CASE REPORT

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A case of primary cutaneous diffuse large B-cell lymphoma, leg type with *MYC* rearrangement and high BCL2 protein expression due to trisomy 18

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ABSTRACT

We report the case of an 80-year-old woman with a medial thigh nodule who was diagnosed with primary cutaneous diffuse large B-cell lymphoma, leg type. Further examination identified it as double-expressor lymphoma with elevated expression of both *MYC* and *BCL2*. This elevated expression has been linked to *MYC* translocation and is likely associated with trisomy 18. Our findings suggest that trisomy 18 plays a significant role in the development of double-expressor lymphoma. While cases of double-expressor lymphoma caused by extra copies of *BCL2* have been reported, we could not find any case of double-expressor lymphoma caused by trisomy 18. Therefore, this is an unique case of double-expressor lymphoma linked to *MYC* rearrangement and trisomy 18. Double-expressor lymphoma typically has a poor prognosis and is often resistant to standard treatments. The current findings may help refine diagnostic strategies for this condition.

Keywords: primary cutaneous diffuse large B-cell lymphoma, leg type, trisomy 18, high BCL2 expression, double-expressor lymphoma

Abbreviations:
PCDLBCL-LT: primary cutaneous diffuse large B-cell lymphoma, leg type
DEL: double-expressor lymphoma

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INTRODUCTION

Primary cutaneous lymphomas are a heterogeneous subtype of extranodal non-Hodgkin lymphomas, with approximately 25% originating from B-cells. They are relatively rare diseases, with an incidence of 4 of 1,000,000 people per year,[1] and are classified into three major entities: primary cutaneous follicle-center lymphoma (PCFCL), primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCL-LT), and primary cutaneous marginal zone lymphoma (PCMZL).[2]

PCDLBCL-LT typically affects elderly patients, mostly with lower limb lesions. PCLDBCL-LT cells highly express bcl-2, likely due to gene amplification,[3,4] as t(14;18) is not observed in PCDLBCL-LT. BCL2 overexpression or double expression of both BCL2 and MYC (DEL, double-expressor lymphoma) is associated with inferior overall survival.[4-6] Lucioni et al[5] reported that 69% of PCDLBCL-LT cases exhibit DEL characteristics.

In this article, we report a case of DEL linked to trisomy 18. There have been some reports of cases of DEL caused by extra copies of the *BCL2* gene, but we could not find any instances of DEL caused by trisomy 18. Therefore, this is a unique case of DEL due to *MYC* rearrangement and *BCL2* overexpression due to trisomy 18.

CASE PRESENTATION

An 80-year-old woman with rheumatoid arthritis was treated with oral methotrexate for more than 13 years, followed by three years of combination therapy with methotrexate and golimumab. She developed a painless, reddish nodule on her left inner thigh and initially consulted a local physician in January 20xx (Fig. 1A). Physical examination revealed a 8 × 8 cm ulcerated, hard nodule. Laboratory examinations performed upon admission revealed mild renal dysfunction (creatinine, 0.87 mg/dL), low serum albumin (3.6 g/dL), anemia (hemoglobin 10.9 g/dL, red blood cell [RBC], 339 × 10^4/μL) and elevated levels of C-reactive protein, lactate dehydrogenase and serum soluble interleukin 2 receptor (1.89 mg/dL, 622 U/L and 3,060 IU/mL, respectively). Other laboratory data were within normal limits. In April, she visited the Orthopedics Department of Hospital A, where a biopsy of the nodule was performed. The pathological findings revealed PCDLBCL-LT, and she was subsequently referred to the Hematology Department of the same hospital. The lymphoma lesions did not shrink significantly after discontinuation of immunosuppressive drugs. An 18F-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) scan revealed FDG uptake in multiple sites, including an irregular mass in the left inner thigh as well as the left inguinal and cervical lymph nodes, and an area extending from the front wall of the mid-pharynx to the left tonsil (Fig. 1B, C). The patient was diagnosed with stage IV PCDLBCL-LT with a poor Revised International Prognostic Index (R-IPI) score. Following five rounds of rituximab (375 mg/m^2), cyclophosphamide (525 mg/m^2), doxorubicin (35 mg/m^2), vincristine (0.98 mg/m^2), and prednisolone (70 mg/body, day1-5) therapy, a computed tomography scan indicated complete remission. The sixth course of the same chemotherapy was administered as outpatient procedures at Hospital B, located closer to the patient's residence.

Histopathology

A pathological review of the left inguinal lymph node showed a loss of follicular structure with the widespread growth of medium-to-large, atypical lymphocytes, exhibiting a distinct fission pattern (Fig. 2). Immunostaining indicated the following pattern of marker expression: CD5 at 0%, CD20 at 100%, c-MYC at 70%, Ki-67 at 95%, BCL2 at 100%, CD10 at 0%, Bcl6 at 10%, and MUM1 at 95% (Fig. 2), representing a non-GCB subtype. EBV-encoded small RNA

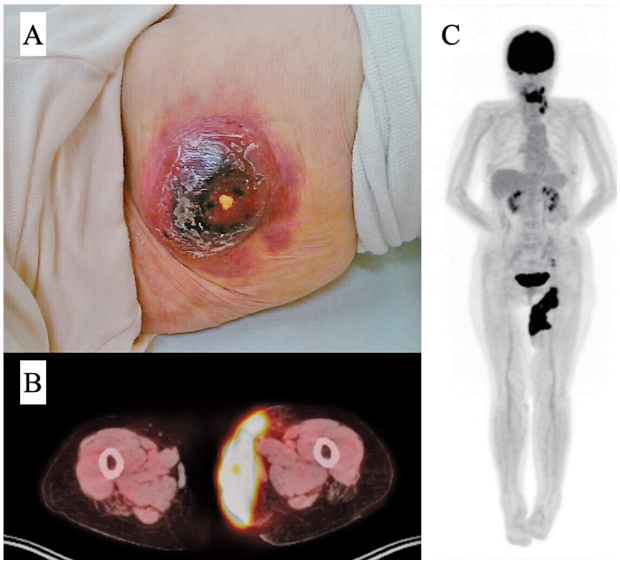

Fig. 1 Macroscopic and 18F-fluoro-2-deoxy-D-glucose–positron emission tomography (FDG-PET) findings
Photograph of the left inner thigh during the initial consultation at Hospital A showing a lesion measuring 8 × 8 cm (A). FDG-PET insights: a mass of 10 cm is observed in the left thigh, showing FDG uptake (B). Whole-body FDG-PET scan: multiple areas of FDG uptake in the left inner thigh, left inguinal and cervical lymph nodes, tonsils, and the anterior wall of the mid-pharynx can be observed (C).

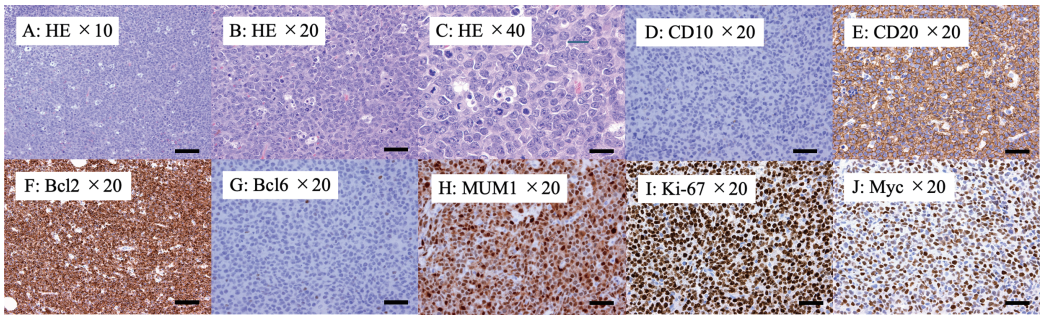

Fig. 2 Pathology of the left inguinal lymph node
Pathological specimens were fixed in 10% formalin and embedded in paraffin. For immunohistochemistry, 3 μm tissue sections were cut from each paraffin block and dried at 60 °C for 1 h. The slides were covered with Bond Universal Covertiles and placed in a Bond III instrument (Leica Biosystems, Ltd, Newcastle, UK). All steps were performed according to the manufacturer's instructions. The antibodies tested included CD5 (clone 4C7), CD20 (clone L26), Ki-67 (clone MM1), Bcl2 (clone bcl-2 /100/D5), CD10 (clone 56C6), Bcl6 (clone LN22), and MUM1 (clone EAU32) from Leica Biosystems (Nussloch, Germany) as well as c-Myc (clone EP121, NICHIREI BIOSCIENCES INC, Tokyo Japan). The typical follicular structure is missed. Instead, there is a widespread proliferation of moderately enlarged, irregular lymphocytes displaying a "starry-sky" pattern (A–C). The immunostaining results are displayed as follows: CD10 at 0% (D), CD20 at 100% (E), BCL2 at 100% (F), BCL6 at 10% (G), MUM1 at 95% (H), Ki-67 at 95% (I), and MYC at 70% (J).
BCL2: B-cell/CLL lymphoma 2
BCL6: B-cell/CLL lymphoma 6
CD: cluster of differentiation
MUM1: multiple myeloma oncogene 1
HE: hematoxylin-eosin

in situ hybridization (EBER-ISH) was negative (data not shown). The mass on the left inner thigh exhibited identical pathological findings.

Flow cytometry

Cell-surface markers were analyzed using the CD45/side scatter gating method. Two-color flow cytometry of the lymphoma cells in the lymph node biopsy tissue revealed CD19 and CD20 positivity with a monoclonal increase in the λ-chain (Fig. 3).

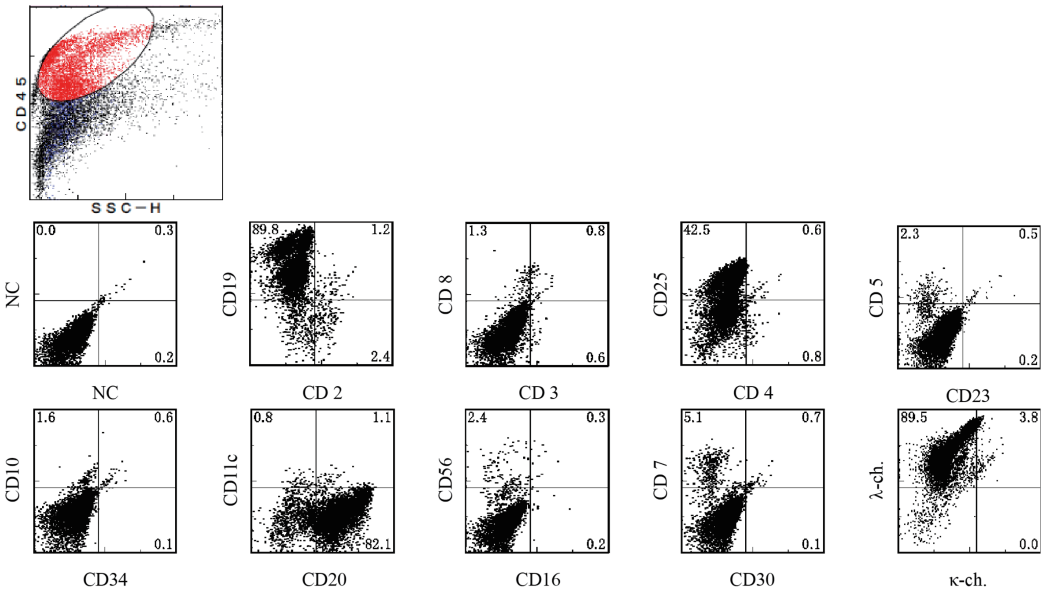

Fig. 3 Two-color flow cytometry of lymphoma cells in left inguinal lymph node biopsy samples
The biopsied lymph node was aseptically minced to prepare cell suspensions, which were then subjected to flow cytometry. Cell-surface markers were analyzed using the CD45/side scatter gating method. The number of cells positive for CD19, CD20, and the λ-chain increased monoclonally.
CD: cluster of differentiation
SSC-H: side scatter-height
NC: negative control

Giemsa-banding and fluorescence in situ hybridization

Giemsa-band analysis revealed a complex karyotype with notable abnormalities including t(8;14)(q24;q32) and trisomy 18 (Fig. 4A). Fluorescence in situ hybridization (FISH) revealed three *BCL2* signals in 54% of the cells with no split signals (Fig. 4B). Similarly, three *MALT1* signals were observed in 42% of the cells with no split signals (Fig. 4C). No split signals for *BCL6* were detected (Fig. 4D), while split signals for *MYC* were observed in 92% of cells (Fig. 4E).

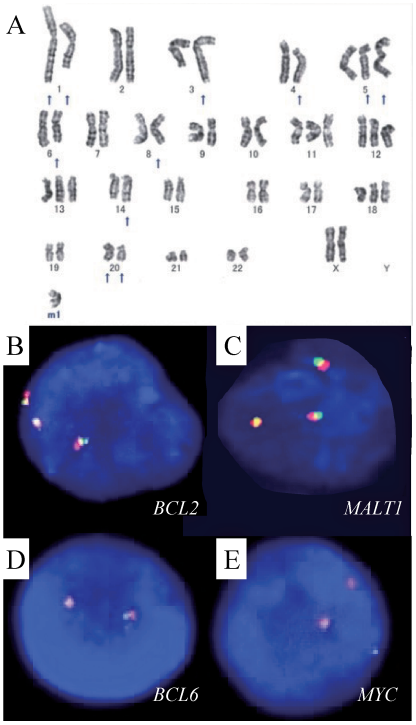

Fig. 4 Cytogenetic analysis of lymph node samples
A, Giemsa-banded karyotype of lymph node upon DLBCL diagnosis includes: 52, XX, add(1)(q42), del(1)(p?), add(3)(q21), add(4)(p11), del(5)(q?), +der(5;7)(q10;q10), del(6)(q?), der(8) add(8)(p11.2)t(8;14)(q24;q32), +11, +12, +13, der(14)t(8;14), +18, add(20)(p11.2), +mar1 [13/13]. B-E, For the fluorescence in situ hybridization (FISH) analysis of *BCL2, BCL6*, and *MYC*, biopsied lymph node slides were air-dried following hypotonic treatment and Carnor's fixation. For the paraffin section–FISH analysis of *MALT1*, 3–4-μm-thick sections section were prepared from paraffin-embedded lymph node tissue. All of the FISH probes were purchased from Abbott Laboratories (Abbott Park, IL, USA). B, Using the Vysis LSI *BCL2* Dual Color Break Apart Rearrangement Probe, three *BCL2* signals are shown without any split signals (54 of 100 cells). C, Using the Vysis LSI *MALT1* Dual Color Break Apart Rearrangement Probe, three *MALT1* signals are shown without any split signals (42 of 100 cells). D, The Vysis LSI *BCL6* Dual Color Break Apart Rearrangement Probe displayed no split signals. E, The Vysis LSI *MYC* Dual Color Break Apart Rearrangement Probe revealed split signals (one orange, one green, and one fusion pattern) in 92 of 100 cells.
BCL2: B-cell/CLL lymphoma 2
BCL6: B-cell/CLL lymphoma 6
MALT1: Mucosa-associated lymphoid tissue lymphoma translocation protein 1

Research ethics

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by the National Center for Geriatrics and Gerontology Institutional Review Boards (No.1632).

DISCUSSION

PCDLBCL-LTs are relatively rare hematological neoplasms that account for 4% of all primary cutaneous lymphomas and 20% of all primary cutaneous B-cell lymphomas.[1,7] PCDLBCL-LT

typically affects women in the seventh decade of life. These lymphomas most often occur in the lower extremities, but 10–15% also appear in other parts of the body.[1]

In reviewing previous studies on the immunohistochemical and cytogenetic profile of PCDLBCL-LT (Table),[4,5,8] it is notable that DEL status is frequent (62–83%).[4,5,8] While extra copies of BCL2 have been identified in 12–18% of PCDLBCL-LT cases, none of these cases[4,5] presents trisomy 18. A previous study with array-based comparative genomic hybridization (CGH) demonstrated that 18q21.31-q21.33 involving *BCL2* and *MALT1* amplification occurs in 67% of PCDLBCL-LT.[9]

Table Immunohistochemical profiles and cytogenetic data of patients with PCDLBCL-LT

	Lucioni et al,[5] 2021 (n = 16)	Menguy et al,[4] 2018 (n = 23)	Schrader et al,[8] 2018 (n = 44)
Sex			
Male, No. (%)	9 (56)	8 (35)	19 (43)
Female, No. (%)	7 (44)	15 (65)	25 (57)
Median age, years (range)	77 (65–98)*	82 (61–95)	78 (49–92)
Phenotype			
BCL2 expression, No. (%)	15 (94)	23 (100)	42 (95)
MYC expression, No. (%)	12 (75)	19 (83)	29 (67)†
MUM1 expression, No. (%)	13 (81)	23 (100)	36 (84)†
BCL6 expression, No. (%)	8 (50)	20 (87)	27 (61)
Mean proliferation index Ki-67 (%)	80	86	NA
DEL, No. (%)	11 (69)	19 (83)	28 (62)
FISH analysis			
BCL2 translocation, No. (%)	2 (12)	0 (0)	0 (0)
BCL6 translocation, No. (%)	8 (50)	4 (18)	2 (4)
MYC translocation, No. (%)	7 (44)	1 (5)	14 (32)
BCL2 extra copy, No. (%)	2 (12)	4 (18)	NA
BCL6 extra copy, No. (%)	NA	3 (14)	NA
MYC extra copy, No. (%)	1 (6)	0 (0)	NA
DHL, No. (%)	3 (19)‡	0 (0)	2 (4)‡
THL, No. (%)	1 (6)	NA	NA
DEL + DHL, No. (%)	3 (19)	0 (0)	1 (2)
DEL + THL, No. (%)	1 (6)	NA	NA

* Mean age, years (range)
† Data are missing for one case.
‡ Double-hit lymphoma occurred only in combination with MYC and BCL6 translocation.
PCDLBCL-LT: primary cutaneous diffuse large B-cell lymphoma, leg type
BCL2: B-cell/CLL lymphoma 2
BCL6: B-cell/CLL lymphoma 6
MUM1: multiple myeloma oncogene 1
DEL: double-expressor lymphoma
DHL: double-hit lymphoma
THL: triple-hit lymphoma
FISH: fluorescence in situ hybridization
NA: not applicable

In the presented case, high BCL2 expression was detected through immunostaining, trisomy 18 was genetically confirmed, and FISH identified three *BCL2* signals in 54% of the cells (Fig. 4). BCL2 gain (defined as 3–4 copies) was strongly associated with increased messenger RNA (mRNA) and protein expression.[10] This indicates that trisomy 18 could have triggered BCL2 overexpression in this case.

Trisomy 18 is found in 10–20% of cases of DLBCL,[11,12] and BCL2 overexpression has been reported in 52–59% of the 80 DLBCL cases examined.[13] Among 34 cases analyzed with comparative CGH, 15% showed chromosome 18 amplification, all of which demonstrated BCL2 overexpression.[13]

MALT1 may be responsible for the BCL2 overexpression in trisomy 18. *BCL2* is located on 18q21.33 and *MALT1* on 18q21.32. The *MALT1* gene is a transcriptional regulator of nuclear factor-kappa B (NF-κB), which is itself a transcriptional regulator of the *BCL2* gene; thus, BCL2 is highly expressed when *MALT1* is activated. *MALT1* gain has been associated with the increased expression of 18q genes at the RNA level.[14] Therefore, it is possible that the gain of *MALT1* and *BCL2* owing to trisomy 18 results in increased BCL2 expression. Previous reports have indicated that the target gene of NF-κB is predominantly present in the DLBCL ABC type.[15]

In summary, our findings suggest that the overexpression of MYC and BCL2 could be attributed to *MYC* translocation and trisomy 18, resulting in diagnoses of PCDLBCL-LT and DEL. Although cases of DEL with MYC rearrangement and additional BCL2 copies have been reported, this is the first documented case of PCDLBCL-LT with both *MYC* rearrangement and trisomy 18. Since PCDLBCL-LT is a relatively rare disease, further accumulation of cases and investigation into tumor pathogenesis are warranted.

AUTHOR CONTRIBUTIONS

AK and MH substantially contributed to the conceptualization of the study. YM, AK and MH collected and evaluated all data. YM and KT wrote the initial draft of the manuscript. AK supervised the conduct of this study. FO, KM, AA, ST, KY, TM, MA, and YN reviewed the data and actively participated in discussions. All authors critically reviewed the revised manuscript and approved the final version for submission.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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