Rare transformation to double hit lymphoma in Waldenstrom's macroglobulinemia

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Waldenström macroglobulinemia (WM) is a lymphoproliferative lymphoma that is characterized by monoclonal immunoglobulin M (IgM) protein and bone marrow infiltration. Its incidence is rare and rarer still is its ability to transform to a B-cell lymphoma, particularly the aggressive diffuse large B-cell lymphoma, which bodes a poor prognosis. When transformation includes mutations of MYC, BCL-2 and/or BCL-6, it is known as a 'double hit' or 'triple hit' lymphoma respectively. This paper presents a rare case of WM with mutations positive for MYC and BCL2, making it a case of double hit B-cell lymphoplasmacytic lymphoma with plasmatic differentiation without morphological transformation to aggressive histology like DLBCL. The paper also broadens to include discussions on current topics in the classification, diagnosis, possible causes of transformation, and treatment of WM, including transformation to double hit lymphoma. The significance of this case lies in that the presence of double hit lymphoma-like genetic mutations in WM have not been previously described in the literature and potentially such changes are harbinger of extra-nodal presentation, aggressive growth, and possibly poor prognosis, if data from other double-hit lymphoma are extrapolated.

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Background & epidemiology

Waldenström's macroglobulinemia (WM) is a lymphoplasmacytic lymphoma (LPL) associated with monoclonal immunoglobulin M (IgM) protein [1]. WM represents 1–2% of all hematologic malignancies and it has an annual incidence rate of five cases per one million [1]. There are many cytogenetic abnormalities and mutations frequently found in WM patients; common abnormalities are: del(6q) (50%), somatic hyper-mutation in immunoglobulin heavy-chain variable, t(9;14) (p13;q32) (50%), and trisomy 4 (20%). Among these, myeloid differentiation primary response gene 88 L265P (*MYD88*^{L265P}) and CXC chemokine receptor type 4 WHIM (*CXCR4*^{WHIM}) somatic mutations were found in more than 90% and 30–35% of WM patients, respectively, and have been shown to play an important role in WM tumorigenesis [2,3]. WM is characterized by: IgM monoclonal gammopathy of any size, bone marrow infiltration in intertrabecular patterns with small lymphocytes, plasmacytoid lymphocytes or plasma cell greater than or equal to 10%, and the presence of symptoms (Table 1) [4]. WM cells are positive for IgM, CD19, CD20, CD22, CD25, CD27, FMC7 and negative for CD10, CD23, CD103 and CD138 by immunophenotypic testing [4].

WM can transform in approximately 5–10% of patients, and the most common morphological transformation is to aggressive lymphomas like diffuse large B-cell lymphoma (DLBCL) [5,6]. Spontaneous new mutations in the original B-cell clone, mutations secondary to prior therapy with nucleoside analogs (NAs) and infections with Epstein–Barr virus play a role in the transformation to more aggressive histology [7,8]. The 2016 revision of the WHO classification of lymphoid neoplasms includes a new category of high grade B-cell lymphoma that include rearrangements of *MYC* and *BCL2* and/or *BCL6*, sometimes referred to as 'double-hit' or 'triple hit' lymphomas, respectively [9]. Double-hit lymphoma (DHL) is most commonly defined as BCL2+ and MYC+ (60–90% of

Table 1. Diagnostic criteria of Waldenström's macroglobulinemia and differential diagnosis.

Waldenström macroglobulinemia [4]	Smoldering Waldenström macroglobulinemia [4]	IgM MGUS [4]
1. IgM monoclonal gammopathy of any concentration 2. Bone marrow (BM) infiltration by small lymphocytes with plasmacytoid or plasma cell differentiation ≥10% 3. Intertrabecular patterns of BM infiltration 4. Surface marker – Positive: IgM, CD19, CD20, CD22, CD25, CD27, FMC7 – Negative: CD10, CD23, CD103, CD138 5. Presence of symptoms	1. Meet the criterial of WM 2. Absence of symptoms, anemia, organomegaly, lymphadenopathy or hyperviscosity	1. Serum IgM monoclonal protein <3000 mg/l 2. BM lymphoplasmacytic infiltration <10% 3. No evidence of end-organ damage, anemia, hyperviscosity, lymphadenopathy or hepatosplenomegaly from the underlying lymphoproliferative disorder
BM: Bone marrow; MGUS: Monoclonal gammopathy of undetermined significance; WM: Waldenström macroglobulinemia.

Figure 1. There is a bulky retroperitoneal adenopathy with the largest conglomerate of lymph nodes on the right measuring 9.7 × 10.9 cm (previously 8.8 × 9.9 cm). This mass encompasses the right renal artery and displaces the inferior vena cava, right kidney and aorta. The right kidney demonstrates no perfusion abnormality. There are additional lymphoma implants posterior to the right kidney. There is a 7.1 × 5.7 cm mass arising from the left adrenal gland.

DHL cases). Another form of DHL is MYC+ and BCL6+, which accounts for about 5% of cases [10]. Triple-hit lymphoma accounts for 8% of aggressive translocation cases, which is characterized by translocation pattern of BCL2+/BCL6+/MYC+ [10]. MYC is a proto-oncogene, whose activation leads to cellular proliferation, increased metabolism, protein synthesis and increased differentiation, while BCL2 is a proto-oncogene that leads to cell survival due to its antiapoptotic effects [10,11]. BCL2 translocations are present in 20–30% of cases of DLBCL and MYC translocations have been seen in 14% DLBCL and more frequently in unclassified B-cell lymphoma [6,12]. BCL-6 is a transcription factor in germinal center B cells, which stops the expression of genes involved in the cell cycle, apoptosis, proliferation, differentiation and DNA damage response [6,10].

We present a rare case of WM with mutations positive for MYC and BCL2, making it a case of double hit B-cell lymphoplasmacytic lymphoma with plasmatic differentiation without morphologic transformation to aggressive histology like DLBCL.

Case presentation

A 69-year-old Southeast-Asian male with a history of Type II diabetes mellitus presented to clinic with complaints of early satiety, poor appetite and intermittent abdominal pain for 2–3 weeks. Physical examination was unremarkable, however laboratory exam revealed microcytic hyperchromic anemia (Hemoglobin 11.7 g/dl), mild monocytosis in the setting of normal white blood cell count and elevated IgM level at 2738 mg/dl. Abdominal CT scan with contrast revealed a 7.1 × 5.7 cm sized mass arising from the left adrenal gland and a 9.7 × 10.9 cm sized retroperitoneal mass with the largest conglomerate of lymph nodes on the right side. The masses encompassed the right renal artery and displaced the inferior vena cava, right kidney and the aorta (Figure 1). Subsequent biopsy from the retroperitoneal mass demonstrated diffuse proliferation of predominantly small CD20, PAX5 and CD5 positive B cells intermixed with a small number of large B-lymphocytes and numerous plasma cells (Figure 2).

Figure 2. Biopsy from the retroperitoneal mass. Pathologic exam of retroperitoneal mass shows B-cell non-Hodgkin lymphoma with plasma cell differentiation (A, 4x). Eccentric nuclei with perinuclear hof are observed in cells with plasma cell differentiation (B, 40x).

Figure 3. Flow cytometry shows a monotypic kappa light chain restricted B cells that showed dim expression of CD5 and some CD23.

Fluorescence *in situ* hybridization with kappa and lambda mRNA showed positive expression of kappa light chains in both lymphocytes and plasma cells. Flow cytometry identified a monotypic kappa light chain restricted B cell that showed dim expression of CD5 and CD23 (Figure 3). Cyclin D1 was negative and Ki-67 was positive in approximately 50% of the cells. Bone marrow biopsy revealed a hypercellular marrow with several small lymphoid aggregates and small clusters of CD138 positive plasma cells with kappa light chain predominance. Fluorescence *in situ* hybridization results were positive for MYC rearrangement (11.5%) and gain of BCL2 (7.0%). Collectively, these findings were consistent with MYC positive/BCL2 positive double hit B-cell lymphoplasmacytic lymphoma with plasmacytic differentiation. Diagnosis was consistent with WM with the presence of elevated IgM paraprotein.

The patient was treated with rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) chemotherapy for total one cycle (mainly due to insufficient tissue on first fine needle aspiration, urgency to treat due to inferior vena cava compression and renal vasculature involvement, rapid disease progression on repeat imaging, and repeat biopsy was pending before CHOP based therapy was initiated). This was followed by five cycles of DRC regime (dexamethasone 20 mg IV followed by rituximab 375 mg/m² IV on day 1, and cyclophosphamide 100 mg/m² orally twice a day on days 1–5) every 21 days for 6 months. The patient achieved complete remission at that time. He then received two cycles of four, weekly rituximab infusions due to relapse detected 6 and 12 months after induction (based on M protein and imaging showing one area of regional relapse).

Table 2. Search data on Waldenström's macroglobulinemia transformation to double hit lymphoma.

PubMed search yields only six articles discussing transformation of WM to DLBCL	<ol style="list-style-type: none"> 1. Kida T, Tanimura A, Ono A <i>et al.</i> Lymphoplasmacytic lymphoma accompanied by transformed diffuse large B-cell lymphoma with the MYD88(L265P) mutation. <i>Rinsho Ketsueki</i>. 2017; 58(2), 155–160. 2. Castillo JJ, Gustine J, Meid K, Dubeau T, Hunter ZR, Treon SP. Histological transformation to diffuse large B-cell lymphoma in patients with Waldenström macroglobulinemia. <i>Am. J. Hematol.</i> 2016; 91(10), 1032–1035. 3. Hayashi Y, Sata H, Akuta K <i>et al.</i> Diffuse large B-cell lymphoma occurring in a Waldenström macroglobulinemia patient with central nervous system infiltration. <i>Rinsho Ketsueki</i>. 2015; 56(11), 2351–2356. 4. Shiseki M, Masuda A, Watanabe N <i>et al.</i> Development of diffuse large B-cell lymphoma in a patient with Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma: clonal identity between two B-cell neoplasms. <i>Hematol. Rep.</i> 2011; 3(2), e10. 5. Owen RG, Bynoe AG, Varghese A, de Tute RM, Rawstron AC. Heterogeneity of histological transformation events in Waldenström's macroglobulinemia (WM) and related disorders. <i>Clin. Lymphoma Myeloma Leuk.</i> 2011; 11(1), 176–179. 6. Lin P, Medeiros LJ. High-grade B-cell lymphoma/leukemia associated with t(14;18) and 8q24/MYC rearrangement: a neoplasm of germinal center immunophenotype with poor prognosis. <i>Haematologica</i> 2007; 92(10), 1297–1301.
Histologic transformation of WM to DLBCL is rare, only about 5–10% [7]	<p>20 of 1466 were noted in Castillo study [7]</p> <ul style="list-style-type: none"> • 5-year incidence of transformation: 1.0% • 10-year incidence of transformation: 2.4% • 15-year incidence of transformation: 3.8% <p>EBV appears to play a role in transformation of WM to DLBCL [8] Median survival after histologic transformation 2.7 years [7]</p>
PubMed search for transformation of WM to DHL yields 0 results	<p>Although one of the six articles listed above (Castillo) notes that poor outcomes in cases of transformed WM may be due to increased expression of BCL-6 and MYC, indicating possible development of DHL [7] The specific genetic changes that underlie these transformation events in the original B-cell clone, remain unknown in WM [5]</p>
BCL: B-cell lymphoma; DHL: Double-hit lymphoma; DLBCL: Diffuse large B-cell lymphoma; EBV: Epstein-Barr virus; WM: Waldenström's macroglobulinemia.	

Discussion

The significance of this case lies in the presence of two mutations seen in DHL without morphological features of DLBCL and extra-nodal presentation with a higher Ki67 score of 50% at the time of presentation. The presence of DHL like genetic mutations in WM was not described in the literature before and potentially such changes are harbinger of extra-nodal presentation, aggressive growth and possibly poor prognosis if data from other DHL are extrapolated [6].

In a study by Castillo *et al.*, 20 out of 1466 WM patients morphologically transformed to DLBCL in an average time span of 4.4 years after diagnosis [7]. Cumulative incidence of histological/morphological transformation was about 2% at 10 years from diagnosis [7]. Transformation of WM has been shown to have pathological heterogeneity [5]. Most patients with transformation to DHL present with extra-nodal disease and high-risk features coupled with a median survival of less than 3 years [7]. The cumulative incidence rates of transformation at 5, 10 and 15 years are 1, 2.4 and 3.8%, respectively [7]. In the same study, 13 of 20 WM patients with histological transformation were previously exposed to rituximab, 11 received alkylating agents, seven were exposed to NAs, six were exposed to proteasome inhibitors and two patients developed spontaneous transformation [7].

Owen *et al.* describe five cases of WM transformation, and showed that WM transformations are pathologically heterogeneous and include clonally unrelated events (Table 2) [5]. Alternatively, Shiseki *et al.* found that in a patient with transformation of WM to DLBCL, a common complementarity-determining region, CDR3 sequence, was found in the tumor cells of both WM and DLBCL indicating that the tumors are clonally identical [13] (Table 2). Literature suggests that prolonged exposure to nucleoside-containing regimens can lead to disease transformation. A study by Leblond *et al.*, using chlorambucil versus fludarabine for patients with untreated WM and closely related disorders, found a 6-year cumulative incidence of transformation to large cell lymphoma of 7.7% in those who received fludarabine versus 11.1% in those who received chlorambucil, though the $p = 0.48$ was not statistically significant [14]. Another group looked at transformation rates in WM following chemotherapy. The study contained 193 patients with WM who had been treated with NAs; 12 patients in their study had transformation or later development of t-myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) (eight transformed to DLBCL, three developed t-MDS/AML and one transformed to Hodgkin's disease) [15].

The 2016 revision of the WHO classification of lymphoid neoplasms sought to better define DLBCL and B-cell lymphoma unclassified distinctly. Better characterization of DHL and triple hit lymphoma were addressed and all B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements are now included in a single category designated as high grade B-cell lymphomas, with *MYC* and *BCL2* and/or *BCL6* rearrangements [9]. Preferably, all DLBCL should be tested for the detection of *MYC*, *BCL2* and *BCL6* rearrangements [9]. No new upgrades were made regarding LPL/WM in the 2016 WHO revisions, but the 2008 classifications state that cases with increased transformed cells/immunoblasts may be associated with an adverse prognosis, and that transformation to DLBCL occurs in a small proportion of cases [16].

Conclusion

Regarding therapy recommendation, WM when present as an indolent disease should be followed but no active treatment is needed until symptoms are present [17]. As the disease progresses, it can lead to serum hyperviscosity. Plasmapheresis is the preferred treatment at that time, which will reduce the serum IgM levels and decrease the symptoms related to hyperviscosity [18]. Chemotherapy is used in more severe cases of WM and first line treatments include rituximab, cyclophosphamide and dexamethasone. Common regimens used to treat previously untreated WM patients include dexamethasone, rituximab, cyclophosphamide (DRC), bendamustine, rituximab (Benda-R), bortezomib-based combination therapy, carfilzomib-based therapy and BTK inhibitor ibrutinib [17]. Symptomatic patients that have been previously treated are commonly re-treated with the prior therapy or will receive agents like ofatumumab, ibrutinib, NAs, everolimus, immunomodulatory agents, and very rarely autologous or allogeneic stem cell transplantation [14]. WM patients with DHL mutation but without histological transformation are postulated to have poor prognosis. Due to lack of data in this group, there are no established recommendations. Treatment options can be inferred from studies in relapsed and refractory DLBCL patients, which is not optimal. R-CHOP is the main treatment for DHL at this time, with some regimen variation [10]. We conclude that WM is a rare malignancy that can morphologically transform to other B-cell lymphoma subtypes and through this report we make a case to further test LPL cases for *MYC*, *BCL2* and/or *BCL-6* mutations in cases with atypical presentation, extra-nodal disease and aggressive clinical behavior even without morphological transformations.

Future perspective

In light of the connection between transformation of WM to high grade non-Hodgkin lymphoma, including DHL, in individuals with previous exposure to NAs we may see higher numbers of histological transformation in the coming years. Due to limited literature on treatment, patients with WM DHL are ideal candidates for clinical trials, which may eventually shed light on new treatment options.

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Ethical conduct of research

Consent to publish has been obtained from the participant to report individual patient data.

Executive summary

- Waldenström's macroglobulinemia (WM) is a lymphoplasmacytic lymphoma associated with monoclonal immunoglobulin M (IgM) protein.
- WM morphologically can transform in approximately 5–10% of patients, and the most common transformation is to aggressive lymphomas like diffuse large B-cell lymphoma (DLBCL). Historically DLBCL with rearrangement of *MYC* and *BCL2* and/or *BCL6*, known as 'double-hit' or 'triple hit' lymphoma respectively bode a very poor prognosis with a median survival of less than 3 years.
- There is evidence that patients with transformation with previous exposure to chemotherapies such as alkylating agents and/or nucleoside analogs have a higher chance of this transformation.
- WM/lymphoplasmacytic lymphoma cases can show mutations for *MYC*, *BCL2* and/or *BCL-6* even without morphological transformations.

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