Cryoglobulinaemia related to Sjögren’s syndrome or HCV infection: differences based on the pattern of bone marrow involvement, lymphoma evolution and laboratory tests after parotidectomy

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Abstract

Objective. The relationship of cryoglobulinaemia with lymphoproliferation of mucosa-associated lymphoid tissue (MALT) as risk factors for lymphoma evolution in SS remains to be clarified. The different biologic background of SS-related cryoglobulinaemia as compared with cryoglobulinaemia linked to HCV infection was clarified by different clinical and biologic approaches.

Methods. B-cell clonal expansion was analysed in the bone marrow of 27 consecutive cases with primary SS and mixed cryoglobulinaemia, HCV unrelated, in comparison with 55 HCV-related patients with cryoglobulinaemic vasculitis (CV) without SS. The results were related to the possible occurrence and localization of B-cell lymphoma in the single case. Secondly, the prevalence of mixed cryoglobulinaemia was investigated in 41 unselected patients with primary SS showing either parotid myoepithelial sialadenitis (MESA) or a frank B-cell non-Hodgkin’s lymphoma. Thirdly, the levels of serum cryoglobulins and RF were followed in one patient with primary SS, CV and parotid B-cell lymphoma of MALT after bilateral subtotal parotidectomy.

Results. A polyclonal pattern of B expansion in the bone marrow was significantly more frequent in SS-related (19/27 cases) than in HCV-related cryoglobulinaemia (19/55) (P = 0.003). Cryoglobulins were positive in a fraction of patients with SS and malignant lymphoma or with parotid MESA (13/18 and 7/23, respectively), whereas MALT involvement by the lymphoproliferative disorder was the rule. Finally, the levels of serum cryoglobulins and RF markedly decreased in the SS patient undergoing bilateral subtotal parotidectomy.

Conclusion. Lymphoproliferation of MALT appears as the biologic background of cryoglobulinaemia in SS, differently from HCV-related cryoglobulinaemia.

Key words: cryoglobulinaemia, Sjögren’s syndrome, lymphoma, hepatitis.

Introduction

SS is an autoimmune and lymphoproliferative disorder primarily involving the salivary and lacrimal glands, leading to glandular damage, dysfunction and sicca syndrome, and to an increased risk of development of B-cell non-Hodgkin’s lymphoma (NHL) [1, 2]. The presence of serum-mixed cryoglobulinaemia, i.e. serum positivity of cryoglobulins, with or without concomitant clinical manifestations of cryoglobulinaemic vasculitis (CV), occurs in ~10–15% patients with SS [1], and it consistently increases the risk of developing a B-cell NHL [2–4]. B-cell
NHL is also a well-known complication of the most frequent subset of CV, i.e. CV secondary to HCV infection, lacking SS [5]. Thus two different diseases (i.e. SS HCV unrelated and CV HCV related) are associated with mixed cryoglobulinaemia and predispose to B-cell NHL.

Besides cryoglobulinaemia, other risk factors for lymphoma evolution have been identified in SS, persistent parotid swelling in particular [4, 6-9]. A history of major salivary gland swelling was recorded in ~90% of the largest series of lymphomas in SS [10], and the use of molecular analyses of B-cell expansion in parotid biopsy lesions with evidence of SS-associated myoepithelial sialadenitis (MESA) better dissected a higher risk in cases with more aggressive pathological features and evidence of tissue persistent monoclonal B-cell expansion [4, 7].

While lymphomas complicating the course of HCV-related CV usually involve the bone marrow [5, 11], B-cell NHL complicating the course of SS usually involves mucosa-associated lymphoid tissue (MALT) sites [4, 9, 10]. Cryoglobulinaemia, as a well-established risk factor for the lymphoma evolution in SS, might then be linked to MALT lymphoproliferation in SS, and therefore SS-linked cryoglobulinaemia might present a different biologic background as compared with cryoglobulinaemia linked to HCV infection.

We herein report data from three different approaches employed by our group to better address this issue. First, molecular analyses of B-cell clonal expansion were performed in the bone marrow from consecutive SS cases with mixed cryoglobulinaemia, HCV unrelated, in comparison with classical HCV-related CV patients without SS, in conjunction with an extensive clinical and pathological characterization of the patient. In both the series, the molecular results were related to the possible occurrence and localization of B-cell NHL in the single case. Secondly, the prevalence of mixed cryoglobulinaemia was investigated in a series of consecutive, unselected patients with SS showing either persistent parotid swelling with MESA at parotid biopsy (pre-lymphomatous lesion), or a frank B-cell NHL.

Finally, one patient with SS, CV and parotid B-cell NHL of MALT was investigated in detail. The patient underwent bilateral parotidectomy, any medical treatment remaining unchanged, and the levels of serum cryoglobulin and RF were followed.

The three sets of data were concordant in indicating that mixed cryoglobulinaemia has a different biologic background in SS, if compared with cryoglobulinaemia linked to chronic HCV infection. In SS, mixed cryoglobulinaemia is associated with MALT B-cell lymphoproliferation and not with B-cell clonal expansion in the bone marrow, consistent with a primary role of chronic inflammation and lymphoproliferation of MALT as a predisposing factor to lymphoma in this disease. In contrast, HCV-related mixed cryoglobulinaemia is mainly a bone marrow and liver B-cell autoimmune and lymphoproliferative disorder [11, 12], and MALT lymphoproliferation is rarer in this setting [13, 14]. Overall, from a biologic point of view, MALT lymphoproliferation characterizes SS and appears as the background predisposing factor to B-cell NHL.

Materials and methods

All the patients included in the present study were followed in the Clinic of Rheumatology, University of Udine, Udine, Italy. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (Ethics Committee of the University Hospital ‘Santa Maria della Misericordia’ of Udine); all patients gave written informed consent. All the patients with SS (48 cases) suffered from primary SS, classified according to the American-European classification criteria [15], associated with mixed cryoglobulinaemia (27 cases) and/or with B-cell lymphoma or parotid MESA diagnosed by tissue biopsy (41 cases). Their median follow-up was 3 years (range 1-18 years). Controls with HCV-related CV were included in the first section of the study, as reported below.

All the patients and controls underwent an extensive clinical, instrumental and laboratory investigation to detect a possible lymphoma, usually due to the presence of persistent parotid swelling or positive serum cryoglobulins [4]. Briefly, parotid biopsy was performed in all the patients with persistent parotid swelling, always preceded by neck ultrasonography. Thorax and abdomen CT scan was also performed. Finally, the patients were also subjected to bone marrow biopsy and aspiration as a standard procedure to investigate the involvement of the bone marrow by lymphoproliferation. Laboratory tests included serum lactate dehydrogenase and β2-microglobulin assessment, and serum protein electrophoresis and immunofixation. Serum cryoglobulins were repeatedly searched, characterized by standard laboratory procedures [16], and, when present, an associated CV was classified according to the recently proposed international criteria [16]. HCV infection was excluded in all the SS patients by the absence of anti-HCV antibodies by last-generation ELISA.

Bone marrow expansion in SS-related cryo vs HCV-related cryo

In this first part of the study, 27 consecutive, unselected patients with primary SS and mixed cryoglobulinaemia [24 females and 3 males, mean age 51.9 (13.8) years, range 19-73 years] and 55 controls with HCV-related CV [42 females and 13 males, mean age 61.3 (11.6) years, range 24-79 years] were investigated for the presence of B-cell clonal expansion in the bone marrow. Their median follow-up was 3 years (range 1-11 years). In 19/27 SS cases, serum-mixed cryoglobulinaemia (11 type II, 8 type III) was accompanied by features of systemic vasculitis, i.e. CV. In contrast, 8/27 SS patients had serum-mixed cryoglobulinaemia (5 type II, 3 type III) without CV.

Controls with HCV-related CV had type II mixed cryoglobulins in 53 cases and type III in 2 cases. Their median follow-up was 9 years (range 2-30 years). They were
positive for anti-HCV antibodies by ELISA testing confirmed by recombinant immunoblot assay, and positivity of serum HCV RNA had been documented in all by PCR. All remained positive for HCV RNA during the whole follow-up period, when anti-viral treatment was not used. They also underwent extensive characterization to exclude a concomitant lymphoma, and bone marrow biopsy and aspiration was performed in all.

Amplification of immunoglobulin (ig) variable-diversity joining (V(D)J) gene segments was carried out in duplicate with third framework protocol on DNA purified from bone marrow lymphocyte [7, 17]. Clonal B expansion was identified by the presence of discrete, reproducible narrow bands within the predicted size range, whereas a polyclonal pattern was defined by a ladder of bands with similar intensities or by the presence of weakly dominant bands, not reproducible in repeated amplifications, as previously reported [7, 17].

Cryoglobulinaemia in SS-related B-cell lymphoma or MESA

In this second part of the study, the presence of serum cryoglobulinaemia was investigated (by at least three determinations in all) in 41 consecutive, unselected SS patients with either B-cell lymphoma (18 patients) or parotid MESA (23 patients; parotid biopsy performed in all due to persistent parotid swelling; all lacking lymphoma). Overall, these were 35 females and 6 males, with a mean age of 50.3 (14.0) years and a median follow-up of 3 years (range 1–18 years). The positivity of serum cryoglobulins was related to the histotype and the tissue localization of lymphoma, if present. All the SS-studied patients were negative for HCV infection.

The course of cryoglobulinaemia after bilateral parotidectomy in SS

A 51-year-old woman suffered from primary SS from 6 years, based on subjective and objective dry mouth and dry eye manifestations, positive anti-Ro/SSA and anti-La/SSB antibodies and positive minor salivary gland biopsy. HBV and HCV virus infections were absent. In July 2002, due to persistent enlargement of the right parotid, she underwent parotid biopsy showing a pathological picture of MESA. Molecular analyses of B-cell expansion revealed a B-cell monoclonal proliferation in the parotid tissue. In September 2003, persistent bilateral parotid gland swelling, as well as purpura and paraesthesias on the lower limbs, with a perimalleolar skin ulcer in the right leg, were recorded. EMG demonstrated a mild sensitive axonal polyneuropathy on the lower limbs. Serum RF, cryoglobulins and complement fraction C4 levels were 9190 IU/l (normal value <20 IU/l), 3256 and 7 mg/dl (normal value 10–40 mg/dl), respectively, confirming the diagnosis of CV associated with SS. A second right parotid biopsy showed a MALT-type B-cell NHL, and stage IE was documented. In November 2003, four weekly infusions of rituximab 375 mg/m² were administered without any clinical and laboratory improvement [18]. Then the patient also failed other therapies (oral daily and pulse CYC, then AZA), and a third repeated right parotid biopsy documented the persistence of MALT-type lymphoma (July 2004). Immunosuppressive therapy was discontinued. In February 2005, the patient underwent subtotal bilateral parotidectomy. Histopathological analyses showed a B-cell MALT-type lymphoma in both the parotid glands. No treatment (in particular, neither immunosuppressors nor glucocorticoids) was given from 5 months before to 3 months after parotidectomy, and the clinical manifestations and laboratory tests were strictly followed. This patient was also included in the previous two parts of the study.

Statistics

Chi-square test was used to compare categorical variables.

Results

Bone marrow B-cell expansion in the course of cryoglobulinaemia, either SS or HCV related

A polyclonal pattern of B-cell clonal expansion was shown in most bone marrow biopsies from patients with SS and mixed cryoglobulinaemia, i.e. in 19/27, whereas a clonal pattern (oligo- or monoclonal) was detected in 8/27 cases. This polyclonal pattern was more prevalent both in patients with type II and type III mixed cryoglobulinaemia (10/16 and 9/11, respectively; P = 0.4). No difference in the rate of bone marrow clonal expansion was noticed between SS patients with cryoglobulinaemia with CV (14/19 polyclonal vs 5/19 oligo- or monoclonal) and SS patients with cryoglobulinaemia without CV (5/8 polyclonal vs 3/8 oligo or monoclonal; P = 0.66 Fisher’s exact test). In contrast, a clonal pattern was demonstrated in the majority of bone marrow biopsies from HCV-related CV patients, i.e. in 36/55 cases (65.4%), whereas a polyclonal pattern was noticed in the remaining 19/55. Thus a pattern of B-cell oligo/monoclonal expansion in the bone marrow was significantly more frequent in HCV-related CV than in SS-related cryoglobulinaemia (P = 0.004, Pearson’s chi-square test; P = 0.02 when limiting analysis to the subset of patients with type II mixed cryoglobulinaemia, HCV or SS related). Of note, this occurred despite the fact that B-cell NHL was present in fewer patients with HCV-related CV (5/55 cases; 9.1%) than with SS-related cryoglobulinaemia (10/27 cases 48.1%) (P = 0.004, Fisher’s exact test).

Cryoglobulinaemia in SS-related B-cell lymphoma and SS-related MESA

Among SS patients with B-cell NHL, cryoglobulinaemia was detected in 13/18 (72.2%), 5/13 with CV. Among SS patients with parotid MESA without lymphoma, cryoglobulinaemia was detected in 7/23 (30.4%), and 6/7 showed a CV. Thus a significantly higher prevalence of mixed cryoglobulinaemia was detected in SS patients with lymphoma than in SS patients with MESA (P = 0.01, Fisher’s exact test).
All the SS-related B-cell lymphomas associated with cryoglobulinaemia had a marginal zone histotype (11 salivary gland lymphomas, 1 gastric lymphoma, 1 thymic lymphoma, and 1 nodal marginal zone lymphoma in a patient with parotid swelling, not subjected to parotid biopsy at the time of nodal lymphoma detection). By bone marrow biopsy, a bone marrow involvement by lymphoma was noticed in 2/13 (15.4%) of these SS-related lymphomas associated with cryoglobulinaemia. Thus the bone marrow was infrequently involved in SS-related lymphomas with cryoglobulinaemia, opposite to the usual bone marrow involvement observed in B-cell lymphomas linked to HCV-related CV (also noticed in four of five of our HCV-related controls with CV and B-cell lymphoma; data not shown). In all seven SS patients with parotid MESA and cryoglobulinaemia, the bone marrow showed non-neoplastic features. Overall, mixed cryoglobulinaemia was linked to malignant lymphoproliferation of MALT in SS, but was also detected, though significantly less frequently, in patients with SS and MALT pre-lymphomatous lesions, i.e. MESA.

The course of cryoglobulinaemia after bilateral parotidectomy in SS

The reported patient with SS, CV and parotid B-cell NHL underwent bilateral subtotal parotidectomy in February 2005. A skin ulcer in the right leg (about 4 × 6 cm in size) and purpura were present. On the day before parotidectomy, the values of serum RF and cryoglobulins were 8520 IU/ml and 700 mg/dl, respectively. One month after parotidectomy, a decrease of the size of the leg skin ulcer by two-thirds was observed for the first time during the course of this CV manifestation. The laboratory course is shown in Fig. 1, with marked decrease in serum RF and cryoglobulins. After initial response, however, the skin ulcer increased in size during the third month after parotidectomy, while purpura was still present. Furthermore, serum RF and type II cryoglobulins were 3390 IU/l and 440 mg/dl, respectively. At the beginning of the fourth month after parotidectomy, plasmapheresis was started. Subsequent follow-up and additional data are reported in detail elsewhere [18].

Discussion

Two different diseases, i.e. SS and chronic infection by HCV, may be associate with mixed cryoglobulinaemia, with or without signs of vasculitis (called CV or syndrome), and may predispose to B-cell NHL [1–5]. Different lines of evidence in this study, when added to previous information, underscore that the biologic background of cryoglobulinaemia in SS is the lymphoproliferation of MALT, different from cryoglobulinaemia in the course of HCV infection, mainly linked to liver and bone marrow B-cell proliferation [5, 12].

Fig. 1. Course of RF (A) and type II mixed cryoglobulins (B) after bilateral subtotal parotidectomy in the reported patient with SS and parotid B-cell NHL. Black arrows indicate the time of parotidectomy. BL: baseline.
Information on the pattern of B-cell expansion in the bone marrow, i.e. in a key target organ of cryoglobulinaemia-associated lymphoproliferation based on present knowledge, was very limited up to now in SS-related cryoglobulinaemia [4], whereas more extensive data are available in HCV-related cryoglobulinaemia [19, 20]. The two settings were never directly compared, an important step to better understand biologic similarities or differences and to better interpret cryoglobulinaemia in SS.

Bone marrow molecular analyses in this study confirmed that cryoglobulinaemia is not associated with bone marrow lymphoproliferation in SS. This is in sharp contrast with the usual pathological and molecular evidence of B-cell lymphoproliferation in bone marrow of patients with HCV-associated cryoglobulinaemia [19, 20], herein confirmed. Conversely, it is well known that lymphoma associated with SS involves the MALT tissue, usually the salivary glands [10]. If the model of Helicobacter pylori-related B-cell lymphomagenesis of MALT is considered [21], the salivary glands or the glandular epithelium in general represent the likely source of antigenic or autoantigenic chronic stimulation sustaining the autoimmune and lymphoproliferative disease in SS [9].

SS mainly involves the exocrine glands and has been defined as an autoimmune epithelitis [1], i.e. SS is primarily a MALT disorder, and persistent parotid swelling is a well-recognized clinical risk factor for lymphoma evolution [4, 6–9]. Consistently, all the SS cases with lymphoma where cryoglobulinaemia was detected showed a marginal zone lymphoma histotype in the present series, very frequently with a salivary gland involvement. In a large published series of SS-related lymphomas, whereas ~50% of SS patients with lymphoma presented cryoglobulinaemia, 90% had a positive history for parotid swelling [10]. Of note, B-cell lymphoma occurs also in the lack of cryoglobulinaemia in SS [10]. Cryoglobulin-negative lymphomas were observed in 5/18 SS cases in the present series and even more frequently, i.e. in about one-half of the cases, in the large study by Voulgarelis et al. [10], arguing against a possible centre-related bias in this study. These data support the concept that it is the MALT disorder that predispose to lymphoma evolution in SS. From a clinical perspective, on the other hand, cryoglobulinaemia, when present, represents a very useful red flag for either the presence of lymphoma or an increased risk of lymphoma development in SS [3, 4, 22]. The laboratory determination of cryoglobulinaemia may lead to false negatives, for different reasons [16], whereas the detection of cryoglobulinaemia proves extremely important to optimize the follow-up of SS. The importance of the quality of laboratory determination of cryoglobulins is then underscored. In this series, cryoglobulin positivity was significantly more frequent in SS-related B-cell lymphoma than in SS-related pre-lymphomatous disorder of MALT, i.e. parotid MESA, suggesting a link between cryoglobulinaemia and more advanced stages of B-cell lymphoproliferation in SS. In contrast, cryoglobulinaemia is detected in ~40% of cases with HCV infection, while B-cell lymphoma occurs much more rarely [5]. Thus cryoglobulinaemia appears as a marker of advanced stages of lymphoproliferation more in the course of SS than in the course of HCV infection. From a clinical perspective, cryoglobulinaemia then represents a major warning for lymphoma evolution or for lymphoma already present in SS.

Cryoglobulinaemia as a secondary event to MALT lymphoproliferation in SS is finally supported by the history of the patient reported, showing a marked decrease in her RF and cryoglobulin serum levels after subtotal bilateral parotidectomy. Since concomitant medical treatment remained unchanged before and after surgery, the parotid glands, where a B-cell NHL of MALT was present, reasonably were a source of RF and cryoglobulins. Previous studies already demonstrated the mixed cryoglobulinaemia cross-reactive idiotype Wa in SS salivary lesions, as well as the production of IgM-x RFs in these lesions [23–27]. Furthermore, by DNA sequencing we demonstrated that the lymphomatous parotid B-cell clone from our reported patient was identical to the pre-lymphomatous monoclonal B-cell expansion in the first MESA sample and employed Ig heavy and light chain genes (IgHV1-69*01, IgHD6-13*01, IgHJ4*02; KV3-20*01, KJ1*01) (data not shown), identical to those employed by B-cell lymphomas in the course of cryoglobulinaemia [19, 20]. Further work is in progress to obtain the lgs expressed by the neoplastic B-cell clone from this lymphoma and to conclusively demonstrate its RF specificity.

Overall, different lines of evidence in this study better clarify that the biologic background of cryoglobulinaemia in SS is different from that of cryoglobulinaemia in the course of HCV infection.

This may be of value to better understand the pathobiology of SS-associated lymphoproliferation, as well as in clinical practice. Cryoglobulinaemia occurring in a patient with primary SS can be considered as secondary to the MALT autoimmune and lymphoproliferative disorder, and MALT lesions are confirmed as the key target tissue for the investigation of B-cell lymphomagenesis in this disease. Patients with SS should undergo surveillance for lymphoma, but definitive guidelines must be defined. We suggest that parotid biopsy and tissue molecular analyses of B-cell clonal expansion in synchronous and metachronous lesions are important to define the risk of lymphoma evolution in SS patients with parotid swelling [4, 7]. SS patients with cryoglobulinaemia should also be carefully investigated for the risk of MALT involvement by lymphoproliferation rather than bone marrow involvement.

One final consideration should be devoted to the sialotropism of HCV and the possible coexistence of HCV infection, clinical features of SS including positive lip biopsy and anti-SSA/SSB antibodies, mixed cryoglobulinaemia and MALT lymphoma, repeatedly reported in different mixtures in a minority of patients [5, 28, 29]. Rather than generating confusion, in our opinion these are interesting cases of overlap between the two usual conditions, i.e.
SS, an HCV-unrelated MALT disorder with cryoglobulinaemia present in a minority of cases, and HCV-related cryoglobulinaemia, infrequently associated with overt B-cell lymphoproliferation of MALT.

**Rheumatology key messages**
- The biologic background of cryoglobulinaemia is different between SS and HCV infection.
- MALT inflammation and lymphoproliferation is the main predisposing factor to lymphoma in SS.
- HCV-related cryoglobulinaemia is mainly a bone marrow and liver autoimmune and lymphoproliferative disorder.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**
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