of pro-inflammatory cytokines by T cells and macrophages. The putative role of these cytokines in regulating the intensity of the inflammatory response in GCA has not been investigated.

Objective: To determine plasma concentrations of IL-10, IL-13 and TGFβ and their relationship with the intensity of the systemic inflammatory response in patients with GCA.

Patients and Methods: Circulating levels of TGFβ, IL-10 and IL-13 were determined in 56 untreated patients with biopsy proven GCA and in 15 healthy controls. Four parameters were used to evaluate the intensity of the systemic inflammatory response (fever, weight loss, ESR ≥ 85 mm, and Hb < 11 gm/dL). Patients were considered to have a weak inflammatory response when had 2 or less inflammatory parameters (group 1) and a strong inflammatory response when 3 or 4 parameters were present (group 2).

Results: Twenty-three patients had a weak (group 1) and 23 a strong (group 2) initial systemic inflammatory response. No differences in IL-10 levels among GCA patients and controls were observed, but IL-10 concentrations were high-

er in group 2 (4.2 ± 3.1 pg/mL) compared with group 1 (1.4 ± 2.5 pg/mL), p=0.002. Circulating TGFβ levels were significantly higher in GCA patients (962 ± 589 pg/mL) than in controls (744 ± 791 pg/mL) (p=0.04). Although TGFβ levels in group 1 were lower (866 ± 574 pg/mL) than in group 2 (1100 ± 596 pg/mL), differences were not significant (p=0.1). Circulating IL-13 levels were undetectable in most patients and controls.

Conclusions: GCA patients with a weak systemic inflammatory response do not have higher concentrations of anti-inflammatory cytokines TGFβ, IL-10 and IL-13 than patients with a strong acute phase reaction. The limited increase in TGFβ and IL-10 levels observed in GCA patients suggests that these cytokines do not significantly down-regulate the intensity of the systemic inflammatory response in this disease.

FIS 98/0443, FIS 00/0689, Fundació Pedro Pons

37-069

TISSUE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES (IL-1β, IL-6 AND TNFα) IN GIANT CELL ARTERITIS (GCA) PATIENTS: CORRELATION WITH THE INTENSITY OF THE SYSTEMIC INFLAMMATORY RESPONSE

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Background: The systemic inflammatory response is mediated by pro-inflammatory cytokines, mainly IL-1β, IL-6 and TNFα which are synthesized mostly by activated macrophages. TNFα, IL-1β and IL-6 mRNAs have been detected in temporal arteries from patients with GCA, a disease characterized by a remarkable acute phase reaction.

Objective: To assess the relationship between tissue expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) in temporal artery biopsies from GCA patients and the intensity of the systemic inflammatory response.

Patients and Methods: Temporal artery sections from 50 GCA patients with a similar degree of histologic involvement were immunostained with antibodies against IL-1β, IL-6 and TNFα. Ten normal temporal arteries from patients in whom a surrogate diagnosis was obtained were also studied. Four inflammatory parameters were considered to evaluate the intensity of the systemic inflammatory response (fever, weight loss, ESR ≥ 85 mm, and Hb < 11 gm/dL). Immunostaining was blindly quantitated using a predefined score considering the percentage of cell staining at the intima/media junction (1: <25%, 2: 26-50%, 3: 51-75% and 4: 76-100%).

Results: Tissue expression of IL-1β, IL-6 and TNFα was intense and occurred mainly within the granulomatous reaction at the intima/media junction. Cytokine expression was variable among patients even displaying a comparable degree of inflammatory changes. No cytokine expression was observed in control samples. Patients with a strong systemic inflammatory response (4 parameters) exhibited significantly higher scores for IL-6 (5 vs 8 patients, p=0.034) and for TNFα (7 vs 12 patients, p=0.025) than patients with a weak systemic inflammatory reaction (0 parameters). IL-1β expression also tended to be stronger in patients with a strong inflammatory response but the difference was not statistically significant.

Conclusions: Tissue expression of pro-inflammatory cytokines IL-1β, IL-6 and TNFα is prominent in full-blown giant-cell arteritis lesions. IL-6 and TNFα expression correlates with the intensity of the systemic inflammatory response.

FIS 98/0443, FIS 00/0689, DAKO, Fundació Pedro Pons, Hospital Clinic Research Award

38-129

DOES ANCA FORMATION RESULT FROM AN ANTIGEN-TRIGGERED IMMUNE RESPONSE IN WEGENER'S ENDONASAL INFLAMED TISSUE?

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Purpose: PR3-specific antibodies (PR3-ANCA) occupy a pathogenetic role in Wegener's granulomatosis (WG). The detection of germinal center-like B lymphoid infiltrates in association to endonasal WG granuloma raised the question, whether an antigen-driven immune response which finally leads to the ANCA associated generalized vasculitis is initiated in the upper respiratory tract. B lymphocytes from nasal tissue of patients with localized and generalized WG were analyzed for distribution and mutational pattern of antibody-encoding genes in order to draw nearer the suspected antigens.

Materials and methods: Cryosections from immediately snap-frozen endonasal biopsies were screened for B lymphocytes by CD20-staining (APAAP). Tissue was protein-digested, DNA was extracted and subjected to a polymerase chain reaction (PCR) of 35 cycles using six VH- and a mix of JH-family-specific oligonucleotides as primers. PCR products were cloned and sequenced. Nucleotide and amino acid sequences were analyzed for mutations and compared to all accessible sequences from gene databases.

Results: Rearranged immunoglobulin genes were detected for all VH families (VH1-6) indicating a polyclonal repertoire. By sequence analysis of a hundred bacterial colonies derived from the cloned PCR products, 66 individual rearrangements of V-D-J segments could be determined. The sequence analysis revealed a high frequency of mutations with amino acid substitutions and a biased repertoire of represented genes indicating selection by an antigen. Three particular VH genes were overrepresented that had been found in PR3-ANCA producing cells. Furthermore, amino acid replacement often led to negatively charged residues characteristic for the binding-site to PR3.

Conclusions: Besides the immunopathogenetic role of neutrophils, T-lymphocytes and monocytes in WG these findings indicate an involvement of B-lymphocytes in the autoimmune mechanism comparable to other rheumatic diseases like RA and SLE. In WG this probably happens through the generation of high-affinity ANCAs by contact to PR3 or a cross-reacting microbial epitope in the inflamed endonasal tissue.

Pathogenesis—Possible Role of ANCA and AECA in Selected Forms of Systemic Vasculitis

39-017

ABNORMAL GALACTOSYLATION OF POLYCLONAL IgG IN ANCA-ASSOCIATED SYSTEMIC VASCULITIS PATIENTS

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IgG-anti-neutrophil cytoplasmic antibodies (ANCA) are implicated in the pathogenesis of small vessel vasculitides, such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). We have analyzed the oligosaccharide profiles of the intact polyclonal IgG, isolated from the serum of 20 ANCA positive patients at the time of acute presentation. For patient samples 40-75% of released oligosaccharides were devoid of galactose (G0-IgG), compared to values of 23-30% for age and sex matched controls. In the absence of galactose the terminal sugar residues are N-acetylglucosamine. Increased levels of G0 IgG have been reported for several inflammatory diseases (rheumatoid arthritis, juvenile arthritis, etc) and have been associated with disease progression/outcome. Possible contributing mechanisms are suggested by the demonstration that G0-IgG can activate the complement cascade through activation of mannan binding lectin and can enhance antigen presentation by uptake through the mannose receptor on dendritic cells.

Thus hypogalactosylation could impact on the inflammatory response and immune regulation of autoantibody production.

40-018

CONTRIBUTION OF ABNORMAL DIFFERENTIATION AND FUNCTION OF NEUTROPHILS TO MPO-ANCA PRODUCTION: ANALYSIS OF ICSBP-KO MICE

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Half populations of splenocytes were morphologically observed as neutrophils, because abnormalities in the maturation of myeloid cells have been shown in interferon consensus sequence-binding protein (ICSBP)-/- mice. We measured dysfunction of granulocytes in spleens of these mice. Higher production of an auto-antibody MPO-ANCA (MPO specific anti-neutrophil cytoplasmic antibody) was accompanied by aging of the mice, whereas anti-double-stranded DNA antibody titer was not detected. Release of myeloperoxidase (MPO) from the purified neutrophils from splenocytes was 64% of that from neutrophils from peripheral blood, and O2- production was 53%. In addition, cells adhering to a slide glass were round by microscopic examination, and white granules were seen in the cells by transmission electron microscopy. Interestingly, ICSBP-/- mice showed defective eosinophils in their peripheral blood due to suppression of mRNA of eosinophil peroxidase in bone marrow. These results suggest that dysfunction of neutrophils in spleens of ICSBP-/- mice relates to an increase of MPO-ANCA titer with aging, and that severe suppression of eosinophil peroxidase mRNA expression in the bone marrow of ICSBP/IRF-8 permits circulation of abnormal eosinophils.

41-025

NEUTROPHIL ACTIVATION AND LEVELS OF PROTEINASE 3 IN PATIENTS WITH ANCA-ASSOCIATED SYSTEMIC VASCULITIS

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Background: In the ANCA-associated systemic vasculitides, patients form autoantibodies against neutrophil granular proteins. Some correlation is seen between ANCA titer and disease activity, but whether this is cause or consequence is still unknown. Our theory is that dysfunctional leukocytes, e.g., constantly somewhat activated neutrophils with increased production and/or leakage of granular proteins, lead to an increased amount of circulating antigen and hence predisposition to autoimmunity. In order to address this, proteinase 3 (PR3), one of the main ANCA antigens, stored in azurophil granules, and NGAL (neutrophil gelatinase-associated lipocalin), a specific marker of neutrophil degranulation, localized in secretory granules, were measured. CRP and Cystatin C were measured as markers of inflammation and renal function, respectively.

Methods: Both PR3 and NGAL were measured in plasma by means of ELISA technique. The NGAL ELISA was a sandwich method using affinity purified rabbit-anti-NGAL. In the PR3 ELISA we used anti-PR3 monoclonals as capture-antibodies and affinity purified rabbit-anti-PR3 for detection. PR3-ANCA, MPO-ANCA and capture-PR3-ANCA were measured by Wieslab AB.

Results: PR3 was significantly raised ($p<0.0001$) in ANCA patients (690±470, n=59) compared to healthy blood donors (350±110, n=30) as well as disease controls (422±200, n=46). The patients had a tendency to divide into two groups, one with normal PR3 levels (410±130, n=32) and one with raised levels (1050±660, n=27). No correlation was seen with disease activity, inflammation or renal function. Nor did we see any correlation between capture-PR3- or MPO-ANCA and PR3. Negative correlation was, however, seen with PR3-ANCA ($r=-0.4$, $p=0.01$). The raised NGAL levels correlated

strongly with decreased renal function (r=0.8, p<0.001). After correction for this, slightly increased levels (120±55, n=59) were seen compared to the healthy blood donors (92±56, n=26), but not compared to the disease controls (128±38, n=48). In the disease controls there was a significant correlation between NGAL and PR3 (r=0.3, p<0.05), but this was not the case in the ANCA patients. Whether patients had PR3- or MPO-ANCA was of no significance.

Conclusions: In our measurements, we found significantly raised levels of proteinase 3 in plasma from patients with ANCA-associated vasculitis, regardless of ANCA specificity. This was due to neither decreased renal function, nor ongoing inflammation, nor neutrophil activation. Plausible mechanisms, in demand of further research, include defects in the reticuloendothelial system, genetic factors and selective neutrophil degranulation or leakage.

42-026

PHOSPHATIDYLINOSITOL-3-KINASE CONTROLS ANCA-INDUCED RESPIRATORY BURST IN HUMAN NEUTROPHILS

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ANCA activates human PMN primed with TNF-α in vitro. PI-3 Kinase (PI3-K) and the protein Akt have been implicated in the control of the phagocyte respiratory burst. We tested the hypothesis that PI-3 Kinase controls the ANCA-induced respiratory burst in human PMN. TNF-α-primed PMN were stimulated with a monoclonal antibody (mab) to MPO, and with human PR3- and MPO-ANCA, respectively. Activation of Akt was assessed by Western blotting with phospho-specific antibodies. Superoxide release was measured by the ferricytochrome assay, and translocation of ANCA antigens by FACS. The effect of TNF-α and MPO-ANCA on the composition of the Akt signaling complex was studied using immunoprecipitation and GST pull-down assays. Western blotting revealed a rapid, but transient, Akt phosphorylation during TNF-α priming and a second phosphorylation after addition of ANCA. Inhibition of PI3-K by LY294002 blocked both Akt phosphorylation and superoxide generation. 20.2±3.4 nmol O_2^-/0.75x10^6 PMN/45 min were released after stimulation with PR3 ANCA, and 5 μM LY294002 decreased this amount to 0.3±2.6 nmol (n=10, p<0.05); these values were 23.3±2.9 versus 1.6±3.6 for MPO-ANCA (n=10, p<0.05). Interestingly, p38 MAPK inhibition with 10 μM SB202190 that also decreases ANCA-induced superoxide generation, prevented S473 phosphorylation of Akt in response to TNF-α and to ANCA. However, SB202190, but not LY294002 abrogated TNF-α-mediated surface translocation of ANCA antigens demonstrating that superoxide generation and ANCA antigen translocation proceed by disparate mechanisms. Characterization of the Akt signaling module showed that Akt, PAK1 and Rac1 exist in complex in resting PMN cytosol. TNF-α stimulation caused increased association of PAK1 with Akt. Consecutive stimulation with a mab to MPO did not cause additional change in the Akt signaling complex. Our data demonstrate the importance of PI3-K for the ANCA-induced oxidant production by human PMN. Pharmacological inhibition of this kinase may control ANCA-induced inflammation.

43-043

ANTI-PR3-ANTIBODIES INDUCE THE RELEASE OF PROCOAGULATORY FACTORS FROM ISOLATED MONOCYTES—ROLE OF NF KAPPA B ACTIVATION

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Capillary thrombosis is one of the early pathologic features of ANCA-associated vasculitis. As ANCAs are capable of activating neutrophils and monocytes in vitro, we investigated the effect of these autoantibodies on the release of activators and inhibitors of the fibrinolytic system from isolated human monocytes. Human monocytes were purified by countercurrent centrifugal elutriation, and were stained for PR3 surface expression after the isolation procedure. Incubation of monocytes with murine monoclonal anti-PR3-antibodies, but not with mouse control IgG, resulted in a time- and dose-dependent release of plasminogen-activator-inhibitor type 2 (PAI-2) into the cell supernatant. In contrast, release of tissue-plasminogen-activator (TPA) was diminished in the presence of anti-PR3 antibodies. These responses were equally observed upon monocyte incubation with c-ANCA-IgG, but not with normal human IgG. In the presence of caffeic acid phenylethyl ester (CAPE), an inhibitor of the activation of the transcription factor NF-kappaB, the anti-PR3-induced release of PAI-2 was dramatically reduced. When analyzed by EMSA gel shift assay, nuclear translocation of NF-kappaB was observed in monocytes stimulated with anti-PR3, but not with control antibodies. We conclude that ANCA activate the secretion of the main inhibitor of fibrinolysis, PAI-2, and inhibit the release of the anticoagulatory factor TPA from human monocytes. Activation of NF-kappaB dependent signaling pathways seems to be centrally involved in these processes. The ANCA-induced alterations of coagulatory activity may contribute to the development of capillary thrombosis in ANCA-associated small vessel vasculitis.

44-046

THE PROTEIN C PATHWAY AND VASCULAR INFLAMMATION

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The endothelial protein C receptor (EPCR) is a member of the protein C pathway that accelerates protein C activation on the surface of endothelial cells. This provides an on-demand source of activated protein C, shown by multiple clinical studies to be required for coagulation control and the host response to inflammation. A soluble form of EPCR (sEPCR) is released from the endothelium as a result of thrombin-induced metal-

loproteinase activity. sEPCR normally circulates in plasma at about 100 ng/ml and at much higher levels in patients with sepsis, systemic lupus erythematosus or Wegener's granulomatosis. Recent biochemical studies demonstrated that sEPCR binds to the surface of PMA-activated neutrophils (*J Immunol* 2001, 165:4697-4703). sEPCR binding to neutrophils is supported by proteinase-3 (PR3), the Wegener's autoantigen, and by a beta-2 integrin, probably CD11b/CD18. Less sEPCR binds if neutrophils are pre-incubated with PR3-ANCA from some, but not all, of a small cohort of Wegener's patients. However, the nature of neutrophil PR3 expression and support of sEPCR binding to neutrophils is not well understood.

Results: PR3 purified from neutrophils was labeled in the active site with a chloromethylketone derivative of fluorescein (FITCcmk-PR3). Efficient labeling was judged by the >99% loss of activity. Neutrophils activated with phorbol myristate acetate bound FITCcmk-PR3, suggesting that PR3 released as a consequence of neutrophil granule mobilization can return to the neutrophil and bind to the membrane. Flow cytometry data demonstrate that TNF/fMLP-treated neutrophils also bind PR3-ANCA and an anti-PR3 monoclonal antibody, consistent with the presence of surface PR3 antigen. The importance of the membrane surface in PR3 expression was supported by studies using synthetic phospholipid vesicles. The amidolytic activity of neutrophil PR3 was determined in the presence of sEPCR and liposomes of varying phospholipids and molar ratio compositions. The nature of the anionic phospholipid head group (sigmoidal rates with phosphatidylcholine:phosphatidylserine vesicles) and bilayer organization (effect of phosphatidylethanolamine) was an important influence on PR3 substrate recognition (Km') and sEPCR effects.

Conclusions: The results suggest that the neutrophil membrane environment is an important contribution to PR3 accessibility, either through endogenous expression or by local rebinding events. This is dependent on neutrophil activation and does not require the PR3 active site. Furthermore, the neutrophil lipid microenvironment effects PR3 activity and sEPCR interactions, thus potentially modulating subsequent PR3-ANCA binding and inflammatory events.

45-074

REDUCTION OF MONOCYTE TRANSENDOTHELIAL MIGRATION BY C-ANCA

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Background: While the interaction of anti-PR3-antibodies with neutrophils has been extensively studied in vitro, their interaction with monocytes is less characterized. In the present study, we investigated the influence of anti-PR3-antibodies on monocyte adhesion and transendothelial migration.

Methods: Monocytes were isolated by counterflow centrifugal elutriation. For transmigration studies monocytes were allowed to migrate across endothelial cells, grown to confluence on transwell inserts, in response to the chemoattractant MCP-1 (monocyte chemoattractant protein-1). Monocyte adherence was analyzed by coincubating fluorescence labeled monocytes with endothelial cells in a microplate assay.

Results: Incubation of monocytes with monoclonal anti-PR3-antibodies in the transwell chamber assay caused a significant reduction of transendothelial monocyte migration. This effect could be reproduced by IgG fractions from patients with active Wegener's granulomatosis, whereas an isotype matched control IgG or IgG fractions of healthy controls were ineffective. On the other hand, monocytes adherence to unstimulated or TNF-α stimulated endothelial cells was not altered in the presence of anti-PR3-antibodies. As it has been reported that c-ANCA inhibit the proteolytic activity of PR3, we then investigated whether proteolytic active PR3 is required for successful monocyte transmigration. Serine protease inhibitors with activity against PR3, like alpha-1-antitrypsin or α-ketooxadiazole-inhibitor, caused a distinct reduction in monocyte transmigration similar to the reduction caused by anti-PR3-antibodies. On the contrary, secretory leukocyte protease inhibitor (SLPI) with activity against cathepsin G and elastase, but not against PR3, did not modify monocyte transendothelial migration.

Conclusion: We conclude that anti-PR3-antibodies reduce monocyte transendothelial migration by interaction with the proteolytic activity of PR3. The retention of monocytes in the lumen of microvessels could contribute not only to the development of vascular lesions, but also to granuloma formation in Wegener's granulomatosis.

46-075

INTERFERENCE OF PR3-ANCA WITH THE ENZYMATIC ACTIVITY OF PR3

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Introduction: Anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3) are strongly associated with Wegener's granulomatosis (WG) and are thought to be involved in its pathogenesis. In vitro functional effects of these antibodies have been suggested to correspond better to disease activity than levels of PR3-ANCA.

Methods: To further investigate the relation between functional effects of PR3-ANCA and disease activity, we tested IgG samples from sera of 43 WG patients and four controls for their capacity to interfere with the proteolytic activity of PR3. Blood was drawn either during active disease or during remission of WG. Moreover, sera of seven patients were analyzed before, during and after relapse. The enzymatic activity of PR3 was determined using a small synthetic substrate (MeSuc-AAPV-pNA), casein, and by complexation of PR3 with its natural inhibitor alpha-1-antitrypsin (alpha-1-AT).

Results: Most of the IgG samples from WG patients inhibited the enzymatic activity of PR3 and the complexation of PR3 with alpha-1-AT. A difference in the capacity to interfere with the proteolysis of casein and with the complexation of PR3 with alpha-1-AT was observed between samples taken during active disease and during remission of WG, but this was not observed for the hydrolysis of MeSuc-AAPV-pNA. How-

ever, PR3-ANCA titers giving fifty percent inhibition of the PR3/alpha-1-AT complexation and the proteolytic activity of PR3 for the hydrolysis of MeSuc-AAPV-pNA were lower for remission samples compared to samples during active disease, indicating a relatively higher inhibitory activity in the former samples. PR3-ANCA titers correlated with the inhibitory activity both for patients with active disease and for patients during remission.

Conclusion: With a fixed amount of IgG, PR3-ANCA-containing IgG from patients with active disease had a higher inhibitory capacity towards the proteolytic activity of PR3 than did PR3-ANCA-containing IgG from patients during remission of WG. However, when correcting the results for the PR3-ANCA titer, PR3-ANCA of patients during remission had a relatively higher inhibitory capacity towards the proteolytic activity of PR3 than did PR3-ANCA of patients during an active phase. These results may indicate that PR3-ANCA of patients with active disease recognize different epitopes on PR3 than do PR3-ANCA of patients during remission of WG. These findings may have relevance for the pathogenicity of the antibodies.

47-077

IgG-MEDIATED ACTIVATION OF LEUKOCYTES IS INDEPENDENT OF Fc-GAMMA RECEPTOR POLYMORPHISM

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Introduction: Ligation of Fc-gamma receptors for IgG (FcgR) can trigger potent effector cell responses. Genetic polymorphisms of these receptors have been shown to modify IgG binding and influence internalization of immune complexes. Indeed, in patients with infectious or autoimmune diseases, skewing towards low-binding FcgR alleles has been demonstrated. The objective of this study was to investigate the influence of FcgR polymorphism on leukocyte activation.

Methods: We analyzed activation of neutrophils and monocytes stimulated by aggregated or solid phase-coated IgG1, IgG2, and total IgG. Neutrophil donors were selected based on their FcgR genotype and homozygous for either FcgRIIa-H131/FcgRIIIb-NA1 (HH-NA1/1) or FcgRIIa-R131/FcgRIIIb-NA2 (RR-NA2/2). Monocyte donors were homozygous for either FcgRIIa-H131/FcgRIIIa-V158 (HH-VV) or FcgRIIa-R131/FcgRIIIa-F158 (RR-FF). Binding of immunoglobulins to lymphocytes was determined by flow cytometry. Activation of neutrophils was measured as the production of reactive oxygen intermediates (ferricytochrome c reduction), degranulation (lactoferrin release), and cytokine production (IL-8). TNF-alpha secretion was used as a measure of monocyte activation.

Results: As determined by flow cytometry, IgG1 aggregates firmly bound to neutrophils of both types of donors, albeit more avidly to donors expressing HH-NA1/1 alleles. In contrast, IgG2 aggregates firmly bound to HH-NA1/1 FcgR neutrophils only. This binding could be blocked by pre-incubation of neutrophils with FcgRIIa and FcgRIIIb blocking antibodies. Despite the differences in binding of IgG subclasses to HH-NA1/1 and RR-NA2/2 neutrophils, we observed no differences in their activation as measured by oxygen radicals production, lactoferrin release and IL-8 production. Activation of both types of neutrophils with IgG1 or IgG2 aggregates could be at least partially blocked by the addition of FcgR blocking antibodies. Similar to neutrophils, HH-VV and RR-FF monocytes were not distinguishable in their response to IgG, IgG1, and IgG2 as measured by TNF-alpha release, although RR-FF monocytes do not bind IgG2 complexes.

Conclusion: We conclude that although IgG-mediated activation of leukocytes is dependent on FcgR, it does not appear to be influenced by FcgR polymorphisms. These results are in favor of a new mechanism for IgG-mediated leukocyte activation, in which even a short interaction between IgG and FcgR is sufficient to generate an appropriate inflammatory response. This may have important implications for inflammatory responses in infectious and autoimmune diseases.

48-080

EFFECT OF TNF-α ON Fcγ RECEPTOR IIA (FcγRIIA) AND β_2-INTEGRIN DISTRIBUTION ON NEUTROPHIL SURFACE ANALYZED BY CONFOCAL LASER SCANNING MICROSCOPY

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Background: Tumor necrosis factor-α (TNF-α) is essential for the induction of the neutrophil activation induced by anti-PR3 or anti-MPO antibodies. We found that Fcγ receptor IIa (FcγRIIa) and β_2 integrins are involved in this reaction (1). Additionally, we suggested that the requirement of TNF-α is probably not only due to an effect of TNF-α on the surface expression of antigens, and another or additional role of TNF-α should be considered (2).

Aim of the study: To assess a possible effect of TNF-α on FcγRIIa and β_2-integrin distribution on neutrophil surface analyzed by confocal laser scanning microscopy.

Results and Discussion: The confocal results exactly match our previous activation results. The experiments showed that TNF-α (2 ng/ml) induced clustering (but not increased surface expression) of FcγRIIa, indicating that FcγRIIa signaling might be enhanced, and induced colocalization of FcγRIIa with β_2 integrins. Moreover, the blocking CD18 mAb MHM23 prevented the ANCA-induced respiratory burst as well as the FcγRIIa clustering. Thus, the FcγRIIa clustering seems to be essential for the induction of the burst, and the colocalization of FcγRIIa with β_2 integrins is probably involved in this process. In conclusion, TNF-α exerts a direct effect on neutrophil signal transduction induced by ANCA by inducing FcγRIIa clustering and possibly by colocalizing the relevant receptors for this process.

(1) Reumaux et al, *Blood* 1995.
(2) Reumaux et al, submitted.

49-084

THE ANCA TARGET ANTIGEN BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN (BPI) IS EXPRESSED IN HUMAN DERMAL FIBROBLASTS

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The ANCA target antigen BPI is an antibiotic, endotoxin-neutralizing and antiangiogenetic protein found in granules of neutrophil granulocytes. Since small molecular neutrophil proteins like defensins were recently detected in epithelial cells, the aim of our study was to determine expression of BPI in non-hematopoietic cells. Cell cultures of human dermal fibroblasts were examined for BPI expression on mRNA and protein level using a BPI-specific RT-PCR, capture-ELISA with murine BPI-specific monoclonal antibodies and IIF after fixation. Cells were stimulated with TNFα, IL4 the active metabolite of cyclophosphamide, dexamethasone and microbes (*S. aureus, P. aeruginosa, C. albicans*). BPI is constitutively expressed on mRNA and protein level in fibroblasts. The expression of BPI is upregulated by proinflammatory cytokines like TNFα and IL4 ranging from 2 to 20 ng/10^6 cells. Immunosuppressive drugs like cyclophosphamide, but not steroids, and *Staphylococcus aureus* down-regulate BPI expression. The ubiquitous presence of BPI outside neutrophil granulocytes indicates an important function in first-line defense against microbes and suggests a role in the local limitation of endotoxin-triggered inflammation. Interaction with BPI-ANCA may impair these functions and facilitate an increased inflammatory response. Moreover, downregulation by immunosuppressive drugs or *S. aureus* may cause a gap in the local spectrum of innate antibiotics making vasculitis patients prone to infections with gram-negative bacteria.

50-085

ANCA SPECIFICITY OF PROTEINASE-3 IN WEGENER'S GRANULOMATOSIS

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The overwhelming percentage of Wegener's granulomatosis (WG) patients with antineutrophil cytoplasmic antibodies (ANCA) suggests that these autoantibodies may play a significant role in this vasculitic disease. ANCA titers often modulate with disease activity indicating that these antibodies might have a direct causative role in the systemic damage observed in patients. One of the major antigenic targets of ANCA in WG is the proteinase-3 (PR-3) protein; however, little is known about how the relationship between PR-3 and ANCA could induce or perpetuate disease. Currently, it is believed that ANCA may induce the release of cytokines from neutrophils and monocytes resulting in hyper-activation and subsequent over-expression of PR-3 which, if not properly inhibited, could cause injury to surrounding tissues. Recently, PR-3 has been shown to bind the soluble endothelial protein C receptor (s-EPCR) suggesting that these proteins are possibly involved with coagulation and inflammatory responses. ANCA could further cause damage in WG patients by binding with PR-3 thus interfering with its interaction with s-EPCR.

Thus, the binding relationship between ANCA and PR-3 should be exactly mapped in order to analyze whether or not ANCA can actually interfere with PR-3 and its natural functions in vivo. This study seeks to determine the common antigenic targets of ANCA on proteinase-3 through sequential epitope-mapping. Overlapping octapeptides of PR-3 were synthesized on derivatized, polyethylene solid phase supports. Ten WG patients, previously determined to have ANCA by immunofluorescence and anti-PR-3 by ELISA, were tested for reactivity with the PR-3 octapeptides. The average binding of all ten patients to the proteinase-3 protein revealed that ANCA reactivity to the proteinase-3 protein occurred at six commonly bound epitopes. Seven out of the ten patients bound epitope 1 (MAHRPPSPAL), seven out of ten patients bound epitope 2 (AQPHSRPYMAS), five out of ten patients bound epitope 3 (SLQMRGNPGSHF), seven out of ten patients bound epitope 4 (VLGAHNVRTQ), five out of ten patients bound epitope 5 (AMGWGRVGA), and five out of ten patients bound epitope 6 (TLRRVEAKGRP). The results of these experiments show that ANCA do in fact bind to linear portions of the proteinase-3 protein which might lead to the disruption of in vivo binding between proteinase-3 and its natural substrates.

51-105

ANCA BINDING TO MONOCYTES ACTIVATES COMPLEMENT

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Antineutrophil cytoplasmic antibodies (ANCA) found in the sera of patients with vasculitis are known to crosslink ANCA antigen to Fcγ receptors on the surface of monocytes and neutrophils with consequent activation of inflammatory effecter mechanisms. We hypothesized that complement and complement receptors may also be targets for activation. We investigated this possibility in vitro, using human monocytes from healthy donors, isolated by centrifugation on ficoll, followed by adherence on plastic. 5 x 10^6 monocytes were incubated for 20 minutes at 37°C with 2 ng/ml TNFα. Subsequently 200 µg of isolated human IgG containing anti-myeloperoxidase (MPO) or anti-proteinase 3 (PR3) antibodies or normal human IgG (nhIgG) were added together with 50 µl of normal human serum (nhs) for 45 minutes at 37°C. Saturating doses of FITC labeled rabbit anti-human C3d or anti-C4c or an irrelevant antibody anti-IgE was added to the monocytes and incubated in the dark for 45 minutes at 4°C. The cells were then washed twice in PBS and resuspended in PBS/1% BSA/1% formaldehyde and stored at 4°C in the dark until FACS analysis. FACS analysis was performed using a flow

cytometer using an argon laser at excitation wavelength of 488 nm and emission wavelength of 530 (+15) nm. Additional experiments were performed with heat inactivated nhs (heated at 56°C for 30 minutes to inactivate complement), EGTA treated nhs (20 mM EGTA/0.8 mM Mg2+) to inhibit the calcium dependent classical pathway in the presence of the magnesium dependent alternative pathway, and C1q deficient serum. Incubation of normal human monocytes with nhs and anti-MPO or anti-PR3 IgG led to a dose and time dependent deposition of the complement breakdown products C3d and C4c. Both anti-MPO and anti-PR3 IgG led to significantly higher C3d deposition (median: range log fluorescence intensity 213:208-217 and 215:209-220, respectively) than incubation with nhIgG (152:142-158) ($p<0.05$). Similarly monocyte C4c deposition following anti-MPO or anti-PR3 IgG (214:209-223 and 210:205-213, respectively) was significantly higher than with nhIgG (157:154-161) ($p<0.05$). ANCA IgG-induced monocyte C3d and C4c deposition was completely abolished by decomplementation of nhs (by heat inactivation); following calcium depletion with EGTA; and following incubation with C1q deficient serum. Incubation of TNFα primed human monocytes with anti-MPO or anti-PR3 IgG followed by nhs leads to complement activation as determined by monocyte deposition of C3d and C4c. This complement activation occurs via the classical pathway as it is abrogated by C1q deficient serum, calcium depletion and heat inactivation of nhs. Deposition of complement components on monocytes or bystander endothelium may augment inflammation in ANCA positive vasculitis.

52-114

ANTI-ENDOTHELIAL CELL ANTIBODIES (AECA) RECOGNIZE A 100 kDa ANTIGEN IN MICROSCOPIC POLYANGIITIS (MPA) BUT NOT IN WEGENER'S GRANULOMATOSIS (WG), CHURG-STRAUSS SYNDROME (CSS) OR POLYARTERITIS NODOSA (PAN)

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Objective: To analyze the repertoire of reactivities of AECA in small and medium sized artery vasculitis.

Methods: Using a quantitative immunoblotting technique on extracts of cultured human umbilical vein endothelial cells (HUVEC), we analyzed the reactivities of serum IgM and IgG from patients fulfilling the ARA and Chapel Hill criteria for the diagnosis of PAN related or not to hepatitis B virus (HBV), WG, MPA, or CSS. Blood samples were obtained from 20 patients with non-HBV cPAN and 10 patients in each other group at the time of diagnosis and before treatment, 10 patients with chronic active hepatitis B without PAN and 60 age- and sex-matched healthy controls. Their sera were tested at the same IgG (200 µg/ml) and IgM (20 µg/ml) concentrations.

Results: MPA patients' IgM reacted with numerous HUVEC extract protein bands, with the two most important being of 100 and 65 kDa. In contrast, IgM from healthy controls and the other patients bound predominantly to one 65-kDa band and a few other minor bands. MPA patients' IgG reacted with 6-8 protein bands, mainly of 100 and 65 kDa, whereas IgG from healthy controls and the other patients reacted with 3-4 protein bands (including the 65-kDa band).

Conclusion: These results provide evidence that a specific 100 kDa antigen is recognized by AECA from MPA patients.

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53-120

HUMAN AND MURINE PR3: FUNCTIONAL AND ANTIGENIC DIFFERENCES WITH POTENTIAL RELEVANCE FOR THE STUDY OF ANCA-ASSOCIATED VASCULITIS

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Rationale: PR3 is the target antigen for C-ANCA in ANCA-associated vasculitis (AAV) and an azurophil granule constituent also expressed on the PMN surface under inflammatory conditions. PR3-ANCA are thought to be pathogenic in AAV. In preparation of a murine model for PR3-ANCA associated vasculitis (PR3-AAV), we compared enzymatic activity, inhibitor spectrum and antigenicity of human PR3 (hPR3) and its murine homolog (mPR3).

Methods/Results: Recombinant hPR3 and mPR3 were expressed in HMC-1 cells which process granule serine proteases. HrPR3 was purified from HMC-1 by sequential anion- and cation-exchange chromatography. MrPR3 requires ethanol precipitation and subsequent binding of the 80% ETOH precipitate to phenyl-superose followed by elution with 2-propanol. Catalytic activity of mrPR3 per unit (t-boc-Ala) for substrate N-MeO-succ-AAPV-pNa is 6-fold higher than that of human PR3. a1-PI inhibits hrPR3 and mrPR3, but eglin C only inhibits mrPR3. These data indicate that mPR3 is more human elastase-like than hPR3. Polyclonal rabbit antibodies raised against hrPR3 and mrPR3 don't crossreact by immunoprecipitation (IP), Western blot or ELISA. Less than 10% of high-titer PR3-ANCA positive sera from AAV patients showed very weak crossreactivity with mrPR3 by IP or ELISA.

Conclusions: Functional similarities and differences in substrate and inhibitor spectrum as well as antigenic differences of human and murine PR3 exist which need to be understood for an appropriate interpretation of murine models of PR3-AAV. The differences can also be exploited for PR3-ANCA-epitope mapping as well as for structure-function analysis of PR3.

Epidemiology of Vasculitis

54-006

DRUG ALLERGY IS ASSOCIATED WITH PRIMARY SYSTEMIC VASCULITIS (PSV)

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Background: Allergy has been associated with PSV (Wegener's granulomatosis [WG] and Churg Strauss Syndrome [CSS]) and is one of the classification criteria for CSS. This is supported by reports of raised IgG levels and Th2 predominant cytokine profiles in CSS and active WG. We examined the evidence for allergy in a case-control study.

Methods: Detailed histories (including a validated questionnaire[1] were taken from 75 adult PSV patients, 220 age/sex matched non-disease hospital controls, 19 systemic rheumatoid vasculitis and 34 age/sex matched asthma controls. Details included: type (skin, drug, insect, plant, food), date and cause of allergy; allergic rhinitis; asthma; family history of allergies/asthma; vaccination or steroid withdrawal in the preceding 6 months; smoking history; TB exposure; hepatitis and blood transfusion. Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated by conditional logistic regression. Total PSV and subgroups (47 WG, 26 CSS, 12 microscopic polyangiitis (mPA), 30 cANCA/PR3 positive, 19 pANCA/MPO positive) were compared to non-disease controls. PSV and CSS were also compared to disease controls.

Results: ORs (95% C.I.) were significantly raised for combined allergy [2.21 (1.30-3.77)], drug allergy [3.38 (1.81-6.29)] and asthma [4.96 (2.49-9.88)] but not other allergy types or rhinitis. Significant ORs (95% C.I.) were found for drug allergy in WG [3.46 (1.63-7.12)], mPA [3.70 (1.02-13.45)] and cANCA [4.60 (1.99-10.61)] but no other groups. Antibiotic allergies (predominantly penicillin) gave significant ORs for PSV [4.15 (1.99-8.65)], WG [4.42 (1.92-10.18)], CSS [4.02 (1.16-14.01)] and cANCA [5.89 (2.28-15.19)] in contrast to other drug allergies. As expected, steroid withdrawal, asthma and rhinitis was higher for CSS vs non-disease but not asthma controls, and PSV had fewer blood transfusions [0.23 (0.08-0.69)] than SRV. Other allergies, family history, smoking, TB exposure, hepatitis and vaccinations were not associated with total PSV or any subgroup.

Conclusions: PSV (especially WG and mPA) was associated with antibiotic allergy. Drug allergies are heterogeneous but, in penicillin allergy, beta-lactam specific T-cells are reported to have a Th2 skewed cytokine profile. One results support the potential role of this type of allergic response in the pathogenesis of PSV.

1. Cuadrado et al, BJR, 1994, 33: 749-753

55-007

PRIMARY SYSTEMIC VASCULITIS—MORTALITY IN A POPULATION-BASED COHORT

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Background: Immunosuppression has greatly reduced mortality in PSV but poorer prognosis has been reported in patients with microscopic polyangiitis (mPA), renal disease, radiographic pulmonary infiltrates and increasing age. Most reports, from tertiary referral centers, are likely to be affected by selection bias. We investigated mortality in an unselected population-based cohort.

Methods: 97 PSV patients, resident in the Norwich Health Authority (NHA) were identified by a prospective vasculitis register (50 Wegener's Granulomatosis-WG, 28 mPA, 19 Churg Strauss Syndrome-CSS). Age at diagnosis, sex, ANCA type, presence or absence of renal/respiratory disease, cause of death and comorbidity were obtained by case note review. For each year incident and prevalent cases and deaths were recorded. Norfolk City Council data were used to obtain population and mortality figures for the 1994 NHA population. Standardized mortality ratios (SMR) were calculated by indirect standardization for 90 PSV patients, diagnosed January 1989-December 1998, compared to the NHA population. A poisson distribution was assumed. SMR's were compared between age-groups, sex and diagnoses using *z*-values. A Cox proportional hazards model compared survival by diagnosis, sex, age, ANCA type and presence/absence of comorbidity and renal or respiratory disease for all cases (May 1988-May 2000).

Results: The SMR (95% C.I.) for PSV was 4.78 (2.98-6.59), higher for men than women [5.94 (3.11-8.76) vs 3.05 (1.16-6.59) p=0.09]. Differences were not significant between age groups or diagnoses. 1 year survival was similar for WG, mPA and CSS (85.5%, 82.7% and 83.2%) but 5 year survival differed: WG=75.9%, mPA=45.1%, CSS=68.1%. Mean survival for PSV was 51.5 months (1-144 months). Survival was less for >65 vs <65 year olds (Log rank, p=0.009) and mPA compared to other diagnoses (Log rank, p=0.07). Hazard ratios [HR (95% C.I.) showed significantly increased risk with age [>61 years, HR=9.22 (2.02-42.0), p=0.03] and mPA [vs CSS, HR=2.52 (0.89-7.15), p=0.077] but no significant differences with ANCA type, comorbidity, renal or respiratory involvement.

Conclusion: The association of increased mortality with age in PSV is due to the expected difference in mortality between age-groups rather than more severe disease as previously suggested.[1] mPA has poorer late prognosis than other diagnoses.

1. Vassallo M et al, JRCP, 1997, 31(4): 396-400

56-008

ARE ENVIRONMENTAL FACTORS IMPORTANT IN SYSTEMIC VASCULITIS?

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Background: The aetiology of Primary Systemic Vasculitis (PSV) is unknown. Potential risk factors include infection, silica, solvents, metal fumes and rural residence.[1] We carried out a case-control study to further explore these and other environmental factors.

Methods: 75 PSV patients (from a prospective vasculitis register), 220 age/sex matched non-disease hospital controls,

19 systemic rheumatoid vasculitis and 34 age-sex matched asthma controls were interviewed using a modified version of a previously used questionnaire.[2] Details included: social class, occupational and residential history, silica, smoking, pets and detailed farm exposure in the year prior to symptom onset (Index Year). Jobs were coded by the Standard Occupational Classification 2000. Job exposure matrices were used to assess levels and duration of silica, solvent and metals exposure. Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated by conditional logistic regression. Total PSV and subgroups (47 Wegener's (WG), 12 microscopic polyangiitis (mPA), 16 Churg-Strauss syndrome (CSS), 19 pANCA/MPO & 30 cANCA/PR3 positive) were compared to controls.

Results: Significantly raised ORs (95% C.I.) were found for a number of factors including farm exposure in the Index Year in PSV [3.15 (1.70-5.83)] and WG [3.59 (1.83-7.03)]. Exposure to livestock (cows, sheep, chickens) was significantly associated with PSV [3.78 (1.17-12.22)]. Working in high silica exposure jobs in the Index Year gave raised ORs for PSV [3.62 (1.41-9.31)], WG [3.45 (1.16-10.25)] and CSS [5.6 (1.34-23.46)]. A history of a high solvent exposure occupation was significantly associated with PSV [2.35 (1.03-5.37)], WG [3.69 (1.54-8.85)] and cANCA [3.43 (1.22-9.68)]. There was no trend to increasing PSV risk with duration of exposure to silica or solvents. There were no significant differences for other items investigated.

Conclusions: This is the first study to report an association between farm exposure and PSV. The association with exposure to livestock may suggest an infectious aetiology, but no single animal is implicated. Results also support a role for silica and solvent exposure in PSV.

1. Watts RA, BJR, 1998; 37: suppl., 86
2. Duna GF, Clin Exp Rheum, 1998; 16: 669-674

57-009

SEASONAL AND PERIODIC VARIATION IN PRIMARY SYSTEMIC VASCULITIS (PSV)

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Background: Some previous studies report that PSV is more common in the winter and may show a periodic fluctuation over many years.[1,2] This suggests that an infectious trigger may be important. We studied seasonal and annual fluctuations in PSV over a ten-year period in an unselected, U.K. population and compared annual fluctuation with common infections.

Methods: All PSV cases diagnosed in the Norwich Health Authority (NHA) between Jan 1989-July 2000 were identified by a prospective vasculitis register. The date of first symptom of PSV (Index date), date of diagnosis, ANCA type and disease classification (Wegener's Granulomatosis-WG, microscopic polyangiitis-mPA, Churg-Strauss Syndrome-CSS) were determined by case note review. Details of the annual fluctuation in mycoplasma pneumonia, parvovirus and chlamydia for the Eastern region (U.K.) were obtained from the Public Health Laboratory Services, London. Annual fluctuations were compared using the poisson distribution and seasonal differences by the chi-squared test.

Results: Of 96 NHA residents diagnosed with PSV between Jan 1989-July 2000, 88 had an Index date between Jan 1989-Dec 1998. There was a trend towards higher onset of PSV in winter and lower in summer, especially in WG and cANCA positive patients (table). There were no significant annual peaks and troughs in the onset of PSV. Annual peaks of infections did not correspond to non-significant fluctuations in PSV.

TABLE 1.
SEASONAL VARIATION IN PSV AND SUBGROUPS (%)

	PSV	WG	mPA	CSS	cANCA	pANCA
Winter (Dec-Feb)	29.9	25.5	42.9	27.8	29	35
Spring (Mar-May)	25.3	23.5	17.1	38.9	19.4	30
Summer (Jun-Aug)	17.2	15.7	17.1	16.7	9.7	15
Autumn (Sept-Nov)	27.6	35.3	22.9	16.7	41.9	20

Conclusions: Data weakly support an autumn/winter peak and summer dip in WG and cANCA. There was no evidence for a cyclical fluctuation in PSV over 10 years or an association of peaks of influenza, mycoplasma, parvovirus or chlamydia.

1. Raynauld J, J Rheumatol, 1993, 20(9): 1524-6
2. Tidman M, J Intern Med, 1998, 244(2): 133-41

58-014

CLINICAL AND EPIDEMIOLOGICAL ANALYSIS OF GIANT CELL (TEMPORAL) ARTERITIS FROM A NATIONWIDE SURVEY IN 1997 IN JAPAN: THE FIRST GOVERNMENT SUPPORTED NATIONWIDE SURVEY

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Objective: To elucidate epidemiological and clinical manifestations of Japanese patients with giant cell arteritis (GCA), the first nationwide survey for GCA was performed in 1997 in Japan.

Methods: The questionnaire on the patients with GCA who had been seen in 1997 was sent to 10,717 departments in Japan. One hundred seventy-seven patients were reported from 6,835 divisions. The answers to the questionnaires detailed in the clinico-epidemiological features on 77 patients were obtained and analysis was conducted on 71 GCA patients.

Results: Prevalence in patients 50 years of age and older in 1997 was 1.47 per million population in Japan. The averaged age at onset was 71.5 years old. The male: female ratio was 1:1.7. The association with visual loss (6.5%), jaw claudication (14.7%), and polymyalgia rheumatica (PMR) (28.2%) were low in frequency compared to those reported from other countries. More than half of the patients were treated with prednisolone less than 40 mg/day with the efficacy of 90.2%. Only three (4.5%) patients were reported as deceased due to other causes.

Conclusion: It revealed that the prevalence of GCA in Japan is extremely low compared to other countries. The clinical findings of visual loss, jaw claudication, and PMR were low in frequency among Japanese patients with GCA. We assumed that the low prevalence of GCA in Japan is due to low frequency in HLA-DNA typing of DRB1*0401,0404 among the Japanese population.

59-024

EXPOSURE TO SILICA AND ANCA-ASSOCIATED VASCULITIS

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Introduction: Exposure to silica is considered among etiological factors of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV). A study is carried out to investigate the association between ANCA and occupational exposure to silica dust.

Methods: 123 patients (122 men and one woman) exposed to silica were examined (mean age 67.6 y, mean exposure 20.0 y). ANCA were tested by immunofluorescence, its specificity by ELISA for proteinase 3 (PR3), lactoferrin, bactericidal-permeability increasing protein (BPI), and myeloperoxidase (MPO). Laboratory and clinical data were collected and analyzed. 27 men represented age-matched control group.

Results: ANCA among silica-exposed persons was detected 21x (17.1%), 2x anti-MPO, 4x anti-BPI, 3x anti-PR3, 1x anti-LTF. No patient suffered from AAV. ANCA were found significantly less frequently (4.9%) in the group of persons with history of SiO_2 exposure without signs of silicosis (risk of silica, RS) than in the group with simple silicosis (SS) (28.6%) or complicated silicosis (CS) (29.6%). Frequency of ANCA+ in controls was 3.6%. Kidney function impairment was found more frequently in ANCA+ patients. Odds ratio for ANCA positivity and the relative risk estimate for patients with both forms of silicosis was highly significant. Predictor factor for ANCA positivity was silicosis, history of tuberculosis, and higher serum creatinine level.

Conclusion: Exposition to silica itself is not associated with increased frequency of ANCA. Risk factors for ANCA positivity are silicosis, tuberculosis, and kidney function impairment. However, the presence of ANCA is not associated with vasculitis in silicotic patients. Other factors must be involved in the triggering of vasculitis in ANCA-positive patients.

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60-036

GERMAN VASCULITIS REGISTER: RESULTS OVER THE FIRST THREE YEARS

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Background: Little has been published on the epidemiology of primary systemic vasculitis (PSV). Much of the data comes from referral centers or covers only small areas, leading to referral or selection bias. This prompted us to establish a Vasculitis Register for North and South Germany to determine the incidence of PSV in a population-based study in a large region (nearly 5 million habitants) at 1/1/1998.

Methods: Data on all new cases of PSV (as defined by the CHCC) are obtained from the following sources: (1) all departments of all hospitals, including their outpatient clinics, (2) all departments of pathology, and (3) reference immunological labs. At three-month intervals all sources were asked by mail (up to three times) to screen for newly diagnosed cases of PSV.

Results: Over the first three years (1998 - 2000) 597 PSV patients were identified. The incidence of PSV was 45 to 54 cases/million/year without differences between north and south Germany. The incidence of ANCA-associated PSV (WG, CSS, MPA) was 9-12/mio/year. The most frequent ANCA-associated PSV was the WG with an incidence of 7 new cases/mio/year. Over the whole period 84 patients with newly diagnosed WG were registered. Their median age at diagnosis was 60 years, conspicuously higher than described in large WG cohorts. On the other hand, the time between the first WG symptoms and diagnosis was only 3 months.

Conclusion: Compared to other countries, in Germany the incidence rate of WG was similar to that in Norway (8) and US (8), but higher than in Spain (5), and lower than in UK (11). If these results reflect a real north-south difference similar to that found for the GCA or whether be caused by differences in the case finding methods (population based study in a large region vs. studies from referral centers or small regions) remains unclear.

61-039

POLYARTERITIS NODOSA IN CHILDHOOD

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Introduction: This study describes the clinical, histological, and angiographic features of polyarteritis nodosa (PAN) presenting in childhood.

Methods: Retrospective review of case notes of patients diagnosed with PAN. Only patients who satisfied 3 or more of 10 classification criteria as defined by the American College of Rheumatology (ACR) were included. Angiography was reviewed independently by 2 blinded radiologists.

Results: Between 1971 and 1998, 38 children satisfied 3 or more of 10 ACR classification criteria for PAN. There was a male preponderance of 1.9:1. Mean age was 7.9 years (range

0.3-14.4 years). All had fever and elevation of acute phase reactants. Additional clinical features included rash (61%), renal impairment (24%), hypertension (34%), myalgia (79%), weight loss (79%), testicular pain (20% of males), peripheral neuropathy (13%), cerebral involvement (8%), and sub-arachnoid hemorrhage (3%). No patient had evidence of hepatitis B infection. 9/12 skin biopsies revealed vasculitis. Renal biopsy was performed in 9 patients and revealed crescentic glomerulonephritis (GN) (4/9), mesangio-proliferative GN (3/9), and focal segmental sclerosis (1/9). Vasculitis was also demonstrated on biopsy of the liver, temporal artery, and gut. 35/38 patients had abnormal visceral angiography. A spectrum of angiographic findings was documented and included aneurysms, renal perfusion defects, collateral renal arteries, arterial cut-off, and pruning of the renal arteries. Overall, the mortality for PAN was 8%.

Conclusion: PAN has a wide spectrum of presentation and is a great imitator of many pediatric conditions. Often the diagnosis remains elusive unless specifically sought, and visceral angiography and tissue biopsy play a key diagnostic role.

62-040

SYSTEMIC NECROTIZING VASCULITIS AND INFLAMMATORY BOWEL DISEASE OF CHILDHOOD

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Systemic necrotizing vasculitis (SNV) can mimic inflammatory bowel disease (IBD). The differentiation between primary SNV and IBD can be clinically testing, however it is important to distinguish these disorders since their treatment and outcomes are different. The aims of this study were therefore to describe a series of children with SNV who initially presented with clinical features suggestive of IBD. 7 children (5 boys, mean age 8.6 years, 2.5-14 years) presenting between 1993-98 satisfied inclusion criteria. All had abdominal pain, failure to thrive, diarrhea (4/7 bloody), and laboratory evidence of a severe acute phase response. Mean colitis score was 4.7 (3-7). Other clinical features included renal impairment (1/7), vasculitic rash (5/7), myalgia (6/7), testicular pain (1/5), and polyarthritis (3/7). pANCA was present in 3/7. Anti-enterocyte antibodies were present in 2/5 patients. Labelled white cell scan showed increased gut uptake in 5/6 patients. Visceral angiography was suggestive of vasculitis in 6/6 studies performed, with renal (5/6), and mesenteric or hepatic (6/6) vascular bed involvement. Endoscopy was abnormal in 6/7, with patchy loss of normal mucosal vascular patterns and areas of sharply demarcated disease activity at watershed areas as noted features. Gut histology revealed indeterminate chronic inflammatory changes in all 7 patients. Treatment comprised systemic steroid (7/7), cyclophosphamide (4/7), azathioprine (7/7), mycophenolate (2/7), cyclosporin (1/7), ASA derivatives (4/7), colchicine (1/7), and plasma exchange (1/7). At mean follow-up of 4 years, all patients are currently in remission although have had a relapsing clinical course, and one patient is off all treatment. Primary SNV can mimic IBD in its clinical presentation. Serology including ANCA and anti-gut antibodies do not help to discriminate between the two groups of diseases. Extra-intestinal manifestations, and acute phase responses, which are disproportionate to the degree of intestinal inflammation, may provide clues to the presence of a primary SNV.

63-042

INCIDENCE AND CLINICAL FEATURES OF WEGENER'S GRANULOMATOSIS IN OLMSTED COUNTY, MINNESOTA, 1990-1999

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Objective: To assess the incidence and clinical features of Wegener's granulomatosis (WG) in a population based cohort of patients since the introduction of antineutrophil cytoplasmic antibody (ANCA) testing.

Methods: Case ascertainment of patients with WG was performed by retrospective medical history review of 231 patients with the diagnosis of systemic vasculitis in Olmsted County, during the years 1990-1999. For completeness, the medical histories of all 699 patients from Olmsted County undergoing ANCA testing in this period were also reviewed to ensure that no cases of WG were missed by the medical record review.

Results: ANCA testing was performed in 49 patients with a diagnosis of vasculitis. Of these, 6 had a positive c-ANCA (all of whom had WG), 11 had a positive p-ANCA, and in the remaining 32, ANCA was negative. A total of 8 incident cases of WG occurred (3 men, 5 women); median age 60.5 yrs. (range 40-81). The overall age and sex adjusted annual incidence of WG was 0.83 cases/100,000 population (95% CI 0.25-1.42). For the population age ≥18 years, the age and sex adjusted annual incidence was 1.1 per 100,000 (95% CI 0.33-1.9). Two patients died during the follow-up period. C-ANCA was positive in 6, and p-ANCA (myeloperoxidase) was positive in the other. Half (4) of the patients were diagnosed with WG prior to obtaining ANCA results; in the other 4 ANCA results were available prior to a final diagnosis of WG and were useful in disease classification.

At the time of diagnosis, the median Birmingham vasculitis activity score (BVAS) was 23.5 (range 18-34, and the median BVAS/WG was 10 (range 5-15); activity scores correlated well between these scales. The frequency of organ involvement in these patients (n,%) was: ears/nose/throat 5 (62.5); lung 6 (75); kidney 6 (75); muscle/joint 6 (75); eye 1 (12.5); peripheral nervous system 3 (37.5); central nervous system 0 (0); gastrointestinal 2 (25); heart 2 (25); skin 5 (62.5); malaise 5 (62.5).

Conclusions: Since the advent of widespread ANCA testing, WG continues to be rare. The incidence of WG as seen in this population based study performed in Olmsted County is similar to that seen in older hospital based studies, suggesting that availability of ANCA testing has not lead to a marked increase in the numbers of patients diagnosed with this disease. The organ involvement in this study is similar to that of older hospital based series. A positive c-ANCA was 100% specific for the diagnosis of WG in this population.

64-060

CLINICAL FEATURES OF SYSTEMIC VASCULITIS IN SANTIAGO, CHILE: A TEN-YEAR STUDY

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Objective: To describe the clinical features of microscopic polyangiitis (MPA), polyarteritis nodosa (PAN), and Wegener's granulomatosis (WG) in a Chilean cohort of patients.

Methods: Case ascertainment was performed by retrospective review of medical records, of 173 patients with the diagnosis of systemic vasculitis from 1990 to 2001. The diagnoses were made according with the ACR and Chapel Hill criteria. Thirty-two patients were excluded because they did not fulfill these criteria. Therefore, we included 65 MPA, 18 PAN, and 58 WG patients.

Results: The mean follow-up (months) for MPA was 15 (1-120), PAN 24 (2-60), and WG 20 (1-120). The median age (years) at diagnosis for MPA was 61 (19-82), PAN 44 (17-83), and WG 50 (20-82). Gender distribution was similar among the three groups (male: 68%, 67%, and 57% respectively). The main clinical features for MPA were renal involvement (68%) (characterized by elevated plasmatic creatinine levels and inflammatory urinalysis), peripheral nervous system involvement (57%), pulmonary hemorrhage (28%), and skin disease (32%). For PAN were cutaneous involvement (45%), peripheral nervous system involvement (39%), hypertension (22%), abdominal pain (22%), myopathy (28%), and renal disease (17%). For WG were alveolar hemorrhage (62%), renal involvement (78%), ENT compromise (65%), and ocular disease (26%). In both, MPA and WG, creatinine levels above 2.0 mg/dl were associated with higher mortality ($p < 0.01$). MPA patients with pulmonary hemorrhage had significantly higher levels of creatinine compared to those without it (6.8 vs 3.4 mg/dl, $p < 0.01$). ANCA by immunofluorescence was performed in 56 MPA (77% ANCAp, 3% ANCAc, 20% negative) and in 55 WG patients (17% ANCAp, 76% ANCAc, 7% negative). The majority of PAN patients were ANCA negative (88%). Global mortality for each group was 18%, 14%, and 17%, and the major causes of death were infections.

Conclusion: The clinical features of our patients are similar to other published data. In our WG and MPA patients the main predictor for death was renal disease with a creatinine above 2 mg/dl. In the MPA cohort the presence of pulmonary hemorrhage is also a significant predictor of death.

65-067

ANCA NEGATIVE POLYARTERITIS NODOSA IN LUND 1990-2001

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The aim of this study was to characterize all patients with polyarteritis nodosa (PAN) seen at our units during the last twelve years and to analyze their outcome. Patients with signs of small vessel vasculitis, such as presence of ANCA or crescentic glomerulonephritis, were excluded. Nine patients (five male and four female) were found to meet the criteria of PAN and were studied retrospectively in detail. Five were diagnosed by angiography and four by biopsy. The median age at diagnosis was 45 years (range 8-77). The time from first symptom to diagnosis varied from 2 weeks to 38 months (median 4 months). Only one patient was found to have hepatitis that could have contributed to the development of PAN.

Organ involvement at diagnosis:

abdominal	6	muscle	3
renal	5	joint	2
skin	5	testicle	2
hypertension	5	eye	1
peripheral nerve	3	lung	1

The patients were followed for 0.5-17 years (median 3 years). Five patients had altogether seven relapses. The median time from diagnosis until the first relapse was 3 years (range 1-6). One patient died after five months. Two patients developed end stage renal failure and started treatment with hemodialysis seven and eight years after diagnosis. Both had malignant hypertension at diagnosis.

During the twelve year period five new cases were diagnosed among patients living in our local catchment area of 300,000 inhabitants, which gives an annual incidence of 0.5 per million. PAN is a rare disease in Sweden, but should not be forgotten in patients with systemic symptoms without ANCA.

66-115

PREVALENCE OF POLYARTERITIS NODOSA (PAN), MICROSCOPIC POLYANGIITIS (MPA), WEGENER'S GRANULOMATOSIS (WG) AND CHURG-STRAUSS SYNDROME (CSS) IN A FRENCH URBAN POPULATION IN 2000: A CAPTURE-RECAPTURE ESTIMATE

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Objective: To estimate the prevalences of PAN, MPA, WG and CSS in an urban multiethnic population.

Methods: Cases were collected in Seine–Saint-Denis *Département*, a northeastern suburb of Paris, which has 1,093,515 adults (≥15 yr), 28% of whom are of non-European ancestry. The study period encompassed the entire calendar year 2000. Cases were identified by general practitioners, the departments of all the public hospitals and 2 large private clinics, and the Public Health Insurance System. The Chapel Hill nomenclature was used to define MPA, and ACR criteria to define WG and CSS; PAN was diagnosed based on clinical, laboratory, histological and/or angiographic findings. Only histologically and/or angiographically documented cases were retained. Three-source capture–recapture analysis (CRA) was performed to correct for incomplete case ascertainment.

Results: A total of 65 confirmed cases were identified; among 18 non-verifiable cases, 7 additional cases were estimated to be true cases. CRA estimated that 21 cases had been missed by any

1 of the 3 sources. Accordingly, prevalences per 1,000,000 adults (CI 95%) was estimated to be 31.8 (23-41) for PAN, 18.9 (11-27) for MPA, 23.0 (16-30) for WG and 10.2 (5-16) for CSS. The overall prevalence was 1.9 times higher for subjects of European ancestry than for non-Europeans (p = 0.02).

Conclusion: This study provides the first prevalence estimates for these 4 vasculitides for a multiethnic and urban population. The significantly higher prevalence observed for Europeans may infer a genetic susceptibility of Caucasians. Compared to previous estimates based mostly on rural populations, the higher frequency of PAN and the lower frequency of WG might suggest specific environmental etiological factors.

67-127

HIGH-DOSE INTRAVENOUS IMMUNOGLOBULINS IN ANCA-ASSOCIATED SYSTEMIC VASCULITIS (AASV)

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New drugs are regularly tested in AASV to reduce disease activity and adverse events associated with corticosteroids and immunosuppressives. Intravenous immunoglobulins (IVIg) are mainly used in patients who are resistant to usual therapeutic regimen. We report on the results of IVIg therapy in patients with AASV who have relapsed.

Five relapses occurring in 4 patients (Wegener's disease: 2 patients, microscopic polyangiitis: 2 patients) were treated by 6 monthly courses of IVIg (0.5 mg/kg/d during 4 days), and prednisone (0.5 g/kg/d). One patient was on azathioprine (50 mg/d). Disease activity was assessed by the Birmingham vasculitis activity score (BVAS). ANCA levels (IF, ELISA) were tested before each course of IVIg and at the end of the study.

At the time of the relapse, the median BVAS was 11 (3-15). Two out of 5 relapses were major (cerebral and renal angiitis). ANCA were positive in each relapse (MPO-ANCA n=4, PR3-ANCA n=1). At the end of the study, complete remission was achieved in every case (BVAS=0). ANCA levels decreased in 2 cases (1/1 PR3-ANCA, 1/4 MPO-ANCA). Acute renal failure due to osmotic tubular injury occurred during one course of treatment, followed by complete recovery without dialysis. Other side effects were minor (n=7). Three new relapses occurred from 2 to 18 months after the last course of IVIg.

We conclude that IVIg may be an alternative to conventional immunosuppressive drugs for inducing remission in relapsing AASV. However, a high rate of relapses occurred after IVIg withdrawal, underscoring the need for additional maintenance therapy.

68-128

VASCULITIS: ARE CURRENT CLASSIFICATIONS USEFUL IN CLINICAL PRACTICE?

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Introduction: Systemic vasculitis is a heterogeneous group of clinical manifestations of unspecific etiology, with or without cutaneous compromise. The gold standard test is the histopathological study. In this descriptive study we reviewed the histological studies with confirmed vasculitis diagnosis and made a correlation with the current classifications.

Materials and Methods: In a retrospective way, we studied all the confirmatory histological studies processed between 1953 and 1990 and prospectively from 1991 to 1997 at Hospital San Juan de Dios (Bogota, Colombia) that had a confirmatory result for vasculitis. Then, we classified them using both Chapel Hill and Lie's classifications in order to determine their accuracy and usefulness in clinical practice.

Results: In this descriptive trial, we found 304 histopathological studies with documented vasculitis of 140,717 that were made in this period. We found an annual incidence of 22 per 10,000 in this population group; the mean age of presentation was 36 (range: 10 to 82); the female:male ratio was 2:1; skin (69%) and muscle (15%) were the most important organs processed. It was possible to classify only 40 histological studies (13%) using the Chapel Hill consensus and its different subsets. The most frequent vasculitis found was that of median vessels (24 of 40, 60%), and all of them corresponded to nodosum poliarteritis. With Lie's classification we could find a correlation in 121 plaques (40%). In this case, primary vasculitis was the most frequent diagnosis (78 of 121, 64%), the miscellaneous group being the most common (38 of 78, 49%) represented by Bnerguer disease. Secondary vasculitis occupied the second place (43 of 121, 35%), represented by connective tissue diseases (27 of 43, 63%), mainly SLE and dermatopolimyositis (14 of 43 in each case). Leucocitoclastic (67 of 183, 37%) and linfomonocitic (49 of 183, 27%) vasculitis conformed the most frequent unclassified groups.

Conclusions: Considering that the histopathologic study is the gold standard for diagnosis of vasculitis, we found that current vasculitis classifications are incomplete and let many pathologies out of these categories. Therefore, further efforts should be made in order to create more complete and comprehensive tools to classify these diseases tending to facilitate clinicians practice and scientific trials.

Diagnostic Modalities, Surrogate Markers of Disease Activity, and Tools for Outcome Measurements

69-002

PROTEINASE 3 IS THE MAJOR AUTOANTIGEN IN HEPATITIS C VIRUS INFECTION

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Hepatitis C virus (HCV) infection has been found to be

TABLE

	CHOP patients	Literature patients	Summary
Number of subjects (females, males)	6 (5, 1)	136 (107, 29)	142 (112, 30)
Mean age, yrs (range)	8.6 (1.6-17)	11.5 (3-17)	11.4 (1.6-17)
Reported signs and symptoms	Fever (4/6), stroke (1/5), arthralgias (2/5), fatigue (2/5), skin nodules (3/6), anorexia/wt loss (3/6), claudication (2/5), chest pain (2/5), back pain (2/5), palpitations (2/5), headache (1/5), vomiting (1/6)	Fever (20/54), stroke (4/24), arthralgias (5/30), fatigue (2/5), skin nodules (5/26), abd pain (18/97), vomiting (30/120), claudication (17/107), chest pain (7/75), palpitations (25/104), anorexia/wt loss (15/99)	Fever (24/60), stroke (5/29), arthralgias (7/35), fatigue (4/10), skin nodules (8/32), abd pain (18/97), vomiting (31/126), claudication (19/112), chest pain (9/80), palpitations (27/109), anorexia/wt loss (18/105)
% with HTN	67% (4/6)	89% (121/136)	88% (125/142)
% with ESR ≥20	83% (5/6)	59% (72/121)	61% (77/127)
% with HTN and ESR ≥20	67% (4/6)	70% (48/69)	69% (52/75)
% with cardiomegaly on CXR	50% (2/4)	55% (30/55)	74% (25/34)
Imaging results	Angiography in 5 pts; aorta, subclavian, vertebral, carotid, cerebral, celiac, splenic, renal arteries involved; MR in 4 pts (2 with gadolinium); thickened aortic, carotid, renal, celiac, subclavian walls or pseudoaneurysm of the iliac artery	Angiography in 118 pts; involvement of the aorta, subclavian, carotid, superior mesenteric, celiac, pulmonary, coronary, splenic, hepatic, vertebral, brachiocephalic, renal, and cerebral arteries	100% of patients who had imaging done showed abnormalities of the aorta and vessels of the thorax, abdomen, or head

strikingly associated with autoimmune phenomena. The aim of the present study was to investigate the presence of various autoantibodies in patients with HCV infection.

ANCA, anti-E3 antibody, and RF were positive in 278/516 (55.6%), 276/516 (53.3%), and 288/516 (56%) patients with HCV infection, respectively. Positivity for ANA was present in 15.8%, anti-ssDNA in 15.6%, anti-dsDNA in 8.5%, aCL in 5%, anti-SS-B/La in 4.1%, anti-SS-A/Ro (60 kD) in 3.9%, anti-E2 in 3.3% and anti-SSA/Ro (52 kD) in 1.2 %, anti-MPO in 4.8%, anti-Topo II and anti-actinin in 0%. All sera with ANCA showed c-ANCA pattern and contained anti-PR3 specificity. HCV patients with ANCA showed a higher prevalence of skin involvement, anemia, abnormal liver functions and α-Fetoprotein (α-FP). The prevalence of autoantibodies was not affected by the treatment of interferon-alpha (IFN-α)

In conclusion, autoantibodies are commonly found in patients with HCV infection. There is a high prevalence of anti-E3, ANCA, and RF in these patients. Proteinase 3 is the major target antigen in HCV infection.

70-021

TAKAYASU ARTERITIS IN CHILDREN

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Takayasu arteritis (TA) is a rare, chronic idiopathic granulomatous vasculitis of the aorta and its branches, predominantly affecting young women (<40 yrs old).

Objective: To determine the diagnostic features of children diagnosed with TA.

Methods: We identified 136 patients <18 yrs old from published reports on TA using the National Library of Medicine PubMed system. We systematically analyzed demographic and clinical data at presentation. We then identified 6 patients with TA cared for at CHOP in the last 10 years and compared them to published cases.

Results: See Table at top of page.

Discussion: Failure to recognize the early signs of TA in children leads to a delayed diagnosis (19 mos vs. 10 mos for adults), more severe hypertension, more congestive heart failure (66%), and higher mortality (30-35% vs. 5-15% in adults). Hypertension and elevated ESR are found in most patients with TA and should merit further screening for TA. MRI/MRA of the thoracic and abdominal aorta and great vessels, using gadolinium contrast to image stenotic arteries with thickened enhanced vessel walls is emerging as a non-invasive tool to diagnose TA.

71-032

ARE C-ANCA ALWAYS USEFUL IN LONG-TERM FOLLOW-UP OF WEGENER'S GRANULOMATOSIS?

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Wegener's granulomatosis (WG) is a necrotizing vasculitis seriously involving mostly upper airways, lungs and kidneys, requiring a long-term therapy with several side effects. It is well established that c-ANCA are very useful in diagnosing the disease.

In order to evaluate the reliability of c-ANCA not only in the diagnosis, but also in the long-term follow-up of WG we considered eight patients (seven males and one female), aged at diagnosis from 20 to 65, followed up at our Clinic for a period of time of five to ten years. The patients were evaluated at least twice per year with clinical examination and laboratory and radiological tests. All patients had their diagnosis made at least five years ago by histological examination of a bioptic tissue specimen (from nose or lung or kidney) and all of them had highest titers of c-ANCA (1:640 to 1:10,240), always confirmed by ELISA test.

All patients were treated with steroids and cyclophosphamide with good response. In particular, c-ANCA became negative in six months-one year. Two patients have done very well even after therapy had been discontinued. Their c-ANCA are always negative. Two other patients are still on therapy: they presented three and four relapses in five and eight years, respectively, but their c-ANCA titer raised only minimally and the autoantibodies rapidly disappeared. The other four patients had a clinical relapse (especially with pulmonary nodules and cavitations), with increase of C reactive protein, but absence of c-ANCA, and rapid response to immunosuppressive therapy.

It is noteworthy that c-ANCA titer is likely to follow the severity of the disease, especially in those patients for whom recovery seems harder. On the other hand c-ANCA do not seem highly reliable in detecting a relapse of WG and probably in the long-term follow-up of patients with WG the suspicion of a relapse should be based mostly on the clinical setting because the reappearance of c-ANCA, even after reduction of doses or discontinuation of therapy, is not constant.

72-034

INCREASED SPECIFICITY FOR SYSTEMIC VASCULITIS WITH CAPTURE ELISA FOR MPO?

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Background: High levels of MPO-ANCA are usually found only in patients with systemic vasculitis. Low levels are less specific and can be found also in many non-vasculitic conditions. This pilot study investigates two different capture-MPO-ANCA assays concerning their specificity for the diagnosis of small vessel vasculitis compared to standard ELISA.

Methods: Patients whose first test exhibited a low or moderately elevated value in standard MPO-ANCA ELISA were included in this study. Patient records were reviewed and a diagnosis was established using the Chapel Hill nomenclature. Sera were tested using two different capture assays based on the monoclonal antibodies 2B11 and 099. These antibodies are known to react with different non-overlapping epitopes on the MPO molecule. If the result in the capture assay yielded a value that was less than 30% of the value in the standard assay, it was considered a significant reduction.

Results: Out of 36 patients with low MPO-ANCA, 27 were diagnosed as having small vessel vasculitis and 9 patients were found to have other diagnoses. For patients with other diagnoses than vasculitis the result of the 2B11 assay was significantly reduced in 55% (5/9) of the cases. Only 7% (2/27) of the vasculitis patients showed reduced results with the 2B11 assay. With the 099 assay corresponding figures were 45% (4/9) and 37% (10/27).

Conclusion: A capture assay based on Mab 2B11 seems to be more specific for the diagnosis of small vessel vasculitis as compared to standard ELISA and the other capture ELISA. Antibodies against the 2B11 epitope may be irrelevant for the diagnosis of small vessel vasculitis.

73-050

FIVE DISTINCT CLINICAL SUBSETS AND THEIR PROGNOSTIC IMPLICATIONS IN MPO-ANCA ASSOCIATED VASCULITIS

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Aim: Clinical subsets in MPO-ANCA associated vasculitis were classified and were examined on the association of these subsets to prognosis.

Method: 50 patients were studied on the clinical subsets and prognosis. Five clinical subsets, which were renal limited type, pulmorenal type, systemic vasculitis type, pulmonary type, and non-pulmorenal type, were identified. The prognosis of life and renal function in these types was studied more than 3 years.

Results: 50 patients were classified into 24 cases with renal limited type, 13 cases with pulmorenal type, 7 cases with systemic vasculitis type, 2 cases with pulmonary type, and 4 cases with non-pulmorenal type. The death cases over 3 years observation period occurred in 8% with renal limited type, in 31% with pulmorenal type, in 57% with systemic vasculitis type, in 50% with pulmonary type, and in 0% with non-pulmorenal type. Hemodialysis was performed in 50%, 77%, 100%, 0% and 0% in each group, respectively. The main causes of death were GI tract lesions, respiratory failure, or infections.

Conclusion: Clinical subset identification is very useful to presume the prognosis of survival and kidney function in MPO-ANCA associated patients.

74-056

IS THE CHURG-STRAUSS SYNDROME (CSS) AN ANCA-ASSOCIATED VASCULITIS?

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Background: ANCA (antineutrophil cytoplasmatic autoantibody) is reported to be present in about 10-80% of

patients with CSS and to be useful as a diagnostic tool. This is in contrast to Wegener's granulomatosis and microscopic polyangiitis, where ANCA is reported to be present in much more consistently high percentage. Therefore the clinical value of ANCA in CSS is questionable. Because of the relative rarity of CSS many reports about ANCA in CSS are based on small numbers of patients. This together with different methods applied for ANCA detection in the respective investigations may contribute to these variable results concerning the prevalence of ANCA in CSS.

Objective: To evaluate the prevalence of ANCA in Churg-Strauss syndrome (CSS) using different methods in well-characterized patients.

Patients and methods: We performed a prospective study on sera of 75 patients with CSS. Diagnosis was made according to the ACR and CHC criteria. We used the first sera of 75 patients after the diagnosis was established. If the first presentation was during inactive disease, we additionally screened a second serum at the time of active disease. 24 patients never had active disease. Screening of ANCA was done using an established indirect immunofluorescence technique (IFT). All sera were investigated serially by direct ELISA for common antigen specificities such as PR3, MPO, CG, Lactoferrin and BPI. In addition to these immunoassays we also used new established capture ELISAs for detection of PR3- and MPO-ANCA.

Results: In IFT only six patients were positive (2 x cANCA, 4 x pANCA). In direct ELISA, two had a PR3-ANCA, four had an MPO-ANCA. In capture ELISA we found 8 patients with ANCA, one patient had a PR3 antigen, seven others had an MPO antigen. Of patients with MPO-ANCA positive capture ELISA, four were IFT-negative. In total 10 patients (13.3%) with CSS were ANCA-positive at one point of disease, regardless of the antigen and of the method applied for screening.

Conclusion: Compared with the literature, we found a lower association of ANCA with CSS. Our data do not support the notion that ANCA is of immuno-diagnostic value in CSS.

75-057

ASSESSMENT OF ACTIVITY AND DAMAGE IN ANCA-ASSOCIATED VASCULITIS IN INDIA

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Background: Wegener's granulomatosis, once thought to be uncommon in India, is being recognized with increasing frequency in Indians. In the present study, the assessment of the primary systemic necrotizing vasculitis was done using the Birmingham Vasculitis Activity Score (BVAS) and Vasculitis Damage Index (VDI).

Method: 76 patients with ANCA-associated vasculitis were evaluated using the BVAS and VDI, between January 1990 and June 2001. The diagnosis of Wegener's granulomatosis and Churg Strauss disease were made by 1990 ACR criteria and that of microscopic polyangiitis by Chapel Hill Consensus. ANCA, ANA and anti-DNA were estimated by indirect immunofluorescence & antibodies to PR3 and MPO by ELISA. All other causes for secondary vasculitis and infections were excluded.

Results: There were 40 males and 36 females. The mean age at diagnosis was 43.4. The mean disease duration prior to diagnosis was 3.4 months. The distribution of vasculitis were: Wegener's granulomatosis - 48, microscopic polyangiitis - 10, Churg Strauss - 6 and crescentic glomerulonephritis - 12. cANCA was positive in 48 (63.15%) and pANCA in 21 (27.63%). ANA and anti-DNA were negative in all the patients. The mean BVAS score at baseline was 16.4. The mean VDI system score was 3 and the mean total VDI score was 4.6. Using the Vasculitis Damage Index, the following items of damage were seen: musculoskeletal damage-12 (15.8%); skin damage-16 (21%); ENT damage-28 (36.8%); pulmonary damage-42 (55.3%); cardiovascular damage-34 (44.7%); renal damage-51 (67.1%); peripheral vascular damage-26 (34.2%); ocular damage-21 (27.6%); neuropsychiatric damage-48 (63.2%); and other damage & drug toxicity-14 (18.4%).

Conclusion: 1. ANCA-associated vasculitis was rare in India, present in only 0.001% of hospital admissions. 2. Neuropsychiatric manifestations were common (63%). 3. The BVAS and VDI offer a comprehensive and cumulative measure of disease activity and damage in the serial assessment of vasculitis patients.

76-058

HEPATITIS C VIRUS RELATED CRYOGLOBULINEMIC VASCULITIS IN EGYPT

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Introduction: Cryoglobulinemic vasculitis is strongly associated with hepatitis C virus (HCV), whose prevalence in Egypt is 10-25%. The aim of this study was to determine the clinical and serological features of HCV-cryo patients presenting with vasculitis.

Methods: We identified all cases of HCV vasculitis referred to two rheumatology units in Cairo between 1998 and 2001. Patients underwent standardized clinical evaluation using the Birmingham Vasculitis Activity Score (BVAS), a physician's global assessment (10 cm horizontal line), PCR for hepatitis C, and serology for rheumatoid factor, complement and cryoglobulins.

Results: We identified 28 patients (M 8; F 20; median age 51 years; range 37 - 70, disease duration range 1 month to 15 years) with cryoglobulinemic vasculitis. All cases were HCV +ve by PCR, 19/28 had cryoglobulinemia detected at the onset, whilst a further 2 cases developed cryoglobulins during disease flares (sensory neuropathy in 1 case, nephritis and retinal vasculitis in 1 case). All patients had skin lesions (5 with ulcers). 17/28 had one or more features of neurological involvement (sensory neuropathy in 11; motor neuropathy in 2; mixed sensory/motor neuropathy in 4, seizures in 2 and sensorineural deafness in 1). Renal disease was present in 8 cases. Constitutional symptoms were common (11/28). Four patients

developed deep vein thromboses. Hypocomplementemia was present in 21 cases and RF was +ve in 17/21 tested. PGA and BVAS had a linear correlation (r = 0.73). The absence of cryoglobulins in 7 cases was associated with less severe organ involvement, using PGA (P<0.05), although BVAS levels were not statistically different between the cryo +ve and the cryo −ve group.

Conclusion: Cryoglobulinemic vasculitis associated with HCV in Egypt has a high propensity to cause multi-organ damage. BVAS is a valuable instrument in assessing activity in these patients. Interestingly, a minority of patients without detectable cryoglobulins have similar clinical features to cryo +ve patients. This raises the possibility that HCV may induce a vasculitis independently of the presence of circulating cryoglobulins.

77-059

A CRITICAL EVALUATION OF COMMERCIAL IMMUNOASSAYS FOR ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES DIRECTED AGAINST PROTEINASE 3 AND MYELOPEROXIDASE IN WEGENER'S GRANULOMATOSIS AND MICROSCOPIC POLYANGIITIS

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Objective: To determine the performance characteristics of 11 commercial enzyme-linked immunoassay (ELISA) kits for the detection of antineutrophil cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3) and myeloperoxidase (MPO) in defined patient groups (Wegener's granulomatosis=WG and microscopic polyangiitis=MPA)

Patients and Methods: Serum samples were derived from patients with histological and clinical diagnosis of WG (n=50), MPA (n=42), SLE (n=15), RA (n=15) and healthy controls (n=30). Each of these sera was tested for the presence of ANCA by indirect immunofluorescence technique (IFT) and PR3- and MPO-ANCA by 11 commercially available ELISA kits. In addition, in-house PR3- and MPO-ANCA capture ELISAs were performed.

Results: Using PR3-ANCA as a diagnostic test for WG there were considerable differences in sensitivity (from 22% to 70%) and negative predictive values (NPV) (from 43% to 70%) among the different ELISA kits, while specificity (from 93% to 100%) and positive predictive values (PPV) (from 93% to 100%) varied only modestly. The highest sensitivity (74%) and specificity (100%) for PR3-ANCA were obtained with the in-house capture ELISA. Similar differences and trends were observed for MPO-ANCA assays. Diagnostic sensitivity was more than 60% in 4 ELISA kits and at least 50% in 6 of 10 kits. The PPV varied from 84% to 100% and the NPV varied from 58% to 70%. Only one MPO-ANCA ELISA kit and in-house capture ELISA were the best assays for detecting MPA (sensitivity 62% and specificity 100%). In WG and MPA, maximum sensitivity for ANCA was obtained with IFT (80% and 70%, respectively).

Conclusion: PR3-ANCA and MPO-ANCA determined with commercial available direct ELISA kits were of poor sensitivity for WG and MPA and the immunofluorescence remains the superior method for ANCA detection in these diseases. The in-house PR3 and MPO-ANCA capture ELISAs perform better than direct ELISAs because combine a higher specificity with a comparable sensitivity.

78-076

A POSITIVE PR3-ANCA TITER AT SWITCH TO AZATHIOPRINE THERAPY IS ASSOCIATED WITH A DISQUIETING RELAPSE RATE IN ANCA-RELATED VASCULITIS

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The CYCAZAREM study showed that switching cyclophosphamide to azathioprine after 3 months of remission does not lead to more relapses within 18 months after diagnosis compared to continued cyclophosphamide therapy in patients with ANCA-related vasculitis. Although long-term data are not available, this regimen is widely adopted in the treatment of these patients, also at our center. We had the impression, however, that ANCA titers in patients switched to azathioprine rose early and that they relapsed more frequently during longer follow-up. We, therefore, analyzed patients diagnosed with ANCA-related small-vessel vasculitis between 1990 and 2000 at our center, ≥1 year follow-up, and treated with cyclophosphamide only (1990-1996) or switched to azathioprine after 3 months of remission (1997-2000).

Included were 128 patients of whom 44 (34%) switched to azathioprine. Fifty-three patients (41%) relapsed. Actuarial disease-free survival at 2 and 4 years was 76% and 65% in the cyclophosphamide group compared to 76% and 51% in the azathioprine group (log-rank test: RR 1.4, 95% CI 0.8-2.7; p=0.20). Relapses were more frequent in patients with PR3 (n=93) as compared to MPO-ANCA (n=35) specificity (RR 3.2, 95% CI 1.4 - 4.4). In PR3-ANCA associated vasculitis a positive as compared to a negative ANCA titer at 12 months tended to be associated with relapse (RR 1.7, 95% CI 0.9-3.0). In patients with PR3-ANCA associated vasculitis switched to azathioprine (n=33) a positive PR3-ANCA titer at the moment of treatment switch was significantly associated with relapse (RR 2.6, 95% CI 1.1 - 8.0). In patients with a negative ANCA titer at treatment switch disease-free survival at 2 and 4 years was 80% and 62%, and nearly identical to patients treated with cyclophosphamide only. In patients ANCA positive at switch disease-free survival was only 58% and 17%.

We conclude that our data, although retrospective and not from a controlled randomized trial, seriously question the safety of switching cyclophosphamide to azathioprine after 3 months of remission in patients with PR3-ANCA associated vasculitis who are still ANCA positive at treatment switch.

79-098

PATIENTS WITH P-ANCA/ANTI-MPO POSITIVE MICROPOLYANGIITIS (MPA) ARE AT HIGHER RISK TO DEVELOP HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

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In our center 2/5 patients newly exposed to heparin because of hemodialysis in year 2000 who develop HIT had p-ANCA/anti-MPO positive MPA. Moreover among the few published cases with HIT because of heparin exposure due to hemodialysis 5 had ANCA-positive vasculitis (Roe SD, NDT, 1998: 3226. Burdese M, Giornale Italiano di Nefrologia, 2001; S18: S25). HIT clinical diagnosis can be confirmed with the positive test for antibodies against the PF4/heparin antigenic complex. We tested sera from 41 pts with p-ANCA/anti-MPO positive MPA. 12 pts with C-ANCA positive vasculitis were also tested. Tests were performed by ELISA (HPIA-Diagnostica Stago, France). To exclude interferences on the test by high titers of ANCA, tests were performed either in pts not exposed to heparin or in pts in the inactive phase of the vasculitic disease. 26/41 pts with p-ANCA/anti-MPO positive MPA were exposed to heparin because of hemodialysis and/or plasmafiltration, 9 (35%) had a positive test for anti-PF4/heparin antibodies, all in the group with active disease (19 pts). None of 15 pts with p-ANCA/anti-MPO positive MPA not exposed to heparin had a positive test. None of 12 C-ANCA/anti PR3 positive pts (8 exposed and 4 unexposed to heparin) had a positive test. The 9 positive pts (3 males and 6 females, age 15-84) were all exposed to heparin because of hemodialysis.Two pts were also exposed because of plasmafiltration. Thrombocytopenia (< 50% of the initial platelet count) developed in all pts, 6-30 days after the first exposure and was generally not severe. Mild to moderate thrombocytopenia was the only manifestation in 4 pts. 3/ 9 pts developed repeated clot formation in the dialyser and extracorporeal circuit despite adequate doses of heparin. Two pts died, one because of severe pulmonary embolism complicating iliac-femoral thrombosis of the leg where the catheter for hemodialysis was placed; the other pt had subarachnoid hemorrhage and died because of stroke complicating severe cerebral vasospasm. The 2 pts who died had very high titers of anti-PF4/heparin antibodies.

In conclusion, pts with p-ANCA/anti-MPO positive MPA, in the acute phase of disease, when exposed to heparin for hemodialysis and/or plasmafiltration, are at risk to develop HIT. The risk seems to be higher than in non-vasculitic uremic pts. In some of these pts, HIT can be responsible or contribute to substantial morbidity.

80-099

THREE PATIENTS WITH SPLEEN NODULES AND NEGATIVE ANCA TEST: ATYPICAL MANIFESTATION OF WEGENER'S GRANULOMATOSIS?

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Wegener's granulomatosis (WG) is a systemic vasculitic disease potentially affecting any organ system, therefore its clinical presentation is highly variable. The presence of positive ANCA test is of great value for the diagnosis, especially in atypical presentations. Spleen involvement has been described in WG, usually in patients with typical generalized disease. We report three patients (pts), two female and one male, 32, 31 and 19 years old, all ANCA negative, who presented spleen nodules as main manifestation. Spleen appeared slightly enlarged with several, hypoechogenic areas, 1 to 3.5 cm in diameter, solid or colliquated. The nodules tended to increase in number and size during time. Besides spleen involvement, patients presented painful subcutaneous nodules on legs and arms (3 pts), vulva (1 pt), and scrotum (1 pt). Lymph node involvement was present in two pts (mediastinal associated with peritoneal in 1 pt and retroperitoneal in 1 pt) and rapidly evolved in colliquated masses. A single liver nodule, 3.5 cm in diameter, with central necrosis, was found in 2 pts. Two pts showed lesions in sites typically involved in WG, represented by multiple cavitating lung nodules in 1 pt and by transient rhinitis with crusting in the other. All three patients presented high grade fever with remittent course, profuse night sweats, weight loss and increase of inflammation indexes. Multiple biopsies from the organs involved were taken in all three patients. Tissue necrosis was a primary component of the disease. The necrosis had geographic pattern with large, irregular areas extensively replacing the parenchyma. The necrotic centre varied from caseation-like to suppurative. Epithelioid histiocytes and rare multinucleated giant cells were present at the periphery of necrotic foci, with a palisading arrangement. Histological criteria diagnostic for WG (vasculitis, microabscesses and scattered multinucleated giant cells in a highly inflammatory background) were only identified in the nasal biopsy from a single patient. Stains for micro-organisms and search for mycobacterium using PCR resulted invariably negative. Extensive serological test for infectious diseases were all negative. Pts recovered after combination treatment with corticosteroids and cyclophosphamide. Two pts with a follow-up of 11 and 13 years had several relapses at time of tapering or stopping treatment and currently are on continuous low-dose therapy. ANCA persisted always negative. An extensive work-up, developed in many years, couldn't identify a definitive diagnosis in all these cases. Could they represent an atypical form of WG?

81-100

HISTOPATHOLOGICAL FEATURES OF TRACHEAL BIOPSIES IN SUBGLOTTIC STENOSIS

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Subglottic stenosis can be the early or the only manifestation of Wegener granulomatosis (WG). As in other forms of localized WG disease ANCA test can be negative and only histology can provide the diagnosis of WG. According to literature data, only rarely can tracheal biopsies be truly diagnostic. We reviewed the tracheal biopsies related to 33 patients with symptomatic subglottic stenosis admitted to the Center of Respiratory Endoscopy and Laser Therapy. All the biopsies were obtained at the time of

mechanical assisted laser resections. Patients were classified as Wegener granulomatosis (WG)(14 pts) on the basis of ANCA-positive test and/or typical clinical manifestations of WG other than subglottic stenosis such as rhinitis with crusting, orbital disease, bronchial stenosis, with/without histological confirmation. All other pts were classified as idiopathic stenosis (IS) (19 pts). A total of 76 biopsies stained with hematoxylin-eosin, elastic stain, and special stains for microorganisms were analyzed. Histological features were classified as to "diagnostic" for WG (dWG) (microabscesses/necrosis/palisated granulomata; giant cells unassociated with granulation tissue or foreign bodies, and vasculitis), or "non-specific" (NS) (inflammatory infiltrate, microthrombi, granulation tissue with/without giant cells, and fibrosis).

Within the WG group, 8 out of 14 pts (57%) showed at least one dWG feature, and only two of them showed all dWG features. Within the IS group, 4 out of 19 IS pts (21%) showed at least one major feature and a single pt presented all dWG features in his biopsies. Among other histological changes, tissue fibrosis was observed with similar frequency in the two groups (11/14 and 15/19 in WG and IS, respectively); the fibrosis occasionally involved small vessels (vascular "scars") (1/14 in WG and 4/19 in IS).

Conclusions: (1) In WG pts, although tracheal biopsies are less informative than nasal biopsies and rarely show features fully diagnostic for WG, they show in a considerable number of cases at least one diagnostic feature. (2) The majority of IS pts show non-specific changes in their biopsies and remain completely undefined both clinically and pathologically. The occurrence of fibrosis involving the small vessels in a minority of pts suggests that possible vascular damages may occur during the course of the disease, but definite proof of this hypothesis is totally lacking. (3) The small group of IS pts showing one or more dWG features may represent truly real cases of WG with a localized form of the disease.

82-101

RECOMBINANT PROTEINS TO ANALYZE AUTOANTIBODIES TO PROTEINASE 3 IN SYSTEMIC VASCULITIS

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Introduction: Anti-neutrophil cytoplasmic autoantibodies (ANCA) directed to proteinase 3 (PR3-ANCA) are closely associated with the systemic necrotizing vasculitides, in particular Wegener's granulomatosis (WG). The presence of PR3-ANCA in serum is usually detected by ELISA with native PR3 as a substrate. As the isolation of native PR3 from neutrophils is laborious and expensive, development of ELISA with recombinant PR3 might be a good alternative. The aim of our study was to test the usefulness of recombinant PR3 ELISA for the detection of PR3-ANCA in systemic vasculitis.

Methods: We analyzed sera of 114 patients with ANCA-associated vasculitis, including 90 with WG, 12 with microscopic polyangiitis (MPA), 6 with Churg-Strauss syndrome (CSS), and 6 with necrotizing crescentic glomerulonephritis (NCGN). All samples were collected at the moment of diagnosis. Together with sera of 20 healthy controls and 59 disease controls (20 with systemic lupus erythematosus, 20 with ulcerative colitis, 19 with autoimmune hepatitis) they were tested for PR3-ANCA by direct ELISA using native PR3 and two recombinant forms of PR3, and by capture ELISA using native PR3, two recombinant forms of PR3, and a crude extract of azurophilic granules. Recombinant antigens were expressed in the baculovirus system and one of them (recPR3-1) was an enzymatically inactive mutant.

Results: 4-6 of 79 and 3-4 of 79 control sera tested positive in direct and capture ELISA, respectively. Most of the sera positive in direct ELISA were derived from patients suffering from autoimmune hepatitis. In the patient group, we observed a correlation between the results of direct and capture ELISA, which was the case for all antigens used. However, capture ELISA was a slightly more sensitive test for PR3-ANCA than the direct assay (see table).

TABLE.
POSITIVE SAMPLES. CUT-OFF VALUE WAS DEFINED AS MEAN + 2 SD OF DISEASE CONTROL GROUP (N=59)

	Direct ELISA			Capture ELISA			
	Native PR3	RecPR3-1	RecPR3-2	Granule extract	Native PR3	RecPR3-1	RecPR3-2
WG n=90	67	53	47	69	71	57	51
MPA n=12	3	3	2	4	5	4	4
CSS n=6	0	0	0	2	2	2	2
NCGN n=6	1	1	0	0	1	1	1

RecPR3-1 and recPR3-2 performed less well than native PR3 in both direct and capture assay. Despite being an enzymatically inactive mutant, recPR3-1 was more efficient in detecting PR3-ANCA than recPR3-2. The results of capture ELISA obtained with two different monoclonal anti-PR3 antibodies were similar.

Conclusions: In this study, we show that ELISA with native PR3 is more sensitive assay for PR3-ANCA in systemic vasculitis than the same test using recombinant forms of PR3. Irrespective of the antigen used, capture ELISA gives more positive results than direct assay. These results suggest that ELISA with native antigen still remains a method of choice for the detection of PR3-ANCA. However, the further optimization of these assays, especially with recombinant antigens, might lead to improvement of their sensitivity and specificity.

83-103

PERFORMANCE OF THE BIRMINGHAM VASCULITIS ACTIVITY SCORE FOR WEGENER'S GRANULOMATOSIS (BVAS/WG) IN A RANDOMIZED CLINICAL TRIAL

The Wegener's Granulomatosis Etanercept Trial (WGET) Research Group

Objective: To evaluate the performance of a disease-specific activity score for WG in patients enrolled to date in a randomized, double-masked, placebo-controlled clinical trial. To preserve the masking in this ongoing trial, the analyses reported include only comparisons of patients with severe versus limited disease.

Methods: The BVAS/WG is scored by physician-investigators at the baseline, 6-week, and 3-month visits, and then at every 3 months until the common trial closeout. Disease activity of WG is scored in 9 separate organ systems, with additional space for adding other items not listed on the form. Only items attributed to active WG are scored in BVAS/WG (damage, adverse effects of treatment, and intercurrent medical problems are recorded elsewhere). Items scored are categorized as either new/worse or persistent. The BVAS/WG also provides a framework for categorizing patients with WG as "severe" or "limited" based on the presence of organ-threatening disease and the need for cyclophosphamide therapy. A physician global assessment (PGA) of disease activity, scored on a 10 cm visual analog scale, is also performed at each visit.

Results: The mean BVAS/WG at entry among the first 116 patients enrolled was 7.0 (median: 6; Q1-Q3: 4,9). Patients whose disease was classified as severe at trial entry (N = 85) had a mean BVAS/WG of 8.0 (median: 7; Q1-Q3: 6,10), compared with 4.2 (median: 4; Q1-Q3: 3-5) for those with limited disease (N = 31)(P = 0.0001). The differences between the severe and limited patients were reflected in higher PGA scores at baseline. The mean PGA among the severe WG patients was 6.1 cm (median: 6.6; Q1-Q3: 4.8-7.8), compared with 4.3 cm (median: 4.1; Q1-Q3: 2.7-5.6) among those with limited disease. For BVAS/WG and PGA at baseline, the correlation coefficient was 0.60 (P < 0.0001). Mean duration of disease since diagnosis was substantially longer among patients with limited WG (22 months), compared to those with severe disease (2.7 months).

Conclusions: Correlation between the BVAS/WG and PGA of disease activity is high. Patients with limited WG have substantially lower BVAS/WG scores at baseline. This may correlate inversely with disease damage, which likely reflects duration of disease.

84-109

DOES CRYOGLOBULINEMIA REPRESENT AN EXCLUSION CRITERION FOR THE DIAGNOSIS OF POLYARTERITIS NODOSA (PAN)?

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Objective: To analyze the significance of mixed cryoglobulins (MC) detection in patients with PAN.

Method: We retrospectively analyzed 580 patients from the French Vasculitis Study Group. Eight patients who met the ARA criteria for the diagnosis of PAN had histologically documented fibrinoid necrosis of small- and/or medium-sized arteries and type II/III MC; one also had mesenteric and renal arteries microaneurysms.

Results: Three patients had transient low-level (< 100 mg/l) MC III with normal C4 levels and negative rheumatoid factor; 2 patients had PAN with multiple relapses and chronic high-level (≥100 mg/ml) type II/III MC, with low C4 levels and rheumatoid factor; 1 had high-level MC II and lymphoplasmacytic lymphoma; 1 had MC II and was co-infected with hepatitis B and hepatitis C viruses; 1 had HIV-related PAN, low-level MC III and normal C4.

Conclusion: Distinguishing essential MC from classic PAN may be difficult. Fibrinoid necrotic lesions may occur in cryoglobulinemia-related vasculitis and are not specific to PAN. The detection of cryoglobulins during the course of PAN must lead to a search for B-cell lymphoproliferation or chronic viral infection, which can be the cause of vasculitis. We propose that MC > 100 mg/l associated with a low C4 level could represent, in the absence of microaneurysms and viral infection, an exclusion criterion for the diagnosis of PAN.

85-110

CLINICAL STUDY OF PATIENTS WITH SYSTEMIC VASCULITIS ADMITTED TO INTENSIVE CARE UNIT

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Objective: To evaluate presenting features and the abilities of disease-activity scores to predict outcome of patients with systemic vasculitis (SV) admitted to intensive care unit (ICU).

Methods: The medical charts of all patients with SV followed in a University Hospital (Hôpital Avicenne) and admitted to ICU between 1982 and 2001 were retrospectively reviewed for clinical presentation, intensive care severity scores (APACHE II, SAPS II), Birmingham Vasculitis Activity Score (BVAS) and outcome.

Results: Twenty-six patients (M/F: 1.6; age: 45.2 ± 16.2 yr; mean duration of SV: 28.4 ± 74.9 mo) with SV (Wegener's granulomatosis n=4; microscopic polyangiitis n=4; polyarteritis nodosa n=3; HBV-related polyarteritis nodosa n=3; Churg-Strauss syndrome n=7; cryoglobulinemic vasculitis n=2; and others n=3) were admitted to ICU for active vasculitis (n=20; 77%) with predominantly pulmonary (50%) and/or renal (40%) involvement; infection (n=3; 12%) and miscellaneous (n=3; 12%). SV was diagnosed in 11 (42%) patients in the ICU. Four (15%) patients died in the ICU and the total mortality rate was 38% after a follow-up of 30.7 ± 29.9 mo. Mean disease-severity scores at ICU admission calculated for early (ICU) and late (end of follow-up) survivors and non-survivors are reported in the table.

Score at ICU admission	ICU			End of follow-up		
	Survivors	Non-survivors	p[a]	Survivors	Non-survivors	p[a]
APACHE II	15.7±6.7	28.0±9.27	0.01	16.4±7.37	19.89±9.93	0.55
SAPS II	26.0±10.95	50.0±22.05	0.02	25.87±11.82	36.89±19.40	0.21
BVAS[b]	18.0±9.67	31.0±14.85	0.13	14.67±4.62	26.87±12.96	0.02

[a] Non-parametric Kruskal-Wallis test.
[b] Only for patients admitted to the ICU for active vasculitis.

Conclusion: The main reason for ICU admission was active

vasculitis, often the first disease manifestation, leading to its diagnosis. The standard intensive care severity scores were able to predict in-hospital mortality, but not overall mortality at the end of follow-up. For patients admitted to the ICU for active vasculitis, BVAS was associated with long-term outcome.

86-111

MICROSCOPIC POLYANGIITIS (MPA) AND POLYARTERITIS NODOSA (PAN): HOW AND WHEN DO THEY START?

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Objectives: To describe the first clinical symptoms attributable to MPA or PAN, and to determine the time to diagnosis and its impact on outcome.

Methods: We retrospectively reviewed the medical files of 75 patients (mean follow-up: 6.9 yr) with biopsy-proven MPA (n = 37) or PAN (n = 38, including 26 related to hepatitis B virus infection). The first clinical signs attributable to vasculitis, the clinical signs at the time of diagnosis, time to diagnosis, subsequent relapse(s) and survival were recorded. The relapse and mortality rates were also analyzed as a function of the median time to diagnosis. Statistical analyses were performed, when appropriate, with chi-square, Student's t- and log-rank tests.

Results: General symptoms (fever ≥38°C, weight loss, fatigue) were the most common findings at disease onset (71%), followed by myalgias/arthralgias (61%), and neurological (24%), cutaneous (13%) and gastrointestinal manifestations (13%); 8% had only general symptoms. Initial manifestations were similar in both entities except for gastrointestinal symptoms and peripheral neuropathy which were more frequent in PAN ($p = 0.01$ and $p = 0.03$, respectively). The mean time to diagnosis was 269 d (median: 90; range: 7-2550). Overall mortality and relapse rates were 33 and 39%; these rates did not differ significantly between MPA and PAN. Time to diagnosis ≥90 d was not associated with different clinical features at the time of diagnosis or with an increased risk of mortality, but tended to predict a greater risk of subsequent relapses ($p = 0.05$).

Conclusion: It appears that MPA and PAN initially start with non-specific symptoms that frequently last for several months before the diagnosis is made. That a longer time to diagnosis tended to predict a higher relapse rate suggests the existence of a subgroup of patients with a less acute but more refractory disease.

87-122

ANCA STATUS, LEUKOTRIENE RECEPTOR ANTAGONIST USE, AND CHURG-STRAUSS SYNDROME

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Rationale: To review the clinical course of Churg-Strauss Syndrome (CSS) and correlate ANCA status and leukotriene receptor antagonist use with the disease process.

Methods: A retrospective chart review was performed of all patients seen at our institution since 1990, who carried or were assigned the diagnosis of CSS.

Results: 96 patients had symptoms suggestive of CSS. 82 met either the American College of Rheumatology's (ACR) criteria (78/82, 95%), or Lanham's criteria (61/82, 74%), or the Chapel Hill classification scheme (58/82, 71%). Of the 82 patients, 39 were female and 43 male. The average age at diagnosis was 49 years (range 10-77). ANCA testing was performed in 66 patients. 25/66 (38%) were P-ANCA positive and 1/66 was C-ANCA positive. Of patients who were tested for ANCA at the time of initial diagnosis 17/25 (68%) patients were ANCA positive. 11/23 (48%) tested during a vasculitic flare were positive, and 1/40 (3%) tested during remission were ANCA positive. Leukotriene receptor antagonists (LRA) were used by 21/82 (26%) of patients. In 13 they were started before the diagnosis of CSS. Of the 7 cases started after diagnosis, 2 had a vasculitic relapse, and 5 remained in remission. In 1 patient the time course of LRA usage was unclear. All patients received corticosteroids with a further 42/82 (51%) requiring other immunosuppressants, most commonly cyclophosphamide.

Conclusions: The ACR were the most inclusive criteria for the diagnosis of CSS. ANCA if present appears to correlate with disease activity, but it doesn't appear to correlate with specific disease manifestations. The pathogenic role of leukotriene inhibitors in CSS also remains unclear, with almost one quarter of the patients receiving it, in this study, apparently suffering no ill effect.

Treatment and Outcomes

88-004

IS NAILFOLD VASCULITIS (NFV) IN RHEUMATOID ARTHRITIS BENIGN?

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Background: Vasculitis is a well-recognized and potentially serious complication of rheumatoid arthritis. Vessels of all sizes can be affected and as a consequence the clinical manifestations may vary, from isolated NFV due to digital endarteritis through to a necrotizing arteritis with internal organ and peripheral nerve involvement—systemic rheumatoid vasculitis (SRV). NFV is generally considered to be benign, requiring simply observation. We have previously reported a favorable prognosis in a cohort of patients after a mean follow-up of 22 months. The aims of this study were to re-evaluate this cohort of patients after a longer follow-up.

Methods: Clinical details were obtained by retrospective case note review of NFV and SRV patients, previously identified prospectively between 1988-94. SRV was diagnosed using the Scott and Bacon criteria. NFV was not treated with cytotoxic agents.

Results: 29 patients (16 males) with NFV were followed

for a median of 86.5 months from diagnosis of NFV (Table). One patient developed SRV with mononeuritis multiplex 65 months after NFV was diagnosed and died 5 months later. One patient developed a vasculitic leg ulcer and died after 120 months. In neither case was a cause of death available. 14 patients developed extra-articular manifestations (including Sjögren's syndrome - 8, bronchiectasis - 2). There was significant mortality in both groups, but in the NFV group this was not due to active RA.

Conclusions: This study confirms that isolated NFV can be regarded as a benign condition with a low long-term risk of developing SRV. Observation of NFV does not warrant introduction of cytotoxic therapy. Extra-articular disease occurs frequently in this group of patients. The high mortality was not attributable to active RA, but this was an elderly cohort of patients.

TABLE

	NFV	SRV
No. of patients	29	47
Sex (M/F)	16/13	25/22
Median duration of RA (yrs)	10 (<1-40)	12 (<1-40)
Seropositive (%)	97	89
Nodules (%)	73	57
Mortality M/F (%)	56/46	80/57
Median interval diagnosis NFV/SRV to death (mos)	67	45

89-005

PREDICTORS OF REMISSION AND RELAPSE IN WEGENER'S GRANULOMATOSIS

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Aim: To study the course of disease activity in a population-based cohort of Wegener's granulomatosis (WG) patients and describe predictors for complete remission and relapse.

Methods: Retrospective study of 56 WG patients (62.5% males, median age 50 years) of whom 52 survived 3 months and were followed for 45.5 months (6-173). Disease activity was assessed by Birmingham Vasculitis Activity Score (BVAS-1) and permanent organ damage by Vasculitis Damage Index (VDI). Induction therapy consisted of prednisolone (Pred) 0.5-1 mg/kg and cyclophosphamide (CYC) daily orally 2mg/kg (19 patients) or IV pulses 15mg/kg every 2nd week (32 patients). Baseline clinical and laboratory features and cumulative treatment during the first 6 months were recorded. Simple and multiple regression analyses were used to find risk factors (hazard ratio [HR] or odds ratio [OR] with [95% confidence interval] for remission and relapse by Cox proportional hazards model or logistic regression analyses). Data are given as median (range).

Results: There was no baseline difference between the two CYC-treatment groups, except that pulse treated patients had higher BVAS-1 scores (27 vs 23, P=0.02). All patients achieved either complete (85%) or partial remission (15%). Higher baseline BVAS-1 increased the chance of complete remission (BVAS-1 increase by 5 points, HR=1.22 [1.05-1.42]), while cumulative dose of CYC during the first 6 months was associated with increased chance of sustained complete remission (dose increase by 5 gram, OR=1.76 [1.10-2.82]). Relapse occurred in 31 patients (59.6%) after 18 months (4-108). The risk of relapse did not decline over time, but the risk was reduced with longer time on Pred >20mg/day during the first 6 months (increase by 1 month, HR = 0.78 [0.62-0.98]) and increased in patients with heart involvement (HR = 2.87 [1.09-7.58]). Therapy resistance, defined as death within 3 months or never achieving complete remission, was associated with baseline organ damage (VDI increase by 1 point, OR = 1.53 [1.03-2.27]).

Conclusion: Initial high disease activity increased, and the presence of baseline organ damage reduced the chance for complete remission in WG. Sustained remission was associated with more intensive initial treatment in terms of higher CYC doses and longer time on Pred >20mg/day.

90-011

LONG-TERM OUTCOME OF PATIENTS WITH ANCA-POSITIVE VASCULITIS (WEGENER'S GRANULOMATOSIS [WG], MICROSCOPIC POLYANGIITIS [MPA]) AND DOCUMENTED RENAL INVOLVEMENT

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Objectives: Comparison of renal outcome and survival in a large cohort of patients (pat) with WG and MPA during the last 20 yrs: 92 pat with WG (53 m, 39 f; median age 54 yrs [11-79]) and 34 pat with MPA (16 m, 18 f; median age 60 yrs [17-81]). Follow-up: median of 71 months (mo). Initial s-crea ≥3 mg: 38/92 pat with WG and 15/34 pat with MPA. Treatment: (modified) Fauci scheme; median cyclophosphamide (cyc) dosage 36.5 g (0.75-412) within a median of 17.7 mo in 91/92 pat with WG and in 33/34 pat with MPA, followed by azathioprine in 34/91 pat with WG and in 11/33 pat with MPA. Steroid doses: median dosage of 9.7 g (0.75-111.9) for a median of 28 mo (0.2-141).

Results: Clinical remission was obtained in 83/92 pat with WG and 30/34 pat with MPA after a median of 5 mo. Initial dialysis (D) was required in 23/92 pat with WG and in 6/34 pat with MPA. Recovery of renal function was obtained in 22/23 pat with WG and in 3/6 pat with MPA; both groups had initial D during a median of 30 days (1-480). Plasmapheresis was performed in 5/92 pat with WG. End-stage renal failure was observed in 19/92 pat with WG (initial D: 10/19) and in 9/34 pat with MPA (initial D: 5/6). Renal relapse (reappearance of nephritic sediment) within 5 years was seen in 60% of pat with WG and in 56% of pat with MPA. Repeat renal biopsy was performed in 13/91 pat with WG (10/13 de novo IgA, 1/13 de novo M Goodpasture) and in 7/34 pat with MPA. Death due to disease activity was observed in 3/16 pat with WG and 1/6

pat with MPA, due to infection in 2/16 pat with WG and 1/6 pat with MPA. Malignancies were observed in 8/92 pat with WG (1 leukemia, 4 bladder-carcinoma (ca) in pat with cyc treatment >2 yrs (3 cured), 1 colon-ca, 2 prostate-ca) and in 2/34 pat with MPA(both squamous-cell ca [both cured]). Side effects of steroid therapy were: cataracts in 14% of pat with WG and 8.8% with MPA; osteoporosis and/or aseptic osteonecrosis in 14.1% of pat with WG and 11.8% with MPA; diabetes mellitus (inherited in 9/25 pat with WG and in 6/10 pat with MPA) in 27.2% of pat with WG and 29.4% with MPA.

Conclusion: The renal relapse rate of patients with ANCA-positive vasculitis was high. Reappearance of nephritic sediment in WG was not always indicative for relapse of ANCA-positive vasculitis. Mortality in the elderly population was mainly related to non-vasculitic causes. Renal outcome in patients with MPA was not different from that of WG.

91-020

PAUCI-IMMUNE NECROTIZING CRESCENTIC GLOMERULONEPHRITIS IN THE ELDERLY

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Aim of the study: to determine clinical, biological and histological characteristics of pauci-immune glomerulonephritis in patients older than 70 years.

Methods: Retrospective study including 46 patients from 5 nephrologic centers suffering from microscopic polyangiitis (39), Wegener's granulomatosis (6) and Churg and Strauss syndrome (1). Statistical analysis was performed using a chi-square test of Pearson or Fisher exact model to test the association of categorical variable, whereas a t-test was used for continuous variables.

Results: Mean age was 76 +/- 4.6, sex ratio was 1.2 (F/M). Interval between onset of symptoms and diagnosis was 10.7 +/- 18.5 months. Number of organs involved (kidney included) was 2.3 +/- 1.1. Constitutional symptoms (fatigue, weight loss and fever) were common. Extra-renal symptoms were less frequent: lung involvement (39.1%), purpura (23.9%) and arthralgias (13.4%). Laboratory signs of inflammation were constant (CRP = 68.8 +/- 55.92 g/l, leukocytosis = 11481 +/- 5886/mm^3, Hb = 8.5 +/- 1.56 g/l). ANCA were found in 20/21 patients. Hematuria and proteinuria (1.6 +/- 1.5 g/24h) were always detected. Mean serum creatinine level at the time of diagnosis was 525.1 +/- 303.6 µmol/l. Renal biopsies usually showed irregular glomerular lesions of various ages. Necrotic lesions were more often segmental than global. Percentage of normal glomeruli was 18.3 +/- 18.7%. Percentage of global sclerotic glomeruli was 44.7 +/- 25.8%. Interstitial fibrosis (scored 0 to 2) was 1.3 +/- 0.77. Treatment included corticosteroid therapy (40/46) and cyclophosphamide (30/46). Six patients under dialysis and without extra-renal symptoms were not treated.

Twelve months after the diagnosis, outcome was chronic renal failure (45.7% of patients; mean creatinine clearance: 32.73 ml/min), chronic dialysis (23.9%), and death (30.4%). Causes of early death were infection (6 patients), cardiovascular complications (4), neoplasm (1) and interruption of renal replacement therapy (2). The predictors of renal survival were entry serum creatinine value (>500 µmol/l; P=0.01) and proteinuria (>1.5 g/24h; P=0.01). The predictor of patient survival was albuminemia (<28 g/l; P=0.01).

Conclusion: Pauci-immune necrotizing crescentic glomerulonephritis in the elderly is a slowly progressive disease, more often restricted to the kidney, with delay in diagnosis and therefore delay in instituting effective treatment. Early diagnosis (detection of hematuria, ANCA and renal biopsy) and early treatment may improve the disastrous outcome of this disease.

92-033

EFFECTIVE THERAPY FOR SUBGLOTTIC STENOSIS IN WEGENER'S GRANULOMATOSIS

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Background: Subglottic stenosis (SGS) occurs in ~20% of pts with WG. In general, systemic therapy for SGS has been ineffective. Tracheostomy has been required in ~50% of cases. One previous study (Langford et al, A&R 1996) demonstrated utility of intralesional depocorticosteroid injection and dilation (ILGCS-D) for WG-SGS lesions. Until now, other studies have not been performed to confirm or refute the utility of ILGCS-D.

Patients and Methods: 14 patients with WG and critical SGS (<3-4 mm patency), dyspnea or stridor, received ILGCS-D by the same surgeon (IE). SGS was visualized under general anesthesia (jet ventilation, suspension laryngoscopy). Aliquots of 40-80 mg depomethylprednisolone were injected into the stenotic lesion in each of 4 quadrants, followed by progressive mechanical dilation. Repeat ILGCS-D was provided if symptoms recurred or severe "silent" restenosis was noted on follow-up examination.

Results: In 8 patients in whom ILGCS-D was the first intervention, tracheal patency was maintained after 1 or 2 dilatations (mean F/U=24.5 months, range = 8 months-6 years). In 6 patients who had prior procedures, including 3 tracheostomies, extensive scar formation led to less satisfactory results. Tracheostomy closure not was achieved in the 3 patients and 3 required multiple (up to 3) dilations before patency was maintained. There were no complications associated with therapy. Hospital discharge was possible on the day of the procedure.

Conclusions: SGS intralesional depo-GCS injection and dilation are effective combined therapies in WG, especially when applied to newly recognized lesions. Although results are less encouraging when applied in the setting of prior scarifying therapies, treatment may be useful in select cases. Our experience is the first to confirm that previously reported in a similar

cohort. We propose that intralesional depo-GCS and dilation be considered as 1st line therapy for WG-SGS.

93-035

AN AUDIT OF INITIAL ASSESSMENT IN WEGENER'S GRANULOMATOSIS

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Background: Accurate initial assessment of organ involvement in Wegener's granulomatosis (WG) is essential to determine immediate and future management.

Objectives: We audited the initial assessment of patients referred to the Rheumatic Diseases Unit (RDU) subsequently diagnosed with WG. As no published gold standards for assessment exist, the following essential items were chosen following consensus discussion: blood pressure (BP), urinalysis (and microscopy if hematuria present), serum creatinine, chest radiograph (CXR) and tissue biopsy.

Methods: A search of the RDU computer database identified 35 patients with WG excluding those with incomplete medical records and re-referrals. Medical records and CXRs of these patients were reviewed to establish if basic investigations had been performed and, if so, their results.

Results: 19 F:16 M. Mean age 53, range 13-85. Features at presentation (%): Ear, nose and throat (94), arthralgia (66), cutaneous (51), renal (40), ocular (29), pulmonary (20) and neurological (17). Results of basic investigations are presented in the table. In 29% (10/35) of patients at least one test was not performed. 11% (4/35) of patients had hypertension (diastolic BP > 99) and in none were recommendations made for BP to be rechecked or treatment to be commenced. 6% (2/35) of patients with microscopic hematuria did not have urine microscopy or a renal biopsy performed. All patients with an elevated creatinine were further investigated. 9 patients had an abnormal CXR: 1 had evidence of previous tuberculosis, 4 had active pulmonary disease and 4 were further investigated.

Conclusion: 71% of patients referred to a tertiary rheumatology centre and later confirmed to have WG received adequate basic investigation at presentation. Patients found to be hypertensive should have their BP repeated and those with hematuria should have urine microscopy performed. These results indicate that we should be more vigilant in performing basic tests in patients with suspected WG. In this regard, these data have been presented to our department and the audit will be repeated in 2005 to reassess the recording of basic investigations in patients with WG.

	BP	Urine	Creatinine	CXR	Biopsy
Test performed (no. of patients)	33/35	33/35	31/35	31/35	30/35
Test performed (% of patients)	94	94	89	89	86
Test abnormal (% of patients)	11	46	23	26	87

94-044

THE USE OF INTERFERON-ALFA-2B/PEGYLATED INTERFERON AND RIBAVIRIN TREATMENT IN RENAL DISEASE AND CHRONIC HEPATITIS C INFECTION WITH OR WITHOUT CRYOGLOBULINEMIA – A CASE SERIES

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Background: Hepatitis C virus (HCV) infection is associated with extrahepatic manifestations as membranoproliferative glomerulonephritis (MPGN) with or without cryoglobulinemia, membranous glomerulonephritis (MGN) and FSGS. Standard treatment for HCV is interferon and ribavirin, but in renal insufficiency ribavirin has been contraindicated due to fear of side effects.

Patients and methods: 7 patients, 2 with cryoglobulinemia and vasculitic manifestations as skin and oral ulcers, neuropathy, cerebral vasculitis, arthralgia, fever and glomerulonephritis (GN), 3 with MPGN, 1 with MGN and 1 with FSGS were treated with a combination of interferon and ribavirin. 2 patients were given pegylated interferon (PEG) and ribavirin. Most patients had renal insufficiency at presentation, with GFR between 10-75 mL/min. One patient had HCV genotype 1, the remainder 2 and 3. Duration of therapy was according to genotype (6-12 months). Ribavirin in plasma was monitored by HPLC throughout the treatment to avoid overdosing, aiming at a target concentration of 10-15 µmol/L. The main side effect of ribavirin, hemolytic anemia, was monitored closely with Hb controls.

Results: 7/7 patients became HCV-RNA-PCR negative and 3/7 (MPGN and FSGS) have maintained both renal and virological remission. One vasculitis patient responded with complete remission, but relapsed virologically and has had a minor vasculitic flare after 9 months. The other, who recently received PEG, improved with regard to vasculitic symptoms and proteinuria, but still has low-grade CRP of unclear significance. The MGN patient currently treated with PEG is in virological remission and improving. Finally, one MPGN patient didn't tolerate interferon, but is in renal remission with low-dose ribavirin. Only one vasculitis patient had low-dose immunosuppression in addition to anti-viral therapy. Average daily ribavirin dose was 200-800 mg. Hb was maintained in all patients with adequate iron stores and erythropoietin up to 20,000 IU/week.

Conclusions: Interferon and ribavirin can, with ribavirin monitoring, safely be used in HCV-related vasculitis and glomerulonephritis irrespective of renal function. Patients with cryoglobulinemia and vasculitis might benefit from longer treatment than indicated by HCV genotype only.

95-045

ANTI-TUMOR NECROSIS FACTOR (ANTI-TNF) THERAPY IN TAKAYASU'S ARTERITIS (TA) RESISTANT TO CONVENTIONAL THERAPY

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TA may cause stenosis, dilatation or aneurysms of large vessels. Many patients (pts) fail to sustain remission despite glucocorticoid (GCS) and cytotoxic therapy.

Goal: Assess anti-TNF therapy in TA.

Methods: Pilot study in 5 pts. Treatment: Etanercept 25-50 mg BIW. Infliximab: 1 pt, up to 7 mg/kg Q8wks.

Results: 1M, 4F, 20-38 y.o. TA duration pre-anti-TNF = 5.2 yrs (M). Prior relapses: 1-12 (M=5.5). Before study, relapses when prednisone <21 mg QD (M). Previously also failed methotrexate, azathioprine, cyclosporin, tacrolimus or mycophenolate. After anti-TNF, time to unprecedented improvement <2mos. Follow-up: anti-TNF therapy = 23.6 (M, 18-27) mos. 1 pt refused GCS re-treatment. 3 others tapered and discontinued GCS. 5th in remission on 5 mg of prednisone QD. Duration remission (M) = 13.4 mos. Remission off GCS (4pts) = 6.8 mos.

Conclusion: Anti-TNF therapy appears to be a useful adjunct to GCS in TA.

96-054

BLOCKING TNF-ALPHA WITH INFLIXIMAB RESULTS IN SUCCESSFUL TREATMENT OF CYCLOPHOSPHAMIDE-REFRACTORY WEGENER'S GRANULOMATOSIS

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Objective: To study the effect of the chimeric monoclonal anti-TNF-α antibody infliximab in Wegener's granulomatosis (WG) refractory to standard treatment with cyclophosphamide and high-dose corticosteroids for the induction of remission.

Patients and Methods: 6 patients with active, generalized, biopsy-proven WG refractory to standard treatment were followed prospectively. Infliximab was administered in addition to standard therapy with cyclophosphamide and high-dose corticosteroids at a dosage of 3 mg/kg in two patients and at a dosage of 5 mg/kg in four patients with a 2-week interval after the first administration and a 4-week interval for the consecutive infusions.

Results: Disease manifestations refractory to standard cyclophosphamide and corticosteroids were: imminent visual loss due to progressive retroorbital granulomas in 3 patients, rapidly progressive glomerulonephritis in 1 patient, pulmonary-renal syndrome in 1 patient, and progressive cavitating pulmonary granulomas (also biopsy-proven) in 1 patient. Addition of infliximab resulted in a rapid and significant improvement of vasculitis activity in 5 patients. Corticosteroid doses could be tapered in these 5 patients. Acute-phase responses (e.g., CRP) normalized. C-ANCA titers were no longer detectable. The BVAS was reduced to zero in these patients. 1 patient was withdrawn because of a suspected systemic infection. The higher dosage (5 mg/kg) was more effective. One patient continues on TNF-α blockade. One patient relapsed after 10 months and remission was reinduced by addition of infliximab. Three patients have remained in remission during follow-up for 12 to 18 months.

Conclusion: TNF-α blockade successfully induces remissions in refractory WG. Infliximab appeared effective and safe. Close monitoring for side effects such as infections is mandatory. Infliximab may appear as a more specific treatment in WG, where TNF-α has been demonstrated to play an important role in the induction of vasculitis and granuloma formation. Maintenance of remission after infliximab treatment still means another challenge to our therapeutic efforts.

97-061

SAFETY AND EFFICACY OF TNFα BLOCKADE IN RELAPSING VASCULITIS

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Blockade of tumour necrosis factor alpha (TNFα) using infliximab, a chimeric monoclonal antibody against TNFα, is an effective treatment in rheumatoid arthritis and Crohn's disease. Sight-threatening Behçet's disease has also been successfully treated with infliximab. A preliminary study has also reported clinical improvements in the primary systemic vasculitis, Wegener's granulomatosis, with the soluble TNFα receptor etanercept.

We report the compassionate treatment of six patients with refractory vasculitis using infliximab. Diagnoses were: Wegener's granulomatosis (3), microscopic polyangiitis (3). Three were PR3-ANCA and 1 MPO-ANCA positive. Four were female, mean age was 58 years (range 23-77 yrs) and mean disease duration was 3.5 yrs. All had suffered at least three clinical relapses and had received prolonged corticosteroids and at least four immunosuppressive drugs. Vital organ involvement at the time of infliximab included eye (4) and lung (3); in addition, five had profound constitutional symptoms. Mean prednisolone dose was 17 mg.

Three intravenous doses of infliximab 200 mg were administered at monthly intervals for three months. One patient complained of fatigue, myalgia and blurred vision 24 hours after the first infusion which did not recur on rechallenge. Infliximab was otherwise well tolerated. Five patients had remission of their disease, four within two weeks of treatment. This allowed steroid withdrawal in three and reduction by more than 50% in two. Disease activity assessed by the Birmingham Vasculitis Activity Scores (BVAS) improved from a mean of 6.3 to 0.8 at three months (**Figure 1**). One receiving continued infliximab for six months relapsed when the treatment interval was extended to two months. Mean falls in ESR and CRP were 17 mm/hr and 13, respectively. ANCA status was unchanged.

Anti-TNFα therapy heralds a new wave of specifically targeted biological interventions of potential value in the treatment of vasculitis. It offers the hope of improved therapeutic efficacy over current agents and the possibility of reducing exposure to steroids and immunosuppressives.

Further studies are warranted to confirm these observations and explore the role of infliximab as a component of initial protocols.

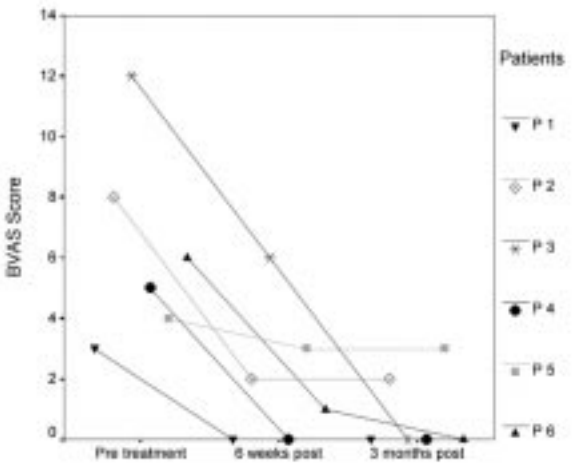

Figure 1. BVAS scores for the six infliximab-treated patients (P).

98-062

RENAL RELAPSE IS AN IMPORTANT DETERMINANT OF RENAL SURVIVAL IN PATIENTS WITH PR3-ANCA ASSOCIATED VASCULITIS WITH RENAL INVOLVEMENT

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Severe renal disease is a feature of ANCA-associated small-vessel vasculitis. We evaluated patient and renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement at diagnosis during long-term follow-up.

Seventy-three patients were diagnosed between 1982 and 1995 and followed until 2000 allowing > 5 years of follow-up. All patients were treated with prednisolone, cyclophosphamide and, if necessary, plasmapheresis. Survival curves were estimated, and univariate (log-rank test) and multivariate (Cox proportional hazards) analysis with patient and renal survival as dependent variables was performed.

Of 73 patients included, 16 (22%) died within 1 year after diagnosis. Of 24 patients (33%) dialysis dependent at diagnosis, 2 remained and 2 again became dialysis dependent < 1 year; 9 died early without renal recovery. Risk factors for death occurring within one year in univariate analysis (RR, 95% CI) were age > 65 years (4.4, 2.0-16.7) and dialysis dependency at diagnosis (2.9, 1.2-10). In multivariate analysis CRP level (1.02, 1.01-1.04) was also associated with worse prognosis. Eighteen patients died > 1 year, with male gender (5.0, 1.1-23) and developing dialysis dependency during follow-up (4.0, 1.3-13) associated with this outcome.

Risk factor for renal failure within one year was dialysis dependency at diagnosis (30, 8.4-101). Of 53 patients dialysis independent 1 year after diagnosis, 9 patients became dialysis dependent during follow-up. A renal relapse was strongly associated with development of renal failure in long-term follow-up (29, 3.2-260).

In conclusion, early death and failure to recover renal function in PR3-ANCA associated vasculitis is associated with age > 65 years and dialysis dependency at diagnosis. Long-term renal survival is determined by renal relapses during follow-up only. In contrast to MPO-ANCA related vasculitis slow, progressive renal failure without relapses is rarely observed in this group.

99-063

OUTCOME OF RENAL VASCULITIS IN LONDON: 1995-2000

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The outcome of renal vasculitis in 313 new patients diagnosed 1995-2000 was determined. Diagnoses were: ANCA-associated systemic vasculitis (AASV) (246), Henoch-Schönlein purpura (25), cryoglobulinemic vasculitis (7), polyarteritis nodosa (17) and anti-GBM disease (18).

Of those with AASV, diagnoses: 38% microscopic polyangiitis (MPA), 27% Wegener's granulomatosis (WG), 11% renal-limited vasculitis (RLV) and 4% Churg-Strauss (CSA). Mean length of prodromal symptoms, 4 months; median age 66. 57% were male and 83% Caucasian. ANCA were present in 92%. RLV/MPA: 65% P-ANCA/MPO, 25% C-ANCA/PR3; WG: 83% C-ANCA/PR3, 12% P-ANCA/MPO. Initial creatinine was higher in RLV and MPA ($p=0.002$).

Survival at 1 and 5 yrs was 82% and 76%, respectively (standardized mortality rate of 242%). Mortality was associated with age >60 ($p<0.001$), end stage renal failure (ESRF) ($p<0.001$), initial creatinine >200 mmol/l ($p=0.01$) and sepsis ($p=0.048$). 28% developed ESRF, of whom 47% died. 54 patients presented with creatinine >500 mmol/l; 29 achieved dialysis independence. There was no association of death or ESRF with gender, diagnosis or ANCA status. Relapse was most common in WG ($p=0.048$) and C-ANCA/PR3 specificity, and was not associated with age or creatinine. Leukopenia occurred in 41% and was associated with sepsis ($p<0.001$). Other major adverse events included cardiovascular (9.5%), bone (4.5%) and malignancy (4.5%). Functional status as assessed by Karnofsky score was low (mean 60). Treatment regimens varied with respect to the dose and route of administration of cyclophosphamide, cumulative steroid exposure and duration of remission therapy with azathioprine.

Within AASV, diagnosis and ANCA subgroup is unimportant in terms of major outcomes. ESRF and death in renal vasculitis are closely related to creatinine at presentation, thus diagnostic delay may have a major influence on outcome. Leukopenia should be avoided due to the close association with sepsis and death. Future therapeutic regimens should address the toxicity and partial efficacy of current treatment of particular importance in the elderly.

100-065

15-DEOXYSPERGUALIN IN PATIENTS WITH INTRACTABLE ANCA-ASSOCIATED SYSTEMIC VASCULITIS

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The combination of cyclophosphamide (CYC) and oral corticosteroids (OCS) is effective in the majority of patients with ANCA-associated vasculitis. However, it carries substantial risk of drug-related morbidity and mortality. New regimens are desired especially in refractory cases. The immunosuppressant 15-deoxyspergualin (DSG) is effective in autoimmune disease and transplantation in animal models as well as in acute kidney transplant rejection in humans. To assess the efficacy and tolerability of DSG we conducted an open label multicenter pilot trial in patients with intractable ANCA associated systemic vasculitis who were either unresponsive or had contraindications for standard immunosuppressants. The patients included 19 cases of Wegener's granulomatosis (WG) and one case of microscopic polyangiitis (MPA). Eighteen of 20 patients had received CYC before and 8 of them had received CYC immediately before study entry. During the study only concomitant steroid usage was allowed. DSG (0.5 mg/kg/day) was given s.c. for 2 to 3 weeks until the WBC count dropped to 3,000/ml followed by a rest until at least a count of 4,000/ml WBC was recovered. This was repeated up to 6 cycles. Remission rate by DSG treatment was 70% (6 cases of complete remission and 8 cases of partial remission). The therapeutically prospective leukopenia occurred in each patient in a regular pattern during the cycles and was transient without exception. No mortality or septicemia was observed. Mild to moderate bacterial infections mainly in the respiratory tract, mucosal candida infections and one herpes zoster infection were observed but resolved under adequate treatment without sequel. We conclude that DSG can successfully treat patients with refractory WG under careful monitoring of WBC count.

101-066

15-DEOXYSPERGUALIN AND CYCLOPHOSPHAMIDE, BUT NOT MYCOPHENOLATE MOFETIL, PROLONG SURVIVAL AND ATTENUATE RENAL DISEASE IN SCG/KJ MICE

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15-Deoxyspergualin (DSG) is an immunosuppressant with a unique mode of action currently undergoing a phase II trial for treatment of patients with Wegener's granulomatosis. We compared here the efficacy of DSG to cyclophosphamide (CYC) and mycophenolate mofetil (MMF) in scg/kj mice, an inbred mouse strain, that develops crescentic nephritis, systemic necrotizing vasculitis and pANCA spontaneously. Mice were randomly assigned to i.p. treatment with either DSG (2 mg/kg/day, n=25), CYC (1.8 mg/mouse/week, n=25), MMF (60 mg/kg/day, n=12 or 100 mg/kg/day, n=15) or vehicle (VEH, glucose 5% 0.3 ml/day, n=25) beginning at disease onset at the 9 week of life and lasting until the death of the animals. ANCA, BUN, proteinuria and hematuria were determined in all animals every 14 days. Sera were analyzed for the presence of pANCA by IIF, proteinuria was determined quantitatively and hematuria semi-quantitatively. Survival was calculated using the Kaplan-Meier method analyzing differences with log-rank testing. 50% survival in VEH treated animals was 123 days. At that point survival was 100% in CYC or DSG treated animals (log-rank $p<0.001$). However, mean survival in both MMF groups was not significantly different from VEH treated animals (MMF60: 117 days [95% CI 108 to 127], MMF100: 117 days [95% CI 110 to 124]). Proteinuria remained on baseline levels in the CYC and DSG groups and was significantly reduced when compared to controls (8 week: CYC 0.4±0.12 mg, DSG 0.5±0.21 mg, VEH 0.4±0.12 mg; 18 week: CYC 0.5±0.21 mg, DSG 0.5±0.33 mg, VEH 3.9±2.38, $p<0.05$ each). However, MMF did not reduce proteinuria significantly (18 week: MMF60 6.87±10.8 mg, MMF100 6.51±12.9 mg, VEH 3.9±2.38). Hematuria, BUN and ANCA titers were significantly decreased in CYC and DSG treated mice when compared to controls, however MMF showed no effect. Thus DSG and CYC, but not MMF, prolong life, limit renal damage and prevent autoantibody formation in scg/kj mice.

102-073

ANTI-THYMOCYTE GLOBULIN (ATG): A THERAPEUTIC OPTION FOR WEGENER'S GRANULOMATOSIS (WG) UNTREATABLE WITH CONVENTIONAL THERAPIES

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Introduction: A subset of WG patients does not respond to daily oral cyclophosphamide (oCyc) and glucocorticosteroids (GC) or suffers of intolerable side effects. Anecdotal data suggest that ATG may be a treatment option for these patients. We now describe the follow-up of 12 patients treated with ATG for refractory WG.

Patients and Methods: 12 patients with histologically proven active WG (7 unresponsive to oCyc, 5 intolerant of oCyc) were treated with ATG Merieux within or according to the SOLUTION protocol designed by the European Vasculitis Study Group (EUVAS) for refractory systemic vasculitis.

Results: Before ATG administration, patients had received a mean of 4 (2 to 6) different therapeutic approaches including oCyc in all and experimental therapies in 5, without control of disease activity (3.5 +/- 2 relapses during a disease duration of 72 +/- 52 months). 11 of 12 patients showed a favorable response to ATG with partial (9) or complete (2) remission of disease activity. During a follow-up of 20 +/- 12 months, 4

patients relapsed after 12 (2-29) months. Seven patients are free of relapse for 12 (3 - 31) months. Although further immunosuppressive treatment was required in all, a dose reduction or a change to a less aggressive regimen could be achieved in 10 cases. One patient died due to active WG 3 days after ATG administration. Side effects of the ATG treatment were mild with fever and chills during the first administration, serum sickness (1 case) and infections (3 cases, not life threatening).

Conclusion: ATG seems to be a treatment option for severe WG refractory to cyclophosphamide and glucocorticosteroids.

103-086

CONTENT AND EVALUATION OF AN INTERDISCIPLINARY PATIENT EDUCATION PROGRAM IN SYSTEMIC VASCULITIS (PEPVAS)

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Background: Standardized patient education programs are effective as an additional therapy in chronic diseases, eg, rheumatoid arthritis, systemic lupus erythematodes, and are able to reduce disease activity and depression. Most patient education programs are based on cognitive behavioral interventions and are supposed to improve the patient's self-efficacy.

Purpose: (1) To develop, establish and standardize an inter-disciplinary patient education program (PEPVAS) for patients with primary systemic vasculitides (PSV). (2) To evaluate the therapeutic effect of the program with a prospective study in a pre/post design.

Methods: In past years, interdisciplinary seminars on disease, therapies, side effects, coping strategies, nutrition and physiotherapy were developed in our center for patients with PSV. This unstandardized approach was revised according to the guidelines of the German Rheumatology Society and the new version was implemented.

Results: Our newly designed patient education program comprises 5 modules each conceived for 90 minutes interactive training based on information presented on transparencies by the participating disciplines (physicians, psychologists, nurses, dieticians, physiotherapists). To evaluate the program and measure the therapeutic effect, a documentation system with physician- and patient-administered questionnaires assessing different aspects of health status as health-related quality of life (SF-36), disease extent (DEI) and activity (BVAS), laboratory parameters (ESR, CRP, ANCA), employment status, disability, knowledge and self efficacy was developed and completed before and 1 and 6 months after participating in the program. Knowledge and all components of health-related quality of life improved.

Conclusions: To our knowledge this is the first standardized patient education program for PSV. We present the content of five modules and first results of the evaluation of 20 participating patients. The practicability and acceptance of the program were high. These early results indicate an effect of PEPVAS.

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104-087

PATIENT OUTCOME IN WEGENER'S GRANULOMATOSIS AND MICROSCOPIC POLYANGIITIS WITH RENAL INVOLVEMENT

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Patients with ANCA-associated small vessel vasculitides such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) did suffer from an extremely bad prognosis until the introduction of cyclophosphamide and corticosteroids according to the scheme introduced by Fauci. However, during the last decade some reports have focused on the problems with cancer and other side effects of therapy in these patients. We have previously published data on a five-year follow-up on 123 consecutive patients with WG or MPA with renal involvement, and documented an increased risk for malignancy, particularly after one year of cyclophosphamide treatment. We therefore aimed at a longer follow-up time on this cohort of patients, investigating the patient survival data and frequency of cancer.

This study comprised 117 consecutive patients, 43 women and 74 men, with a Wegener's granulomatosis or microscopic polyangiitis. All patients had a biopsy-proven renal involvement and were followed up for 8.4 years (range 0.1-336 months). Six patients out of the 123 were thus lost to follow-up. The cumulative relative survival was analyzed according to Hakulinen, comparing the present cohort to the general Swedish population, matched for age and gender. Cancer incidence data were obtained from the South Swedish Regional Tumor Registry.

The cumulative relative survival 10 years after diagnosis of the vasculitis was 73% for the whole group. Analyzing men and women separately revealed a lowered cumulative relative survival rate for men. This was not the case for women who survived the first year after the diagnosis of vasculitis with renal involvement. One new case with cancer was registered since last follow-up; in total there were 16 cases with cancer during the whole follow-up period. Urinary bladder cancer was registered in four patients, all men, with a standardized morbidity ratio of 7.1, 95% CI 1.9-18.2.

In conclusion, men surviving the first year after the diagnosis of a WG or MPA with renal involvement have a lowered cumulative relative survival, compared to the general male population.

105-088

ANTI-β-GLUCAN ANTIBODY IS USEFUL AS AN INDICATOR OF IMMUNOLOGICAL COMPETENCE AND A PREDICTOR OF THE OCCURRENCE OF DEEP MYCOSES DURING IMMUNOSUPPRESSIVE THERAPY FOR ANCA-ASSOCIATED VASCULITIS

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Aim: Patients with antineutrophil cytoplasmic antibody-associated (ANCA) vasculitis (AAV) sometimes recover after treatment with potent steroids and immunosuppressive agents. However, deaths do occur as a result of opportunistic infections. β-Glucans are present diffusely in nature among fungi, bacteria and plants. Since β-glucans are not immunogenic, the ability of the human body to produce antibodies against β-glucans in the cell wall has not been sufficiently clarified. The present study was performed to establish a method to analyze serum titers of anti-β-glucan antibodies to predict the occurrence of infectious diseases supervening AAV and also to identify an indicator of immunological competence of the host. We also examined the clinical significance of the method.

Methods: Serum antibody titers induced against β-glucan in solubilized *Candida* cell wall (anti-CSBG antibody) were analyzed by ELISA. Subjects were 22 healthy adults, 77 patients with RA and 35 patients with AAV.

Results: Anti-CSBG antibody recognized the β-1,6 structure of normal chains of β-glucans. Mean antibody titers were 5,527 ± 1,686 U and 838 ± 546 U in the healthy and RA groups, respectively. Mean antibody titer was 687 ± 543 U in the AAV group before treatment (in the active phase), but this value decreased significantly to 533 ± 432 U after immunosuppressive treatment ($P < 0.01$). Serial analyses of anti-CSBG antibody titers in individual patients showed that whereas the titer increased in cases with remission of AAV, it decreased in those with deep mycoses. Analysis of anti-CSBG antibody in cases of AAV is useful to estimate immunological competence during nonspecific immunosuppressive therapy and to predict the occurrence of deep mycoses.

106-089

HEALTH-RELATED QUALITY OF LIFE IN SYSTEMIC VASCULITIS: VALIDATION OF THE GENERIC INSTRUMENT SHORT FORM 36 (SF-36)

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Purpose: Measurement of physical, mental and social function is essential for the evaluation of patients (pts) with rheumatic diseases. The purpose of this study is the assessment of health-related quality of life (HRQL) with the multi-dimensional generic questionnaire Short Form 36 (SF-36) in a university-based rheumatology department, the evaluation of its psychometric properties, acceptance and practicability in daily use and the comparison of pts with primary systemic vasculitides (PSV), connective tissue diseases (CTD) and fibromyalgia (FM) with a healthy population.

Methods: The 36-item questionnaire measures the dimensions physical function (PF), role physical (RP), pain (P), vitality (V), mental health (MH), role emotional (RE), social function (SF), and general health (GH). Scales range from 0-100 with high figures indicating high HRQL. Laboratory parameters were examined to assess convergent validity.

Results: In this cross-sectional study 279 consecutive patients admitted to the department of rheumatology with PSV (n=172), connective tissue diseases (n=96) and fibromyalgia (n=11) completed the SF-36 within three days of admission. The mean age was 57.2, 51 and 58.8 years, respectively. All patient groups estimated their HRQL lower than an age-adjusted reference population. Pts with PSV estimated their HRQL in all aspects higher than pts with CTD or FM. Values between .84 and .94 for Cronbach's alpha indicate high internal consistency. CRP and HRQL correlate statistically significantly.

Conclusions: Pts with PSV estimate their HRQL significantly higher than pts with CTD in the physical dimensions (PF, V, P) despite older age. Reasons, which will be elucidated in longitudinal studies, may be slightly lower disease activity and effects of patient education in our PSV cohort. The SF-36 represents a valid and reliable instrument that shows high acceptance and practicability in daily clinical use. Further studies with patient focus groups may be useful to identify disease-specific aspects of health-related quality of life in PSV that are not covered by the SF-36.

107-094

TRIMETHOPRIM-SULFAMETHOXAZOLE MONOTHERAPY FOR ACTIVE LOCO-REGIONAL OR LIMITED WEGENER'S GRANULOMATOSIS

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Standard therapy for Wegener's granulomatosis (WG) consists of cyclophosphamide and corticosteroids. At the costs of significant morbidity and even mortality, this therapy leads to remission of disease activity in most patients. Incidental reports suggest that WG limited to the upper and lower airways can be treated with trimethoprim-sulfamethoxazole (TS).

Since 1993 we have performed a cohort study with TS monotherapy 2 × 160/800 mg in untreated patients presenting with biopsy-proven active WG limited to the upper and lower airways. Complete remission (CR) was defined as the total absence of symptoms or signs attributable to active WG (BVAS 0) in combination with a normal serum CRP level, partial remission (PR) as an improvement in disease activity score and CRP without fulfilling the criteria for CR. Treatment failure was the need for alternative or additional treatment to control WG, or relapse of disease activity during TS.

Included were 31 patients (age 29-86 years; 10M/21F), 25 at diagnosis, 6 at relapse. In 14 patients disease activity was confined to the ENT region (loco-regional WG), 17 patients had in addition to ENT activity arthralgias, episcleritis or pulmonary lesions (limited WG). All patients had nasal mucosal abnormalities with necrotizing granulomatous inflammation with or without vasculitis on nasal biopsy. ANCA were detected in 26 patients (84%; PR3-ANCA 20, MPO-ANCA 6). Treatment with TS was successful in 27 patients (87%, 95% CI 70-96%), with CR in 18, and PR in 9 patients. Time to maximal treatment response was 3 months (1 to 15). TS was stopped in 2 patients due to side effects, 2 patients had disease progression after 1-2 months of therapy. Eleven patients relapsed 14 months (2 to 32) after start of TS treatment, 5 of 9 (56%) after partial and 6 of 18 (33%) after complete remission (RR 5.8; 95% CI 4.6-21.3). All were treated with

cyclophosphamide and prednisolone. Disease-controlled survival with TS monotherapy was 70% (95% CI 53-87%), 60% (95% CI 41-79%), and 36% (95% CI 14-58%) at 12, 24, and 36 months.

Given the observed response rate, initial therapy with TS may obviate the need for more toxic conventional treatment for prolonged periods in patients presenting with loco-regional or limited Wegener's granulomatosis. Especially in patients with initially a complete response on trimethoprim-sulfamethoxazole, long-term control of the disease seems possible.

108-095

INTRAVENOUS IMMUNOGLOBULIN (IVIG) TREATMENT OF MPO-ANCA-RELATED MICROSCOPIC POLYANGIITIS

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Anti-neutrophil cytoplasmic antibody (ANCA)-related microscopic polyangiitis (MPA) requires strong immunosuppressive treatment including steroid and cyclophosphamide; however, such treatment is not always applicable to elderly or immunocompromised patients. For these patients, intravenous immunoglobulin (IVIG) treatment is an alternative because IVIG can modulate the immune system without severe side effects. Eight patients with myeloperoxidase (MPO)-ANCA positive MPA (age, 61-84 y.o.; average, 73 y.o.), histopathologically proven by renal biopsy, received IVIG treatment in our hospitals. In seven of the eight patients, the IVIG treatment has provided significant amelioration of the following ANCA-related symptoms and signs: high fever (3 cases), appetite loss (3 cases), elevated CRP values (6 cases), and progressive renal dysfunction (3 cases). In only one case was steroid pulse therapy required, but in other cases relatively low dose of oral steriod with minimal cyclophosphamide was enough to suppress exacerbation of glomerulonephritis (GN) as the maintenance therapy. IVIG seems to exert a convincing effect for patients with MPO-ANCA-related MPA, especially those who cannot endure strong immunosuppressive treatment.

109-097

CLINICAL STATUS OF 897 PATIENTS WITH TAKAYASU ARTERITIS IN JAPAN

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Patients with Takayasu arteritis (TA) are still increasing in Japan. However, progress of medical tools and social care permit early diagnosis and therapy, thereby improving prognosis.

We surveyed the clinical status of 897 patients with TA. 30% of patients (Group I) no longer require steroid therapy. 40% (Group II) are in a stable clinical state on a small dose of steroid therapy with or without immunosuppressant therapy. 5% (Group III) are in an unstable state with recurring relapses despite medical and/or surgical treatment.

20% (Group IV) are suffering from various complications, such as aortic regurgitation, hypertension, ischemic heart disease, pulmonary infarction, strokes and cataracts, all of which are adequately manageable. 5% (Group V) require strict medical attention for serious complications, such as congestive heart failure, arrhythmia, heart attack and/or renal failure.

These data suggest that diagnosis of TA at an early stage is essential for improved prognosis.

110-106

OUTCOMES OF VASCULAR INTERVENTION (SURGERY AND ANGIOPLASTY) IN PATIENTS WITH TAKAYASU ARTERITIS

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Background: Takayasu arteritis (TA) can be disabling or life-threatening. Stenoses are the most frequently encountered lesions. Symptoms usually result from ischemia to organs supplied by diseased vessels. Optimal management of TA involves appropriate medical therapy for inflammatory aspects of large-vessel disease on one hand, and revascularization procedures (surgical or endovascular) for anatomic lesions, before irreversible damage is sustained. Despite advances in angioplasty and surgery, significant morbidity continues to occur because of limitations in treatment of established anatomic lesions.

Objective: To provide an analysis of outcomes of vascular interventions in 18 TA patients who received care at the Cleveland Clinic Foundation between 1979 to 2001.

Patients and Methods: Retrospective chart review. Coronary artery revascularization procedures were excluded from the review for patients older than 35 years old since it was felt that ability to discriminate between atherosclerosis and TA might not be possible beyond this (granted arbitrary) age. The primary outcome measure was patency of vessels treated by angioplasty, bypass or intravascular stent placement. Patency was assessed by repeat invasive angiography or magnetic resonance angiography. Secondary outcomes included complications from the procedure, morbidity and mortality.

Setting: Tertiary care referral center.

Intervention: Revascularization procedures included balloon angioplasty, stent placement, or vascular surgery with graft anastomosis.

Results: Data are available for 18 patients. Mean age at first revascularization procedure was 34.1 years. A total of ***48*** revascularization procedures were performed in 18 patients. 33 bypass procedures were performed. Thirty percent (10/33) of all grafts restenosed or reoccluded over 2 to 194 months after surgery. Eight percutaneous balloon angioplasties were performed, with a 50% (4/8) rate of restenosis/occlusions at 2-22 months; 7 stents were installed, of which 3 reoccluded (42.9%) at 2-22 months. There were no deaths associated with revascularization procedures.

Conclusion: Despite providing short-term benefit, revascularization procedures adapted for atherosclerotic disease are associated with a higher failure rate in patients with TA.

111-107

LONG-TERM REMISSION OF POLYARTERITIS NODOSA (PAN) ASSOCIATED WITH MYELODYSPLASTIC SYNDROME AFTER INTRAFAMILIAL ALLOGENEIC BONE-MARROW (BM) TRANSPLANTATION

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Objective: To test the efficacy of bone marrow allograft in a patient with PAN refractory to treatment and myelodysplastic syndrome.

Patient: A 33-year-old man presented in 1993 with fever, weight loss, polyarthritis, abdominal pain, digital ischemia and low neutrophil count at 1,000/mm^3. Retinal angiography detected vasculitic lesions; renal and mesenteric artery arteriographies revealed multiple microaneurysms. ANCA were negative. PAN was diagnosed and the disease worsened despite prednisone (1 mg/kg/day). Methylprednisolone, cyclophosphamide, intravenous immunoglobulin, methotrexate and cyclosporine were prescribed successively, without prolonged efficacy. In 1995, large granular lymphocytes, corresponding to polyclonal T lymphocytes, were identified and, in 1996, sideroblastic anemia was diagnosed. Blood transfusions were frequently required and systemic vasculitis remained uncontrolled despite prednisone (30 mg/day) and methotrexate (20 mg/wk). In February 1998, an intra-familial bone marrow allograft was performed. Conditioning regimen combined total body irradiation and melphalan (140 mg/m^2).

Result: Four years post-transplant, complete remission of systemic vasculitis was obtained and karyotypic analysis of BM showed complete chimerism, with no evidence of myelodysplastic syndrome. However, persistent skin and liver graft-vs-host disease is treated with mycophenolate mofetil.

Conclusion: Bone marrow allograft may cure PAN in patients with associated or premalignant or malignant hemopathy.

112-113

TREATMENT OF POLYARTERITIS NODOSA (PAN) AND MICROSCOPIC POLYANGIITIS (MPA) WITH POOR PROGNOSIS FACTORS: A PROSPECTIVE TRIAL COMPARING STEROIDS (CS) AND 6 OR 12 CYCLOPHOSPHAMIDE (CYC) PULSES IN 65 PATIENTS

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Background: The reference treatment for severe PAN without virus infection and MPA comprises CS and pulse CY, but optimal CY treatment duration has not been established. We conducted a trial to determine whether 6 or 12 CY pulses given in combination with CS could cure the disease.

Methods: Upon inclusion in this trial, organized by FVSG, 65 (18 PAN, 47 MPA) patients were randomized to receive 12 (n=34) or 6 (n=31) CY pulses combined with CS. None had received prior treatment for vasculitis. PAN and MPA were histologically proven or met ACR criteria. CS were administered as follows: a daily 15 mg/kg pulse for 3 days, then 1 mg/kg/d orally for 3 weeks. CS were then progressively tapered and definitively stopped after 1 year. CY pulses were administered every 2 weeks for 1 month, then every 4 weeks. No maintenance treatment was given after stopping CY. The endpoint of the study was the number of events (relapses and/or deaths) occurring in each group, analyzed according to an intention-to-treat strategy. The outcome was evaluated by Cox proportional hazards analysis.

Results: The main baseline clinical manifestations—poor condition 60/65, arthralgias and/or myalgias 38, peripheral neuropathy 37, glomerulonephritis 34, vascular nephropathy 12, renal insufficiency 30, gastrointestinal involvement 26, and cardiomyopathy 7—were similar for both groups. Mean five factor score (FFS) at entry was 1.8 ± 0.8; mean BVAS was 21.8 ± 7.7; mean follow-up was 32 ± 21 months. Comparing the 12- and 6-pulse groups, respectively, complete remissions were obtained in 88% and 84%; relapses occurred in 24% and 54%, and 18% and 26% died; 35% and 65% experienced an event during follow-up. Survival analysis showed a significantly lower relapse probability ($P = 0.02$; hazards ratio [HR] = 0.34) and higher event-free survival ($P = 0.02$; HR = 0.44) for the 12 CY-pulse group while the mortality rates were similar for both groups ($P = 0.47$).

Conclusion: These results suggest that 6 CY pulses are less effective than 12 CY pulses to treat severe PAN and MPA, particularly with respect to the risk of relapses.

113-116

CUTANEOUS PANARTERITIS NODOSA IN CHILDREN

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Cutaneous panarteritis nodosa (PAN) is rare vasculitic disorder in children. The main histologic feature is the necrotizing vasculitis small- and medium-sized arteries of the skin without visceral involvement. Infectious triggers of disease onset, ie, Strep and HBV, have been discussed. Ocular involvement has been noted in adults.

Objective: Define the clinical and laboratory features of the disease onset, the treatment and the outcome of cutaneous PAN in children.

Methods: A retrospective chart review of all patients seen over an 11-year period (1991–2001) at HSC was performed. Twelve patients were identified to have cutaneous PAN. The clinical presentation and laboratory data at onset, treatment and outcome were reviewed.

Results: Twelve patients (8m/4f) with a mean age at diagnosis of 7.5 yr (range: 1.2-13.4 yr) were included. The clinical presentation was skin lesions in terms of painful nodules and local swelling (12/12), fever (11/12), local pain (12/12), lym-

phadenopathy (9/9), arthritis (10/12), and splenomegaly (8/10). The skin lesions were noted on the legs (10/12), feet (8/12), hand/wrists (2/12), thorax/neck (2/12). 2 patients developed uveitis. 2 patients had severe vessel involvement leading to gangrene. 6/12 patients were pre-treated with antibiotics for a q. Strep infection. Lab features at onset included raised ESR (mean: 102, range: 52-132), high WBC (m: 20.5, r: 9.8-40.0), elevated Polys (m: 10.5, r: 8.26-33.6), anemia (10/12), thrombocytosis (9/12), low albumin (8/11) and high C4 complement levels (6/9 patients). 4/12 patients had significant ANA titers of >80, all showed speckled patterns, no other specific auto-antibodies were found, when tested. 7/12 patients had positive ASOT (3/12 high titers >160, 4/12 low titers, 5/12 neg). No other positive serology (HBV, EBV) was detected. The treatment was prednisone in 10/12 patients, plus penicillin 3/10, penicillin alone 2/12, or additional cyclophosphamide (2/12 for initial non-responsiveness, 1/12 relapse treatment). The mean duration of prednisone treatment was 35 months (range 4-120 months). 2/12 children relapsed after remission (1 skin manifestation, 1 uveitis relapse).

Conclusion: Cutaneous PAN in children is a rare but well-characterized disease entity, which has the typical clinical onset feature of painful skin lesions plus fever, lymphadenopathy, arthritis, and splenomegaly. Lab tests reveal severe inflammation including raised ESR, WBC, and Poly count, anemia, thrombocytosis and elevated C4 complement levels. More than 50% of the vasculitides are associated with Strep. Treatment mainly consists of prednisone as immunosuppression for a mean duration of 35 months. Cyclophosphamide is effective either in the case of non-responsiveness or as relapse treatment. 10/12 patients had a monocyclic course of the disease. Ocular involvement was seen in 2/12 patients, both with significant ANA titers.

114-117

BROAD SPECTRUM OF VASCULITIS AFFECTING THE AORTA

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Background: The spectrum of vasculitis affecting the aorta requiring surgical repairs is not well documented.

Methods: Data and routine histological examination with H&E and elastin stains were collected prospectively on patients undergoing 403 ascending aorta aortic arch operations; 87 of the patients were found to have inflammatory vasculitis of the aorta. Forty were male and 47 were female. Coronary artery disease was present in 34, aortic dissection in 33, peripheral vascular disease in 22, and 16 had a history of stroke. Twenty had emergency surgery and 26 urgent surgery with rupture being present in 14. Macroscopic atheroma in the aorta was present in 57.

Results: In addition to the aortic repair, 54 patients had aortic valve replacements and 13 had the entire or nearly entire aorta replaced. Specimens were available in 75 of the 87 patients. The associated findings were giant-cell arteritis in 13 (4 with temporal arteritis, 3 with polymyalgia rheumatica, and one of each of the following: TB or myasthenia gravis or cocaine abuse). Takayasu was present in 6, severe rheumatoid arthritis in 5, history of chest radiotherapy for carcinoma in 3, severe osteoarthritis in 3 (one with ankylosing spondylitis), Buerger's in 2, one of each with Behcet's, relapsing polychondritis, lupus, Cogan's, Hashimoto's, Erdheim-Chester, and 1 chronic myeloid leukemia. Past history of chronic, non-active infections included 7 bacterial endocarditis, 3 likely syphilis, 3 TB, 2 rheumatic fever, 1 chronic bronchiectasis and 1 chronic dental carries with aphthous ulcers. Two patients had systemic malaise and fatigue of unknown etiology treated by steroids. In 12 patients with aortitis, no other associated findings were present. Three had Marfan syndrome. For the 403 patients, the 30-day survival rate was 98% and for the 87 aortitis patients 95% (84/87). Three suffered a stroke (3.5%).

Conclusion: The incidence of inflammatory vasculitis associated with diverse etiologies is not as uncommon as thought when the aortic histology is examined and the patients are carefully questioned for associated pathologies.

115-124

MYCOPHENOLATE MOFETIL (MMF) THERAPY FOR ANCA-ASSOCIATED VASCULITIS (AAV)

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Rationale/Methods: Induction and maintenance of remission of AAV can be limited by drug toxicity or inefficacy. MMF has recently been introduced as an alternative agent for the treatment of AAV. To determine the efficacy and side effect profile in AAV patients, we performed a retrospective analysis of the 14 AAV patients treated with this drug at our institution.

Results: In three patients, MMF was part of a post-renal transplant regimen. In three patients, MMF was used for remission induction because other agents were contraindicated or had failed. In the remaining eight, MMF was used for remission maintenance, also primarily because of initial agent failure or contraindication. Twelve patients had Wegener's granulomatosis; two were diagnosed with microscopic polyangiitis. Seven (88%) of the maintenance patients remained in remission for an average of 17 months (range 11-26 mo), four (57%) without concomitant prednisone therapy. One relapsed after 24 months, but was found to have subtherapeutic drug levels. All of the maintenance patients in remission had therapeutic drug levels. One transplant patient suffered a meningeal flare, prompting cyclophosphamide therapy. Of the three patients for whom induction of remission was intended, relapse occurred within an average of 4 months, one episode occurring during taper of steroids. Lymphocyte counts did not seem to predict disease course. MMF was generally well tolerated, with only mild possible drug-related adverse effects including leukopenia, thrombocytopenia and gastrointestinal upset.

Conclusion: MMF appears to be an effective agent with few side effects when used for remission maintenance in patients with AAV. However, it may not be effective for induction of remission in AAV. Based on this encouraging preliminary data, further prospective controlled trials to define the exact role of MMF in the therapy of AAV seem warranted.

116-126

EFFICACY AND SAFETY OF LF 15-0195 (ANISPERIMUS) IN PERSISTENT OR RELAPSING PRIMARY SYSTEMIC VASCULITIS

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An open-label, non-controlled, multicenter phase II study evaluating the efficacy and safety of repeated administration of LF 15-0195 (anisperimus) was performed in 18 patients with persistent or relapsing primary systemic vasculitis. Immunosuppressives were withdrawn at entry and prednisolone dose adjusted according to clinical status. LF 15-0195, 0.025 mg/kg/day, was administered by subcutaneous injection on five consecutive days at four-week intervals for four cycles. Those in remission after two cycles reduced to 0.0125 mg/kg/day. From four months to the study end at 12 months, patients continued on prednisolone alone. Immunosuppressives were allowed to be restarted in case of relapse.

Of 9 patients completing the study to date, mean age at entry was 51 years and disease duration 6 years. Diagnoses were: Wegener's granulomatosis 7, microscopic polyangiitis 1, and polyarteritis nodosa 1. Seven were receiving immunosuppressives up to the time of entry (cyclophosphamide 3, azathioprine 3, methotrexate 1). By the end of the treatment period 4 patients achieved full remission (BVAS = 0), 3 partial remission (BVAS <50% baseline) and 2 had no response. At 12 months, of the 7 responders, 5 had had a minor and 2 a major relapse. Mean prednisolone doses fell from 34 mg/day at entry to 10 mg/day after three months. No severe adverse reactions associated with the trial medication were reported. Minor infections occurred in 3 and neutropenia in 1. Immunosuppressives were restarted during the follow-up phase in 5 (cyclophosphamide 2, methotrexate 1, leflunomide 1, infliximab 1).

These preliminary results in refractory vasculitis indicate potential efficacy of LF 15-0195 without major toxicity. However, remission was not sustained after the treatment period by prednisolone alone. Further results will be available in 2002.

Treating the Permanent Sequelae of Vasculitis

117-001

NASAL CAVITY SQUAMOUS CELL CARCINOMA IN WEGENER'S GRANULOMATOSIS

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Wegener's granulomatosis (WG) is well known for its chronic, debilitating nature, multiple organ system involvement, variable course, and myriad of complications causing morbidity and mortality. Although the occurrence of a variety of malignancies has been recognized in patients with long-standing WG undergoing cyclophosphamide therapy, only one case of squamous cell carcinoma (SCC) of the upper aerodigestive tract has been recorded. We report two cases of nasal cavity/sinus cavity SCC diagnosed in patients with quiescent WG. Both patients presented with progressive facial discomfort which did not respond to therapies directed to treat possible WG recurrence or infection. Tissue diagnosis was eventually positive for SCC. Because the nasal and sinus findings in WG may conceal the overt appearance of malignancy, diagnosis is very difficult and may be delayed, resulting in a suboptimal clinical outcome. Physicians who manage patients with WG should recognize that worsening nasal and sinus symptoms might not only be due to disease exacerbations or secondary infection but may less commonly signal the development of carcinoma.

118-048

BRONCHOSCOPIC FINDINGS IN WEGENER'S GRANULOMATOSIS (WG)

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Background: Airway involvement leading to chronic, irreversible lesions occurs in up to 20% of WG patients.

Objective: To describe findings and propose early bronchoscopy in patients with WG and airway involvement.

Patients and Methods: 7 of 32 WG patients (21.8%) underwent 15 bronchoscopic procedures (range 1-5).

Results: They relate to the number of procedures. Airway symptoms (prior at least one month) were found in all except 3 occasions (80%): dyspnea, 60%; stridor and dysphonia, 53%; and cough, 20%. Mean BVAS score was 4.6 ± 3.3 (0-12). Prior nasofibrolaryngoscopy was performed in 8/15 showing stenosis ≥50% in 5. Fibrobronchoscopy was performed in 13/15 procedures, rigid bronchoscopy in 2/15 and both in 2/15. In all but 4, subglottic and/or tracheal stenosis ≥50% was found. An active lesion in the left bronchial carina was seen in one procedure. On follow-up evaluation of this lesion, a 70% chronic stenosis was seen which after mechanical dilatation was reduced to 30%. Mechanical dilatation was done in 11/15 procedures (including both tracheal), being progressive in 7, and not possible or not done in 2 each. Biopsies were performed in 7/15 (5 tracheal, 5 subglottic). Chronic inflammation and/or fibrosis was seen in 3 tracheal and 4 subglottic biopsies, acute inflammation and necrosis in 3 tracheal. All patients with acute inflammation received systemic treatment (cyclophosphamide in 2, methotrexate in one). Dilatation and intralesional steroid injection, including one on the main left bronchus, was done in 3/15 according to the technique by Langford et al, with successful results in all three. One of the patients subject to this technique was lost to follow-up at the time a second procedure was planned and had subglottic restenosis when reevaluated. Complications were observed in 2/15 procedures. On the first patient (with 95% subglottic stenosis), tracheoplasty was performed at the same time of first bronchoscopic evaluation; on the second, after successful pro-

gressive dilatation, the patient developed sudden laryngeal edema which required tracheostomy. Laryngotracheomalacia was observed in two patients. Mean follow-up is 18.8 ± 20.2 months (3-61).

Conclusions: We found both chronic and new lesions coexisiting in 3 patients, requiring both local and systemic therapy. The procedure proposed by Langford et al seems effective in both settings. In general, BVAS was not useful to predict the nature (acute or chronic) of the lesions observed. We therefore propose fibrobronchoscopy for evaluation of all the airway (as we also found significant tracheal lesions) to be performed in patients with both new and past airway involvement to precise nature of the lesions and decide on early treatment.

119-092

TREATMENT OF ENDOBRONCHIAL WEGENER'S GRANULOMATOSIS WITH LOCAL APPLICATION OF HUMANIZED ANTI-LYMPHOCYTE ANTIBODY

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Wegener's granulomatosis is a form of necrotizing small-vessel, systemic vasculitis which commonly involves the upper airways, lungs, and kidneys.[1] A minority of patients with Wegener's granulomatosis develop tracheal and bronchial stenoses, which may occur in the absence of systemic disease.[2] These pose a significant therapeutic challenge in that they are often unresponsive to standard immunosuppressive agents. We would like to report a series of cases treated with mechanical dilatation and intra-mucosal injection of anti-CD52 monoclonal antibody. Systemic anti-CD52 antibody has previously been found to be effective in the treatment of refractory systemic Wegener's granulomatosis.[3]

Five patients with Wegener's granulomatosis with tracheal and/or bronchial stenoses were treated between 1997 and 1999. Patient details: 4 female, 1 male. Mean age: 50 years (34-63 years). Endobronchial involvement: subglottic stenosis + unilateral bronchial stenosis (n=1), subglottic stenosis + bilateral bronchial disease (n=2), single bronchial stenosis (n=2). Patients were treated with 20 mg anti-CD52 monoclonal antibody injected submucosally and concurrent mechanical dilatation. Patients received between 1 and 9 treatments. Following treatment, stenoses were noted to be more amenable to dilatation. All patients had improvement in their pulmonary function tests, and in one patient there was resolution of the bronchial stenosis.

Complications of treatment included lower respiratory tract infection (n=4) and goiter with associated thyrotoxicosis (n =1).

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120-123

MANAGEMENT OF ACUTE AND CHRONIC MANIFESTATIONS OF TRACHEOBRONCHIAL WEGENER'S GRANULOMATOSIS

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Purpose: To evaluate the clinical spectrum and course of endobronchial lesions in Wegener's granulomatosis (WG), and to assess the efficacy of bronchoscopic intervention in providing long-term airway patency.

Methods: Review of medical records of patients with WG who underwent initial bronchoscopy between January 1990 and November 2001. Specific data assessed included symptoms, frequency of bronchoscopy, pulmonary function testing, and treatment.

Results: 90 patients with a diagnosis of WG underwent bronchoscopy. Acute manifestations of tracheobronchial WG included ulcerating tracheobronchitis, inflammatory pseudotumor, and mucosal cobblestoning. Systemic disease activity did not correlate with endobronchial disease activity. Chronic manifestations included luminal narrowing or occlusion from endobronchial scarring. Eight patients underwent dilatation for symptomatic airway narrowing, and four patients had airway Silastic stents placed due to refractory symptoms related to the severity of their stenosis. Mean follow-up for patients receiving stents was 6.2 years. Patients with stents underwent bronchoscopy approximately every 8 to 12 months for inspection of stent function, replacement and evaluation of disease progression. Stent migration or mucoid impaction were the most common reasons for stent replacement. One patient died two months following stent placement, of an unrelated cause. One patient had two stents placed: a tracheal stent that was removed after 2 years, and a right mainstem bronchus stent that was removed after 4 years. After 6 years of follow-up, there have been no recurrent symptoms or stenosis requiring further intervention. One patient had a left mainstem bronchus stent removed after 4 years. Recurrent symptomatic stenosis necessitated replacement of the stent 2 years later. One patient had a left mainstem bronchus stent placed due to severe stenosis. Progressive stenosis of the right mainstem bronchus necessitated placement of a Y-stent 6 months later. There were no reported complications due to the stents in any of the patients.

Conclusions: Tracheobronchial manifestations of WG can result in significant airway stenosis necessitating bronchoscopic intervention. Bronchoscopic dilatation and stent placement can provide long-term symptomatic and functional improvement in patients with WG and endobronchial disease.