Combined therapy with rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP) for Sjögren’s syndrome-associated B-cell aggressive non-Hodgkin’s lymphomas

M. Voulgarelis, S. Giannouli, D. Anagnostou and A. G. Tzioufas

Objective. To determine the safety and therapeutic response of a regimen consisting of cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP) plus rituximab in patients with Sjögren’s syndrome (SS) and aggressive non-Hodgkin’s lymphoma (NHL).

Methods. Four SS patients with aggressive marginal zone NHL were enrolled in this trial. All patients were classified according to the newly proposed revised European–American classification of lymphoid neoplasms. Three out of four patients also had mixed cryoglobulinaemia (MC) of type II. They were treated every 3 weeks for eight cycles of CHOP. Patients also received rituximab, at a dose of 375 mg per square metre, on day 1 of each of the eight cycles of CHOP. Four weeks after completion of the eighth course of CHOP plus rituximab and every 6 months thereafter, patients were re-evaluated for response.

Results. Complete remission of lymphoma was achieved in all four patients. The lymphoma patients remained in remission for a period of 23, 15, 12 and 10 months respectively, while certain signs and symptoms of MC type II (purpura, peripheral neuropathy and arthralgias) significantly improved with treatment. In addition, the titres of circulating cryoglobulins and RF decreased, while C4 levels returned to normal.

Conclusion. CHOP plus rituximab was well tolerated and proved effective in SS patients with aggressive NHL. Our observations may warrant a larger controlled trial to assess the effectiveness of this regimen in such patients.

KEY WORDS: Sjögren’s syndrome, Lymphoma, Rituximab, Cryoglobulinaemia.

Non-Hodgkin’s lymphoma (NHL) is the most serious complication of Sjögren’s syndrome (SS). Most of these lymphomas are extranodal, related to mucosa-associated lymphoid tissue (MALT) and characterized predominantly by an indolent course. However, NHLs of high or intermediate grade are occasionally found in SS patients, are diagnosed at advanced clinical stage and are typically associated with poor survival [1]. The cyclophosphamide/doxorubicin/vincristine/prednisone regimen (CHOP), combining three of the most effective antilymphoma drugs, has been used for more than 25 yr in the treatment of aggressive NHL with cure rates ranging from 30 to 50%. Clinical trials comparing CHOP with more intensive and toxic treatments have shown no greater benefit when compared with the newest regimens [2]. Rituximab (R), a chimeric immunoglobulin (Ig) G1 monoclonal antibody that specifically targets CD20 surface antigen on B cells (from early pre-B to mature lymphocyte), induces both complement-mediated and antibody-dependent cytotoxicity and sensitizes the chemoresistant lymphoma cells to cytotoxic agents [3]. In previous studies, the combination of R-CHOP had a good safety profile, inducing responses in over 90% of patients with indolent or aggressive lymphoma [4]. Prompted by these observations, we undertook the present study to assess the therapeutic response and toxicity of R-CHOP in four female SS patients with aggressive NHL.

Patients and methods

Eligibility criteria

SS patients were eligible for entry if they had a histological diagnosis of high-grade marginal zone NHL of the MALT or non-MALT type according to the newly proposed revised European–American classification of lymphoid neoplasms [5]. All patients had previously received immunosuppressive therapy that included methotrexate, cyclophosphamide and prednisone. No patient had liver or renal impairment. Patients at the onset of the study were required to have a performance status of 0–2 according to the Eastern Cooperative Oncology Group scale [6] and no active local or systemic infection. All patients who participated in our study were fully informed of the aim of the study and provided written consent to their participation and their agreement that the results of this study may well be presented or published, solely in the interests of science, provided that their anonymity is maintained. We also confirm that this study was...
approved and authorized by the Scientific Board of Athens University Medical School Clinical-Pathological Division and conforms to standards defined by our University authorities.

**Pretreatment evaluation**

All patients fulfilled the European American classification criteria of SS [7]. For each individual, a special Sjögren’s record was completed and included the exocrine and systemic manifestations, haematological and biochemical studies, serum and urine protein electrophoresis and immunofixation, immunoglobin quantitation, β2-microglobulin, circulating cryoglobulins, serum levels of the complement components C3 and C4, ANA, autoantibodies to Ro/SSA and La/SSB, bone marrow biopsy, and computed tomography (CT) scans of the thorax and abdomen. Clinical examination of the oropharynx, gastroscopy and cerebrospinal fluid analysis were also included in staging procedures. All patients were staged by the Ann Arbor and International Prognostic Index scoring systems [8, 9]. Neurological assessment included strength evaluation, electromyography, motor and sensory nerve conduction velocity and sensory-evoked potential. Electromyography confirmed the diagnosis of chronic polyneuropathy in two of two patients tested at baseline, which was consistent with axonal plus myelin damage and detected a sensory and a mild motor involvement in both. Neuropathic symptoms (pain and paraesthesias) were also graded according to a patient-scored visual analogue scale (VAS) (range 0–10).

**Response criteria for lymphoma patients**

Tumour response was graded as (a) complete response (CR), (b) partial response, (c) no response or (d) progressive disease. CR was defined as complete disappearance of all measurable disease parameters for at least 4 weeks on physical examination and chest, abdomen and pelvis CT scans; B symptoms (fever, night sweats and weight loss), if present, had to be abated. Partial response was defined as a 50% reduction in the sum of the products of the cross-sectional diameters of all known lesions for at least 4 weeks. No response was defined as less than a partial response, while progressive disease was defined as an increase of ≥25% in measurable disease.

**Treatment plan**

Patients received the combination of 750 mg/m² of cyclophosphamide on day 1; 50 mg/m² of doxorubicin on day 1; 1.4 mg/m² of vincristine, up to a maximal dose of 2 mg, on day 1; and 40 mg/m² of prednisone per day for 5 days. They were treated every 3 weeks for eight cycles of CHOP. Patients also received rituximab, at a dose of 375 mg/m², on day 1 of each of the eight cycles of CHOP. No radiation therapy was given or recommended at the end of treatment. All the patients received central nervous system prophylaxis, which consisted of an intrathecal infusion of 12.5 mg of methotrexate. Four weeks after the completion of the combined R-CHOP chemotherapy, staging was repeated for all patients to assess response. Treatment was withheld between two courses of chemotherapy until the neutrophil or platelet counts reached 1000 and 100 000/μl respectively. No reduction in dose was done in subsequent courses.

**Follow-up**

All patients were followed up monthly with blood cell counts and biochemistry profile during the entire treatment period of 24 weeks. Four weeks after completion of the eighth course of R-CHOP and every 6 months thereafter, the patients were re-evaluated for response (exocrine and non-exocrine manifestations, cryoglobulinemia, autoantibody profile etc.) using the same diagnostic and imaging techniques as those used before treatment. A response in peripheral neuropathy was considered present if a decrease in VAS greater than 25% was noted. Electromyography was repeated whenever possible.

**Toxicity**

The standard criteria of the Eastern Cooperative Oncology Group were used for the evaluation of haematological and non-haematological toxicities [8]. Grade III and IV toxicities were considered significant.

**Results**

The demographic, clinical and serological data of patients, the Ann Arbor staging and the histological type of lymphoma are shown in Tables 1 and 2. All patients displayed unusually high levels of serum β2-microglobulin. Patients developed lymphoma 2, 5, 10 and 3 yr after the initial SS diagnosis. Three of the four patients had extranodal marginal zone B-cell lymphoma of the MALT type; the diagnosis was established by parotid gland biopsies and open lung biopsy. The lymphoma of case 2 was classified as nodal marginal zone lymphoma after a cervical lymph node biopsy. Malignant B cells expressed a CD20- , CD21-, CD35- and IgM-positive phenotype, but lacked reactivity for CD5, CD10 and IgD. All cases were characterized by high-grade transformation.

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**Table 1. Demographic, clinical characteristics and laboratory parameters of the patients**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>62</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td><strong>Exocrine signs/symptoms</strong></td>
<td>BPGE, ED</td>
<td>BPGE, ED, OD</td>
<td>UPGE, ED, OD</td>
</tr>
<tr>
<td><strong>Systemic manifestations</strong></td>
<td>Arthralgia, purpura, peripheral oedema, lymphadenopathy</td>
<td>Arthralgia, purpura, neuropathy, anaemia, lymphadenopathy</td>
<td>Arthralgia, anaemia, lymphadenopathy</td>
</tr>
<tr>
<td><strong>ANA titre</strong></td>
<td>1/2560</td>
<td>1/640</td>
<td>1/160</td>
</tr>
<tr>
<td><strong>Anti-Ro/La positivity</strong></td>
<td>−/+</td>
<td>−/+</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>RF (IU)</strong></td>
<td>640</td>
<td>640</td>
<td>80</td>
</tr>
<tr>
<td><strong>C3/C4 (mg/dl)</strong></td>
<td>102/2.8</td>
<td>87/16</td>
<td>126/24</td>
</tr>
<tr>
<td><strong>Cryoglobulinaemia</strong></td>
<td>Type II IgMx</td>
<td>Type II IgMx</td>
<td>−</td>
</tr>
<tr>
<td><strong>(type, mg/dl)</strong></td>
<td>150</td>
<td>320</td>
<td>40</td>
</tr>
<tr>
<td><strong>Previous immunosuppressive steroid therapy</strong></td>
<td>Steroid</td>
<td>Steroid, cyclophosphamide</td>
<td>Steroid</td>
</tr>
</tbody>
</table>

BPGE, bilateral parotid gland enlargement; UPGE, unilateral parotid gland enlargement; ED, eye dryness; OD, oral dryness; MTX, methotrexate. *Given during the disease course for the extraglandular manifestations prior to lymphoma development.
with sheets of large B-cells that left only small foci of low-grade disease, in such a way that the MALT component was barely detectable. Karyotypic analysis did not reveal chromosomal translocations or hyperdiploidy. Disseminated disease was common in our patients, with involvement of multiple mucosal (case 3) and non-mucosal sites such as the bone marrow (cases 1, 3 and 4). In the three cases with bone marrow involvement, bone marrow biopsies revealed infiltration by lymphoma cells, predominately in the form of intertrabecular nodules, and to a lesser extent with the pattern of interstitial infiltrates.

Three patients had significant bilateral parotid enlargement, while all patients had systemic manifestations, including lymphadenopathy, purpura, peripheral neuropathy, anaemia (haemoglobin <11.5 g/dl) and low C4 levels. Monoclonal immunoglobulin in serum electrophoresis was present in two of four patients. Mixed monoclonal cryoglobulinaemia (MC) type II was detected in three patients.

All four patients received the planned dose of chemotherapy, achieved CR after 8 cycles of R-CHOP therapy and remained in CR after 23, 15, 12 and 10 months. $\beta_2$-Microglobulin and lactate dehydrogenase (LDH) levels were also normalized. Monoclonal cryoglobulins, purpura and arthralgias disappeared after four courses of therapy in patients 1 and 4 and after five courses in patient 2. In all patients with MC type II, the RF titre decreased and a significant increase in the serum levels of C4 was observed; ANA titres and anti-Ro/La expression were unchanged (Table 3). An improvement in subjective symptoms of sensory peripheral neuropathy and a marked improvement in muscle strength were noticed in patients 2 and 4. In patient 4 the electromyography pattern was unchanged with respect to baseline 5 months after completion of therapy. Serum and urine monoclonal bands disappeared in patients 1 and 2.

The therapy-related complications were not severe and the treatment was well tolerated, only one patient requiring granulocyte colony-stimulating factor (G-CSF)/antibiotics. Noticeably, peripheral neuropathy in patients 2 and 4 was improved in spite of the use of the potentially neurotoxic agent vincristine. The number of CD20+ B-lymphocytes remained low after therapy for 6–7 months. Although infusions of rituximab were accompanied by complete elimination of CD20+ B-cells, severe infections and cytokine release syndrome were not observed. Red blood cell transfusions were not required, while nadir platelet counts remained above 100 000/μl. Two episodes of chills and fever were recorded; both were related to the infusion of rituximab and disappeared after the temporal discontinuation of the infusion.

### Discussion

SS is a chronic autoimmune disease that is at the crossroads of systemic autoimmunity and malignancy. Four to five per cent of SS patients, most often those with particular extraglandular manifestations, such as palpable purpura, peripheral neuropathy, anaemia, low C4 and lymphadenopathy, develop NHL [1, 10, 11]. Therefore, treatment of SS-associated NHL should target both the autoimmune and the neoplastic nature of the disease.

In a multicentre European study, the majority of SS-associated NHLs were primarily low-grade, extranodal, marginal zone B-cell lymphomas of the MALT type. In this study, three out of 33 of the cases showed a high-grade transformation, which suggests that lymphomas in SS patients potentially evolve towards a less differentiated cell type in some patients [1]. Moreover high-grade transformation to diffuse large B-cell lymphomas has been seen in some MALT lymphomas, in which the clonal relation between the high- and low-grade component has been confirmed by molecular analysis [12]. SS-NHL patients with high or intermediate grade lymphoma treated with CHOP alone have a rather poor outcome, with an estimated median overall survival of 21 months [1]. Rituximab is used for the treatment of a wide variety of lymphomas, including diffuse large B-cell lymphomas, but single-agent therapy with rituximab had only moderate activity in patients with advanced extranodal, marginal zone B-cell lymphomas [13]. These observations, together with data indicating that R-CHOP had a significant clinical effect in diffuse large B-cell lymphomas [14], encourage the use of this regimen in SS-NHL.
lymphoma, increasing both response rate and survival compared with CHOP alone [4], prompted us to use this regimen in four SS patients with aggressive NHL. The major point of our study was that R-CHOP induced sustained CR in all SS patients with aggressive B-cell NHL for a follow-up period that ranged from 10 to 23 months (mean 15 months). Moreover, the extranodal manifestations of our patients, such as peripheral neuropathy and skin vasculitis, disappeared after eight cycles of R-CHOP. The remission of these symptoms and signs was accompanied by a decrease in the circulating mixed monoclonal cryoglobulins as well as an increase in C4 levels. Thus, R-CHOP appears to be effective in controlling both the autoimmune and the neoplastic process in these patients. In a recent study, patients with aggressive lymphomas receiving intensive conventional chemotherapy with the ACVBP regimen (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) seemed to have better event-free and overall survival with this regimen compared with CHOP [14]. Therefore, the association of rituximab and ACVBP in SS patients with high-grade lymphomas should also be evaluated.

There is strong evidence that B-cell clones that are responsible for the myoepithelial sialadenitis in SS produce Ig with RF activity. Secreted by these proliferating RF-positive B-cell clones, type II mixed cryoglobulins are frequently detected in SS patients [13]. Rituximab efficiency in the type II, hepatitis C virus (HCV)-related cryoglobulinaemia has already been demonstrated [16]. The findings that rituximab was effective against RF-positive B-cell clones provided the trigger for using it for selective B-cell blockade in SS-related type II MC. The significant decrease in serum RF levels and cryoglobulins and the clinical improvement of purpura and peripheral neuropathy in our patients further support these data.

Previous studies suggested that rituximab selectively inhibits IgM-positive CD20+ plasma cells, which produce autoantibodies [17]. In our study, the titre of ANA and anti-Ro/La antibodies did not change; this observation indicates that the combination treatment with R-CHOP did not have any effect on antibody-producing B-cell clones, such as populations of long-lived plasma cells [18]. These cells, most likely sheltered in the splenic red pulp, bone marrow and inflamed tissues, secrete autoantibodies and therefore supply the antibody-mediated autoimmune memory in the absence of any detectable memory B cells [18]. The inability of R-CHOP to fully suppress autoimmune responses that are mediated by long-lived plasma cells gives further insight into the pathogenetic mechanisms governing B-cell expansion and autoantibody production.

In conclusion, we found that combination of R-CHOP was a well tolerated and efficient regimen for the treatment of the aggressive SS-associated lymphoma in our small group of patients. However, the effectiveness of this treatment should be further assessed by larger controlled, multicentre trials.

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References