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High efficacy of Rituximab in indolent HCV-related lymphoproliferative disorders associated with systemic autoimmune diseases

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ABSTRACT

Objective. *The evidence of an increased frequency of B-non Hodgkin's lymphomas (NHL) in patients with HCV and systemic autoimmune diseases suggests a close relationship between infection, autoimmunity and cancer. Choosing the best therapy for patients affected either by HCV-related lymphoma or autoimmune disorders is not easy; in fact, some treatments may be accompanied by an excessive hepatic toxicity and may be followed by a reactivation of hepatitis. There is growing interest in the search for an ideal therapy for this kind of patient. Thanks to its mechanism of action and good toxicity profile, Rituximab could prove to be an attractive therapeutic option: it has been reported to be highly active in low-grade NHLs and has been proposed for the management of autoimmune diseases.*

Results. *In this paper we evaluate the role of anti-CD20 monoclonal antibody in mono-therapy in 10 patients with either indolent HCV-related lymphoma or autoimmune disease. A very high rate of response, of both NHL and of the associated autoimmune disease, was observed (100% of clinical response), with no significant hepatic and extra-hepatic toxicity.*

Conclusion. *Thus, although the number of patients was small, our data strongly support the use of anti-CD20 in this patient setting.*

Introduction

The etiologic factors of non-Hodgkin's lymphomas are still unknown. Several infective agents are suspected to be potential causes of this group of neoplasias (1). Epidemiologic studies have proposed that Hepatitis C virus (HCV), a hepatotrophic virus involved in the development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma, may contribute to the pathogenesis of lymphoproliferative syndromes (hepatitis C virus and lymphoproliferative disorders (2).

In fact, HCV infection is frequently associated with both non neoplastic and neoplastic B-cell lymphoproliferative disorders, including mixed cryoglobulinemia (MC) and B-cell indolent lymphomas. MC is a systemic vasculitis which is thought to progress to B-NHL in 5-10% of cases (3, 4). Forty to 100% of patients with MC are infected with HCV, thus reinforcing the potential link between HCV and NHL, particularly indolent lymphomas. Moreover, a direct involvement of HCV in lymphomagenesis has been subsequently supported by molecular results showing a monoclonal B proliferation in the bone marrow of patients with these disorders (5).

Recent data have shown the presence of HCV-related proteins and/or HCV replicative particles in peripheral lymphocytes and biopsy samples of lymphoma tissue by immunochemistry, reverse transcription-polymerase chain reaction and *in situ* hybridization. The role of HCV in the pathogenesis of B-cell lymphoproliferative disorders is unclear; it is probably due to an HCV-antigen driven process: chronic HCV antigen stimulation may be responsible for the activation and proliferation of B-cell clones.

The involvement of HCV in lymphomagenesis is further supported by evidence regarding the effects of antiviral treatment. It has been shown that HCV-related splenic lymphoma patients may achieve a complete hematological response after therapy with interferon-α (IFN-α), while no such effect was observed in HCV-negative patients (6). Moreover, a molecular response (negativity for IgH rearrangement) was also obtained in HCV-related lymphomas after treatment with IFN-α (7).

On this basis, there is growing interest in demonstrating a relationship between chronic HCV infection, systemic autoimmune disorders and lymphoproliferative neoplasias, and in the search for the best treatment for these patients. It should be noted that chemotherapy may be accompanied by an excessive hepatic toxicity and may be followed by hepatitis reactivation in HCV patients.

Rituximab, an unlabeled monoclonal chimeric antibody against the pan-B antigen CD20, is an effective treatment for indolent and aggressive NHLs. It eliminates most circulating B-lymphocytes, suggesting its suitability for au-

toantibody mediated diseases as well, by depleting the autoreactive B cells (8). Many reports have demonstrated its efficacy in refractory or resistant autoimmune disorders using the same schedule employed in lymphomas (9, 10). Thanks to its good toxicity profile, Rituximab could be an attractive therapeutic option in HCV-positive patients affected either by lymphoma or by autoimmune disorders. Thus, the main purpose of this study was to evaluate the role of Rituximab in this subset of patients.

Material and methods

Ten patients affected by HCV-positive indolent NHL, according to REAL-WHO classification, were enrolled in this study. Eligibility criteria were: histological diagnosis of indolent non-Hodgkin's Lymphoma (B-cell small lymphocytic lymphoma, lymphoplasmacytic lymphoma, follicular lymphoma-grade I or II), HCV positivity, performance status 0-3 (ECOG), age ≥ 18 years, normal cardiac function (ventricular ejection fraction ≥ 50%); normal renal defined as serum creatinine < 124 μmol/l; HIV negativity. Moreover, only patients affected by a systemic autoimmune disease were eligible for the study.

The protocol was approved by the Ethical Committee of our Institution and written informed consent was obtained from each patient.

All cases were affected by indolent NHL and HCV infection. Patients'clinical characteristics are shown in Table I. Four of 10 patients had been previously treated (two with oral cyclophosphamide, one with clorambucil and one with CHOP regimen). All cases presented bone marrow involvement (Stage IV); splenomegaly was present in four patients and lymphoadenopathies in one. All patients showed positivity for IgH rearrangement at molecular assay before treatment.

All cases were also affected by autoimmune disease (6 by rheumatoid arthritis (RA), 3 by mixed cryoglobulinemia (MC), and one by both RA and MC). Diagnosis of RA was made according to the 1987 revised criteria from the classification of RA (11). Patients with MC presented vascular lesions (12). Median values of ESR, CPR and rheumatoid factor (RF) were 27 (range 13-33 mm/h), 21.6 (range 0-79 mg/dl) and 157 (range 57-291 IU) respectively.

Staging included a full history and clinical examination, complete serum biochemistry, bone marrow biopsy, bone marrow molecular analysis, chest and abdomen and pelvic computed tomographic (CT) scan, dosage of some indicators of inflammation (C-reactive protein-CPR, erythrocyte sedimentation rate-ESR) and autoimmune markers such as cryoglobulins, antinuclear antibodies (ANA), anti-extractable nuclear antigen antibodies (ENA), rheumatoid factor (RF), and quantitative evaluation of HCV RNA.

The patients were evaluated for response 1 month after the end of treatment, every 3 months during the first 2 years and then every 6 months. Response was evaluated according to the International Working Group Recommendations (Cheson et al, 1999). WHO criteria (WHO, 1979) were used to assess toxicity.

The patients were treated with Rituximab (375 mg/m^2 once a week, for a total of four doses). Anti-CD20 monoclonal antibody was infused over a 6-8 h period on an outpatient basis. Patients were pre-medicated with diphenhydramine (40 mg orally) and acetominophen (1 g orally).

Molecular assays

PCR analyses were performed on mononuclear cells separated by Ficoll/Hypaque gradient from bone marrow samples. High-molecular-weight DNA was extracted and suitable aliquots were utilized for PCR tests after spectrophotometric quantitative evaluation.

Two consensus primers were designed for the VH-DH-JH regions of the immunoglobulin heavy chain gene (IgH), as previously described; the downstream primer was 5' labeled with 6-FAM fluorochrome. Every PCR procedure included distilled water instead of DNA as a negative control and DNA carrying monoclonal IgH rearrangement as a positive control. PCR-amplified products were resolved by 2.5% agarose gel and then by capillary electrophoresis.

Samples were prepared for analysis by mixing 1 μl of PCR products with 15 μl of deionized formamide (Amresco) and 0.5 μl Gene Scan ™500 Tamra-labeled internal standard (Applera). Capillary electrophoresis and fluorescence detection with a virtual filter C was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystem).

Genescan 2.1 software was then used to analyze the PCR products, with accurate sizing and quantification of the peak areas (13).

Results

During the period February 2002 to June 2004, 10 patients (8 female and 2 male; median age 70 years, range 53-80 years) were enrolled in the study. All cases were evaluable for clinical response. Five of the 10 patients (50%) achieved a complete hematological response (CR) and the remaining ones showed a partial response (PR). Molecular analysis after treatment was performed in 8/10 patients. Four of these (50%) achieved IgH-negativity.

After a median follow-up of 14 months (range, 10-33) all the patients survived with a median progression-free sur-

Table I. Clinical characteristics of patients.

	No.	Range
Median age (years)	55	39 – 70
Sex (M:F)	10: 0	
BM median infiltration	50%	25 – 80
Median Hb value (g/dl)	12.4	10.1 – 17.2
Median WBC value (x10^3/mcl)	3720	2200 – 16850
Median platelet value (x10^3/mcl)	85000	47000 – 172000
Splenectomy (no. pts)	1	
Splenomegaly (no. pts)	6	

vival (PFS) of 12 months (range, 8-31). Interestingly, the same patients who had achieved hematological CR (50% of cases), also achieved the complete clinical and laboratory disappearance of concomitant autoimmune disorders. Disappearance of purpura and weakness arthralgia, and decline of RF and cryglobulins was observed. The remaining 50% showed a clinical and laboratory improvement of the autoimmune disease. No further treatment for autoimmune symptoms was administered during the following 12 months. Monitoring of serum HCV RNA levels, performed in 8/10 patients, revealed no significant change; in fact, no patient presented an increase of HCV RNA levels and there were no alterations of hepatic function.

Interestingly, anti-CD20 toxicity, evaluable in all 10 cases, was very mild (grade 1-2 WHO), and restricted to the infusion time. One patient experienced chills and another allergic rhinitis, rapidly resolved by temporary cessation of the infusion and by the administration of steroids. Fever, nausea or vomiting were not recorded. No infections were observed.

Discussion

HCV prevalence in B-non Hodgkin's lymphoma patients is approximately 15% higher than that observed in the general population (1.5%) and in other hematologic malignancies (2.9%) (14). The geographic variation in this association may be due to genetic and/or enviromental factors.

Moreover, an increased prevalence of systemic autoimmune diseases is often observed in patients affected by HCV infection. Hepatitis C virus is one of the viruses most frequently associated with autoimmune features (15). A dysregulation of immune response during the course of infection seems to be one mechanism supporting the expansion of auto-antibody-producing B-cells.

The focus on HCV as a potential cause of lymphoproliferative disorders was prompted by data from MC Type II (14). HCV antigens may stimulate the expansion of mono- and oligoclonal B-cells. Envelope protein E2 is considered the main antigen involved in B lymphocyte activation and proliferation. Several authors have proposed a dual signalling model for B-cell activation: BCR-mediated and CD81 mediated pathways (16). In fact, it has been suggested that the E2 envelope protein is able to bind to CD81, a cell surface protein on B-cell, enhancing B-lymphocyte stimulation. These processes could favor the appearance of somatic hypermutations in Ig heavy/light chains (Vh region) and subsequently of pathologic B-cell clones (17). The evidence for an increased frequency of B-NHLs in patients with HCV and systemic autoimmune diseases underlines strong links between infection, autoimmunity and cancer (18).

The therapeutic choice in lymphoma anti-HCV positive patients is not often easy, due to the risk of reactivating an HCV infection. Aggressive treatments are frequently accompanied by an unacceptable toxicity, and conservative treatments rarely achieve adequate response in patients affected by low-grade NHL. Thus, there is an urgent necessity for a treatment that is non-toxic but able to induce a substantial number of complete remissions. Moreover, an ideal treatment should be able to control the autoimmune diseases that are frequently associated with NHL patients.

Rituximab appears to be a possible candidate: it has been reported to be highly active in low-grade NHLs and has been proposed in the management of autoimmune diseases (19-23). In this paper we report a very high rate of response of both NHL and the associated autoimmune disease. In particular, MC appears to be extremely sensitive to anti-CD20. Interestingly, and in contrast with some authors (24), our patients did not show any significant hepatic and extra-hepatic toxicity. When evaluated, viremia did not increase during or after treatment.

Rituximab has been reported to be very active in low-grade NHLs, but a significant rate of clinical and molecular responses has been described when associated with chemo-immunotherapy (25, 26). In HCV-related NHLs we reported a surprisingly effective activity of Rituximab in mono-therapy, with 100% of clinical response and 50% of complete response. When evaluated, the molecular response appeared to be concomitant to hematological remissions. Moreover, median failure-free survival appeared to be relatively long, being 1 year.

Various data reported in the literature have shown the efficacy of Rituximab either in lymphoproliferative disorders or in autoimmune diseases. Nevertheless, the use of anti-CD20 monoclonal antibody in patients with HCV-related lymphomas and concomitant autoimmune syndromes has not yet been exhaustively explored. Thus, even if drawn from a relatively small number of patients, our data strongly support the use of anti-CD20 in this patient setting.

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