Birmingham vasculitis activity score, disease extent index and complement factor C3c reflect disease activity best in hepatitis C virus-associated cryoglobulinemic vasculitis

P. Lamprecht, F. Moosig, A. Gause, K. Herlyn, W.L. Gross

Poliklinik für Rheumatologie, Universitätsklinikum Lübeck, Medizinische Universität zu Lübeck, and Rheumaklinik Bad Bramstedt, Bad Bramstedt, Germany

Abstract

Objective

Clinical measures of vasculitis activity (Birmingham vasculitis activity score = BVAS) and disease extent (Disease Extent Index = DEI), serological and immunological parameters were evaluated for the monitoring of hepatitis C virus (HCV)-associated cryoglobulinemic vasculitis (CV), treated with either cyclophosphamide or interferon-α2b depending on disease severity.

Methods

Serial serum samples of 15 patients with HCV-associated CV were analyzed, and BVAS, DEI, serological and immunological parameters were recorded at diagnosis and during therapy. Eight patients were treated with interferon-α2b and 7 patients with cyclophosphamide.

Results

A complete or partial response of the CV was seen in both treatment groups. BVAS, complement factor C3c, cryoglobulinemia, and rheumatoid factor significantly decreased in both treatment groups during 6 months ($p < 0.05$). DEI decrease was significant in the cyclophosphamide group ($p < 0.05$), and there was a trend in the interferon-α2b group ($p = 0.06$). BVAS and DEI were significantly positively correlated, and both parameters were significantly negatively correlated with C3c levels in both treatment groups (interferon-α2b/ cyclophosphamide: $r = -0.89$, $p = 0.001$ versus $r = -0.87$, $p < 0.001$, respectively) whereas other parameters were not, e.g. ESR and CRP.

Conclusions

Patients with different degrees of disease severity, treated with either cyclophosphamide or interferon-α2b depending on their disease activity, achieved remission of their CV. BVAS, DEI and C3c were especially useful in the follow-up of HCV-associated CV. C3c correlated with BVAS and DEI during therapy and provided additional information about vasculitis activity that was not reflected by other serological or immunological parameters, e.g. ESR or CRP.

Key words

Cryoglobulinemic vasculitis, hepatitis C virus infection, BVAS, DEI, C3c.

Peter Lamprecht, MD; Frank Moosig, MD; Angela Gause, MD; Karen Herlyn, MD, MPH; Wolfgang Ludwig Gross, MD.

Please address correspondence and reprint requests to: Peter Lamprecht, MD, Poliklinik für Rheumatologie, Universitätsklinikum Lübeck, Medizinische Universität zu Lübeck, and Rheumaklinik Bad Bramstedt, Oskar-Alexander-Str. 26, 24576 Bad Bramstedt, Germany.
E-mail: bibliobb@aol.com

Introduction

Hepatitis C virus (HCV)-associated cryoglobulinemic vasculitis (CV) is an immune complex mediated vasculitis predominantly affecting small vessels (1, 2). CV is a sequel to chronic hepatitis C, usually evolving after 20 years or more of chronic disease (2). The typical clinical aspects of HCV-associated CV such as palpable purpura, arthralgia/arthritis, weakness, polyneuropathy, and cryoglobulinemic glomerulonephritis are usually confined to type II mixed cryoglobulinemia, which is composed of a monoclonal IgM, usually with rheumatoid factor activity, and polyclonal IgG (3).

The Birmingham vasculitis activity score (BVAS) (4) and the Disease Extent Index (DEI) (5-7) have been used to assess vasculitis activity and to denote the disease extent in primary systemic vasculitides (8). Neither measure has yet been used for the follow-up of biopsy proven HCV-associated CV nor have they been compared with standard serological and immunological parameters. In this study we have evaluated BVAS and DEI as well as serological and immunological parameters for the follow-up of patients with HCV-associated CV during therapy.

Materials and methods

Patients

Between January 1996 and January 1999 HCV-associated CV was newly diagnosed in 15 patients on the basis of the criteria of the GISC (Italian Group for the Study of Cryoglobulinemias), i.e. 6-months duration of symptomatic cryoglobulinemia, presence of at least 2 symptoms from Meltzer's triad (purpura, arthralgia, weakness), detection of high rheumatoid factor (RF) activity and/or low complement factor C4, and no coexistence of autoimmune, lymphoproliferative or other infectious disease (except for HCV infection) (9-12). Furthermore, the Chapel Hill Consensus Conference definition of "essential" CV (1) was applied, and histological proof of immune-complex mediated vasculitis affecting small vessels was sought in each patient.

Diagnosis of chronic hepatitis C infection was based on standard criteria, i.e. history, laboratory abnormalities, serology and liver histology as described elsewhere (13). All patients were examined by a team of clinicians consisting of specialists in ophthalmology, neurology and rheumatology. Additional imaging procedures included chest X-ray, ultrasound of the abdomen, echocardiography and magnetic resonance imaging (MRI) of the head as previously described for other vasculitides by our group (14).

Vasculitis activity was denoted by applying the Birmingham vasculitis activity score (BVAS). This score is a clinical index of the degree of vasculitis activity in nine separate organ-based systems, i.e. systemic, cutaneous, mucous membranes/eyes, ear, nose and throat, 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, e.g. purpura, ulcer and digit gangrene indicating cutaneous vasculitis activity. The activity index provides a total score of clinical disease activity and improves with remission of the vasculitis. The maximum score is 63 points for present symptoms and 32 points for new symptoms or symptoms which had worsened within the previous weeks (4).

The disease extent was measured by applying the Disease Extent Index (DEI). This index scores the organs involved in a vasculitis regardless of the onset and thus describes the number of organ systems involved, e.g. skin and peripheral nerves. Every organ system affected by a vasculitis (i.e. ear, nose and throat, upper respiratory tract, lung, eye, kidney, heart, gastrointestinal tract, skin, peripheral nervous system, central nervous system, rheumatic complaints) is given two points with the exception of constitutional symptoms which is allocated one point. A total of 21 points can (theoretically) be reached (5-7).

Laboratory testing

Routine blood chemistry, antibody detection and urine sediment analysis were performed at the time of diagnosis of the HCV-associated CV and every 3 months after treatment was started. Patient sera were routinely tested for serum trans-

aminases, gGT, AP, concentrations of serum bilirubin, total protein, albumin, IgG, IgA, IgM, creatinine, complement factors C3c and C4, total hemolytic complement CH50, and rheumatoid factor (RF) by standard methods. Anti-nuclear antibodies (ANA), antibodies against extractable nuclear antigens (ENA), e.g. anti-SSA, anti-mitochondral-antibodies (AMA), antibodies associated with autoimmune liver disease, e.g. anti-soluble liver antigen antibodies (SLA), and anti-cardiolipin IgM and IgG antibodies (ACLA) were detected by current techniques. Anti-neutrophil cytoplasm antibodies were detected by established and evaluated techniques (cANCA/pANCA by IFT and specificities by ELISA) (15). Urine sediment analysis was routinely done.

Cryoglobulins were detected and classified according to Brouet *et al.* (16) as described elsewhere (13). IgG antibodies to HCV were determined with an ELISA-test (Sanofi Pasteur) using two recombinant antigens produced in *E. coli*. One of the antigens is located in the structural and the other in the non-structural (NS3) area of the virus genome. Sample preparation, amplification and detection of HCV-RNA was performed with a kit commercially available according to the description of the manufacturer (Amplicor, Roche).

Therapy

After the diagnosis of HCV-associated CV, therapy was started according to current treatment recommendations (17-19). Patients were allocated to one of the following two treatment groups with regard to the severity of their CV: (1) severe life-threatening vasculitic manifestations, e.g. rapid deterioration of renal function, symptomatic CNS involvement or cardiac involvement, and acute flares of HCV-associated CV were treated with cyclophosphamide p.o. (2 mg/kg body weight); (2) if leukopenia became a prevailing problem under cyclophosphamide p.o. therapy, the patients were treated with cyclophosphamide i.v. (15 mg/kg body weight every 3-4 weeks; Endoxan®, ASTA Medica AWD, Frankfurt). Plasmapheresis had to be added in two patients in order to overcome their severe, disabling polyneuropathy. Glucocorticosteroid medication (starting with ca. 0.5 - 1 with consecutive dose reduction; prednisolone, Decortin H®, Merk, Darmstadt) accompanied cyclophosphamide therapy.

Chronic phases of the disease and mild organic manifestations were treated with interferon-α2b (Intron-A®, Essex Pharma, Munich) at the individually tolerated maximal dose (9-30 million units/week s.c.). Glucocorticosteroid therapy accompanying the interferon-α2b therapy was allowed. After allocation of the patients to one of the two treatment groups, the patients were prospectively followed. All patients gave informed consent for the collection of their data.

Criteria of remission of CV and response of HCV infection to therapy

Complete remission of CV was diagnosed when clinical and serological investigations as well as imaging procedures revealed no evidence of vasculitic manifestations. Partial remission of CV was defined as partial improvement of the disease activity or as an arrest of the disease progression. A relapse of CV was defined as the re-emergence of vasculitis activity after prior remission of the vasculitis. This was done in accordance with similar definitions of remission in primary systemic vasculitides (20). Furthermore, the biochemical (i.e., normalization of the serum alanine transaminase) and virological (i.e., disappearance of HCV-RNA from serum) response was determined during treatment of the HCV-associated CV in the same way as for patients with chronic hepatitis C without extra-hepatic manifestations. A sustained response was assumed in patients with a biochemical and virological response during therapy and for at least 6 months follow-up after therapy. A breakthrough was defined as the re-appearance of HCV-RNA and transaminase elevation after a treatment period of at least 3 months. A relapse of chronic hepatitis C meant a re-emergence of HCV-RNA and/or transaminase elevation subsequent to discontinuation of therapy in patients who had had a biochemical and virological response during treatment (21).

Statistics

The Mann-Whitney U test was applied to determine differences among clinical, serological and immunological parameters between patients treated either with interferon-α or cyclophosphamide. The Wilcoxon matched pairs signed rank test was used to test for significant differences in the parameters at the time of diagnosis as compared to after 6 months of treatment. Spearman rank order correlations were determined to assess the associations of clinical, serological and immunological parameters.

Results

Clinical and immunological findings, treatment

None of the patients were known to have HCV-associated CV before being referred to our unit. All but 2 patients had been given glucocorticosteroids on the assumption of an immunological disorder - most probably vasculitis - prior to admission to our unit. The main clinical and immunological findings are given in Table I and Table II. Hypocomplementemia, type II mixed cryoglobulinemia with monoclonal IgM and polyclonal IgG, HCV-antibodies and HCV-RNA were present in all patients. Diagnosis was supported by the results of one or more biopsies in all but one patient, who refused a biopsy. Cutaneous leukocytoclastic vasculitis with immune complex depositions in small vessels was demonstrated in 6 patients; necrotizing vasculitis of small to medium-sized vessels of the muscle in 4 patients; necrotizing vasculitis of small vessels in a nervus suralis biopsy in 2 patients; intracapillary glomerulonephritis in 1 patient and mesangioproliferative glomerulonephritis with immune complex depositions in 1 patient; necrotizing vasculitis of small to medium-sized vessels of the adnexe and omentum in 1 patient, and necrotizing vasculitis of small to medium-sized vessels in the gallbladder in 1 patient. All patients had biopsy-proven chronic hepatitis C with a histological appearance ranging from minimal fibrosis to portal fibrosis with few septa, and mild to moderate activity. Liver cirrhosis was not present.

After the diagnosis of HCV-associated CV, the patients were treated with cyclophosphamide or interferon-α2b, depending on the severity of their vasculitis.

Table I. Clinical manifestations of 15 patients with HCV-associated CV treated either with interferon-α2b or cyclophosphamide according to their vasculitis activity.

Parameters	Interferon-α2b	Cyclophosphamide
No. of patients	8	7
M/F ratio	2/6	2/5
Purpura	5/8	5/7
Arthralgia	4/8	4/7
Weakness	5/8	6/7
Polyneuropathy	6/8	7/7
Glomerulonephritis	2/8	1/7
Sjögren's syndrome	2/8	1/7
Raynaud's phenomenon	2/8	1/7
Other manifestations	Heart (1/8), gastrointestinal (2/8), CNS (1/8)	Heart (1/7), gastrointestinal (2/8), CNS (1/8) *sec. art. temp. (1/7)
Biopsy proven vasculitis	7/8	6/7

*sec. Art. temp. = secondary arteritis temporalis.

Eight patients were treated with interferon-α2b and 7 patients were treated with immunosuppression due to the severity of the CV. Five of the 7 patients treated with cyclophosphamide received cyclophosphamide p.o. (2 mg/kg body weight) and 2 patients cyclophosphamide i.v. (15 mg/kg body weight every 3-4 weeks). Plasmapheresis (6 courses with 2-4 liters exchange per session every 3 days) was initially used in addition to cylophosphamide p.o. in 2 patients with a severe, disabling polyneuropathy.

All the patients in the cyclophosphamide group and 5 patients in the interferon-α2b group were additionally treated with glucocorticosteroids, with the dose being significantly higher in the cyclophosphamide group at the time of diagnosis ($p = 0.05$), but not after 6 months ($p = 0.4$). BVAS was significantly higher in the cyclophosphamide group than in the interferon-α group at the time of diagnosis as well as 6 months later ($p < 0.05$). DEI was also higher in the cyclophosphamide group, but the difference did not reach significance. None of the other parameters were significantly different between the 2 treatment groups at the time of diagnosis. After 6 months ALT levels were significantly higher in the cyclophosphamide group ($p < 0.05$), whereas other parameters (except BVAS, see before) did not differ significantly.

Table II. Vasculitis activity and disease extent as measured by BVAS and DEI. serological and immunological parameters of 15 patients with HCV-associated CV treated either with interferon-α2b or cyclophosphamide according to vasculitis activity as at time of diagnosis.

Parameters	Interferon-α2b	Cyclophosphamide
No. of patients	8	7
Age (years)	58 ± 15	56 ± 13
BVAS	12.2 ± 3.9 *	21.3 ± 6.7 *
DEI	4.6 ± 1.7	5.4 ± 2.4
ESR	28 ± 21	33 ± 10
CRP	0.7 ± 0.6	0.5 ± 0.0
Cryoglobulin	892 ± 735	849 ± 1075
C3c	0.75 ± 0.16	0.72 ± 0.11
C4	< 0.09	< 0.09
CH 50	< 18	< 18
RF	252 ± 237	310 ± 302
ALT	20.9 ± 7.4	18.7 ± 6.5
ANA	3/8	2/8
Anti-SSA	0/8	1/8
AMA	1/8	0/8
Anti-SLA	1/8	0/8
ACLA	2/8	0/8
cANCA	1/8	1/8
Steroids	5/8 (18.4 ± 23.3) *	7/7 (34.6 ± 26.6) *

Laboratory data (except antibodies), BVAS and DEI are expressed as mean ± standard deviation. The normal reference ranges were as follows: ESR ≤ 20 mm/h; CRP < 0.5 mg/dl; ALT < 18 U/l; RF = rheumatoid factor < 30 U/ml; complement: C3c 0.9 - 1.8 g/l; C4 0.1 - 0.4 g/l; total hemolytic complement CH50 20 - 50 U/ml; BVAS = Birmingham vasculitis activity score [4]; DEI = Disease extent index [5, 6, 7]; ANA = anti-nuclear antibodies (ab.); AMA = anti-mitochondral-ab.; Anti-SLA = anti-soluble liver antigen ab.; ACLA = anti-cardiolipin ab.; cANCA = anti-neutrophil cytoplasm ab. with cytoplasmic fluorescence (specificity could not be determined); Cryoglobulin detection threshold for immunofixation ≥ 80 mg/l; Steroids = prednisolone p.o. is expressed as number of patients and dose in mg/day (in brackets).
* BVAS and prednisolone dose were significantly higher in the cyclophosphamide group as compared to interferon-α2b at the time of diagnosis.

Outcome

Interferon-α group: 5 patients achieved a partial remission and 3 patients a complete remission of their CV after 3-6 months of treatment. A biological response was seen in 5 patients, and 4 patients experienced a virological response after 3-6 months. BVAS (12.2±3.9 at the time of diagnosis vs. 4.7 ± 1.5 after 6 months of interferon-α2b), cryoglobulin (892 ± 735 vs. 181 ± 140 mg/l), C3c (0.75 ± 0.16 vs. 1.02 ± 0.10 g/l), ALT (20.9±7.4 vs. 14.5±4.1 U/l) and rheumatoid factor (252±237 vs. 62±26 U/ml) changed significantly within 6 months ($p < 0.05$). There was a trend towards a lower glucocorticosteroid dose (18.4 ± 23.3 vs. 6.0 ± 2.8 mg/d) and DEI (4.6 ± 1.7 vs. 2.0±0.0) (both $p = 0.06$), but ESR (28 ± 21 vs. 22±11 mm/h) and CRP (0.7 ±0.6 vs. 0.5±0.0 mg/dl) were not significantly reduced ($p=0.4$, $p=0.3$, respectively).

Immunosuppression group: 3 patients achieved a partial remission and 4 patients a complete remission of their CV after 3-6 months of treatment. Neither a biological response nor a virological response was observed. BVAS (21.3±6.7 at the time of diagnosis vs. 8.4±5.2 after 6 months cyclophosphamide), DEI

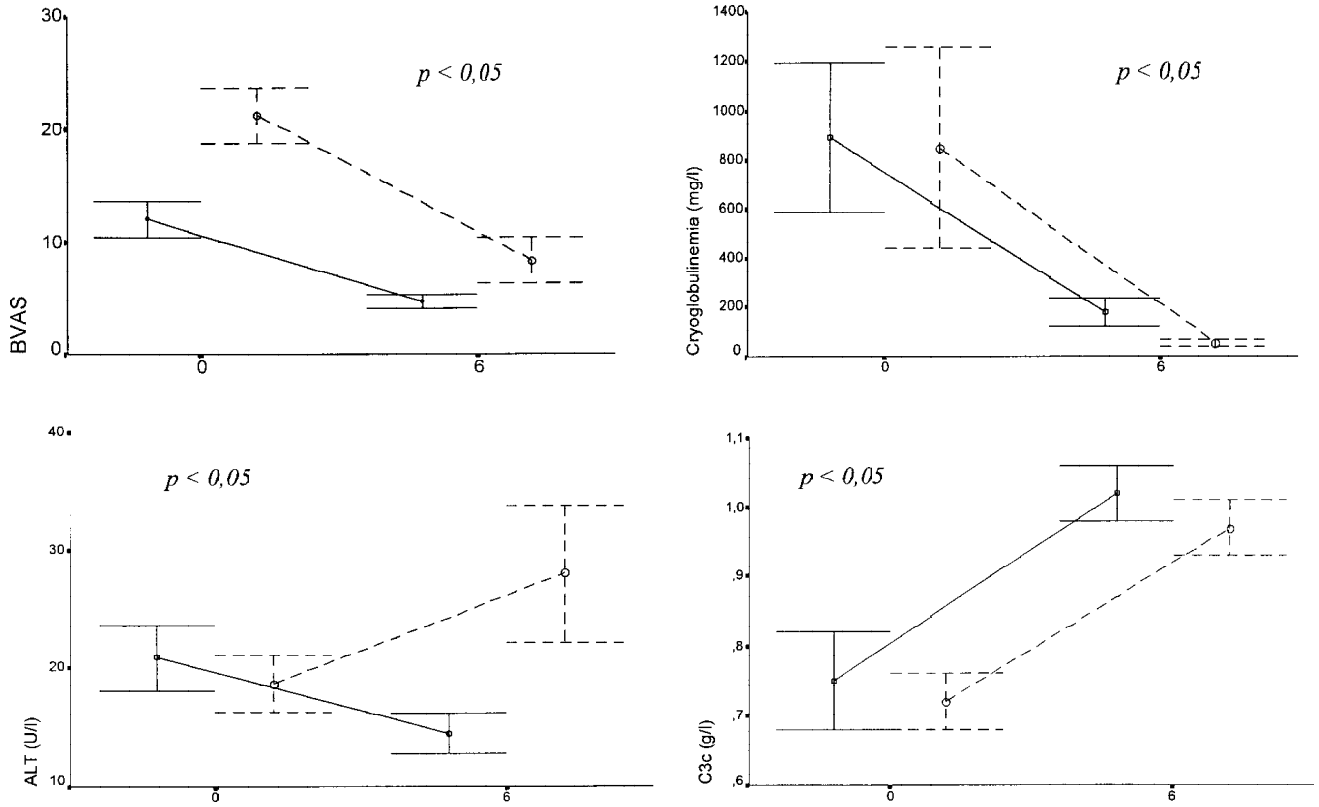

Fig. 1. Significant changes in Birmingham vasculitis activity score (BVAS) [4], cryoglobulinemia, ALT- and complement factor C3c levels during a 6 months period in 15 patients with HCV-associated CV treated either with interferon-α2b (——) or cyclophosphamide (- - -) according to the vasculitis activity. Bars represent mean ± SEM.

(5.4±2.4 vs. 2.0±0.0), cryoglobulin (849 ±1075 vs. 56±37 mg/l), C3c (0.72±0.11 vs. 0.97 ± 0.12 g/l), ALT (18.7 ± 6.5 vs. 28.0 ± 15.4 U/l), ESR (33 ± 10 vs. 21 ± 11 mm/h), rheumatoid factor (310 ± 302 vs. 51 ± 44 U/ml) and glucocorticosteroid dose (34.6 ± 26.6 vs. 7.6 ± 4.5 mg/d) changed significantly within 6 months ($p < 0.05$). The CRP (0.5 ± 0.0 vs. 0.6 ± 0.2 mg/dl) did not change significantly ($p = 0.3$) (Fig. 1).

Spearman rank order correlations were used to assess the association between clinical, serological and immunological parameters. In both treatment groups BVAS and DEI correlated negatively with C3c.

Follow-up

Interferon-α group: Five patients were treated with interferon-α2b for 12-18 months. Three patients are still being treated with interferon-α2b (12-24 months). Among the aforementioned 5 patients, 2 patients were lost to follow-up after 12 months of therapy, 2 relapsed with a severe flare of their CV 2 and 12 months respectively after cessation of the interferon therapy and were treated with oral cyclophosphamide thereafter, and 1 patient continued on a low-dose glucocorticosteroid monotherapy.

Immunosuppression group: Seven patients were treated with either cyclophosphamide p.o. or cyclophosphamide i.v. for 6-12 months and have been followed for 6-12 months since. Two patients are still being treated with cyclophosphamide (6-8 months). 3 patients with contraindications for interferon-α were switched to methotrexate, 1 patient to interferon-α2b, and 1 patient to interferon-α2b and ribavirin for the maintenance of their cyclophosphamide-induced remission.

Discussion

The present study demonstrates that partial or complete responses of an HCV-associated CV during 6 months of either interferon-α2b or cyclophosphamide therapy can be monitored by the follow-up of clinical measures of vasculitis extent and activity which have been used in primary vasculitides, i.e. the Birmingham vasculitis activity score (BVAS) (4) and the Disease Extent Index (DEI) (5-7). The patients were treated either with cyclophosphamide or interferon-α2b, depending on the severity of the CV. Thus, the decision regarding therapy was based on clinical grounds. Interestingly, the significant difference in BVAS between both treatment groups reflected the decision for one or the other therapy. A BVAS above 20 was always followed by intensified therapy, i.e. cyclophosphamide and higher glucocorticosteroid doses. Previously, the scoring system of the GISC (Italian Group for the Study of Cryoglobulinemias) had been used alone or in combination with other scores (e.g. purpura, glomerulonephritis and neuropathy scores) for the follow-up of HCV-associated CV (10, 12, 22). However, single or few scoring items may not correctly reflect vasculitis activity as they may be biased by different responses of vasculitic manifestations of the CV, especially during interferon-α therapy (18, 23), e.g. purpura responds well to interferon-α (10, 22) whereas neuropathy does not (10). BVAS and DEI are currently under evaluation for primary

systemic vasculitides. BVAS significantly declined with both treatments, whereas a significant decrease of DEI was observed in the cyclophosphamide group, and a trend was seen in the interferon-α2b group. This difference may be partly due to the higher range of BVAS and, subsequently, a greater decrease of values of the BVAS as compared to the DEI during therapy.

In our study, clinical improvement was accompanied by a significant decrease of cryoglobulinemia, rheumatoid factor activity, and an increase of complement factor C3c. The consumption of the early serum complement components with a characteristic sparing of C3 is thought to present a typical pattern of HCV-associated mixed cryoglobulinemia and CV and has been attributed to alterations of the control mechanisms of the classical pathway, especially C4-binding protein (24). However, further studies on the role of complement factors and the aforementioned pattern are needed. Serum C4 levels are usually found to be low in HCV-associated mixed cryoglobulinemia and CV, whereas C3 follows the disease activity intermittently (18). We also found generally low levels of C4 and low total hemolytic complement CH 50 throughout the diseases course.

When correlations of clinical, serological and immunological parameters were analyzed, only C3c correlated with BVAS and DEI in both treatment groups. BVAS also correlated with cryoglobulinemia and rheumatoid factor in the cyclophosphamide group. ESR (in the interferon-α2b group) and CRP (in both treatment groups) did not change significantly during the 6 months of therapy. Thus, as C3c levels correlated with BVAS and DEI, C3c provided additional information about vasculitis activity that was not reflected by either ESR or CRP during the following 6 months after initiation of a therapy. A similar situation has been demonstrated in rheumatoid arthritis with regard to TNF and ESR. In rheumatoid arthritis, TNF levels appear to provide information about disease activity which is not reflected by ESR (25).

Once HCV was found to be the principle cause of the formerly so-called “essential" mixed cryoglobulinemia, this decades’ randomized, controlled studies exclusively dealt with the beneficial effect of interferon-α on HCV-associated CV (10, 22, 26-29). Immunosuppressive therapy is recommended for acute flares or seriously deteriorating courses of CV on the basis of uncontrolled reports on small numbers of patients (17, 18). Interestingly, after 6 months of treatment with either interferon-α2b or cyclophosphamide none of the clinical, serological and immunological parameters - except BVAS and ALT - differed significantly between our treatment groups. Thus, viral persistence during cyclophosphamide therapy (30) will not result in a worse outcome of immunological parameters in CV during 6 months of therapy.

The number of patients included in this study was relatively small compared with other studies on interferon therapy (10, 22, 26-29). However, our study included patients with biopsy-proven vasculitis (only one patient refused a biopsy), thus representing a slightly different patient series with probably more severe disease manifestations in some patients.

In conclusion, patients treated with either cyclophosphamide or interferon-α2b depending on their disease activity, achieve remission of CV. Remission is reflected by significant changes of the BVAS, DEI (in cyclophosphamide treated patients), cryoglobulinemia, rheumatoid factor, and complement factor C3c, which correlated significantly with BVAS and DEI in both treatment groups. BVAS, DEI and complement factor C3c may be especially useful in evaluating disease activity during the therapy of HCV-associated CV.

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