Significant efficacy of 2-chlorodeoxyadenosine±rituximab in the treatment of splenic marginal zone lymphoma (SMZL): extended follow-up

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Background: Splenic marginal zone lymphoma with or without villous lymphocytes (SLVL/SMZL) is an indolent lymphoma that typically affects elderly patients and that has a median survival >10 years. It presents with marked splenomegaly. Treatment is required in symptomatic cases. Splenectomy remains one of the first-line options in patients fit for surgery. The best pharmacological strategy has not yet been identified for poor surgical risk cases. Among different possible chemotherapeutic approaches, purine analogs, alone or in association with Rituximab, seem to be a valid therapeutic choice.

Patients and methods: Fifty SMZL patients were treated with Cladribine ± anti-CD20 monoclonal antibody.

Results: Forty-seven of 50 patients were evaluable for response. ORR was 87%; 24 of 47 patients (51%) achieved a complete hematological response (CR), 17 of 47 (36%) a partial response (PR) and 6 (13%) resulted unresponsive. Interestingly, 15 of 24 cases (62%) in CR achieved also a molecular remission. After a median follow-up of 48 months, 7 of 41 responsive cases relapsed and the 5-year PFS was 80%.

Conclusions: These data confirm the efficacy of this schedule emphasizing the impact of minimal residual disease even in the outcome of SMZL patients.

Key words: cladribine, MRD, rituximab, splenic marginal zone lymphoma

introduction

Splenic marginal zone lymphoma (SMZL) is a rare B-cell indolent lymphoproliferative disorder (<2% of all non-Hodgkin’s lymphomas, NHL) that characteristically affects elderly or middle-age patients, with median survival longer than 10 years [1–4].

Malignant cells present features of mature activated B lymphocytes, with expression of CD19, CD20, CD22, CD79b and FMC7, and with light chain restriction. In the majority of cases, neoplastic lymphocytes are negative for CD5, CD23, CD10 and CD103. Bone marrow infiltration pattern is typically
intrasinusoidal, but it can become nodular during disease progression or after splenectomy [5, 6].

SMZL characteristically presents massive splenomegaly, abdominal discomfort, lymphocytosis and cytopenias, often related to hypersplenism. Lymphonodes and/or organ involvement are infrequent at the diagnosis, but they may develop with progression of disease. B symptoms are present in 25% up to 60% of cases, and autoimmune phenomena are not uncommon (15%–20% of patients). Serum paraproteinemia, usually <20 g/l, is observed in about 10%–25% of patients. Association with HCV infection has been registered more frequently in South Europe countries, supporting a role of HCV in lymphomagenesis.

No specific prognostic factors have been still established for SMZL: high tumor mass, hemoglobin level <12 g/dl, increased LDH, albumin <3.5 g/dl and increased β₂-microglobulin at diagnosis have been described as adverse prognostic factors [1, 4, 7, 8].

The overall prognosis of SMZL is quite good in most patients. The percentage of patients surviving 5 years from diagnosis is 65%–75%, often even in the absence of treatment or of a complete response to therapy. There is no clear advantage in a precocious treatment, which is considered indicated only when patients develop significant signs and/or symptoms (severe cytopenias, symptomatic splenomegaly, recurrent infections, systemic symptoms). A ‘watch-and-wait’ policy is also reasonable for asymptomatic patients who have moderate cytopenias and no-bulky splenomegaly.

Two-thirds of patients do not have symptoms at diagnosis, and up to one-third of those may never require antilymphoma treatment [1, 4].

Patients presenting with symptomatic (usually painful) splenomegaly are most often treated by splenectomy. This approach often results in a remission lasting several years. If patients have splenomegaly and are HCV positive, treatment with the anti-hepatitis drugs, such as alpha-interferon and ribavirin may be considered and offers good rate of responses [9, 10].

Patients with advanced disease are candidates for more aggressive therapy; purine analogs, such as fludarabine and cladribine, seem to produce higher response rates, although no standard regimen exists [11–13]. ‘The therapeutic impact of Cladribine (2-chlorodeoxyadenosine-2-chlorodeoxyadenosine) is not yet defined, with different response rates, up to 80%’ [14–16].

Moreover, Rituximab showed significant activity in SMZL. Treatment with Rituximab in symptomatic patients seems to be superior to splenectomy and often leads to normalizing of blood counts and disappearance of splenomegaly. Rituximab is synergistic with chemotherapy, and it is often added to whichever regimen is chosen [17–20].

To further contribute to understanding of the more efficacious treatment, we decided to retrospectively evaluate the activity of 2-chlorodeoxyadenosine with or without Rituximab in 50 SMZL observed at our Center from January 2000 and October 2008. Thirty-two of these 50 cases received anti-CD20 monoclonal antibody; indeed, 15 cases received Rituximab concomitantly to the 2-chlorodeoxyadenosine and other 17 after treatment, as consolidation.

Moreover, the prognostic impact of LDH, β₂-microglobulin, albumin, hemoglobin levels, HCV positivity and molecular status after treatment was also evaluated in this series.

Furthermore, we analyzed survivals of our patients according to Intergruppo Italiano Linfomi (IIL) prognostic score. This risk score system include LDH, albumin and hemoglobin levels identifying three different risk groups: low, intermediate and high [8].

patients and methods

Fifty patients affected by SMZL with or without villous lymphocytes were included in this analysis.

We enrolled all cases consecutively treated in our institution.

Eligibility criteria were histological diagnosis of SMZL, age >18 years; absence of renal, hepatic and respiratory failure; no evidence of active infections, HIV negativity, two or more signs of active disease (symptomatic splenomegaly, constitutional symptoms, severe peripheral cytopenias); written informed consent.

Patient evaluation included a full history and clinical examination, complete serum biochemistry with dosage of LDH and β₂-microglobulin, peripheral blood and bone marrow immunophenotyping, bone marrow biopsy, chest and abdomen and pelvic computed tomographic (CT) scan, serology for HIV, HBV and HCV. IgH and Bcl2/IgH rearrangements were carried out at diagnosis then every 3 months for 2 years, as previously reported [21].

Diagnosis was based on lymphocyte morphology, immunophenotype of peripheral blood and bone marrow samples, bone marrow biopsy and spleen histology when available.

Patients’ clinical characteristics are shown in Table 1. Ten of 50 patients had been previously treated [two with oral cyclophosphamide, four with clorambucil, one with clorambucil and oral cyclophosphamide, one with CHOP regimen, one according to the FLIDA schedule (Fludarabine, Idarubicin and steroid) and one with Rituximab alone]. Seven patients underwent splenectomy. Forty-four of fifty cases presented bone marrow involvement documented by biopsy (stage IV). Four of fifty patients resulted HCV positive.

According to IIL prognostic index, 12 patients resulted in low-risk group, 24 patients in the intermediate group and 14 cases in the high-risk group.

Table 1. Clinical characteristics of patients

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
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<tbody>
<tr>
<td>Median age (years)</td>
<td>64 (33–85)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>M : F = 24 : 26</td>
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</tr>
<tr>
<td>Median LDH value (U/l)</td>
<td>386 (137–1478)</td>
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<tr>
<td>Median Hb value (g/dl)</td>
<td>12.6 (8.5–16.4)</td>
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<tr>
<td>Median albumin value (mg/dl)</td>
<td>4 (3–5)</td>
<td></td>
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<tr>
<td>Median β₂-microglobulin value</td>
<td>2900 (1261–6900)</td>
<td></td>
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<tr>
<td>Splenectomy (n pts)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>HCV-positive patients</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pretreated patients</td>
<td>10</td>
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<tr>
<td>Oral cyclophosphamide</td>
<td>2</td>
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<tr>
<td>Clorambucil</td>
<td>4</td>
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<tr>
<td>Oral cycloph and clorambucil</td>
<td>1</td>
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<tr>
<td>CHOP</td>
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<td>FLIDA</td>
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<td>Rituximab</td>
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Forty-three patients were assessed at diagnosis for IgH and Bcl2/IgH rearrangement; 37 (86%) showed a molecular marker at the diagnosis.

**treatment**

Cladribine was administered IV at a dose of 5 mg/m²/weekly, for a total of six cycles. All patients received acyclovir, fluconazole and cotrimoxazole as anti-infective prophylaxis until 8 weeks after completion of 2-chlorodeoxyadenosine administration.

From 2000 to 2001, 18 patients did not receive Rituximab; after that period, further 32 cases were treated with antibody anti-CD20. In 17 of 32 cases (53%), Rituximab was administered after chemotherapy for four doses; in the remaining 15 cases, the anti-CD20 monoclonal antibody was infused concomitantly with Cladribine for a total of six cycles. Two of four HCV-positive patients received anti-CD20 monoclonal antibody.

Rituximab was infused over a 3–6 h period, on an outpatient basis. Patients were premedicated with diphenhydramine (40 mg orally) and acetaminophen (1 g orally).

Patients were evaluated for response 2 months after the end of treatment, then every 3 months during the first 2 years and every 6 months for further 3 years.

Response criteria were the following: (i) Complete Response (CR) was defined as resolution of organomegaly, normalization of the blood count (Hb >12 g/dl; platelets >100 × 10¹³/l; neutrophils >1.5 × 10¹⁹/l and no evidence of circulating malignant B cells). No evidence of bone marrow infiltration detected by immunohistochemistry. (ii) Partial response (PR) was defined as ≥50% of improvement of all signs and symptoms of the disease (resolution or decrease of spleen size and of lymphoadenopaties, improvement of cytopenias). (iii) Stable or progressive disease were computed as ‘no response’.

CTCAE v.4 criteria were used to assess toxic effect.

**statistical analysis**

All calculations were carried out using the SPSS for Windows, release 17, 2011. Overall survival and progression-free survival (PFS) were estimated using the Kaplan–Meier test. PFS was computed from the beginning of treatment to further disease progression, relapse or death for lymphoma.

**results**

**response rates and survival**

Fifty patients (24 males and 26 females; median age 64 years, range 33–81 years) affected by SMZL and treated between January 2000 and October 2008 at our institution were evaluated in this retrospective analysis. Overall, 47 patients were evaluable for clinical response.

Overall response rate (ORR) was 87%: 24 of 46 of these patients (51%) achieved a complete hematological response (CR), 17 of 46 (36%) a partial response (PR) and 6 (13%) resulted unresponsive. Interestingly, focusing on the 17 cases who received Rituximab as consolidation, the CR rate after Cladribine was 11%, but this percentage increased up to 66% after the anti-CD20 antibody. Moreover, in the whole series, the quality of response was significantly influenced by the addition of Rituximab: CR rate was 62.5% in the subgroup receiving the anti-CD20 antibody versus 21.4% for cases treated with Cladribine alone (P = 0.004). To note that the schedule of Rituximab administration (concomitant or after cladribine) did not condition the achievement of CR.

After the median follow-up of 48 months (range 3–150 months), 7 of 41 responsive cases (14%) relapsed and the 5-year PFS was 80%. PFS was not significantly influenced by sex, age (>65 years), stage of disease (IV versus I-II-III), hemoglobin <12 g/dl, albumin <3.5 g/dl, increased LDH values, β₂-microglobulin >3.5 mg/dl, splenectomy, or presence of a molecular marker at diagnosis. All seven relapsed patients were retreated with Rituximab-based therapy (three patients: Rituximab associated with oral ciclophosphamide; 4 patients: R-CVP). All cases reached a PR.

HCV positivity seems to be a worse prognostic factor: two of four HCV-positive cases did not respond to the therapy. The 5-year PFS was significantly conditioned by the achievement of response, but not by its quality. Indeed, the percentage of patients free from progression was 95% for cases in CR and 87% for cases in PR, versus 0% for no responsive patients (P < 0.001 Figure 1).

Forty-three patients were assessed at diagnosis for IgH and Bcl2/IgH rearrangement; 37 (86%) showed a molecular marker at the diagnosis. Thirty-one were reassessed 3 months after the end of treatment; 15 (48%) achieved PCR negativity. The 5-year PFS was significantly longer for cases achieving PCR-negativity (100% versus 73% P = 0.023 Figure 2).

The 5-year OS was 86%; it was not significantly influenced by sex, age (>65 years), stage of disease (IV versus I-II-III), hemoglobin <12 g/dl, albumin <3.5 g/dl, increased LDH values, β₂-microglobulin >3.5 mg/dl, or HCV positivity. Analogously, OS was not significantly different for cases with or without molecular marker at diagnosis or that underwent splenectomy as part of therapeutic plan. On the contrary, patients that lost molecular marker after treatment showed longer survival compared with cases still PCR positive, even without reaching the statistical significance (5-year OS = 100% versus 60%, P = 0.159).

The only parameter that significantly conditioned the 5-year OS was the response to treatment, even without difference.
between CR and PR; indeed, at 5 years, 95% of responsive patients survived versus 50% in the resistant cohort ($P = 0.045$; Figure 3).

Moreover, both PFS and OS were not significantly influenced by the IIL prognostic index score.

discussion

The advent of immunotherapy, in particular of anti-CD20 monoclonal antibody, has produced a significant improvement in the outcome of several lymphoproliferative disorders. Inevitably, Rituximab has been employed also in SMZL, demonstrating an effective anti-tumor activity either in monotherapy than in combination with chemotherapy [17, 19, 20, 22–25].

Minimal residual disease (MRD) is considered a real prognostic index in predicting hematological relapse in lymphomas. On this light, MRD eradication could be considered an end point for improving quality of response and consequently the outcome even in SMZL.

We evidenced the significant role of anti-CD20 monoclonal antibody in eradicating MRD in HCL patients pretreated with Cladribine, without significant toxic effect [26]. Our group previously evidenced the efficacy and the good safety profile of association Rituximab–Cladribine in MZL (marginal zone lymphoma): ORR 96.5% with 60.3% CR [24, 25].

On this basis, we decided to review 50 SMZL patients treated in our Hospital with Cladribine alone or plus anti-CD20 monoclonal antibody, demonstrating that this association constitutes an effective and safe therapeutic choice for SMZL patients: ORR 87% with 51% CR and 36% PR.

The 5-year PFS was significantly conditioned by the achievement of response, but not by its quality. Indeed, the percentage of patients free from progression was 95% for cases in CR and 87% for cases in PR, versus 0% for no responsive patients ($P < 0.001$) (Figure 2).

Our data showed that Rituximab–Cladribine association, thanks to its good toxic effect profile, could be a valid therapeutic choice in SMZL patients not fit for surgery.

Nevertheless, Anti-CD20 monoclonal antibody improved significantly quality of response increasing the percentage of CR and molecular remissions. Molecular status post-therapy considerably influenced clinical outcome; in fact, patients negative for MRD presented a longer PFS. These results remarked the crucial role of Rituximab in the treatment of SMZL. Moreover, Rituximab alone might be an advantageous alternative.

Further studies, even in controlled clinical trials, are needed to confirm our data.

disclosure

The authors have declared no conflicts of interest.

references