

Immunological and clinical follow up of hepatitis C virus associated cryoglobulinaemic vasculitis

P Lamprecht, F Moosig, A Gause, K Herlyn, E Csernok, H Hansen, W L Gross

Abstract

Objective—To study immunological markers and compare these markers with standard measures for the clinical and immunological follow up of vasculitis activity in hepatitis C virus (HCV) associated cryoglobulinaemic vasculitis (CV).

Methods—Serial serum samples from eight patients with newly diagnosed HCV associated CV were followed during interferon α treatment induced remission of the CV. Vasculitis activity and disease extent were evaluated with the Birmingham vasculitis activity score (BVAS) and disease extent index (DEI). Cryoglobulinaemia, complement levels (C3c, C4, and CH50), rheumatoid factor (RF), autoantibodies such as antinuclear antibodies, soluble interleukin 2 receptor (sIL2r), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble CD30 (sCD30) were determined.

Results—All patients achieved either complete or partial remission of their CV during interferon α treatment. There was a significant reduction in vasculitis activity and disease extent (BVAS, DEI), cryoglobulinaemia, RF, sIL2r, sICAM-1, and sCD30. Complement C3c levels increased significantly during this period. Erythrocyte sedimentation rate and levels of complement C4 and CH50 did not change significantly. Both clinical measures (BVAS and DEI) correlated significantly only with C3c and sCD30.

Conclusions—Although this study was of only a small group of patients, it shows that BVAS and DEI as clinical measures and C3c and sCD30 as immunological markers may be useful in the follow up of disease activity of HCV associated CV. The data indicate that activity of the humoral (cryoglobulinaemia, RF, autoantibodies) and cellular (sIL2r, sICAM-1, sCD30) immune response and endothelial damage (sICAM-1) are found in HCV associated CV.

(Ann Rheum Dis 2001;60:385–390)

Hepatitis C virus associated cryoglobulinaemic vasculitis (HCV associated CV) is an immune complex mediated vasculitis predominantly affecting small vessels.^{1,2} It typically evolves in patients in the presence of type II mixed cryoglobulinaemia consisting of cryoprecipitating monoclonal IgM κ -RF and polyclonal IgG. This disorder is usually found after years of chronic hepatitis C.^{3–5} Mixed cryoglobulinaemia and the detection of rheumatoid factor

(RF) and various autoantibodies are hallmarks of HCV associated CV. These findings have been attributed to polyclonal activation of B lymphocytes and the subsequent evolution of a so called benign lymphoproliferative disorder with oligoclonal or monoclonal B lymphocyte proliferation.^{4–7} Furthermore, increased serum levels of soluble intracellular adhesion molecule-1 (sICAM-1) and soluble interleukin receptor 2 (sIL2r) have been found in acute and chronic hepatitis C without cryoglobulinaemia.^{8,9}

Previously, the correlation of cryoglobulin levels with organ involvement in CV has been shown to be weak.⁵ In a recent study, we showed that HCV associated CV can be clinically monitored by measures of vasculitis activity and disease extent—that is, the Birmingham vasculitis activity score (BVAS) and the disease extent index (DEI). Complement consumption, as indicated by C3c, was found to correlate most closely with the course of the disease. C3c reflected disease activity and thus provided additional information on vasculitis activity that was not reflected by erythrocyte sedimentation rate (ESR).¹⁰ Similar results with regard to the superiority of immunological variables—for example, tumour necrosis factor α or sIL2r—over conventional serum markers of inflammation, such as ESR, have been shown recently for rheumatoid arthritis and Churg-Strauss syndrome.^{11,12} These studies raised the hypothesis that follow up of certain immunological markers is of additional value or superior to follow up of conventional markers in predicting and following disease activity in rheumatic diseases. We chose the immunological markers, sIL2r, sICAM-1, and sCD30, to monitor disease activity in patients with HCV associated CV. All patients were newly diagnosed and treated with interferon α . sIL2r, sICAM-1, and sCD30 have not previously been followed in HCV associated CV. These markers were compared with standard measures such as ESR and complement levels. Clinical disease activity was followed with the BVAS and DEI.

Methods

PATIENTS

HCV associated CV was diagnosed on the basis of the criteria of the GISC (Italian Group for the Study of Cryoglobulinaemias)—that is, six month duration of symptomatic cryoglobulinaemia, presence of at least two symptoms of Meltzer's triad (purpura, arthralgia, weakness), detection of a high RF activity and/or low complement factor C4, and no coexistence of autoimmune, lymphoproliferative, or other

Department of Rheumatology, University of Lübeck, and Rheumaklinik Bad Bramstedt, Germany

P Lamprecht
A Gause
K Herlyn
E Csernok
W L Gross

Second Medical University Clinic and Municipal Hospital Kiel, Germany
F Moosig

Department of Biochemistry, University of Kiel, Germany
H Hansen

Correspondence to: Dr Lamprecht, Department of Rheumatology, University of Lübeck, and Rheumaklinik Bad Bramstedt, Oskar-Alexander-Str 26, 24576 Bad Bramstedt, Germany
gross@medinf.mu-luebeck.de

Accepted 23 August 2000

infectious disease (except HCV infection) between January 1996 and January 1999.^{13,14} Furthermore, the Chapel Hill Consensus Conference definition for “essential” cryoglobulinemic vasculitis was applied,¹ seeking histological proof of cryoglobulin immune deposits affecting small vessels in each patient. Diagnosis of chronic hepatitis C infection was based on standard criteria—that is, history, laboratory abnormalities, serology, and liver histology as described elsewhere.¹⁵ Detection of HCV antibodies and demonstration of HCV RNA by polymerase chain reaction were required for the diagnosis of HCV infection in all patients. All patients were examined by a team of clinicians consisting of specialists in ophthalmology, neurology, and rheumatology in order to document all vasculitic manifestations. Additional imaging procedures such as chest radiograph, ultrasound of the abdomen, echocardiography, or magnetic resonance imaging followed as described previously.¹⁶

Vasculitis activity was denoted by applying the BVAS. This score is a clinical index of the degree of vasculitis activity in nine separate organ based systems—that is, systemic, cutaneous, mucous membranes/eyes, ENT, chest, cardiovascular, abdominal, renal, and nervous system. Items are scored if they are ascribable to current disease activity and are either of recent onset or currently present. Various defined abnormalities are ascribed for each organ system and may be scored if present. The activity index provides a total score of clinical disease activity. The maximum score is 63 points for present symptoms and 32 points for new or worse symptoms within the previous weeks. This score is used and evaluated in several treatment studies of primary systemic vasculitides by the European Vasculitis Study Group (EUVAS).^{17,18} Further information on these studies and BVAS can be obtained from the internet (www.vasculitis.org). Disease extent was measured by applying the DEI. This index score is merely a measure of total organ involvement attributable to the vasculitis regardless of the onset and, thus, describes the number of organ systems involved. Every organ system affected by vasculitis—that is, ENT/upper respiratory tract, lung, eye, kidney, heart, gastrointestinal tract, skin, peripheral nervous system, central nervous system, rheumatic complaints—is given two points, except for constitutional symptoms, which are allocated one point. A total of 21 points can (theoretically) be reached.¹⁹⁻²¹

LABORATORY STUDIES

Blood chemistry, antibody detection, urine sediment analysis, and virological studies were performed at the time of diagnosis of the HCV associated CV and every three months after treatment was started. In brief, patient sera were routinely tested for serum transaminases, γ -glutamyltransferase, alkaline phosphatase, concentrations of serum bilirubin, total protein, albumin, IgG, IgA, IgM, creatinine, complement factors C3c and C4, total haemolytic complement CH50, RF, antinuclear antibodies, antibodies against extractable nuclear antigens—for example, anti-SSA, antibodies associated with autoimmune liver disease—for

example, anti-(soluble liver antigen) antibodies—and anticardiolipin IgM and IgG by standard techniques. Antineutrophil cytoplasmic antibodies were also detected by established and evaluated techniques (cANCA/pANCA by the indirect immunofluorescence test and specificities by the enzyme linked immunosorbent assay (ELISA)).²² Cryoglobulins were detected and classified as described by Brouet *et al.*^{3,15} Cryoglobulinaemia, RF, and autoantibody detection served as markers of B lymphocyte activation. sIL2r and sICAM-1 were chosen as markers of activated (T and B) lymphocytes, monocytes, and/or endothelial cells. In addition, we measured the soluble form of CD30 (sCD30) as an indicator of a (mainly) type 2 T helper lymphocyte (Th2) immune response, which favours the humoral immune response.¹³

IgG antibodies to HCV were determined with an ELISA (Sanofi Pasteur, Berlin Germany) using two recombinant antigens produced in *Escherichia coli*. One of the antigens is located in the structural and the other in the non-structural area 3 of the virus genome. Sample preparation, amplification, and detection of HCV RNA were performed with a commercially available kit according to the description of the manufacturer (Amplicor; Roche Mannheim, Germany). Commercially available ELISAs were used according to the manufacturer's instructions to determine sICAM-1 (sCD54, Cytoscreen; BioSource International, Camarillo, California, USA) and sIL2r (Interleukin-2-Rezeptor Milena; DPC Biermann GmbH, Bad Nauheim, Germany). sCD30 levels were determined by radioimmunoassay using the monoclonal antibody Ki-2 as described previously.¹⁶

TREATMENT

The eight patients included in this study had a non-life threatening manifestation of their newly diagnosed vasculitis, which did not necessitate rescue treatment by plasmapheresis and/or immunosuppressive therapy. Patients with prothrombin time more than three seconds longer than normal, and/or cytopenia, as indicated by a leucocyte count below 3000/ μ l and platelet count below 60 000/ μ l, were excluded. Treatment followed current recommendations.^{5,23} The patients were treated with either interferon α 2b (Intron-A; Essex Pharma, Munich, Germany) or interferon α 2a (Roferon-A; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) at the individually tolerated maximal dose (9–30 million IU/week subcutaneously). Glucocorticosteroid treatment—that is, prednisolone not exceeding 5 mg by mouth (Decortin H; Merck, Darmstadt, Germany)—accompanied the interferon α treatment in six patients. All patients gave informed consent for collection of their data.

EVALUATION OF THERAPEUTIC RESPONSE

Patients were seen as inpatients at the time of diagnosis and either as inpatients or outpatients every three months during the following treatment period and follow up. Complete remission of CV—that is, no evidence of

Table 1 Vasculitic manifestations and antibodies in eight patients with hepatitis C virus associated cryoglobulinaemia vasculitis who were consecutively treated with interferon α

Variables	No
Mean (SD) age (years)	55.8 (14.7)
M/F ratio	1/7
Purpura	7/8
Arthralgia	5/8
Weakness	5/8
Polyneuropathy	7/8
Glomerulonephritis	2/8
Sjögren's syndrome	2/8
Raynaud's phenomenon	2/8
ANA	3/8
Anti-SSA	1/8
ACLA	2/8
cANCA	1/8

ANA = Antinuclear antibodies; Anti-SSA = anti-SSA antibodies; ACLA = anticardiolipin IgG; cANCA = cytoplasmic pattern antineutrophil cytoplasm antibodies on the immunofluorescence test (specificity could not be determined by the enzyme linked immunosorbent assay).

vasculitic activity by clinical and serological investigations as well as imaging procedures—and partial remission of CV—that is, partial improvement of vasculitis activity or arrest of the vasculitic progression—were distinguished. A relapse of CV was defined as re-emergence of vasculitis activity after prior remission of the vasculitis.²⁴ Biochemical—that is, normalisation of the serum alanine transaminase—and virological responses were followed with regard to chronic hepatitis C and HCV infection.^{25, 26} A sustained response was assumed in patients with a biochemical and a virological response during treatment and for at least six months' follow up after treatment.²⁵

STATISTICAL ANALYSIS

The Wilcoxon matched pairs signed rank test was used to test for differences of the variables at the time of diagnosis compared with after six months of treatment. Spearman rank order correlation coefficients were determined to assess associations of clinical, serological, and immunological variables. Because of the small sample size and assumption of a non-normal distribution, non-parametric tests were performed.

Table 2 Clinical, serological, and immunological variables in eight patients with hepatitis C virus associated cryoglobulinaemia vasculitis at the time of diagnosis and after six months of treatment with interferon α

Variable	At diagnosis	Six months interferon α	p Value	Normal range
BVAS	12.3 (1.3)	4.9 (0.5)	<0.05	0
DEI	4.2 (0.5)	2.0 (0.0)	<0.05	0
ESR (mm/h)	27.7 (6.2)	20.5 (3.3)	NS	<20
Cryoglobulin (mg/l)	625 (218)	144 (53)	<0.05	0*
C3c (g/l)	0.79 (0.10)	1.04 (0.04)	<0.05	0.9–1.81
C4 (g/l)	<0.09	<0.09	NS	0.1–0.4
CH50 (U/ml)	<18	<18	NS	20–50
RF (U/ml)	229 (95)	61 (15.6)	<0.05	<30
ALT (U/l)	21.8 (2.4)	15.1 (1.5)	<0.05	<18
sIL2r (U/ml)	1543 (316)	954 (214)	<0.05	<1000
sICAM-1 (U/ml)	205 (22)	128 (14)	<0.05	<295
sCD30 (U/ml)	14.3 (2.8)	7.0 (0.3)	<0.05	<10

Where appropriate, data are expressed as mean (SEM).

*Cryoglobulin detection threshold for immunofixation ≥ 80 mg/l. ESR = erythrocyte sedimentation rate; ALT = alanine aminotransferase; RF = rheumatoid factor; C3c = complement C3c; C4 = complement C4; CH50 = total haemolytic complement; sIL2r = soluble interleukin 2 receptor; sICAM-1 = soluble intercellular adhesion molecule-1; sCD30 = soluble CD30; BVAS = Birmingham vasculitis activity score¹²; DEI = disease extent index.^{14–16}

Results

CHARACTERISTICS OF THE PATIENTS

HCV associated CV was newly diagnosed in eight patients. All patients had typical manifestations attributable to their CV (table 1). Hypocomplementaemia, RF, type II mixed cryoglobulinaemia with monoclonal IgM and polyclonal IgG, HCV antibodies, and HCV RNA were shown in each patient. The diagnosis was supported by the results of biopsies in all but one patient, who refused a biopsy. Cutaneous leucocytoclastic vasculitis with immune complex depositions in small vessels was shown in five patients, and mesangioproliferative glomerulonephritis with immune complex depositions in two patients. All patients had biopsy proven chronic hepatitis C, with histological appearance ranging from minimal fibrosis to portal fibrosis with few septa, and mild to moderate activity. Liver cirrhosis was not diagnosed in any of the patients.

RESPONSE TO INTERFERON α TREATMENT

After the diagnosis of HCV associated CV, the eight patients were treated as described above. Five patients achieved partial remission of CV and a biochemical response. Three patients had complete remission of CV and a virological response after three to six months of treatment. BVAS and DEI as measures of vasculitis activity and disease extent were significantly reduced after six months of interferon α treatment. Cryoglobulinaemia and RF levels (markers of B lymphocyte activation) were also significantly reduced. Levels of C3c were significantly increased during that period. sIL2r, sICAM-1 (markers of activated T and B lymphocytes, monocytes and/or endothelial cells), and sCD30 (indicator of a Th2 immune response) were significantly reduced after six months of interferon α treatment. Serum alanine transaminase levels significantly declined, indicating a response of the hepatitis. ESR and levels of complement C4 and CH50 did not change significantly during this interval. Table 2 summarises the effects of the treatment on the clinical measures (BVAS, DEI), ESR, serological (alanine transaminase) and immunological variables (cryoglobulin, RF, complement levels, sIL2r, sICAM-1, sCD30).

BVAS and DEI showed a significant positive correlation ($r=0.86$, $p=0.001$). Both clinical measures correlated significantly with complement C3c ($r=-0.81$, $p=0.001$ for BVAS; $r=-0.68$, $p=0.014$ for DEI) and sCD30 ($r=0.62$, $p=0.023$ for BVAS; $r=0.63$, $p=0.037$ for DEI) (fig 1), whereas other variables did not.

FOLLOW UP

Two patients who initially responded to interferon α relapsed with a severe flare up of their CV two and 12 months after cessation of the interferon treatment; they were treated successfully with oral cyclophosphamide and intravenous cyclophosphamide bolus respectively. Three patients are still treated with interferon α (12–24 months). One patient continued on low dose glucocorticosteroid monotherapy after cessation of the interferon α

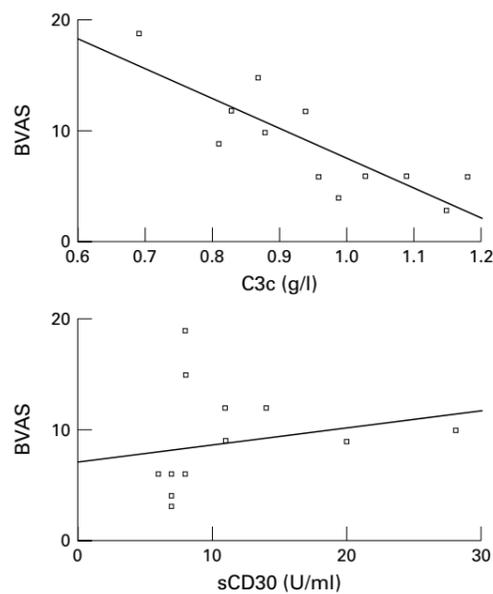


Figure 1 Correlation of the Birmingham vasculitis activity score (BVAS) with C3c complement and soluble CD30 (sCD30) in eight patients with hepatitis C virus associated cryoglobulinaemic vasculitis treated with interferon α . Spearman rank correlation coefficients were $r=0.81$, $p=0.001$ for BVAS/C3c and $r=0.62$, $p=0.023$ for BVAS/sCD30.

treatment. Two patients were lost to follow up after 12 months of interferon α treatment.

Discussion

We sought to follow immunological markers of the humoral and cellular immune response and to compare these markers with standard measures of inflammatory activity—for example, ESR—during follow up of the induction of remission with interferon α . As sIL2r, sICAM-1, and sCD30 have not previously been followed in HCV associated CV, this study may also have shed more light on the pathogenetic mechanisms involved in this vasculitis. In this study, we found a significant reduction in the clinical measures (BVAS and DEI), cryoglobulinaemia, RF, sIL2r, sICAM-1, and sCD30 during six months of interferon α treatment in eight patients with HCV associated CV. Levels of complement C3c significantly increased during that period. All patients achieved either a complete or partial remission of their vasculitis. ESR and levels of complement C4 and CH50 did not change significantly. Both clinical measures (BVAS and DEI) correlated significantly with levels of C3c and sCD30. Although these findings were confined to a small group of patients, the results should encourage further studies on the pathogenetic mechanisms at work in HCV associated CV. Furthermore, monocentric acquisition of patients with HCV associated CV takes time, indicating the need for international cooperation in the study of HCV associated CV.

BVAS and DEI were chosen for the clinical follow up in this study because they are used and are under current evaluation in studies of primary systemic vasculitides.¹⁷⁻²¹ BVAS and DEI have not so far been compared with the

purpura, kidney, and peripheral neuropathy scores of the GISC.²⁷ Treatment with interferon α and additional low dose steroids has been used in other studies on the treatment of HCV associated CV in patients with non-life threatening disease manifestations.²⁸ We tended to use higher doses of interferon α and a lower maximum dose of steroids than other studies on the efficacy of interferon α such as that by Ferri *et al*²⁸ in an attempt to improve the rate of remission in CV and possible HCV elimination. However, we found no principal difference in outcome of our small group of patients from that in the aforementioned study.²⁸

Reduction of cryoglobulinaemia and RF during interferon α induced remission of HCV associated CV has been shown previously.²⁸ A so called benign lymphoproliferative disease, with B lymphocyte activation and oligoclonal or monoclonal B lymphocyte proliferation and mixed type II cryoglobulinaemia, is the cause of this profound alteration of the humoral immune system.⁴⁻⁶ As mentioned above, correlation of cryoglobulin levels with organ involvement in CV has been shown to be weak.⁵ Complement consumption in the presence of cyroprecipitating immune complexes of the mixed type II cryoglobulinaemia shows a typical pattern with generally low complement C4 and low total haemolytic complement CH50. C3 levels seem to fluctuate with the disease course.^{5 29-31} The reasons for this pattern are not well defined, but may involve alterations in regulatory components.³⁰ We also found low levels of C4 and CH50 in this group of patients. Furthermore, we found a correlation between C3c levels and vasculitis activity and extent, as measured by BVAS and DEI, confirming our previous results.¹⁰

We found a significant reduction in sIL2r, sICAM-1, and sCD30 during six months of interferon α treatment. Both clinical measures, BVAS and DEI, correlated significantly with complement C3c and sCD30 levels, whereas other immunological markers did not. Thus these may be more useful for the follow up of disease activity than other variables such as cryoglobulinaemia and RF. Our data confirm the general notion that ESR is not a good variable for the follow up of HCV associated CV. Mean values, range, and standard deviation of the variables such as sIL2r were comparable with those published by other groups investigating different vasculitides.³²

Changes in levels of sIL2r, sICAM-1, and sCD30 may also point to the activation of cellular sources other than B lymphocyte activation. Activated T lymphocytes, B lymphocytes, and monocytes express interleukin 2 receptors. These receptors are shed on activation, and the soluble form of the receptor, sIL2r, indirectly reflects activation of these cells.^{8 32} sIL2r levels are increased in chronic hepatitis C⁸ and in vasculitides such as Wegener's granulomatosis and Churg-Strauss syndrome.^{12 32} In Wegener's granulomatosis, there is a close correlation between sIL2r levels and disease activity.³² In this study, we found a significant difference in sIL2r between active

state sera and sera taken during remission. sIL2r levels did not return to normal levels in three of eight patients. Persistence of sIL2r may suggest continuing subtle immunological alterations during remission of the vasculitis despite clinical and serological (ESR) remission.¹²

sICAM-1 represents a fragment of surface expressed ICAM-1 from mononuclear cells and endothelial cells, which is shed after induction by cytokines, reflecting inflammation and endothelial damage.^{9, 33} ICAM-1 is a ligand for leucocyte function associated antigen-1 on lymphocytes³⁴ and is induced on hepatocytes in the areas of inflammation in chronic hepatitis C.^{9, 34} Serum levels of sICAM-1 are elevated in some, but not all, rheumatic diseases. In systemic lupus erythematosus and rheumatoid arthritis, sICAM-1 concentrations do not change with disease activity,³³ whereas sICAM-1 levels are elevated in active Wegener's granulomatosis^{35, 36} and acute hepatitis C.⁹ In our study, we found a significant reduction in sICAM-1 levels during induction of remission with interferon α , but, in general, sICAM-1 levels remained below the upper limit of normal, making a judgment on its relevance difficult.

CD30, a member of the tumour necrosis factor receptor superfamily, is expressed on activated T lymphocytes, with stronger more sustained expression on Th2 lymphocytes than on Th1 lymphocytes. A splice variant of CD30 is expressed on stimulated myeloid cells and alveolar macrophages. Engagement of CD30 by its ligand CD30L is followed by enhanced shedding of CD30, which leads to increased levels of its soluble form, sCD30.³⁷ sCD30 was shown to be increased in acute viral infections—for example, those caused by hepatitis B virus, Epstein-Barr virus, and HIV^{38, 39}—systemic lupus erythematosus, and systemic sclerosis,^{40, 41} as well as in certain neoplasms such as large cell anaplastic lymphoma and Hodgkin's lymphoma.⁴² Treatment of chronic hepatitis C with interferon α diminishes the Th2 lymphocyte cytokine response—for example, interleukin 4, interleukin 10—in these patients.⁴³ sCD30 has also been found to correlate with disease extent and activity in Wegener's granulomatosis.¹⁶ We found a significant reduction in sCD30 and correlation of sCD30 with BVAS and DEI during induction of remission of HCV associated CV with interferon α . This may indirectly reflect a change in the cytokine balance towards a Th1 profile, with augmentation of the cellular immune response under interferon α treatment.

In conclusion, this study of a small group of patients with HCV associated CV shows that several markers indicating activity of the humoral (cryoglobulinaemia, RF, autoantibodies) and cellular (sIL2r, sICAM-1, sCD30) immune response and endothelial damage (sICAM-1) may be demonstrated in HCV associated CV. BVAS and DEI as clinical measures of vasculitis activity and extent correlated with complement C3c and sCD30 levels and thus may be more useful for follow

up of disease activity than other parameters such as cryoglobulinaemia.

- Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, *et al.* Nomenclature of systemic vasculitides. Proposal of an international conference. *Arthritis Rheum* 1994;37:187-92.
- Guillemin L, Lhote F, Gherardi R. The spectrum and treatment of virus-associated vasculitides. *Curr Opin Rheumatol* 1997;9:31-6.
- Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974;57:775-88.
- Agnello V. Mixed cryoglobulinemia after hepatitis C virus: more or less ambiguity. *Ann Rheum Dis* 1998;57:701-2.
- D'Amico G. Renal involvement in hepatitis C infection: cryoglobulinemic glomerulonephritis. *Kidney Int* 1998;54:650-71.
- Lamprecht P, Gause A, Gross WL. Cryoglobulinemic vasculitis. *Arthritis Rheum* 1999;42:2507-16.
- Ferri C, Zignego AL. Relation between infection and autoimmunity in mixed cryoglobulinemia. *Curr Opin Rheumatol* 2000;12:53-60.
- Simsek H, Kadayifci A. Serum interleukin 2 and soluble interleukin 2 receptor in chronic active hepatitis C: effect of interferon therapy. *J Int Med Res* 1996;24:239-45.
- Yang SS, Tsai G, Wu CH, Chen DS. Circulating soluble intercellular adhesion molecule-1 in type C viral hepatitis. *Hepato-Gastroenterol* 1996;43:575-81.
- Lamprecht P, Moosig F, Gause A, Herlyn K, Gross WL. Birmingham vasculitis activity score, disease extent index and complement C3c reflect disease activity best in hepatitis C virus-associated cryoglobulinemic vasculitis. *Clin Exp Rheumatol* 2000;18:319-25.
- Beckham JC, Caldwell DS, Peterson BL, Pippen AMM, Currie MS, Keefe FJ, *et al.* Disease severity in rheumatoid arthritis: relationship of plasma tumor necrosis factor- α , soluble interleukin 2-receptor, soluble CD4/CD8 ratio, neopterin, and fibrin D-dimer to traditional severity and functional measures. *J Clin Immunol* 1992;12:353-43.
- Schmitt WH, Csernok E, Kobayashi S, Klinkenborg A, Reinhold-Keller E, Gross WL. Churg-Strauss syndrome. Serum markers of lymphocyte activation and endothelial damage. *Arthritis Rheum* 1998;41:445-52.
- Piotelli P, Maldivassi P, Vacca A, Mazzaro C, Mussini C, Migliaresi S, *et al.* GISC protocol experience in the treatment of essential mixed cryoglobulinemia. *Clin Exp Rheumatol* 1995;13 (suppl 13):S187-90.
- Monti G, Galli M, Invernizzi F, Piotelli P, Saccardo F, Monteverde A, *et al.* Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. *Q J Med* 1995;88:115-26.
- Hartmann H, Schott P, Polzien F, Mihm S, Uy A, Kaboth U, *et al.* Cryoglobulinemia in chronic hepatitis C infection: prevalence, clinical manifestations, response to interferon treatment and analysis of cryoprecipitates. *Z Gastroenterol* 1995;33:643-50.
- Wang G, Hansen H, Tatsis E, Csernok E, Lemke H, Gross WL. High levels of the soluble form of CD30 activation molecule reflect disease activity in patients with Wegener's granulomatosis. *Am J Med* 1997;102: 517-23.
- Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, *et al.* Birmingham vasculitis activity score (BVAS) in systemic necrotizing vasculitis. *Q J Med* 1994;87:671-8.
- Bacon PA, Moots RJ, Exley A, Luqmani R, Rasmussen N. Vital assessment of vasculitis. *Clin Exp Rheumatol* 1995;13:275-8.
- DeRemee RA, McDonald TJ, Harrison EG, Coles DT. Wegener's granulomatosis. Anatomic correlates, a proposed classification. *Mayo Clin Proc* 1976;51:777-81.
- Reinhold-Keller E, Kekow J, Schnabel A, Schmitt WH, Heller M, Beigel A, *et al.* Influence of disease manifestation and antineutrophil cytoplasmic antibody titer on the response to pulse cyclophosphamide therapy in patients with Wegener's granulomatosis. *Arthritis Rheum* 1994;37:919-24.
- deGroot K, Gross WL. Wegener's granulomatosis: disease course, assessment of activity and extent and treatment. *Lupus* 1998;7:285-91.
- Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, *et al.* Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. *Kidney Int* 1998;53:743-53.
- Misiani R, Bellavita P. Mixed cryoglobulinemia. A guide to drug treatment. *Clinical Immunotherapy* 1996;5:115-21.
- Gross WL. Systemic necrotizing vasculitis. *Baillière's Clin Rheumatol* 1997;11:233-58.
- Polzien F, Schott P, Mihm S, Ramadori G, Hartmann H. Interferon- α treatment of hepatitis C virus-associated cryoglobulinemia. *J Hepatol* 1997;27:63-71.
- Sharara AI, Hunt CM, Hamilton D. Hepatic C. *Ann Intern Med* 1996;125:658-68.
- Migliaresi G, Tirri. Interferon in the treatment of mixed cryoglobulinemia. *Clin Exp Rheumatol* 1995;13 (suppl 13):S175-80.
- Ferri C, Marzo E, Longombardo G, Lombardini F, La Civita L, Vanacore R, *et al.* Interferon- α in mixed cryoglobulinemia patients. A randomized, crossover-controlled trial. *Blood* 1993;81:1132-6.
- Tarantino A, Anelli A, Costantino A, De Vecchi A, Monti G, Massaro L. Serum complement pattern in essential mixed cryoglobulinemia. *Clin Exp Immunol* 1978;32:77-85.
- Haydey RP, Pataroyo de Royas M, Gigli I. A newly described control mechanism of complement activation in

- patients with mixed cryoglobulinemia (cryoglobulins and complement). *J Invest Dermatol* 1980;74:328-32.
- 31 Corvetta A, Marchegiani G, Salvi A, Chirelli PA, Danieli G. Immune complexes and complement profile in essential mixed cryoglobulinemia before and after plasma exchange. *Int J Artif Organs* 1983;6:65-8.
 - 32 Schmitt WH, Heesen C, Csernok E, Rautmann A, Gross WL. Elevated serum levels of soluble interleukin-2 receptor in patients with Wegener's granulomatosis. *Arthritis Rheum* 1992;35:1088-96.
 - 33 Mrowka C, Sieberth HG. Circulating adhesion molecules ICAM-1, VCAM-1 and E-selectin in systemic vasculitis: marked differences between Wegener's granulomatosis and systemic lupus erythematosus. *Clin Invest* 1994;72:762-8.
 - 34 Imada K, Fukuda Y, Koyama Y, Nakano I, Yamada M, Katano Y, et al. Naive and memory T cells infiltrates in chronic hepatitis C: phenotypic changes with interferon treatment. *Clin Exp Immunol* 1997;109:59-66.
 - 35 Hauschild S, Schmitt WH, Kekow J. Hohe Serumspiegel von ICAM-1 bei der aktiven Wegener'schen Granulomatose. *Immun Infekt* 1992;20:84-5.
 - 36 Cohen Tervaert JW, Kallenberg CGM. Cell adhesion molecules in vasculitis. *Curr Opin Rheumatol* 1997;9:16-25.
 - 37 Gruss HJ, Pinto A, Duyster J, Poppema S, Herrmann F. Hodgkin's disease: a tumor with disturbed immunological pathways. *Immunol Today* 1997;18:156-63.
 - 38 Fattovich G, Vinante F, Giustina G, Morosato L, Alberti A, Ruol A, et al. Serum levels of soluble CD30 in chronic hepatitis B virus infection. *Clin Exp Immunol* 1996;103:105-10.
 - 39 Pizzollo G, Vinante F, Nadali G, Krampera M, Morosato L, Chilosi M, et al. High serum level of soluble CD30 in acute primary HIV-1 infection. *Clin Exp Immunol* 1997;108:251-3.
 - 40 Caligaris-Cappio F, Bertero MT, Converso M, Stacchini A, Vinante F, Romagnani, et al. Circulating levels of soluble CD30, a marker of cells producing Th2-type cytokines, are increased in patients with systemic lupus erythematosus and correlate with disease activity. *Clin Exp Rheumatol* 1995;13:339-43.
 - 41 Giacomelli R, Cipriani P, Lattanzio R, Di Franco M, Locanto M, Parzanese I, et al. Circulating levels of soluble CD30 are increased in patients with systemic sclerosis (SSc) and correlate with serological and clinical features of the disease. *Clin Exp Immunol* 1997;108:42-6.
 - 42 Gause A, Pohl C, Tschiersch A, Da Costa L, Jung W, Diehl V, et al. Clinical significance of soluble CD30 antigen in the sera of patients with untreated Hodgkin's disease. *Blood* 1991;77:1983-8.
 - 43 Cacciarelli TV, Martinez OM, Gish RG, Villanueva JC, Krams SM. Immunoregulatory cytokines in chronic hepatitis C virus infection: Pre- and posttreatment with interferon-alfa. *Hepatology* 1996;24:6-9.