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CASE REPORT

B-lymphoblastic transformation of mantle cell lymphoma/leukemia with "double hit" changes

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Abstract Mantle cell lymphoma is a mature B-cell neoplasm composed of small to medium-sized atypical lymphocytes and has a characteristic t(11;14)(q13;q32) translocation, with a variably aggressive and overall incurable course. More aggressive histologic variants have been described, as well as rare cases of transformation to other large cell lymphomas. Here, we describe a novel case of large cell blastic transformation of mantle cell lymphoma/leukemia at presentation with unusual immunophenotypic and cytogenetic features, most consistent with B-lymphoblastic leukemia. Morphologic findings include sheets of large blasts replacing the bone marrow, as well as occasional small to medium-sized atypical lymphocytes in the background. The blasts express CD19, PAX5, CD10, Cyclin D1, and TdT but are negative for CD5, CD20, and BCL2 by immunophenotyping. Cytogenetic studies show a complex karyotype with t(11;14), monosomy 13, gains of 8q, and *MYC* gene rearrangement and amplification among other changes. This unique case of blastic TdT-positive B-cell leukemia arising from mantle cell lymphoma may represent transformation with complex cytogenetic abnormalities including "double hit" changes. This distinctive presentation may expand our understanding of the biology behind mantle cell lymphoma progression.

Keywords B-lymphoblastic leukemia · Mantle cell lymphoma · Double hit lymphoma · Blastic TdT-positive leukemia

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Introduction

Mantle cell lymphoma (MCL) is a mature B-cell neoplasm composed of monomorphic small to medium-sized atypical lymphocytes arising from naïve mantle zone B-cells, with a generally aggressive clinical course and a median survival of 3–5 years [1]. Morphologic variants have been described including the aggressive blastoid and pleomorphic variants, as well as small cell and marginal zone-like variants, an indolent subtype, and in situ lesions [1, 2]. Very rare transformations to large cell lymphoma have been reported as well, including therapy-related B-lymphoblastic leukemia (B-ALL) [3], Burkitt lymphoma [4], and "diffuse large cell lymphoma" [5].

The hallmark cytogenetic event in mantle cell lymphoma is the t(11;14)(q13;q32) between the *IGH* and *CCND1* genes present in the majority of cases [1]. This results in deregulated cyclin D1 expression and progression through the G1-S cell cycle checkpoint but is not in itself sufficient for the development of MCL [6, 7]. Secondary cytogenetic abnormalities help determine differences in clinical behavior with increased karyotype complexity commonly associated with tumor progression and blastoid variants [7, 8]. In particular, concurrent *MYC* gene rearrangements or amplifications with *CCND1* gene rearrangement represent a "double hit" MCL with transformation and aggressive behavior, similar to the "double hit"

diffuse large B-cell lymphoma (DLBCL) with *BCL2*/*BCL6* and *MYC* gene rearrangements [8].

We describe a novel case of large cell and TdT+ blastic transformation of mantle cell lymphoma/leukemia at presentation with complex immunophenotypic and cytogenetic abnormalities including the double hit genetic aberrations most consistent with B-lymphoblastic leukemia transformation of MCL. B-lymphoblastic leukemia arising from MCL has previously been described in only one therapy-related MCL report [3], but to our best knowledge, neither at the initial presentation nor in the context of the double hit MCLs. This case illustrates the variable pathologic features seen within the aggressive double hit MCL group and may expand our understanding of their biologic behavior.

Clinical history

The patient is a 66-year-old man with no significant past medical history who presents with 2 weeks of abdominal pain and fatigue, as well as 3–5 days of hematemesis and hematochezia. He also complains of cough, night-sweats, shortness of breath, and headache behind his right eye with vision changes. He is a former smoker of 2–3 cigarettes per day, uses no alcohol or other drugs, and has no family history of leukemias or lymphomas.

Physical examination revealed an uncomfortable and sick appearing, somnolent but arousable male with a prominent right-side anterior cervical lymph node, bilateral inguinal lymphadenopathy, and splenomegaly. His admission labs were notable for an elevated white blood cell count of 56,000/μL, hemoglobin of 9.9 g/dL, and platelets of 225,000/μL, with a differential count of atypical/immature lymphocytes of 32 %, promyelocytes of 3 %, myelocytes of 4 %, metamyelocytes of 2 %, band neutrophils of 4 %, segmented neutrophils of 24 %, lymphocytes of 25 %, and monocytes of 6 %. A CT scan showed lymphadenopathy of the retroperitoneal, mesenteric, and inguinal lymph nodes causing ureteral obstruction, and mild tonsillar enlargement slightly greater on the right side with level IB lymphadenopathy.

Fine needle aspiration was performed on the right sided neck mass, as well as a peripheral blood smear review and bone marrow biopsy with flow cytometry and cytogenetic studies. Unfortunately, the patient refused further treatment and left the hospital against medical advice, and was lost to follow-up.

Materials and methods

Histology and immunohistochemistry were routinely performed on peripheral blood smears, bone marrow smears, and formalin-fixed paraffin-embedded tissue (FFPE) sections according to established protocols. Flow cytometric studies were performed using 6-color/8-parameter assay with auto controls and internal controls for determination of antibody binding patterns. Standard bone marrow and peripheral blood cytogenetic studies were done and G-banded metaphase cells were described according to the ISCN 2013 [9]. Fluorescence in situ hybridization (FISH) on cultured peripheral blood cells, and FFPE sections of the bone marrow was performed according to manufacturers' recommendations using commercially available (Abbott-Molecular, IL) direct labeled probes including dual-color dual-fusion *IGH-CCND1* probes, CLL FISH panel probes, and dual-color *MYC* "break-apart" probe sets. Approximately 300 interphase nuclei for each probe set were analyzed using standard fluorescence microscopy.

Results

Morphologic review of the peripheral blood smear shows an atypical lymphocytosis consisting of small to medium-sized atypical lymphocytes with irregular to folded nuclear contours, mildly dispersed chromatin, inconspicuous to prominent nucleoli, and scant cytoplasm. Occasional blasts are seen with vacuolated cytoplasm. Bone marrow aspirate smears reveal sheets of large blasts with irregular nuclei, dispersed chromatin, prominent nucleoli, and scant basophilic cytoplasm containing frequent vacuoles. Admixed are occasional small to medium-sized atypical lymphocytes with similar morphologic features as described in the peripheral blood. Decalcified bone marrow core biopsy sections demonstrate a hypercellular marrow with an approximate cellularity of 70 %, and diffuse involvement by sheets of blasts with similar morphology as described above. The background contains scattered small atypical lymphocytes and occasional megakaryocytes, with significantly reduced erythropoiesis and myelopoiesis (see Image 1).

Flow cytometric studies of the peripheral blood and right neck mass fine needle aspirates reveal identical findings with monotypic B-cells (68–71 % of the total) positive for CD5 (dim), CD19, CD20 (moderate to bright), CD22 (dim), CD45, HLA-DR, and FMC7, and surface kappa light chain restriction (moderate to bright). The neoplastic B-cells are also positive for intracellular CD79a but negative for CD10, CD23, and CD38. Flow cytometric studies of the marrow aspirate reveal distinct small and large neoplastic B-cell populations. The small neoplastic B-cells comprise approximately 30 % of the total, displaying an immunophenotype identical to that seen in the peripheral blood and in the FNA of the right neck mass. The large neoplastic B-cell population comprises approximately 40 % of the total and expresses CD10, CD19, CD22 (dim), CD38, CD45, demonstrates surface kappa light chain restriction (dim), and is essentially negative for CD20 as well as negative for CD5, CD23, and FMC7 (see Image 2).

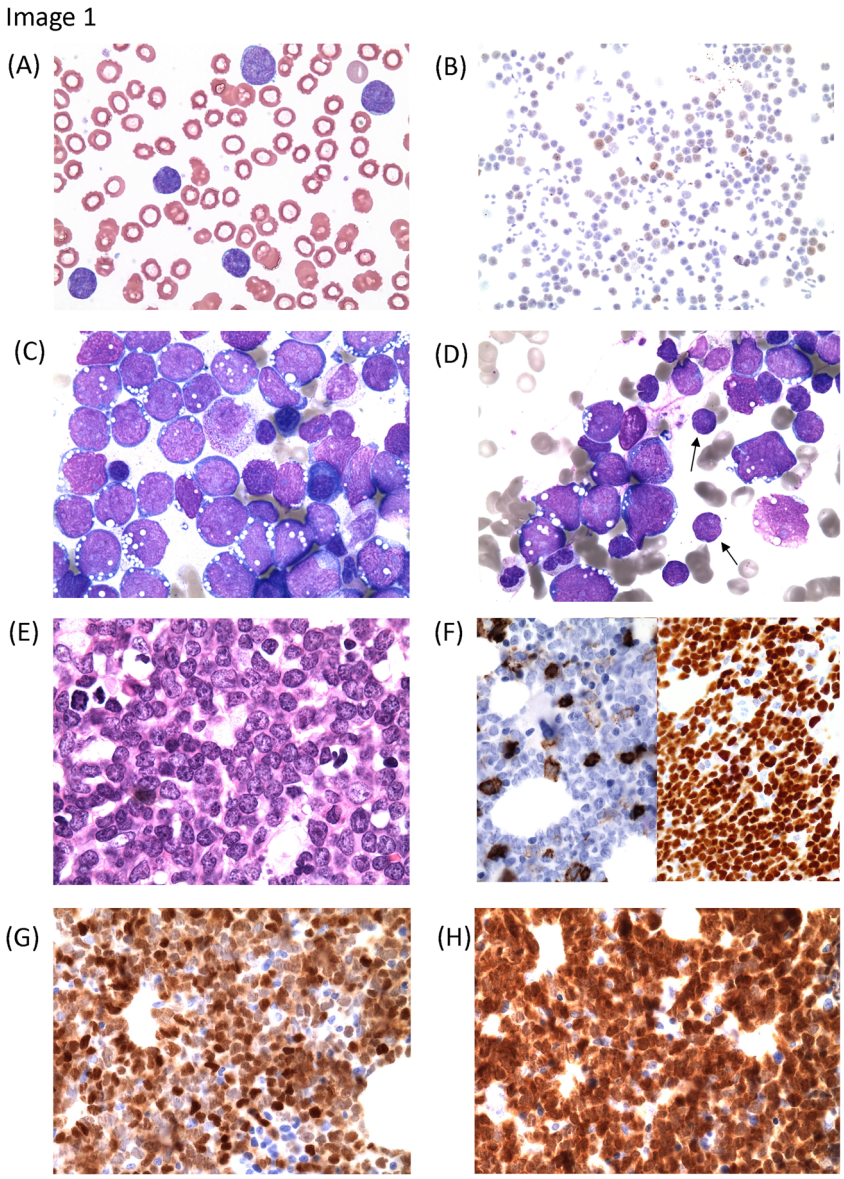

Image 1 Microscopic photographs. **a** Atypical lymphocytes in peripheral blood (Wright-Giemsa stain, 1000× magnification). **b** BCL1 immunostain, fine needle aspiration of right cervical lymph node (400× magnification). **c** Sheets of blasts on bone marrow aspirate smear (Wright-Giemsa stain, 1000× magnification). **d** Bone marrow aspirate smear shows atypical lymphocytes (*arrows*) and blasts (Wright-Giemsa stain, 1000× magnification). **e** Hypercellular bone marrow biopsy with sheets of large blasts (H&E stain, 1000× magnification). **f** Bone marrow biopsy with blasts negative for CD20 (left) and positive for PAX5 (right) (400× magnification). **g** BCL1 immunostain, bone marrow biopsy (400× magnification). **h** TdT immunostain, bone marrow biopsy (400× magnification)

Immunohistochemical stains of the bone marrow core biopsy reveal sheets of blasts positive for PAX-5 (strong), CD10 (strong), CD79a (patchy, weak), Cyclin D1 (strong), TdT (strong), and MYC, with a Ki67 proliferation index of about 90 %. The blasts are negative for CD5, CD20, BCL2, and BCL6, as well as CD3, CD34, CD68, CD117, and myeloperoxidase (see Image 1).

Karyotyping performed on peripheral blood reveals a complex abnormal male composite hypotriploid karyotype exhibiting multiple numerical and structural abnormalities: 64~66<3n>XX, −Y, del(1)(p22), +del(1)(p22), −5, −8, t(11;14)(q13;q32), dic(13;13)(p12;p12), −15, −16, −19, −19, +20, −21, +2~5mar, +r(8)[cp4] (see Image 3a).

Karyotyping performed (at another laboratory) on the marrow was reported to have a complex abnormal male composite hypotriploid karyotype: 64–66, XXY, +add(1)(p22), +add(1)(p22), +3, add(5)(p11.2), −8, −9, −10, t(11;14)(q13;q32), −13, add(13)(p11.2), −15, −16, −17, +20, −21, +3–4mar[cp4]/46, XY [10]. Both BM and PB karyotypes have in common the t(11;14) along with several secondary aberrations. The apparent difference in karyotypes noted between the two tissue types is more a reflection of the genomic instability (composite karyotype) and interpretative differences (ring versus marker chromosome), rather than the presence of clinically significant differences in chromosomal anomalies.

FISH studies on cultured peripheral blood cells show *CCND1-IGH* fusion signals [t(11;14)] in 74.3 % (see Image 3c) and monosomy 13 in 79.7 % (see Image 3d) of nuclei examined. FISH performed on FFPE sections of the bone marrow biopsy showed multiple copies (~3–5) of the *MYC* gene locus at 8q24 in 73.7 % of nuclei examined. Some

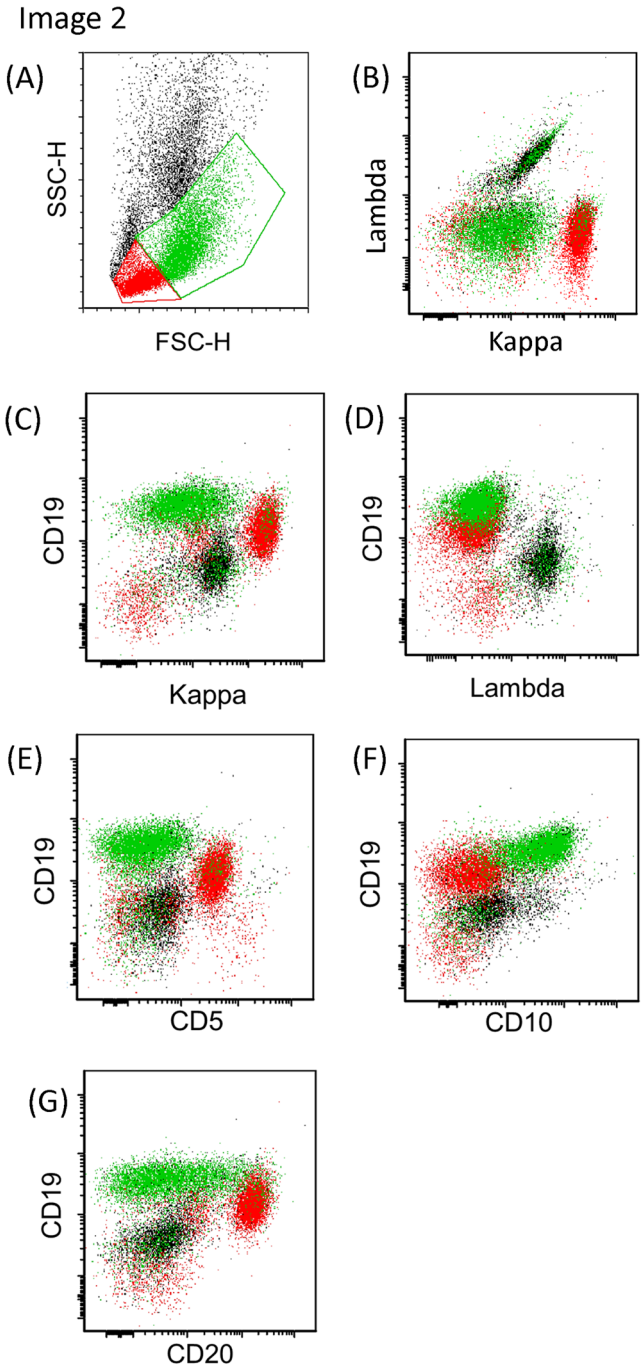

Image 2 Flow cytometry histograms of bone marrow. **a** Small (*red*) and large/blastic (*green*) neoplastic B-cells. **b**–**d** Kappa light chain restriction in small neoplastic B-cells. **b**–**f** Small neoplastic B-cells are CD5+/CD10−, while the large ones show loss of CD5 and granular gain of CD10 expression. **g** The large neoplastic B-cells reveal heterogeneous loss of CD20 expression compared to the small ones

of these copies were localized to the ring chromosome 8 in cultured peripheral blood cells (see Image 3b). Cells of 46.2 % showed several variable signal patterns including split signals along with gains of MYC locus, an unbalanced rearrangement of the *MYC* gene manifested by a loss of the 3′ region (green signal) of the *MYC* dual-color probe, and gains of the 5′*MYC* region (red signal) consistent with *MYC* gene amplification (see Images 3e, f). The presence of cells with t(11;14) both with and without *MYC* gene rearrangements and amplification is highly suggestive of an evolving clone from MCL to transformation.

Discussion

This case shows mostly sheets of blastic cells in the bone marrow with an unusual immature B-cell immunophenotype (CD19+, PAX5+, CD79a+, CD10+, Cyclin D1+, TdT+, dim kappa light chain restriction, CD5−, CD20−, BCL2−), plus a minor component of small atypical lymphocytes (CD20+, CD5+, FMC7+, Cyclin D1+, BCL2+, kappa light chain restriction, CD10−, TdT−). The smaller lymphoid population is consistent with classic mantle cell lymphoma according to the morphology, immunophenotype, and t(11;14) cytogenetic criteria. Transformation to a blastic process rather than de novo or composite lymphoma/leukemia is supported by the overlapping as well as evolving features of the immunophenotype observed in the small neoplastic lymphoid and large blastic populations, including transition from strong to heterogeneously reduced to absent expression of CD20, heterogeneously reduced intensity of the surface kappa light chain restriction, loss of CD5, gradual gain of CD10, and gain of TdT. Flow cytometric data is conclusive that both a MCL and a transformed component are synchronously present. Additional support comes from cytogenetic findings with complex karyotype and *MYC* abnormalities as identified by FISH, in addition to t(11;14), consistent with the double hit MCL category.

We considered the possibility that the larger cell population may represent the blastoid variant of MCL, as the CD5−/CD10+ immunophenotype has been reported in both classic [11, 12] and blastoid variant MCL [13], however, against this interpretation is the strong TdT expression, and lack of CD20 and BCL2. While concurrent Burkitt lymphoma was also considered, transformation to B-lymphoblastic leukemia is favored because of the strong TdT expression, intracellular CD79a, and limited/partial CD20 expression.

This case poses a diagnostic dilemma between blastoid variant MCL and B-ALL arising from classic MCL at initial presentation. Cases have been reported of lymphoblastic leukemia with expression of surface immunoglobulin light chains and *MYC* translocations [14–16]. While *CCND1* expression and even t(11;14) are not specific for MCL and have been reported in other high-grade lymphomas [10, 17, 18], they have not been reported in B-ALL to our knowledge. TdT expression is more in favor of B-lymphoblastic leukemia/lymphoma given the overall phenotype, and has not been reported in MCL to our knowledge [19]. This is the first report, as far as we are aware, of a high-grade blastic neoplasm with simultaneous coexpression of Cyclin D1 and TdT.

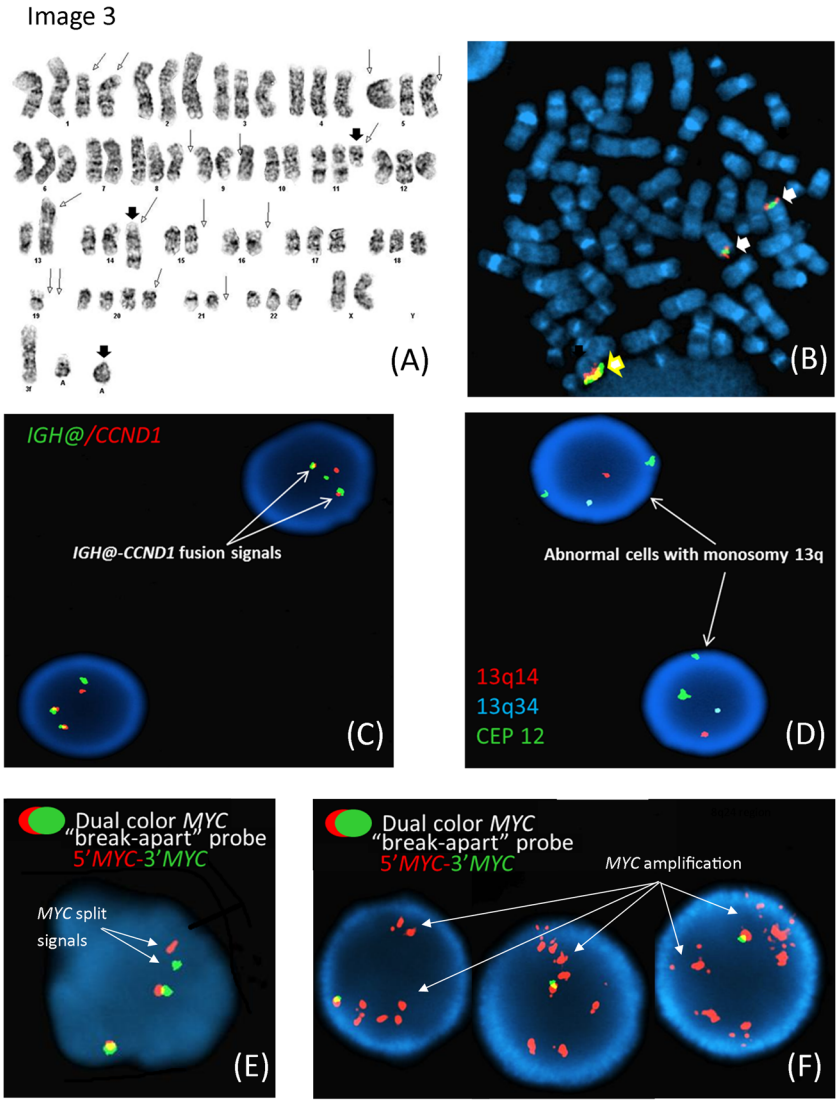

Image 3 Cytogenetic studies of **a**–**d** peripheral blood and **e** bone marrow. **a** A karyogram of an abnormal cell from peripheral blood showing a complex karyotype with several abnormalities including t(11;14) and a ring chromosome (*bold arrows*). **b** A DAPI (4′,6-diamidino-2-phenylindole)-stained metaphase cell from peripheral blood showing dual-colored *MYC* "break-apart" probes on two normal chromosome 8′s (*white arrow*) and multiple copies of the probe on the ring chromosome (*yellow arrow*). **c** Two DAPI-stained interphase nuclei from peripheral blood showing two *IGH@-CCND1* fusion signals (*red/green*) consistent with a reciprocal t(11;14). **d** Two DAPI-stained interphase nuclei from peripheral blood showing a loss of one copy of 13q14 (*red*) and 13q34 (*aqua*) regions consistent with monosomy 13. **e** One DAPI-stained nucleus from bone marrow showing two normal *MYC* probes (*red/green*) and one split *MYC* probe consistent with rearrangement. **f** Three DAPI-stained nuclei from bone marrow showing one normal *MYC* probe (*red/green*) and multiple copies of the red signals representing the 5′*MYC* probe region consistent with MYC amplification

The cytogenetic findings in this case include the t(11;14)(q13;q32) *CCND1-IGH* fusion, and monosomy 13, often seen as a secondary abnormality in some MCL cases. Unbalanced translocations resulting in 1p deletion, gains and losses of several chromosomes, marker and ring chromosomes, plus *MYC* gene rearrangements and amplification in the context of a complex karyotype are all consistent with transformed MCL. The *MYC* gene aberrations in particular would qualify this case as a so-called "double hit" MCL, described and reviewed in Setoodeh et al's recent case series [8]. This rare combination portends a poor prognosis with leukocytosis, extensive marrow involvement, and splenomegaly, shows blastoid or pleomorphic morphology in the majority of, but not all, cases, and warrants aggressive Burkitt-type chemotherapy [8]. A reported case of Burkitt transformation of MCL also featured so-called double hit MCL cytogenetic abnormalities [4].

Juskevicius D et al. report two recent cases of diffuse large B-cell lymphoma with cyclin D1 overexpression, one with the t(11;14)(q13;32) translocation and features typical of DLBCL, and the other with a complex t(4;11;14) translocation and features between MCL and DLBCL [17]. Finally, Holdener et al's

report of a rare therapy-related B-ALL in a patient with indolent MCL also lacks the double hit MCL cytogenetic abnormalities, but may provide precedent for a wider range of possible secondary hematopoietic malignancies in MCL [3].

This case also highlights the necessity of a combined algorithmic approach for diagnosis of hematopoietic neoplasms, as well as the importance of pattern recognition in interpreting flow cytometric studies. Difficult to diagnose lymphomas, such as this case, are made easier by incorporation of information from multidisciplinary modalities, and interpretation should best fit the picture provided by taking all the available information as a whole.

Conclusions

This case poses a high-grade blastic B-cell leukemia/lymphoma, arising from classic mantle cell lymphoma, with simultaneous t(11;14)(q13;q32) and *MYC* gene aberrations consistent with a so-called double hit mantle cell lymphoma. It is the first report of a blastic leukemia/lymphoma coexpressing Cyclin D1 and TdT with additional unusual phenotypic features, creating a diagnostic dilemma between the blastoid variant of mantle cell lymphoma with aberrant TdT expression but lack of CD20 and BCL2, and a B-lymphoblastic leukemia/lymphoma transformation of mantle cell lymphoma. Either scenario represents a new presentation of the aggressive category of double hit mantle cell lymphomas and widens our awareness of the diagnostic gray zones between high-grade non-Hodgkin lymphomas.

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Conflicts of interest The authors have no conflicts of interest to disclose.

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