Central nervous system infiltration of a multiple cytokine-producing double-hit B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma with CC chemokine receptor 7 expression

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To the Editor,

The most recent edition of the World Health Organization (WHO) "Classification of Tumors of Hematopoietic and Lymphoid Tissues" included a new category of lymphomas with histologic, phenotypic, and genetic features similar to both Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL), and described them as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (iDLBCL/BL)". This type of neoplasm, which was previously described as a Burkitt-like lymphoma,

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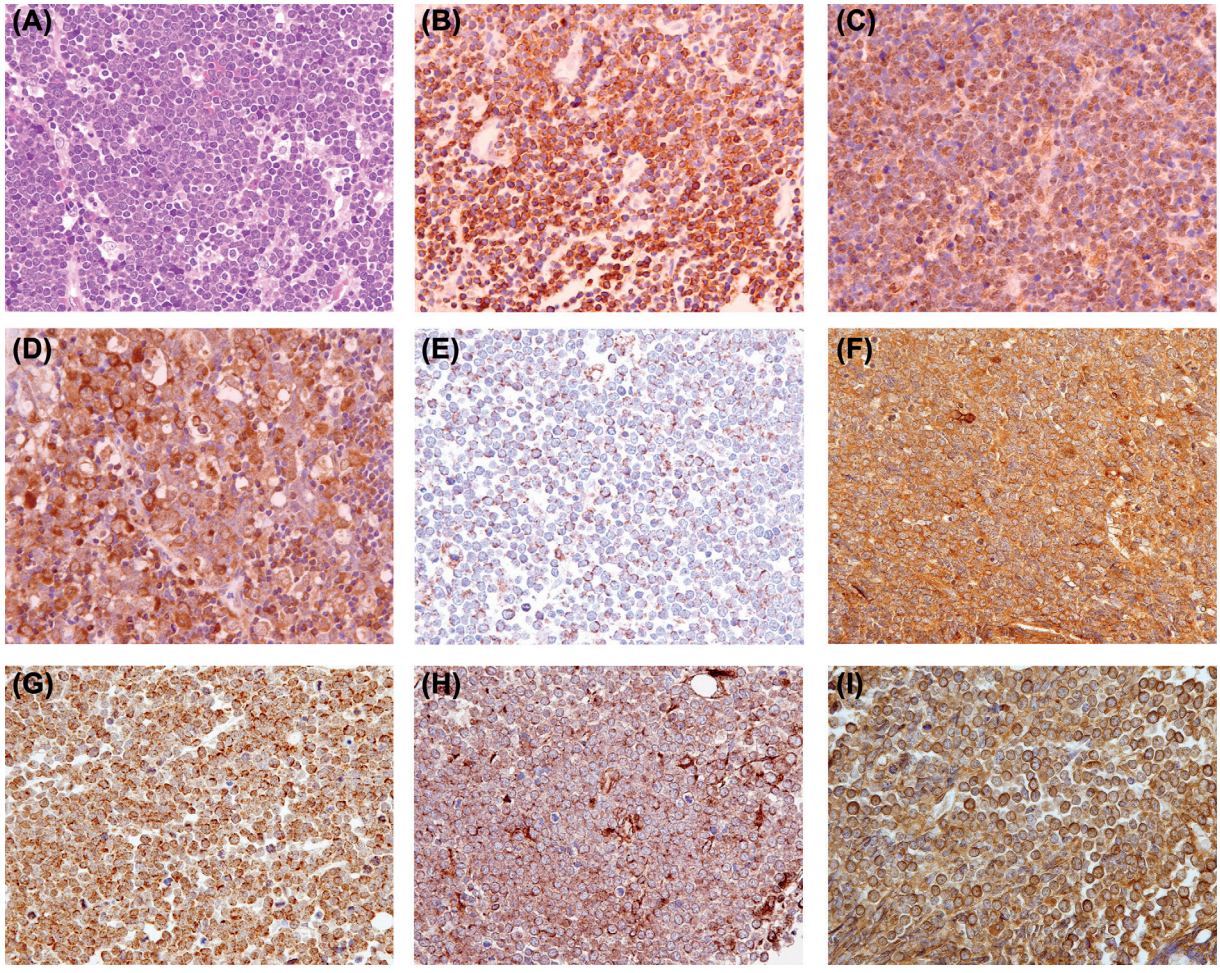

Figure 1. (A) Histological examination showing monotonous proliferation of medium-sized lymphocytes accompanied by a starry-sky appearance (hematoxylin-eosin stain; original magnification, 40×). (B) Lymphoma cells were positive for BCL2 (objective magnification, 40×). (C) Lymphoma cells were positive for MYC (objective magnification, 40×). (D) Lymphoma cells were positive for IL-6 (objective magnification, 40×). (E) Lymphoma cells were positive for TNF-α (objective magnification, 40×). (F) Lymphoma cells were positive for IL-6R (objective magnification, 40×). (G) Lymphoma cells were positive for TNFR1 (objective magnification, 40×). (H) Lymphoma cells were positive for TNFR2 (objective magnification, 40×). (I) Lymphoma cells were positive for CCR7 (objective magnification, 40×).

shares some of the morphological features of BL, but some cells are larger than those that are typical of BL [1]. A classical DLBCL shows rearrangements that involve the immunoglobulin heavy chain (*IGH*) locus (14q32) with different genes such as B-cell lymphoma 2 protein (*BCL2*) (18q21). On the contrary, a classical BL presents translocations joining c-*MYC (MYC)* (8q24) and immunoglobulin genes, usually *IGH*. In some of these cases, concurrent *IGH-BCL2* and *MYC* rearrangement occurs, and these are called double-hit lymphoma (DHL). DHLs are more aggressive and have a higher incidence of central nervous system (CNS) involvement than either BL or DLBCL [2].

A 30-year-old man was admitted to our hospital with an enlarged right cervical lymph node. Computed tomography (CT) scans showed lymphadenopathy of the right cervical, para-aortic, and left inguinal regions. Histological examination of the right cervical lymph node biopsy showed monotonous proliferation of medium-sized lymphocytes accompanied by scattered tangible body macrophages, which gave the tumor a starry-sky appearance (Figure 1A). Immunohistochemical analyses showed that the tumor cells had membrane staining for CD20, CD79a, and CD10, cytoplasmic staining for BCL2 (124; Dako, Glostrup, Denmark) (Figure 1B), and nuclear staining for MYC (C19; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (Figure 1C), but were negative for CD3, CD5, and CD56. Lymphoma cells demonstrated cytoplasmic staining for IL-6 (10C12; Novocastra, Newcastle Upon Tyne, UK) (Figure 1D), and TNF-α (52B83; Santa Cruz Biotechnology) (Figure 1E), and membrane staining for the IL-6 receptor (IL-6R; Deciphargen Biotechnology, Cheshire, CT, USA) (Figure 1F), TNF-α receptor-1 (TNF-R1; H-271; Santa Cruz Biotechnology) (Figure 1G), TNF-R2 (L-20; Santa Cruz Biotechnology) (Figure

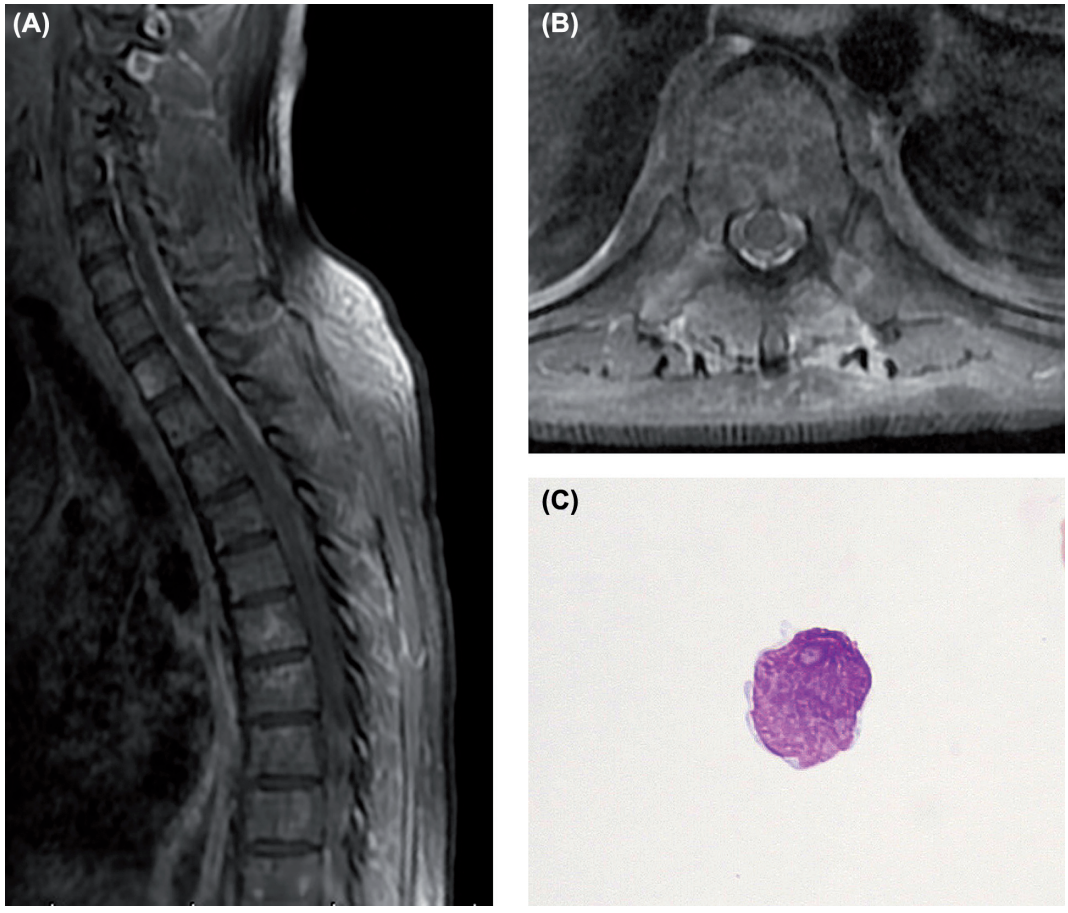

Figure 2. MRI disclosed remarkable enhancement of the meninges in a T1-weighted sequence. (A) Sagittal view section. (B) Axial view section. (C) CSF samples showing lymphoma cells with irregular nuclei with moderately dispersed chromatin and conspicuous nucleoli (May-Giemsa stain, objective magnification 100×).

1H), and CC chemokine receptor 7 (CCR7) (Lifespan Bioscience, Seattle, WA, USA) (Figure 1I). The proliferation index assessed by Ki67 was 90%. Fluorescence in situ hybridization (FISH) analysis revealed fusion between the *heavy chain of immunoglobulin H* (*IgH*) and the *MYC* gene (*IgH*/*MYC*). FISH analysis also revealed fusion between the *IgH* and *BCL2* gene (*IgH*/*BCL2*). A bone marrow aspiration and biopsy showed infiltration of lymphoma cells. These findings were consistent with a new category of iDLBCL/BL, stage IVA, and the International Prognostic Index was categorized as low–intermediate [1]. Following treatment with three courses of R-hyper-CVAD therapy (a regimen of rituximab, hyperfractionated cyclophosphamide, doxorubicin, vincristine, and dexamethasone alternating with a high dose of methotrexate and cytarabine), the patient's clinical symptoms and lymphoma lesions disappeared. However, after the fourth course of chemotherapy, he presented with bone pain. Observation of the bone marrow revealed hypercellular marrow with 86.8% lymphoma cells showing the disease to be progressive. Salvage chemotherapy with fludarabine did not improve the patient's clinical symptoms and the patient became refractory to chemotherapy. Thereafter, he developed progressive paresthesia in his chest, back, and lower limb and weakness of the lower limbs. MRI disclosed remarkable enhancement of the meninges, including the dura mater and leptomeninges, extending from C5 to T3 and T9–L2 (Figure 2A and B). Cerebrospinal fluid (CSF) examination revealed pleocytosis, mainly including abnormal lymphocytes (Figure 2C) (7 cells/mm^3; normal: <5 cells/mm^3), normal glucose (64 mg/dL; normal: 50–80 mg/dL), and elevated protein levels (312.5 mg/dL; normal: 15–45 mg/dL). These clinical findings showed CNS infiltration. He was treated with an infusion of 50 mg etoposide and intrathecal injections of 15 mg methotrexate, 40 mg cytarabine, and 10 mg prednisolone. However, the patient died because the disease was progressive.

The 18q21.3/*BCL2* gene was initially observed by cloning of the chromosomal breakpoint in cases with t(14;18) translocations, which are presumed to result from an error during VDJ rearrangement of the *IG* gene. Neoplastic B-lymphocytes with this

translocation constantly express the BCL2 protein, an apoptosis inhibitor, whereas in normal B-cell differentiation, the BCL2 protein is not expressed in the germinal center (GC). Neoplastic B-lymphocytes with BCL2 overexpression by t(14;18) translocation become apoptosis-resistant and proliferate in the GC [3]. BCL2 was also promoted by TNF-α (3) and IL-6 [4]. The 8q24/*MYC* (*c-MYC*) gene was discovered in the analysis of the chromosomal breakpoint in cases with BL. The *MYC* gene has been reported to be amplified in various types of cancer, and *MYC* aberrations are likely to confer a powerful growth advantage to lymphoma cells [5]. *MYC* is thought to be an oncogene that encodes the MYC protein which is involved in cell proliferation, apoptosis, and cell cycle control. In cases with translocation between the *MYC* and *IG* genes, the MYC protein is constantly expressed in all stages of cell turnover. In addition, MYC expression is stimulated by IL-6 [6] and TNF-α [7]. The basis for the extremely aggressive clinical behavior of DHL is likely to be related to the combination of the MYC-induced growth promotion and the anti-apoptotic effects conferred by BCL2 overexpression [8].

CCR7 is an attractive candidate for recruiting lymphoma cells to CNS, because it is a known regulator of lymphocyte migration, and it has been suggested to be important for the trafficking of lymphocytes participating in CNS immunosurveillance. The single chemokine-receptor interaction acting as a CNS entry signal and the importance of CCR7-mediated T-cell acute lymphoblastic leukemia cell recruitment to the CNS have been shown [9]. However, there have been no reports of CCR7-expressing DH iDLBCL/BL lymphoma with CNS infiltration which may indicate CCR7-mediated DH iDLBCL/BL lymphoma cell recruitment to the CNS. Analysis of the CCR7 promoter sequence revealed two potential binding sites for NFκB [10] and CCR7 up-regulation was shown to be mediated by constitutive NFκB activity [11]. NFκB is a ubiquitously expressed transcription factor that is involved in the activation of genes associated with inflammation and cell adhesion. NFκB activity can be induced by BCL2 and proinflammatory cytokines, such as IL-6 and TNF-α [12,13]. In this case, BCL2 and the cytokines produced by lymphoma cells may promote the aberrant expression of CCR7, resulting in CNS infiltration.

To the best of our knowledge, this is the first case report on the CNS infiltration of multiple cytokine-, multiple cytokine receptor-, and chemokine receptor-expressing DH iDLBCL/BL that showed the immunohistological expression of BCL2, MYC, IL-6, IL-6R, TNF-α, TNFRs, and CCR7 in lymphoma cells. These observations suggest that IL-6- and TNF-α-producing lymphoma cells with IL-6 and TNF-α promoted BCL2 and MYC expression and those with BCL2, IL-6, and TNF-α promoted CCR7 expression, and may show CCR7-mediated efficient migration toward CNS infiltration. DH iDLBCL/BL produce multiple cytokines which may result in the aberrant expression of BCL2, MYC, and CCR7, thereby playing a role in the initiation and enhancement of the recruitment of lymphoma cells to the CNS. These cytokines and the chemokine receptor may play a key role in CNS infiltration in some DH iDLBCL/BL lymphomas.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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