Prevalence and clinical features of cryoglobulinaemia in multitransfused β-thalassaemia patients

R Perniola, C De Rinaldis, E Accogli, G Lobreglio

Abstract

Objective—The aim of the study was to determine the prevalence of cryoglobulinaemia and its clinical features among β-thalassaemia patients.

Methods—Eighty eight multitransfused β-thalassaemia patients were studied. They were physically examined and asked about the presence of cryoglobulinaemia related symptoms. Hepatitis C virus (HCV) serology, HCV-RNA, HCV subtypes, viraemia, serum ferritin, liver and kidney function tests, rheumatoid factor (RF), circulating immune complexes (CIC), complement levels and autoantibodies were all evaluated. The patients were divided into four groups: HCV-RNA positive patients with and without cryoglobulinaemia (groups A and B), HCV-Ab positive/HCV-RNA negative patients (group C), HCV-Ab negative patients (group D).

Results—Cryoglobulinaemia was present in 35 of 53 (66.0%) patients with chronic HCV infection. They had higher viraemia than non-cryoglobulinaemic viral carriers, but no statistical difference relating to sex or HCV subtypes was found. In comparison with the other groups, group A patients were older, had undergone transfusion therapy for a longer period, had received a higher number of transfusions, and had increased levels of RF and CIC, as well as consumption of C4; in addition, they had a higher prevalence of cirrhosis. Cutaneous lesions (purpura, Raynaud’s phenomenon, nodules and leg rash), peripheral neuropathy and sicca syndrome symptoms were present only in group A. Musculoskeletal symptoms (bone pain, arthralgia and myalgia), weakness, splenomegaly, lymphadenopathy, skin ulcers and proteinuria were also commoner in group A, but the difference did not reach statistical significance, possibly because of partial overlap between cryoglobulinaemia and β-thalassaemia syndromes.

Conclusion—Because of its high prevalence in multitransfused β-thalassaemia patients, cryoglobulinaemia needs to be systematically studied and considered in the differential diagnosis of various β-thalassaemia manifestations.


Multitransfused β-thalassaemia patients are at high risk of developing transfusion associated infectious diseases. Hepatitis C virus (HCV) is the main agent of post-transfusion hepatitis; as screening for HCV in blood donors was introduced only at the beginning of the 1990s, HCV antibodies (HCV-Ab) are found in most β-thalassaemia patients, the prevalence being proportional to the number of blood units received before this date. An association between chronic HCV infection and some immunological abnormalities has been ascertainment: a frequent one is the presence, in blood samples from HCV carriers, of cryoglobulins consisting of complexes of polyclonal IgG and monoclonal (type II) or polyclonal (type III) IgM. Purpura, arthralgia and weakness are frequently reported; kidney involvement, splenomegaly, lymphadenopathy, skin ulcers, peripheral neuropathy, Raynaud’s phenomenon and sicca syndrome resembling Sjögren’s sialadenitis are also reported in a variable number of cases. Some of these signs may overlap with those of β-thalassaemia syndromes: the aim of this study was to investigate the prevalence and clinical features of cryoglobulinaemia in a homogeneous group of β-thalassaemia patients.

Methods

Eighty eight multitransfused patients suffering from β-thalassaemia major or intermedia and followed up at the Paediatric Unit were studied. Age at first blood transfusion, duration of transfusion therapy and the number of transfusions received up until the time of the investigation were registered for each patient. For HCV serology, third generation assays were used (ELISA-3, Ortho Diagnostics, Raritan, NJ, and RIBA-3, Chiron, Emeryville, CA, USA); HCV-RNA was detected by a home-made “nested” polymerase chain reaction (with a detection limit of 100 copies/ml) using primers derived from the highly conserved 5' genomic region. Typisation was performed with a line probe assay (InnoLiPa, Innogenetics, Gent, Belgium), and viraemia was quantified with HCV Monitor (F Hoffmann-La Roche, Basel, Switzerland). None of the patients was currently infected with hepatitis B or human immunodeficiency viruses.

Liver biopsy was performed in 34 patients, in 24 using the percutaneous method, and in 10 during laparotomy for splenectomy and/or cholecystectomy. When biopsy specimens were unavailable, diagnosis of chronic liver disease was based on clinical and laboratory data. 
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Cryoglobulinaemia was considered a significant increase in viraemia was found in cryoglobulinaemic viral carriers: a difference in viraemia was found between cryoglobulinaemic viral carriers and non-cryoglobulinaemic ones (159.4 (244.5) (x10^3) copies/ml) and non-cryoglobulinaemic ones (18.9 (31.6) (x10^3) copies/ml, p=0.0158). With regard to the distribution of HCV subtypes, two of three and 13 of 20 patients infected respectively with subtypes 1a and 1b, the patient with coinfection 1a plus 1b, 15 of 23 patients infected with subtype 2a/2c, all three patients infected with subtype 3a, and one untreated patient were cryoglobulinaemic (p=NS). Among these, no statistical difference was demonstrable in either viraemia (subtype 1a: 140.0 (168.3) (x10^3) copies/ml; subtype 1b: 316.5 (300.7) (x10^3) copies/ml; subtype 2a/2c: 70.8 (166.3) (x10^3) copies/ml; subtype 3a: 17.0 (4.6) (x10^3) copies/ml; p=NS) or cryocrit (subtype 1a: 4.0 (4.2%); subtype 1b: 4.5 (3.5%); subtype 2a/2c: 4.6 (4.4%); subtype 3a: 7.7 (3.1%); p=NS). Table 1 gives other demographic, clinical and biochemical features of the HCV-RNA positive patients. Circulating cryoglobulins with very low cryocrit (0.5%) were found in one HCV-Ab positive/HCV-RNA negative patient and in one HCV-Ab negative patient: the presence of HCV-RNA was not investigated in the cryoprecipitate.

Cryoglobulinaemia related clinical and laboratory data

To summarise clinical and laboratory findings, we divided the patients into four groups (table 1): group A: cryoglobulinaemic HCV-RNA positive patients; group B: non-cryoglobulinaemic HCV-RNA positive patients; group C: HCV-Ab positive/HCV-RNA negative patients; group D: HCV-Ab negative patients. Analysis of variance and Kruskal-Wallis test showed a statistical difference in patients’ age, duration of transfusion therapy, number of transfusions received, RF, CIC and C4 levels. Application of multiple comparisons revealed that group A was statistically different from group D alone for patients’ age, from groups C and D for number of transfusions received and CIC and C4 levels, and from groups B, C and D for duration of transfusion therapy and RF levels. In the other comparisons, groups B and C differed from group D for duration of transfusion therapy and number of transfusions received; a difference was also found in C4 levels between group B and groups C and D. Among categorical parameters, a significant result was yielded in the prevalence of
Table 1 Cryoglobulinaemia related clinical and biochemical data

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=35)</th>
<th>Group B (n=18)</th>
<th>Group C (n=20)</th>
<th>Group D (n=15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female patients</td>
<td>23/12</td>
<td>8/10</td>
<td>12/8</td>
<td>11/4</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y)</td>
<td>28.9 (8.5)</td>
<td>23.7 (6.3)</td>
<td>24.0 (12.0)</td>
<td>18.0 (15.3)</td>
<td>0.0100</td>
</tr>
<tr>
<td>Age at first transfusion (y)</td>
<td>3.4 (5.0)</td>
<td>2.2 (4.6)</td>
<td>6.3 (12.8)</td>
<td>6.3 (10.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Transfusion therapy duration (y)</td>
<td>25.7 (6.3)</td>
<td>21.7 (4.6)</td>
<td>18.4 (4.8)</td>
<td>11.9 (6.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Transfusions received (n)</td>
<td>469.8 (136.1)</td>
<td>402.4 (171.5)</td>
<td>340.6 (144.1)</td>
<td>174.2 (112.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cryoglobulinaemia†</td>
<td>35 (100)</td>
<td>2 (5.6)</td>
<td>0</td>
<td>0</td>
<td>0.0092</td>
</tr>
<tr>
<td>Cryocrit (%)</td>
<td>4.8 (3.8)</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0.0170</td>
</tr>
<tr>
<td>Chronic liver disease†</td>
<td>34 (97.1)</td>
<td>16 (88.9)</td>
<td>12 (60.0)</td>
<td>6 (40.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cirrhosis†</td>
<td>11 (31.4)</td>
<td>2 (5.6)</td>
<td>2 (5.0)</td>
<td>0</td>
<td>0.0293</td>
</tr>
<tr>
<td>Serum ferritin (mg/dl)</td>
<td>1216 (1030)</td>
<td>2186 (2303)</td>
<td>1765 (1438)</td>
<td>1551 (1048)</td>
<td>NS</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>16.2 (18.7)</td>
<td>4.6 (4.1)</td>
<td>2.1 (2.3)</td>
<td>1.8 (1.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CIC (mg/ml)</td>
<td>11.4 (16.7)</td>
<td>6.4 (4.8)</td>
<td>3.0 (2.0)</td>
<td>3.0 (2.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>55.1 (13.3)</td>
<td>57.1 (11.9)</td>
<td>59.6 (11.9)</td>
<td>55.4 (9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>15.5 (4.9)</td>
<td>17.7 (4.1)</td>
<td>21.9 (7.1)</td>
<td>22.4 (4.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ANA†</td>
<td>3 (8.6)</td>
<td>1 (5.6)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>ASMA†</td>
<td>9 (25.7)</td>
<td>4 (22.2)</td>
<td>0</td>
<td>0</td>
<td>0.0992</td>
</tr>
<tr>
<td>Cutaneous lesions*†</td>
<td>7 (20.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Skin ulcer†</td>
<td>4 (11.4)</td>
<td>1 (5.6)</td>
<td>1 (5.0)</td>
<td>1 (6.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Musculoskeletal symptoms†</td>
<td>22 (62.9)</td>
<td>11 (61.1)</td>
<td>9 (45.0)</td>
<td>5 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Bone pain†</td>
<td>17 (48.6)</td>
<td>8 (44.4)</td>
<td>5 (25.0)</td>
<td>3 (20.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Arthralgia†</td>
<td>11 (31.4)</td>
<td>6 (33.3)</td>
<td>4 (20.0)</td>
<td>2 (13.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Myalgia†</td>
<td>3 (8.6)</td>
<td>1 (5.6)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Weakness†</td>
<td>19 (54.3)</td>
<td>7 (38.9)</td>
<td>9 (45.0)</td>
<td>4 (26.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Splenomegaly†</td>
<td>6/6 (100)</td>
<td>1/1 (100)</td>
<td>2/2 (100)</td>
<td>12/12 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Proteinuria†</td>
<td>2 (5.7)</td>
<td>1 (5.6)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral neuropathy†</td>
<td>3 (8.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Sicca syndrome symptoms†</td>
<td>2 (5.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Including purpura, Raynaud’s phenomenon, nodules and leg rash. Data shown as mean (SD) or †percentages.

Discussion

Multitransfused β-thalassaemia patients constitute an interesting population for the study of immunological diseases related to transfusion-transmissible infectious agents: despite this, very little has been published on the argument.6–7 Our data show that cryoglobulinaemia is significantly associated with chronic HCV infection even in β-thalassaemia patients. As shown elsewhere,6–11 cryoglobulinaemic viral carriers had a higher prevalence of cirrhosis than non-cryoglobulinaemic ones, were older and presumably had HCV infection of longer duration as a result of having received transfusion therapy for a longer period. Unfortunately, as almost all the HCV-Ab positive patients began blood transfusions in early childhood, and were infected when diagnostic tests for hepatitis C were unavailable, it is hard to determine the real duration of the infection.
It should also be borne in mind that acute hepatitis C is mostly asymptomatic and the cause of increase in serum transaminases is multifactorial in these patients. Nevertheless, the considerable manifestation of blood transfusions per year (up to 35–40) in each patient, and the high prevalence of HCV infected subjects among Italian blood donors, especially in southern regions, are consistent with a linear correlation between the duration of transfusion therapy and that of HCV infection in our patients. The longer exposure to iron overload and the higher viraemia might have contributed to the more extensive immunological alterations and the increased prevalence of cirrhosis in group A; however, viraemia levels fluctuate, and thus its role remains uncertain. With regard to the association between cryoglobulinaemia and sex or HCV subtypes, previous research has given inconclusive results; our findings did not show any relation to these variables.

The prevalence of cryoglobulinaemia among our patients with chronic HCV infection was unusually high: a comparison with the study by Congia et al on Sardinian β-thalassaemia patients is not feasible, as in that report patients with serum positivity for HCV-Ab and/or HCV-RNA were all placed in the same group. Such prevalence of cryoglobulinaemia could be interpreted as a sign of longstanding infection. In contrast, the prevalence of symptoms was low, as shown in table 1. This may be attributable to the following factors: firstly, we performed a screening on a patient population exposed to transfusion associated infections, while in several cases authors investigated patients who had been referred to their observation because they already showed clinical features of cryoglobulinaemia. On the other hand, the prevalence of cryoglobulinaemia related symptoms in our study was not significantly different from that of other studies performed to screen HCV infected or β-thalassaemia patients.

Secondly, the cryocrit was generally low despite efforts to avoid cryoglobulin precipitation before blood coagulated and serum was separated: it has been shown that a low cryocrit reduces the risk of clinical manifestations, this being mainly related to cryoglobulin type; however, because in this study cryoglobulin typing was not performed, interpretation of this aspect of the data is necessarily limited. Lastly, an environmental factor may be involved, as cryocomplexes develop in subependimal vessels of the skin attributable to hydrostatic pressure and cooling; they are then borne away by the blood stream and deposited in internal organs before dissolving. Although this aspect has been poorly investigated, some authors have underlined the importance of exposure to cold for the onset and/or aggravation of cryoglobulinaemia vasculitis. In this regard, it should be remembered that β-thalassaemia subjects generally do not work and spend most of their time at home because of their invalidity: this clearly results in a limited exposure to low temperatures.

Consequently, an interesting aspect of our study was that the prevalence of certain cryoglobulinaemia related clinical signs was higher in patients with circulating cryoglobulins, but often did not reach a statistical significance. This may be attributed to overlapping manifestations of cryoglobulinaemia and β-thalassaemia. Hyperplasia of bone marrow, with consequent thinning of cortical bone and increased prevalence of fractures, is the main cause of osteoporosis and osteoarthritis in β-thalassaemia subjects. Endocrine failures, especially hypogonadotrophic hypogonadism, may worsen the disease. Arthralgia also is a side effect of treatment with deferiprone, a new oral iron chelating agent: among our patients, only one group A subject was receiving deferiprone treatment, and he reported intermittent ankle pain. A clear distinction between bone pain and arthralgia is often unattainable, and in any case this would not help to definitively attribute the cause of the symptoms. Besides chronic anaemia, other causes of weakness may be advanced liver disease and endocrine failures leading to reduction of muscular mass, haemodilution related cardiomyopathy or psychological factors; in addition, non-specific myopathological changes have been observed in β-thalassaemia major. The low prevalence of arthralgia and weakness among group D patients may be attributable to their lower age at cryoglobulinaemia: indeed, in very young β-thalassaemia patients these symptoms are rare and more difficult to record.

Splenomegaly is a key sign of β-thalassaemia syndromes, whereas lymphadenopathy may be found in a variety of transfusion transmitted diseases, such as Epstein-Barr virus or cytomegalovirus infections. Skin ulcers of patients with blood dyscrasias can be provoked by poor tissue oxygenation; these were more frequent in the past, when a greater number of β-thalassaemia subjects were not adequately transfused. At present, skin ulcers are found in β-thalassaemia intermedia patients who do not receive regular transfusions, or in patients suffering from iron overload complications, such as IDDM; the lesions, localised at lower extremities, are typically slow to heal and indistinguishable from those resulting from vascular inflammation, as in the case of cryoglobulinaemia.

Other clinical manifestations need careful evaluation: although infrequent, renal disease in β-thalassaemia may be the result of prolonged iron overload, and an early marker of damage has been recently identified; however, even high dose deferoxamine treatment has been demonstrated to have nephrotoxic effects. On the other hand, MPGN is a well recognised complication of HCV related cryoglobulinaemia, and in fact our only patient with MPGN was cryoglobulinaemic. In the same way, particular attention must be paid to β-thalassaemia patients with symptoms of peripheral neuropathy: we found these features in three cryoglobulinaemic patients, although other studies have reported electrophysiological abnormalities in most cryoglobulinaemic patients. Nevertheless, other causes of neuropathy have to be considered in β-thalassaemia patients: for example, it is well known that spinal cord compression by

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extramedullary haematopoiesis masses may display various degrees of symptomatic neuropathy. When in doubt, appropriate investigations are required to clarify the diagnosis. In contrast, cutaneous manifestations (excluding ulcers) seem to constitute a hallmark of cryoglobulinaemia even in β-thalassaemia, as shown by the significant increase in group A.16 24

The increased levels of CIC and consumption of C4 confirmed the immunological alterations attributable to chronic HCV infection; these findings were more significant in the viral carriers with cryoglobulinaemia, in which high levels of RF were also found, as widely reported.7

In conclusion, cryoglobulinaemia is frequent in multitransfused β-thalassaemia patients with chronic hepatitis C, but some of its clinical signs are hard to evaluate precisely because of the partial overlapping between the two syndromes. Other symptoms require accurate investigation, underlining the need for systematic testing for cryoglobulinaemia in the differential diagnosis of several β-thalassaemia manifestations.

The authors thank Mr G Metcalf for his help in the translation of the manuscript.

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Ann Rheum Dis 1999 58: 698-702
doi: 10.1136/ard.58.11.698

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