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Original Article

Clinical features and prognostic relevance of Waldenström macroglobulinemia: analyses of fifty-eight cases

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Abstract: Objective: To analyze the clinical presentations, laboratory features, outcome and prognostic relevance of Waldenström macroglobulinemia (WM) in China. Methods: The clinical features, survival and prognostic factors were retrospectively analyzed in 58 patients with WM hospitalized in First Affiliated Hospital of Nanjing Medical University from January 2009 to March 2015. The MYD88 L265P mutation was detected by the allele specific oligonucleotide polymerase chain reaction. Results: Twenty-two patients experienced extramedullary disease. Sixteen patients presented with B symptoms. All but two patients presented with anemia. Twenty-nine patients showed bicytopenia or pancytopenia. All patients had an increased erythrocyte sedimentation rate. Elevated β_2-microglobulin (β_2M) level and C-reaction protein (CRP) level were detected in 83% and 67% patients, respectively. The majority of patients were kappa light chain restriction with kappa-to-lambda ratio of 3.46:1. Thirteen of 15 patients (86.7%) with WM harbored the MYD88 L265P mutation. After a median follow up of 23 months from diagnosis, the median duration of overall survival was 81 months. Patients with bicytopenia or pancytopenia had a poorer survival. Other factors including age, higher CRP, higher β_2M, higher lactate dehydrogenase, type of light chain, M-protein concentration >70 g/L, B symptoms, expression of CD138-positive failed to show statistical significance for survival. Conclusion: WM is a rare B-cell lymphoproliferative disorder. It is incurable disease but with indolent clinical course. Patients with bicytopenia or pancytopenia had a poorer survival.

Keywords: Waldenström macroglobulinemia, clinical features, MYD88 L265P mutation, prognosis

Introduction

Waldenström macroglobulinemia (WM) is the lymphoplasmacytic lymphoma (LPL) with monoclonal immunoglobulin M (IgM) [1]. Over 95% of the cases of LPL are WM, while the remainders were consisted of IgA, IgG, and non-secreting LPL. The overall age-adjusted incidence of WM is 0.38 cases per 100,000 persons per year, increasing with age to 2.85 in patients above 80 years [2]. According to the Surveillance, Epidemiology and End Results (SEER) database, approximately 1000-1500 new cases of WM are diagnosed per year in the US [3]. There is race difference in the incidence of WM, which is highest among white people and rare in other populations [4]. The LPL/WM accounted for only 1.9% in 5147 consecutive lymphomas from 18 hospitals in our province [5].

A lymphoplasmacytic infiltrate in the bone marrow and extramedullary sites and elevated IgM levels account for the symptoms related to WM patients. Patients with WM may present with a variety of clinical presentations and symptoms vary in individual patient. Our objective was to perform a comprehensive analysis to investigate the clinical presentations, laboratory features, outcome and prognostic relevance in Chinese WM.

Materials and methods

Patients

Between January 2009 and March 2015, fifty-eight WM patients at the First Affiliated Hospital of Nanjing Medical University were enrolled in this study. Initial work-up included bone marrow

Table 1. Clinical and laboratory data of WM patients at initial diagnosis

n		58
Age (median) range in years		63 (35-83)
Sex (male/female)		44/14
Anemia		56
Bicytopeniaor pancytopenia		29
		Median
Leukocyte counts ($\times 10^9$/L)		4.7
Hemoglobin (g/dL)		7.5
Platelet counts ($\times 10^9$/L)		124
β_2-microglobulin (mg/L)		4.07
C-reaction protein (mg/L)		15.9
M-protein concentration (g/L)		26
Light Chain Type	kappa	45
	lambder	13
Involved sites	Soft tissues	2
	Lymph nodes	12
	Hepatosplenomegaly	10

biopsy and aspiration, chest/abdominal/pelvic CT, serum electrophoresis, immunoglobulin quantification, immunoelectrophoresis or immunofixation of serum, complete blood count, erythrocyte sedimentation rate (ESR), dehydrogenase (LDH), β_2-microglobulin (β_2M), C-reactive protein (CRP), and albumin levels, plasma viscosity, hepatitis B and Epstein-Barr virus (EBV) testing and MYD88 L265P allele specific oligonucleotide polymerase chain reaction (ASO-PCR).

The initial therapy included alkylator, nucleoside analogs containing treatment, rituximab in combination with chemotherapy or thalidomide-based therapy.

Analyses of MYD88 mutations

Genomic DNA was isolated from mononuclear cells using the QIAamp DNA Blood Kits (Qiagen, Düsseldorf, Germany) according to the manufacturer's recommendation. Direct Sanger sequencing was performed to validated the specificity of the ASO-PCR. The DNA was subjected to ASO-PCR to detect the MYD88 L265P mutation. The standardization process was according to the published study [6].

Statistical analysis

Statistical analysis was performed using the software package SPSS version 17.0. Survival curves were constructed with the use of Kaplan-Meier estimates. Correlation between laboratory factors with overall survival (OS) was computed by the Log-rank univariate analysis. All *P* values were two sided. Differences with *P* values of <0.05 are considered significant.

Results

Our analytical cohort included 58 patients with WM. The median age at diagnosis was 63 years (range: 35-83 years). There was a male predominance with a male-to-female ratio of 3.14:1. Twenty-two patients experienced extramedullary disease, such as lymphadenopathy, hepatosplenomegaly or soft tissue tumor. Sixteen patients may present with B symptoms including night sweats, fever and weight loss.

All but two patients presented with anemia at initial diagnosis. The median hemoglobin (Hb) level was 75 g/L. Twenty-nine patients presented with bicytopenia or pancytopenia at initial diagnosis. The median leukocyte counts and platelet counts (Plt) were 4.7×10^9/L and 124×10^9/L, respectively. All patients had an increased ESR (range: ≥41 mm/h). Elevated β_2M level and CRP level were detected in 48 (83%) patients and 39 (67%) patients, respectively. The median level of β_2M and CRP was 4.07 mg/L (normal range: 1.09-2.53 mg/L) and 15.9 mg/L (normal range: 0-8 mg/L), respectively. However, increased LDH level was only detected in 12 patients. CA125 was analyzed in 32 patients and increased in only 9 patients. Another lymphoma-associated biomarker thymidine kinase 1 (TK1) was tested in 30 patients and rose in 13 patients (43%). The median serum M-protein concentration was 26 g/L. The majority of patients were kappa light chain restriction with kappa-to-lambda ratio of 3.46:1. In this study, there was only one patient harboring EBV infection (**Table 1**). Lymphoplasmacytic cell population in the bone marrow was documented by trephine biopsy and aspiration. Ten patients were complicated with myelofibrosis.

Flow cytometric immunophenotyping was performed in 43 patients. The majority of patients typically expressed CD19, CD20, CD22 and CD79a, but lack CD5, CD10 and CD23. CD20

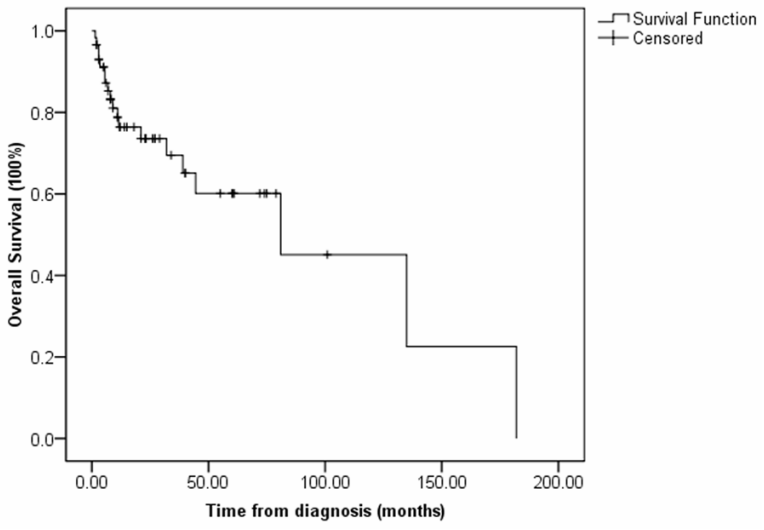

Figure 1. After a median follow up of 23 months from diagnosis, the median duration of overall survival was 81 months.

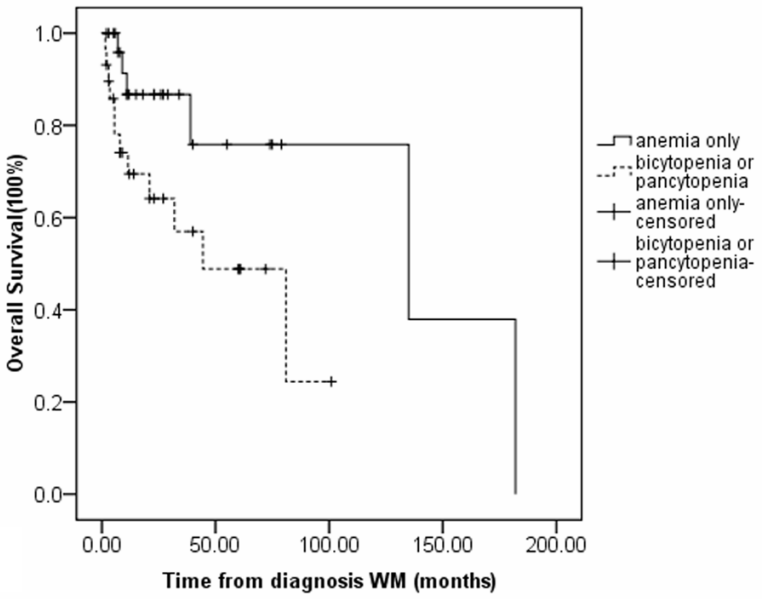

Figure 2. Log-rank univariate analysis showed that patients with bicytopenia or pancytopenia had a poorer survival than patients with anemia only (135 vs 44.5 months, *P*=0.033).

and CD22 were expressed in all cases. Expression of CD5 was detected in 5 patients. The existence of CD138-positive plasma cells was detected in 11 WM patients by flow cytometry.

We tested the MYD88 L265P mutation in 15 patients. Thirteen of 15 patients (86.7%) with WM harbored the MYD88 L265P mutation.

After a median follow up of 23 months (range: 1.5-182 months) from diagnosis, the median duration of OS of patients was 81 months (**Figure 1**). Log-rank univariate analysis showed that patients with bicytopenia or pancytopenia had a poorer survival than patients with anemia only (135 vs 44.5 months, *P*=0.033, **Figure 2**). However, other factors including age, higher CRP, higher β_2M, higher LDH, type of light chain, M-protein concentration >70 g/L, B symptoms, expression of CD138 failed to show statistical significance for survival.

Discussion

WM is a rare B-cell lymphoproliferative disorder. LPL/WM accounted for only 1.9% of all lymphoma types according to a study of a total of 5147 consecutive lymphomas from 18 hospitals in our province from January 2007 to December 2013 and diagnosed according to the WHO classification [5]. Data on WM are limited to a few case reports in China. WM is an incurable disease but with indolent clinical course [7]. Previous studies have evaluated outcomes in patients with WM, particularly in Europe. Median OS has varied in different series, ranging from 60 to 120 months [8-10]. The prognosis of WM patients was improved over time during a 25-year period due to the considerable changes in the therapeutic management of WM, reflecting the use of new agents (such as purine analogs, monoclonal antibodies, thalidomide, and bortezomib-based therapies) [11]. The main aims of this study were to document the clinical and hematological spectrum of changes that occur in WM in the Chinese population.

In this study, we detected that most patients were male, with a male-to-female ratio of 3.14:1. In lines with our result, a male preponderance has also been described in published data [6, 12]. Extramedullary disease in MM was not uncommon. However, WM presents predominantly with bone marrow involvement and only a minority of patients (15-30%) present with extramedullary disease such as lymphadenopathy or hepatosplenomegaly. In this study, 22 patients experienced extramedullary disease. The incidence was 38%, which is higher than previous reports [13].

All but two of the patients with WM exhibited anemia at diagnosis and 29 patients presented with bicytopenia or pancytopenia at initial diagnosis. This symptom can be attributed to bone marrow infiltration with malignant B cells. With regard to tissue infiltration by tumor cells, the replacement of the normal hematopoietic bone marrow with WM cells usually leads to a progressive normochromic or normocyticanemia and, suppression of other blood cell lineages leading, for example, to thrombocytopenia [12]. Some patients presented with myelofibrosis, which may also attribute to anemia, or to a more extent, pancytopenia.

Several studies have evaluated the effects of laboratory variables at baseline on the prognosis. In International Prognostic Scoring System for WM (IPSSWM), 5 covariates (age ≥65 years, hemoglobin ≤115 g/L, platelet counts ≤100×10^9/L, β_2M>3 mg/L, serum monoclonal protein >70 g/L) defined 3 risk groups (low, intermediate, and high risk, respectively) [8]. Besides these factors, increased serum LDH was identified as an additional independent variable, which improved risk assessment beyond the recent WM international prognostic scoring system (IPSSWM) [14]. However, in our study, age failed to show statistical significance for survival. Moreover, higher β_2M and M-protein concentration also did not contribute to poorer survival in this study. In our 58 patients, the majority of patients (83%) showed elevated β_2M (>3 mg/L). Meanwhile, only eight patients presented with higher M-protein concentration (>70 g/L). We speculate this may explain why β_2M and M-protein concentration did not show significance for survival in this study. Furthermore, we also analyzed the association of other factors including cytopenia, higher CRP, higher LDH, type of light chain, B symptoms, and expression of CD138 with survival. The result showed that only patients with bicytopenia or pancytopenia had a poorer survival.

WM is a distinct type of neoplasm resulting from the accumulation of clonal lymphocytes, lymphoplasmacytic cells and plasma cells in bone marrow. The lymphoma cells typically express CD19, CD20, CD22 and CD79a, but lack CD5, CD10 and CD23, which helps to discriminate WM from follicular lymphoma, chronic lymphocytic leukemia and mantle cell lymphoma [15]. However, numerous variations can be observed. CD5 and CD23 are expressed in 5-20% and 35% of the cases, respectively [16]. The expression of CD138-positive plasma cells in WM by either immunohistochemistry or flow cytometry was also documented [17]. However, the phenotype of clonal plasma cells from WM patients is clearly different from that of myeloma patients. The plasma cell clones were associated by a more immature/plasmablastic phenotype of BM plasma cells, as reflected by a greater reactivity for CD19, CD20, CD45 and sIgM), always in the absence of myeloma-related phenotypic aberrations (for example, upregulation of CD56 or absence of CD27) [18]. The WM clone showed some degree of phenotypic overlap with that of other low-grade lymphoma. Thus, besides immunophenotype analysis, other specific biomarkers were needed to help to diagnose WM. The presence of the MYD88 variant might provide additional help to differentiate WM from other lymphoma [13]. In this study, CD20 and CD22 were expressed in all cases. Expression of CD5 was detected in 5 patients. The existence of CD138-positive plasma cells was detected in 11 WM patients, but was not linked to prognosis.

MYD88 has been reported to be mutated (L265P) in the majority of WM patients, using whole genome sequencing, MYD88 mutation has been described in nearly 91% of LPL/WM. It seems that this mutation may be used to define the diagnosis of LPL/WM [19]. In our center, the MYD88 mutation analysis was used in clinic practice since 2014. We tested the MYD88 L265P mutation in 15patients. However, 13 of 15 patients (86.7%) with WM harbored the MYD88 L265P mutation.

In this study, there was one patient harboring EBV (Epstein-Barr virus) infection. Previous

study suggested patients involving EBV infection might be associated with "histological transformation events". This indicated patients with WM progressed to the high-grade lymphoma, diffuse large B cell lymphoma (DLBCL) and usually presented with aggressive clinical course, profound cytopenias, extramedullary disease, and poor outcome [20]. In our study, the patient exhibited pancytopenia at diagnosis, without lymphadenopathy or hepatosplenomegaly. After a follow up of 14 months from diagnosis, the disease was still stable.

In conclusion, WM is a rare B-cell lymphoproliferative disorder. It is an incurable disease but with indolent clinical course. Patients with bicytopenia or pancytopenia had a poorer survival.

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Disclosure of conflict of interest

None.

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