Immunological and clinical follow up of hepatitis C virus associated cryoglobulaemia 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 cryoglobulaemia 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). Cryoglobulaemia, 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), cryoglobulaemia, 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 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 (cryoglobulaemia, RF, autoantibodies) and cellular (sIL2r, sICAM-1, sCD30) immune response and endothelial damage (sICAM-1) are found in HCV associated CV.

Hepatitis C virus associated cryoglobulaemia vasculitis (HCV associated CV) is an immune complex mediated vasculitis predominantly affecting small vessels. It typically evolves in patients with the presence of type II mixed cryoglobulaemia consisting of cryoprecipitating monoclonal IgM-RF and polyclonal IgG. This disorder is usually found after years of chronic hepatitis C, 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. Further serum levels of soluble intracellular adhesion molecule-1 (sICAM-1) and soluble interleukin receptor 2 (sIL2r) have been found acute and chronic hepatitis C without cryoglobulaemia. Previously, the correlation of cryoglobulin levels with organ involvement in CV has been shown to be weak. 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). 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. 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 Cryoglobulaemias)—that is, six month duration of symptomatic cryoglobulaemia, 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
Of 21 points can (theoretically) be reached.19–21 Given two points, except for constitutional nervous system, rheumatic complaints—is respiratory tract, lung, eye, kidney, heart, gastrointestinal tract, and ENT/upper respiratory tract—that is, ENT/upper respiratory tract, lung, eye, kidney, heart, gastrointestinal tract—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 active. Various defined abnormalities are ascribed for each organ system and may be scored if present. The activity index provides a total score for 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). Further information on these studies and BVAS can be obtained from the internet (www.vasculitis.org). Disease extent was measured by applying the DIEL. 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

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 active. Various defined abnormalities are ascribed for each organ system and may be scored if present. The activity index provides a total score for 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). Further information on these studies and BVAS can be obtained from the internet (www.vasculitis.org). Disease extent was measured by applying the DIEL. 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 diagnosis and either as inpatients or outpatients. Complete blood cell counts were excluded. Treatment followed current recommendations.19–21 The patients were treated with either interferon α2b (Intron-A; Essex Pharma, Munich, Germany) or interferon α2a (Roferon-A; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) or interferon α2a (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...
months of treatment with interferon.

Table 1 Clinical, serological, and immunological variables in eight patients with hepatitis C virus associated cryoglobulinaemia vasculitis who were consecutively treated with interferon α.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age (years)</td>
<td>55.8 (14.7)</td>
<td>7–70</td>
<td></td>
</tr>
<tr>
<td>M:F ratio</td>
<td>1:7</td>
<td>1:1</td>
<td></td>
</tr>
<tr>
<td>Purpura</td>
<td>7/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>5/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Weakness</td>
<td>5/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td>7/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>2/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>5/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td>3/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Anti-SSA</td>
<td>1/8</td>
<td>0–2</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>2/8</td>
<td>0–2</td>
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</tr>
</tbody>
</table>

ANA = Antinuclear antibodies; Anti-SSA = anti-SSA antibodies; ACLA = anticonutophil IgG; cDTNCA = cryoprecipitate pattern antinuclear antibodies; C3c = complement C3c; C4 = complement C4; CH50 = total haemolytic complement; sCD30 = soluble CD30; sICAM-1 = soluble intercellular adhesion molecule-1; sIL2r = soluble interleukin 2 receptor; sIL2R = soluble interleukin 2 receptor; sICAM-1 = soluble intercellular adhesion molecule-1; sIL2R = soluble interleukin 2 receptor; ESR = erythrocyte sedimentation rate; DEI = disease extent index.14–16

Two patients who initially responded to interferon α treatment 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 glucocorticoid monotherapy after cessation of the interferon α.

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. Cytokine leucocyte responses in seven patients with chronic hepatitis C and HCV infection were significantly reduced after six months of interferon α treatment. The interferon treatment was well tolerated and had no significant side effects attributable to their CV (table 1).

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.

VASCULARITY AND 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 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 hepatobiliary system. ESР 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), ESР, 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=0.004 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 glucocorticoid monotherapy after cessation of the interferon α.

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primary systemic vasculitides.17–21 BVAS and are under current evaluation in studies of HCV associated CV.

HCV associated CV takes time, indicating the need for monocentric acquisition of patients with HCV work in HCV associated CV. Furthermore, studies on the pathogenetic mechanisms at 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. 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.15 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 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.16

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.15 As mentioned above, correlation of cryoglobulin levels with organ involvement in CV has been shown to be weak. Complement consumption in the presence of cryoprecipitating 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.29–31 The reasons for this pattern are not well defined, but may involve alterations in regulatory components.22 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.17

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.17

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 interferon 2 receptors. These receptors are shed on activation of these cells.17 sIL2r levels are increased in chronic hepatitis C and in vasculitides such as Wegener's granulomatosis and Churg-Strauss syndrome.12 In Wegener's granulomatosis, there is a close correlation between sIL2r levels and disease activity.16 In this study, we found a significant difference in sIL2r between active
Hepatitis C virus associated cryoglobulinemic vasculitis

state sera and sera taken during remission.

Serum levels of sICAM-1 are elevated in patients with systemic vasculitis.

Serum levels of sICAM-1 and sCD30 levels may be more useful for follow

up of disease activity as other parameters such as cryoglobulinaemia.

References:


8. 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.

9. 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, sCD30.10

10. CD30 was shown to be increased in acute viral infections—for example, those caused by hepatitis B virus, Epstein-Barr virus, and HPV.11—systemic lupus erythematosus, and systemic sclerosis,12 as well as in certain neoplasms such as large cell anaplastic lymphoma and Hodgkin’s lymphoma.13 Treatment of chronic hepatitis C with interferon α diminishes the Th2 lymphocyte cytokine response—for example, interleukin 4, interferon γ—10 in these patients.14 CD30 has also been found to correlate with disease extent and activity in Wegener’s granulomatosis.15 We found a significant reduction in CD30 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 measure were correlated with complement C3c and sCD30 levels and thus may be more useful for follow-up of disease activity as other parameters such as cryoglobulinaemia.


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