Anaplastic variant of diffuse large B-cell lymphoma with hallmark cell appearance: Two cases highlighting a broad diversity in the diagnostics

Short running title: Two cases of anaplastic DLBCL

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ABSTRACT

The anaplastic variant of diff use large B-cell lymphoma (A-DLBCL) is morphologically defined but remains an enigmatic disease in its clinicopathologic distinctiveness. Here, we report two cases involving Japanese women age 59 years, both with A-DLBCL with the hallmark cell appearance and both indistinguishable from common and giant cell–rich patterns, respectively, of anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma. Case 1 was immunohistochemically positive for CD20, CD79a and OCT-2 but not for the other pan–B-cell markers, CD30 and ALK. Case 2 showed CD20 and CD30 positivity for 50% and 20% of tumor cells in addition to strong expression of p53 and MYC. Both were positive for fascin without Epstein–Barr virus association. Our cases provide additional support for the earlier reports that A-DLBCL exhibits clinicopathologic features distinct from ordinal diffuse large B-cell lymphoma (DLBCL), and documented its broader morphologic diversity than previously recognized. They also shed light on the unique feature of absent expression of pan–B-cell markers except for CD20 and CD79a,

suggesting that A-DLBCL may biologically mimic a gray zone or intermediate lymphoma between DLBCL and classic Hodgkin lymphoma.

Key words: diffuse large B-cell lymphoma, anaplastic variant, hallmark cell, grey zone lymphoma, classic Hodgkin lymphoma

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease involving morphologic variants, immunophenotypic and molecular subgroups, and distinct disease entities.[1] The anaplastic variant of DLBCL (A-DLBCL) was morphologically defined in the 2017 World Health Organization (WHO) classifications.[1] The clinicopathologic characteristics and biologic behavior, however, have been controversial or even contradictory in the English-language literature compared with non-anaplastic DLBCLs because of the absence of a diagnostic consensus among pathologists.[2-7] Indeed, many of the previously documented cases consisted of a short series or single cases and emphasized the morphologic analogy with Hodgkin/Reed-Sternberg (HRS) cells in classic Hodgkin lymphoma (CHL) and/or CD30 expression in tumor cells with and without a sinusoidal pattern. A-DLBCL diagnosis principally depends on morphologic features but currently has no specific marker with good consensus among pathologists, accounting for 3.4% in the series of Diebold et al.[2] and less than 1% of all patients with newly diagnosed DLBCL in our experience (unpublished data). Because of its rarity, the clinicopathologic

distinctiveness of this disease has long been unproven. Recently, Li et al. reported that A-DLBCL had a high frequency of *TP53* mutation and concurrent *MYC* and *BCL2* and/or *BCL6* genetic abnormalities (translocation or extra copy) in their series of 35 cases, indicating that the clinicopathologic features and aggressive behavior of this disease are distinct from the more common DLBCL.[8]

On the other hand, anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL) is well known as a distinct disease. It is characterized by "hallmark cells" with eccentric horseshoe- or kidney-shaped nuclei and a cytotoxic phenotype in most cases, in addition to uniform expression of ALK activated by a translocation involving the *ALK* gene.[1, 9] Here, we report two A-DLBCL cases with hallmark cells, posing a problem for a differential diagnosis from ALK-positive ALCL. One case was characterized by a number of hallmark cells but lacked conspicuous or pleomorphic HRS cells and pan–B-cell markers other than CD20, CD79a, and OCT-2. The other showed hallmark cells and pleomorphic HRS-like giant cells in a sinusoidal pattern with CD30 expression in 20% of the tumor cells. However, CD20 expression was restricted to approximately half of the

tumor cells despite high positivity of the other pan–B-cell markers. In the past, less attention appears to have been focused on the cytopathologic morphology of hallmark cells and the relevance of the lack of pan–B-cell markers in A-DLBCL cases.

CASE PRESENTATION

Clinical Summary

Case 1

A 59-year-old woman was admitted to a local hospital, presenting with cervical lymphadenopathy and swelling of a left tonsil. Lymph node biopsy indicated a diagnosis of DLBCL with unusual morphologic features. The patient had Ann Arbor stage II disease, no B symptoms, and low international prognostic index (IPI) score. She received immuno-chemotherapy with a regimen of rituximab and CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone), achieved a partial remission, and is now alive with disease 5 months after diagnosis.

Case 2

A 59-year-old woman was admitted to a local hospital, presenting with left cervical, mediastinal, and retroperitoneal lymphadenopathies, with lymph node biopsy indicating DLBCL with marked sinusoidal pattern. The patient had Ann Arbor stage III disease, no B symptoms, and low IPI score.

She received immuno-chemotherapy with a regimen of rituximab and CHOP and achieved a partial remission. She is now alive with disease 4 months after diagnosis.

Pathological Findings

Case 1

The lymph node architecture was diffusely effaced by a number of large tumor cells with eccentric horseshoe-, kidney-, or doughnut-like shaped nuclei (Figure 1). The nuclear chromatin was finely clumped with indistinct nucleoli and amphophilic cytoplasm, and the overall histopathologic appearance of this case was indistinguishable from the common pattern of ALK+ ALCL. The tumor cells were positive for CD20, CD79a, OCT-2, and fascin but negative for CD1a CD3, CD4, CD5, CD8, CD10, CD15, CD21, CD23, CD30, CD56, CD68, CD117, CD138, CD163, PAX5, BOB.1, ALK, BCL2, BCL6, MUM1, MYC, and kappa and lambda light chains. The p53 immunostaining resulted in completely negative. The Ki67 index was 5 to 15%. The tumor cells were not associated with Epstein–Barr virus (EBV) based on EBV-encoded small nuclear early region in situ hybridization (EBER-ISH). PD-L1 (clone SP142) was detected in the microenvironment immune cells, i.e., tumor-infiltrating macrophages, with a small number of PD1-positive background T-lymphocytes. Polymerase

chain reaction (PCR) analysis showed a clonal rearrangement of the *IGH* gene, but not the *TCRγ* gene, using an available paraffin block.

Case 2

The lymph node architecture was well preserved. The tumor cells were distributed in the sinus with preservation of underlying lymphoid tissue but a minimal parenchymal infiltration, consisting of large cells with horseshoe-shaped and multiple wreath-like nuclei (Figure 2). These morphologic features highly suggested the possibility of a pleomorphic giant cell–rich pattern in ALK-positive ALCL. The tumor cells were strongly positive for CD79a, PAX5, BCL2, MUM1, p53, and MYC but negative for CD3, CD5, CD10, CD15, ALK, and BCL6. The Ki67 index was 70%. Approximately 50% of the tumor cells were also positive for CD20, 20% for CD30 and 20% for fascin but were not associated with EBV based on EBER-ISH. BOB.1 and OCT-2 were variable from weak/absent to strong in intensity among tumor cells, having a tendency of the decreased expression on giant cells, contrasted with a consistent strong positivity in background

B-lymphocytes. Tumor cells were negative for PD-L1 (clone SP142) but showed a compartmentalization surrounded by PD-L1-positive microenvironment immune cells. PCR analysis showed no rearrangement of either *IGH* or *TCRγ* genes. Fluorescence in situ hybridization failed to examine *BCL2* and *MYC* genetic alterations because of a limited area affected by the tumor.

DISCUSSION

In the English-language literatures, the preferred diagnosis of A-DLBCL has been made with an emphasis on the co-existence of conspicuous HRS-like cells and their CD30 positivity.[1-7] As a result, this diagnostic category was presumed to include heterogeneous diseases, such as common DLBCL with CD30 expression of varying degrees, ALK-positive DLBCL, EBV-positive DLBCL, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL, and anaplastic transformation of low-grade B-cell lymphoma.[1] Li et al. in their series of 35 patients recently highlighted the distinctiveness of this neoplasm from ordinal DLBCLs in terms of genetic alterations and biologic features.[8] They defined A-DLBCL principally based on morphology, i.e., the presence of polygonal, bizarre-shaped tumor cells with a sinusoidal and/or cohesive growth pattern, which led them to recognize some characteristics in A-DLBCL. These features included a higher incidence of p53 positivity (80%) and expression of both MYC and BCL2 (double expressor) (43%); a high frequency of *TP53* mutation (57%) and concurrent *MYC* and *BCL2* and/or *BCL6* abnormalities (translocation or extra copy) (41%); almost cases (86%) demonstrated non-

germinal center B-cell phenotype; and two morphologic subgroups of numerous bizarre Reed-Sternberg (RS)-like tumor cell type (51%) and ordinary DLBCL type with scattered RS-like cells (49%), respectively. All cases were strongly positive for CD20 and negative for CD3, ALK, and EBER-ISH. CD30 positivity was 51%.[8] Our two cases appear to be within the boundaries of these clinicopathological findings.

Here we have documented two A-DLBCL cases characterized by hallmark cell appearance. Both cases involved 59-year-old women who presented at around the same time, posing the problem of differential diagnosis from ALK-positive ALCL in our routine workup. Conspicuous HRS-like cells, a diagnostic landmark of A-DLBCL in previous studies, were present in case 2 but not in case1. The histopathologic appearance of case 1, which highly overlapped with the common pattern of ALCL without conspicuous HRS-like cells, seems to have gone unrecognized or at least underestimated in earlier studies.[1] Tumor cells in this case were strongly positive for CD20, CD79a, and OCT-2 but were unique in lacking expression of other pan–B-cell markers so far examined, suggesting their association with CHL and leading us to consider the possibility of a gray zone or

intermediate lymphoma between DLBCL and CHL. This disease was first listed in the 2008 WHO classification[1, 10, 11] and was recently suggested to affect patients at a median age of 50 years without mediastinal lesions.[12] Indeed, many of the tumor cells were positive for fascin, a sensitive marker for classical HRS cells,[13] supporting the concept of a gray zone or intermediate lymphoma between DLBCL and CHL. The Ki67 index was lower in case 1 than those in the ordinal DLBCLs, the biologic significance of which remains to be elucidated. Case 2 was considered a typical A-DLBCL involving numerous RS-like cell types as described by Li et al.,[8] with sinusoidal pattern, posing a differential diagnostic candidate from intravascular/intrasinusoidal large B-cell lymphoma.[1, 7, 14, 15] Of interest, CD20 positivity was present in around 50% of the tumor cells despite the strong expression of the other pan–B-cell markers. As in case 1, fascin expression was detected in 20% of the tumor cells. Antigen loss of the neoplastic cells was not a common event in ordinary DLBCLs. This issue in A-DLBCL has previously been reported in a minority (25%) of A-DLBCL cases (n=24) of Haralambieva et al.[4] without additional comment, and also was not mentioned in the cases described by Li et al..[8] Of note, our two cases

shared the loss of pan–B-cell markers and the expression of fascin beyond their morphologic diversity. Carey et al. recently documented the unique topology of CHL in which PD-L1 positive tumor-associated macrophages surround PD-L1 positive HRS-cells and implicate CD4-positive T-cells as a target of PD-1 blockade.[16] The tumor cells of our cases were further negative for PD-L1 but were associated with PD-L1-positive microenvironment immune cells, indicating a possible therapeutic target for the immune check-point inhibitors as well.[17]

In conclusion, our two cases showed a broad diversity of morphologically defined A-DLBCL, shedding light on pan–B-cell antigen loss suggestive of a grey zone/intermediate lymphoma between DLBCL and CHL. These features are expected to contribute to a deeper understanding of this enigmatic disease.

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Conflicts of interest and source of funding

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FIGURE LEGENDS

Figure 1

Case 1. (a) (upper left) The tumor consisted of a number of hallmark cells with eccentric horseshoe-, kidney- or doughnut-like shaped nuclei. (b) (upper right) Higher magnification of hallmark cells with horseshoe-like nuclei. (c) (middle left) Immunohistochemistry for CD20. Tumor cells are strongly positive for CD20. (d) (middle right) Immunohistochemistry for PAX5, showing the complete absence of its expression on tumor cells. (e) (lower left) Immunohistochemistry for PD-L1 (clone SP142). PD-L1 positivity was detected in the microenvironment immune cells but not in tumor cells. (f) (lower right) Immunohistochemistry for Ki67, which is found in 5 to 15% of the tumor cells.

Figure 2

Case 2. (a) (upper left) Tumor cells consisted of large or giant cells with eccentric horseshoe-like or multiple wreath-like nuclei. (b) (upper right) Immunohistochemistry for CD20, which was detected on approximately half

of the tumor cells. (c) (middle left) Immunohistochemistry for p53, highlighting its strong expression in the nuclei of the tumor cells. (d) (middle right) Immunohistochemistry for PD-L1 (clone SP142), showing the compartmentalization of the tumor cells surrounded by PD-L1-positive microenvironment immune cells. (e) (lower left) Immunohistochemistry for OCT-2, the intensity was variable from weak/absent to strong among tumor cells.

Figure 1

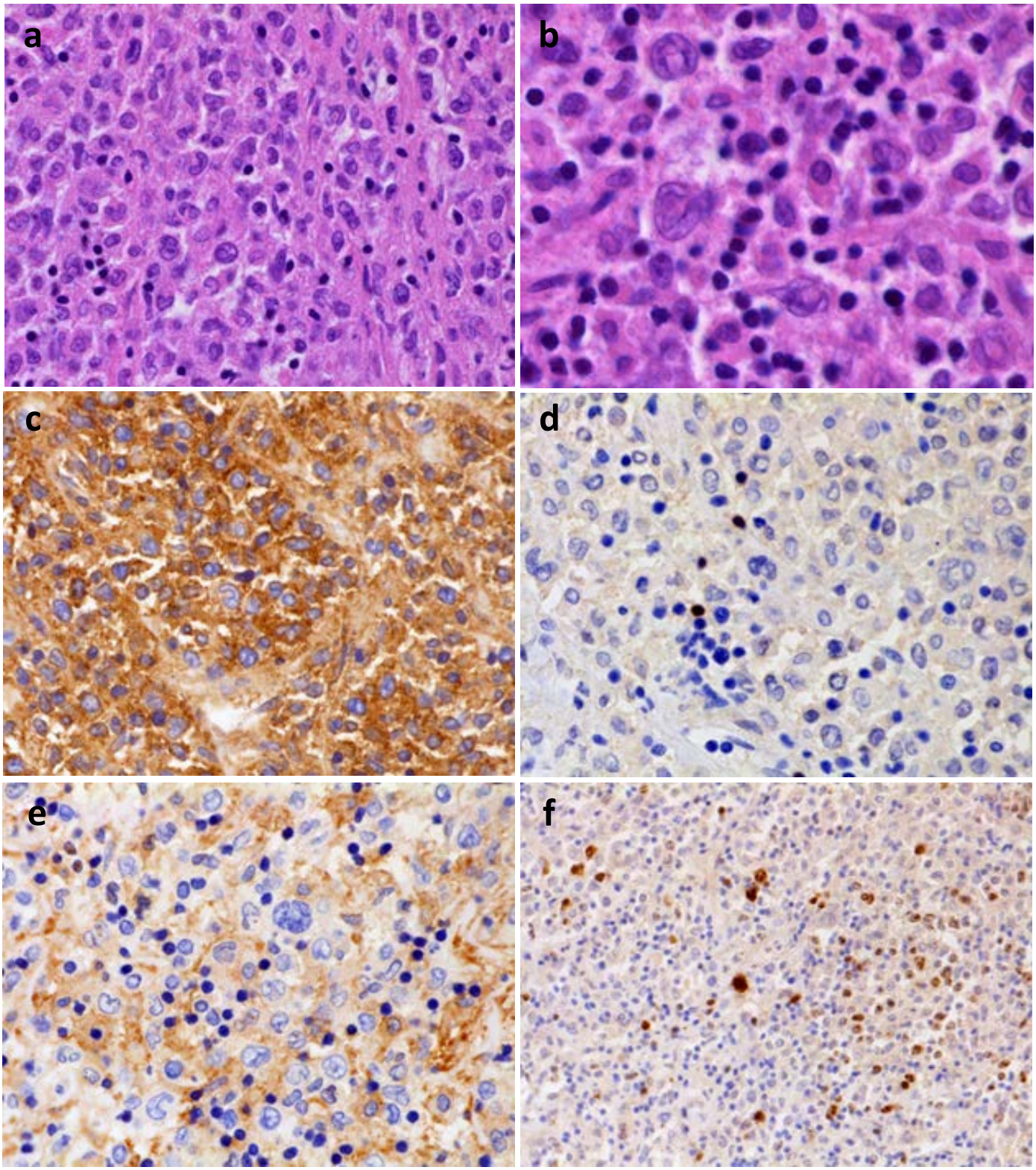

Figure 2