Thrombosis Research 134 (2014) 980–984

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Lupus anticoagulant and thrombosis in splenic marginal zone lymphoma

J. Gebhart [a], K. Lechner [a], C. Skrabs [a], T. Sliwa [b], E. Müldür [c], H. Ludwig [c], T. Nösslinger [d], K. Vanura [a], K. Stamatopoulos [e], I. Simonitsch-Klupp [f], A. Chott [g], P. Quehenberger [h], G. Mitterbauer-Hohendanner [h], I. Pabinger [a], U. Jäger [a], K. Geissler [b,*]

[a] Department of Medicine I, Clinical Division of Hematology and Hemostaseology and the Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria
[b] 5thDepartment of Medicine, Krankenhaus Hietzing, Vienna, Austria
[c] Department of Medicine I, Wilhelminenspital Vienna, Vienna, Austria
[d] Third Medical Department for Hematology and Oncology and the Ludwig Boltzmann Institute for Leukemia Research and Hematology, Hanusch Hospital, Vienna, Austria
[e] Hematology Department, G. Papanicolaou Hospital, Thessaloniki, Greece
[f] Institute of Pathology, Medical University Vienna, Vienna, Austria
[g] Institute of Pathology, Wilhelminenspital Vienna, Vienna, Austria
[h] Institute of Laboratory Medicine, Medical University Vienna, Vienna, Austria

ARTICLE INFO

Article history:
Received 3 May 2014
Received in revised form 30 July 2014
Accepted 21 August 2014
Available online 29 August 2014

Keywords:
Splenic marginal zone lymphoma
SMZL
Lupus anticoagulant
Antiphospholipid antibodies
Thrombosis
Splenectomy

ABSTRACT

Introduction: Splenic marginal zone lymphoma (SMZL) is a rare low-malignant Non-Hodgkin lymphoma (NHL), in which immune mediated paraneoplastic phenomena such as autoimmune hemolytic anemia (AIHA), autoimmune thrombocytopenia (ITP) and C1 esterase inhibitor deficiency are relatively common.
Materials and Methods: We performed a multicenter retrospective study in 70 patients on the prevalence and clinical features of antiphospholipid antibodies (aPLA) in SMZL.
Results and Conclusions: Nine patients (13%) had the diagnosis of a lupus anticoagulant (LA). The occurrence of venous thromboembolic events was significantly higher in LA positive patients compared to LA negative patients (4/9 [44%] vs 5/61 [8%], p = 0.002), especially within 12 months after splenectomy (3/6 [50%] vs 2/28 [7%], p = 0.007). None of the patients with LA had a persistent complete remission of LA after splenectomy, but complete remission of LA was achieved in 2/2 patients after rituximab-bendamustine immuno-chemotherapy. In conclusion, our data show a relatively high prevalence of aPLA in SMZL and an increased risk of postsplenectomy thrombosis in these patients. The fact that rituximab-bendamustine was effective for eradicating LA may be considered as an argument for using immuno-chemotherapy as first line therapy in SMZL patients with LA.

Introduction

Splenic marginal zone lymphoma (SMZL) is a rare low-malignant Non-Hodgkin lymphoma (NHL), which is characterized by splenomegaly, lymphocytosis, anemia, thrombocytopenia (due to splenic sequestration), advanced stage and an indolent course [1]. Immune mediated paraneoplastic phenomena such as autoimmune hemolytic anemia (AIHA), autoimmune thrombocytopenia (ITP) and C1 esterase inhibitor deficiency are relatively common [2,3]. In a few reports the occurrence of antiphospholipid antibodies (aPLA) with or without thrombosis had been described [4]. However, in literature reviews on SMZL [5–7], only two possible cases of aPLA were mentioned in one review [7]. In our clinical routine patients with SMZL we identified a number of patients with a lupus anticoagulant (LA). In view of these discrepancies we decided to perform a systematic study in a larger group of patients on the prevalence and clinical consequences of aPLA in SMZL. We also studied the efficacy of splenectomy and immuno-chemotherapy to eradicate aPLA and the thrombotic risk.

Patients and methods

This was a retrospective cooperative study of four hematological centers in Vienna. We included all patients who had been registered with the diagnosis of SMZL between 1995 and 2011 in the hematology and pathology departments of the Medical University of Vienna (MUV) and three community hospitals in Vienna (Krankenhaus Hietzing, Wilhelminenspital and Hanusch-Krankenhaus). The study was approved by the ethic committees of the MUV (EC Nr 1101/2009) and the City of Vienna (EC Nr 10-025-0310).

Requirements for inclusion of patients in this study were a definite histopathological diagnosis of SMZL and the availability of relevant clinical and laboratory data, including at least one valid LA screening test. Lupus anticoagulant was diagnosed according to the criteria of the International Society of Thrombosis and Hemostasis [8]. The patients' history, laboratory findings, the course and treatment of the lymphoma

* Corresponding author at: 5th Department of Medicine - Oncology/Hematology, Krankenhaus Hietzing, Wolkersbergenstrasse 1, A-1130 Vienna, Austria. Tel.: +43 1801103122; fax: +43 1801102671.
E-mail address: klaus.geissler@wienkav.at (K. Geissler).

http://dx.doi.org/10.1016/j.thromres.2014.08.021
0049-3848/

were evaluated by chart review in all patients and recorded. The occurrence of symptomatic venous thromboembolic events, such as deep venous thrombosis (DVT), pulmonary embolism (PE), and splanchnic vein thrombosis (SVT) was recorded. The diagnosis of venous thromboembolic events had been confirmed with objective methods in all cases (duplex sonography for peripheral DVT and duplex sonography and/or CT in SVT, and spiral CT in cases of PE).

Seventy-two patients with histologically confirmed SMZL were screened for study inclusion. Two patients were not included in this retrospective data analysis as no LA screening test was available.

Forty-six patients were still alive in 2009 and were invited for a follow-up visit. Twenty-three (50%) of these were re-examined in 2009 and 2010. After obtaining written informed consent of these patients, a specific history was taken with particular emphasis on clinical events such as thrombosis, occurrence of immune mediated disorders, treatments such as splenectomy and/or immuno-chemotherapy. The laboratory examinations included all aPLA tests (lupus anticoagulant, anti-ß2GP1 and anticardiolipin (aCL), IgM and IgG antibodies) and in addition factor XII activity and free protein S activity and antigen, besides further routine assessments.

Determination of aPLA

For primary screening a lupus-sensitive activated partial thromboplastin time (APTT; Diagnostica Stago, Asnieres, France) reagent and a diluted Russell´s Viper Venom Test (dRVVT, Life Diagnostics, Clarkston GA, USA) were used. In the case of prolonged APTT the diagnosis of LA was made according to the recommendations of the SCC/ISTH [8]. Anticardiolipin antibodies and anti-ß2GP1 antibodies were determined with commercial ELISAs (Orgentec Diagnostika, Mainz, Germany).

Assays of factor XII and protein S

The activities of the coagulation factor XII were measured as fully automated one-stage assay on a Sysmex CA7000 (Sysmex, Kobe, Japan) in a multi-dilution mode by using Aktin FS as APTT-reagent and the respective factor-deficient plasma (both Siemens Healthcare, Marburg, Germany).

Free protein S antigen and activity were measured by using an automated latex ligand immunoassay (HemosIL Free Protein S; Instrumentation Laboratory, Milan, Italy) and the STA Protein S Clotting assay (Diagnostica Stago), respectively. For the identification of anti- protein S antibodies plasma mixing studies were performed and protein S activity was assessed after 60 minutes incubation.

Nucleotide sequencing

PCR amplification and sequence analysis of IGHV-IGHD-IGHJ rearrangements of lymphoma cells were obtained from peripheral blood or bone marrow samples, and total mRNA or genomic DNA were extracted using standard methods. PCR was performed following the recommendations of the European Research Initiative on CLL using leader primers or framework region 1 primers for the IGHV gene region as described previously [9,10]. Clonal rearrangements were sequenced using the GenomeLab DTCS Quick Start Kit (Beckman Coulter, Brea, CA, USA) on a Beckman CEQ 2000XL DNA analyzer. When direct sequencing was not successful, PCR products were cloned into the pGEM T-Easy vector (Promega, Madison, WI, USA) and transformed into JM109 cells (Promega, Madison, WI, USA). Colony screens were performed using standard m13 forward and reverse primers. Sequencing was carried out as described above. Forward and reverse reads were aligned, edited manually, and sequences were submitted to the IGMT/V-QUEST integrated database for receptor analysis and interpretation.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 19.0, SPSS, Chicago, Illinois, USA). We performed Mann–Whitney U tests to detect differences in age. Comparison of categorical parameters among groups was done by the chi-square test. A two-tailed p-value of less than 0.05 was considered to indicate statistical significance.

Results

Prevalence of lupus anticoagulant

In total 70 patients with an established histopathological diagnosis of SMZL met the inclusion criteria and were analyzed in this retrospective study. The clinical and laboratory characteristics of these patients are shown in Table 1. APTT prolongation was observed in 12 (17%) out of 70 patients with SMZL. Nine of them had the diagnosis of a lupus anticoagulant based on SCC/ISTH criteria [8] with the exception that not all patients had tests on two or more occasions as required. Five out of 7 (71%) LA positive patients were also positive for aCL and 3/6 patients were anti-ß2GP1 antibody positive. All antibodies against aCL or ß2GP1 were of the IgM isotype and all these patients had IgM paraproteinemia. In 1 patient with a prolonged APTT the LA confirmation assay was negative and 2 patients had APTT prolongations due to oral anticoagulant therapy with vitamin K antagonists.

Differences between LA positive and LA negative patients with regard to clinical parameters are delineated in Table 1. LA positive patients were younger ($p = 0.006$). The occurrence of venous thromboembolic events was higher in LA positive patients ($p = 0.002$), especially within 12 months after splenectomy ($p = 0.007$). The observation time was longer in LA positive patients ($p = 0.021$).

Changes of LA during follow up

The clinical courses of the 9 LA positive patients are depicted in Fig. 1. All LA positive patients followed our invitation and were re-examined in 2009 and 2010. Of the 9 LA positive patients, 5 still had LA at the time of

Table 1
Summary of clinical and laboratory data of 70 patients without (n = 61) and with (n = 9) the lupus anticoagulant.

Patients	LA negative N = 61	LA positive N = 9	p-value
N	61 (87%)	9 (13%)	
Male/Female	39/22	4/5	0.262
Age (mean [IQR])	69 [61–75]	57 [50–64]	0.006
HCV antibody positivity	2/47 (4%)	0/9 (0%)	0.217
Spleen size (median [range])	18 [8–30]	16 [11–35]	0.199
Paraproteinemia	21/41 (51%)	5/9 (56%)	0.814
AIHA	5/61 (8%)	2/9 (22%)	0.190
Thrombocytopenia (≤100 G/L)	5/61 (8%)	1/9 (11%)	0.826
Protein S deficiency	1/13 (8%)	3/7 (43%)	0.061
Factor XII deficiency	1/7 (14%)	3/8 (38%)	0.310
Treated	41/61 (67%)	7/9 (78%)	0.524
SX alone	16 (26%)	2 (22%)	0.797
ICT alone	13 (21%)	1 (11%)	0.475
ICT and SX	12 (20%)	4 (44%)	0.099
Venous thromboembolism	5/61 (8%)	4/9 (44%)	0.002
Solitary VTE	3/61 (5%)	2/9 (22%)	
DVT + PE	3	1	
SVT	0	1	
Recurrent VTE	2/61 (3%)	2/9 (22%)	
DVT/PE	1	1	
SVT + DVT	1	0	
SVT	0	1	
VTE after SX (≤12 months)	2/28 (7%)	3/6 (50%)	0.007

HCV: hepatitis C virus; AIHA: autoimmune hemolytic anaemia; SX: splenectomy; ICT: immuno- and/or chemotherapy; VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; SVT: splanchnic vein thrombosis.