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Case Report

A case of immunohistochemical false positive staining caused by incompatibility between a CD4 antibody and an autostainer

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Abstract: Precise immunophenotyping of tumor cells by immunohistochemistry is complementary to morphological examination. It is critical for the correct histopathological diagnosis of lymphomas. In this paper, we report a case of T-cell lymphoma whose histopathological diagnosis was confounded by an immunohistochemical pitfall: a false positive caused by incompatibility between an antibody and an autostainer. In this case, based on CD4 immunohistochemistry of the affected lymph nodes, the T-cell lymphoma was diagnosed as CD4-positive at the onset, while it appeared discordantly to be CD4-negative at the second relapse. We noticed that CD4 antibodies and autostainers of different suppliers (designated as suppliers X and Y) were used in an unqualified combination in immunohistochemistry at the onset: that is, the combination of an antibody supplied by X and an autostainer supplied by Y (designated as X-Y combination) was used at the onset. On the other hand, the Y-Y combination was at the second relapse. At the second relapse, flow cytometry of the affected lymph node showed infiltration of CD4-negative T-cell lymphoma. We reasoned that CD4 immunonegativity obtained by the Y-Y combination at the second relapse was specific, while CD4 immunopositivity by the X-Y combination at the onset was false positive. Immunohistochemical reexamination of the lymph node at the onset proved to be CD4-negative by not only the Y-Y but also X-X combinations, confirming our final diagnosis of nodal relapse of CD4-negative T-cell lymphoma. This case illustrates the importance of using compatible combinations of antibodies and autostainers in diagnostic immunohistochemistry.

Keywords: Immunohistochemistry, false positive, autostainer, CD4, T-cell lymphoma

Introduction

Histopathological diagnosis plays essential roles in the diagnosis of lymphomas. Although important, morphological examination of the tumor cells has its own limitation. To complement this, correct immunophenotyping of tumor cells by immunohistochemistry is critical for the correct histopathological diagnosis of lymphomas.

However, there are some practical pitfalls in the interpretation of immunohistochemical results for the diagnosis of lymphomas. Immunohistochemical results may be misinterpreted when an antigen usually expressed in some cell types is not expressed or downregulated for some reasons. For example, cells of presumed B cell lineage may be stained negatively in CD20 immunohistochemistry in the cases of precursor B lymphoblastic leukemia/lymphoma, plasmacytoma, and other subtypes B-cell lymphoma with CD20 downregulation after rituximab therapy [1]. Another pitfall may be expression of antigens aberrantly and ectopically expressed where they are not usually expressed. Examples of the aberrant expression include CD4 or CD56 expression in myeloid sarcoma, which provide a diagnostic challenge for pathologists. Not only knowledge in immunology but also recognition of these practical pitfalls is essential for correct interpretation of the immunohistochemical results.

In this paper, we report a case of T-cell lymphoma whose histopathological diagnosis was confounded by another immunohistochemical pitfall different from the above two: a false positive

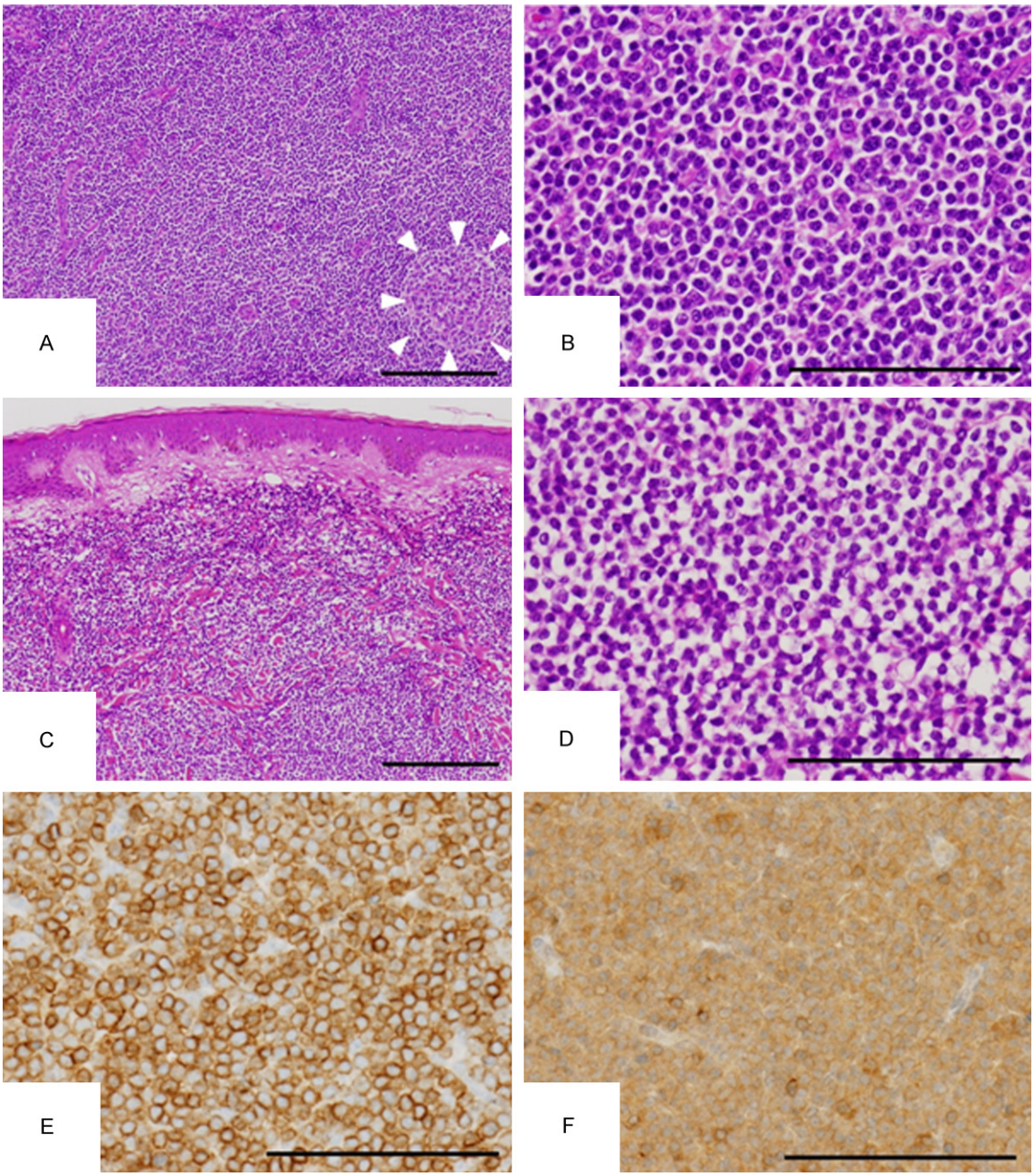

Figure 1. Representative histological images of the T-cell lymphoma at the onset. (A-D) Hematoxylin and eosin (HE) stain. (E, F) Immunohistochemistry. (E) CD3. (F) CD4 (using CD4 antibody purchased from Leica and an autostainer supplied by Ventana; see text for details). In immunohistochemistry, positive cells are stained brown. At low power view in the lymph node (A), the lymphoid tumor cells proliferated diffusely, almost effacing the follicular structure of normal lymph nodes. A residual germinal center was indicated by arrowheads at the right lower corner of the figure. At low power view in the skin (C), they proliferated in the dermis and subcutaneous tissue without epidermotropism, at high power view, the tumor cells were atypical lymphoid cells of medium size (B, D). They were positive for CD3 (E), weakly positive by CD4 immunohistochemistry using antibody purchased from Leica (F). (A, C) Original magnification: × 100, bar: 200 μm. (B, D-F) Original magnification: × 400, bar: 100 μm.

caused by incompatibility between an antibody and an autostainer. In this case, based on CD4 immunohistochemistry of the affected lymph nodes, the T-cell lymphoma was diagnosed as CD4-positive at the onset, while it appeared discordantly to be CD4-negative at the second

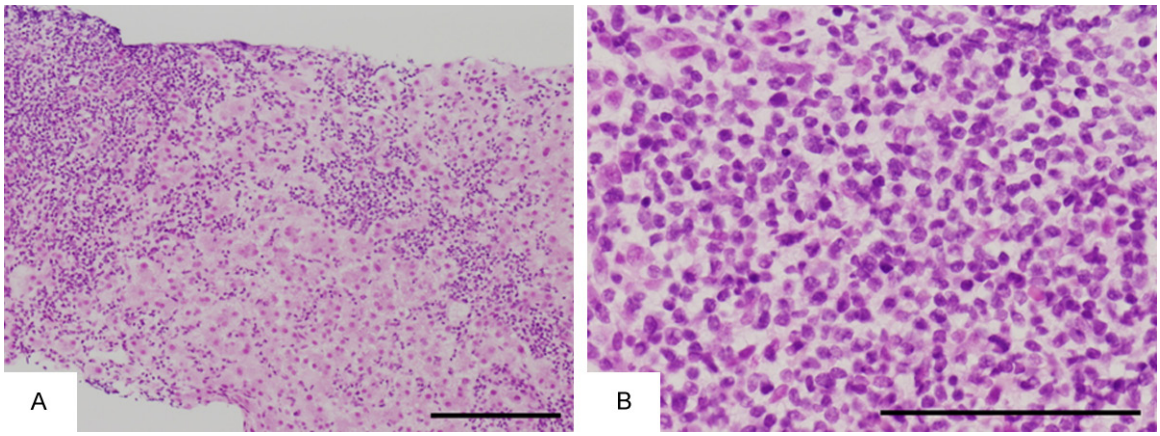

Figure 2. Representative histological images of the T-cell lymphoma at the first relapse in the liver. HE stain is shown. A. Low power view. Sinusoidal infiltration of atypical lymphoid cells was shown. Original magnification: × 100, bar: 200 μm. B. High power view. The infiltrating atypical lymphoid cells were indistinguishable from those at the onset. Original magnification: × 400, bar: 100 μm.

relapse. We noticed that CD4 antibodies and autostainers of different suppliers (designated as suppliers X and Y) were used in an unqualified combination in immunohistochemistry at the onset: that is, the combination of an antibody supplied by X and an autostainer supplied by Y (herein designated as X-Y combination) was used at the onset, while the Y-Y combination was at the second relapse. At the second relapse, flow cytometry of the affected lymph node showed infiltration of CD4-negative T-cell lymphoma. We reasoned that CD4 immunonegativity obtained by the Y-Y combination at the second relapse was specific, while CD4 immunopositivity by the X-Y combination at the onset was false positive.

Case report

A fifty-three-year old Japanese man was admitted to Takarazuka Municipal Hospital for the examination of bilateral swollen inguinal lymph nodes of around 2 cm in size and subcutaneous tumor. Computerized tomography examination of the abdomen revealed swelling of lymph nodes in para-aortic and hepatic portal regions (data not shown). In his past history, intermittent liver dysfunction had been pointed out. The serum biochemistry showed that antibodies against hepatitis B (HB) s and HBc were positive without evidence of hepatitis C infection. Alcoholic liver dysfunction was not suggested clinically (data not shown). The histopathological examination of the resected lymph node by hematoxylin and eosin (HE) stain showed diffuse proliferation of medium-sized atypical lymphoid cells that effaced primary follicular structure of the lymph node (**Figure 1A** and **1B**). The histology of the biopsy of the subcutaneous tumor showed infiltration of atypical lymphoid cells with similar morphology to those of the lymph node (**Figure 1C** and **1D**). Immunohistochemical analysis of the atypical lymphoid cells showed that the tumor cells were stained positive for CD3 (**Figure 1E**), weakly positive for CD4 (**Figure 1F**), and negative for CD20 (data not shown) and CD79a (data not shown). CD8 staining of the medium-sized tumor cells was considered to be negative with associated infiltration of reactive CD8-positive small T-cells (data not shown). The anti-adult T-cell leukemia/lymphoma antibody was negative. The diagnosis of CD4-positive peripheral T-cell lymphoma, not otherwise specified, and its infiltration into the skin was made.

The patient had been administered seven courses of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy during the following five months. One month after the seventh course of the CHOP chemotherapy, he was readmitted to the hospital because of aggravation of his liver dysfunction. The laboratory data on the readmission showed that total bilirubin was 13.0 mg/dl (normal range: 0.2-1.2), aspartate aminotransferase 1789 U/L (normal range: 8-40), alanine aminotransferase 1115 U/L (normal range: 4-45), alkaline phosphatase 402 U/L (normal range: 100-340), and lactate dehydrogenase 410 U/L (normal range 100-210), together with elevated level of soluble interleukin-2 receptor (3256 U/

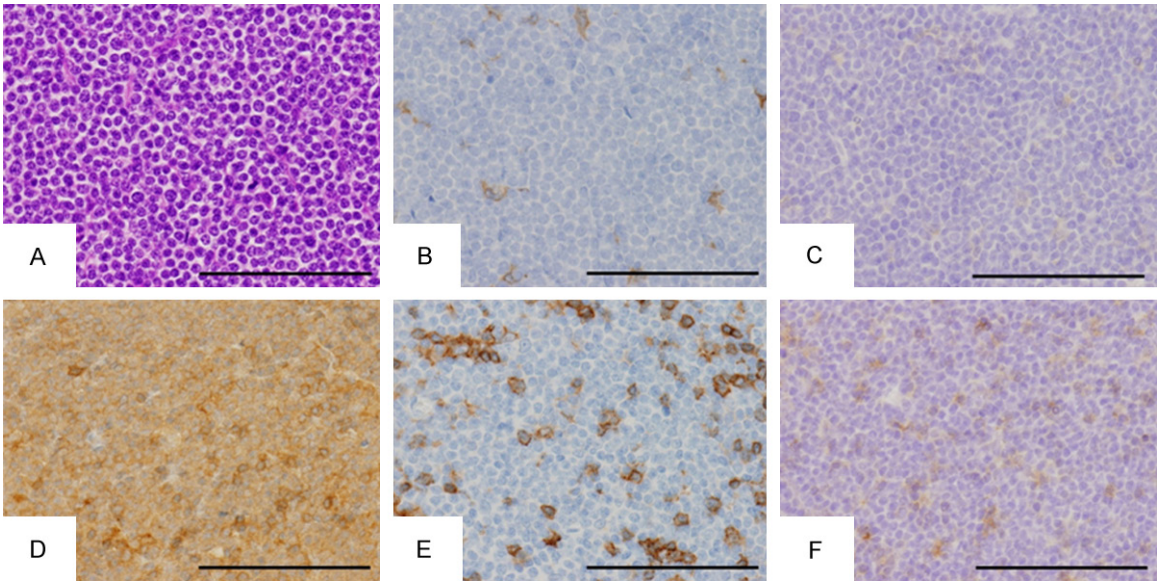

Figure 3. CD4 immunohistochemistry of the T-cell lymphoma at the onset and the second relapse: comparison of the Y-Y, X-X, and X-Y combinations. 'Y-Y, X-X, and X-Y' each represent 'CD4 antibody-autostainer' combinations, where X represents 'supplied by Leica', and Y 'supplied by Roche/Ventana'. See texts for more details. (A-C) At the second relapse. (D-F) At the onset. (A) HE. (B-F) immunohistochemistry. (B, E) Y-Y combination. (C, F) X-X combination. (D) X-Y combination. Positive cells are stained brown. The lymphoma cells were stained diffusely albeit weakly positive by the X-Y combination at the onset (D). However, in fact, the lymphoma cells were stained negative by the Y-Y combination at the onset (E) as well as at the second relapse (B). The lymphoma cells were stained negative by the X-X combination at the onset (F) as well as at the second relapse (C). Original magnification: × 400, bar: 100 μm.

mL; normal range: 145-519). No evidence of reactivation of hepatitis B virus was suggested (data not shown). Although abdominal CT examination showed no remarkable abnormality, histological examination of the needle biopsy of the liver revealed sinusoidal infiltration of atypical lymphoid cells (**Figure 2**). The immunohistochemical analysis revealed that they were CD3 and CD4-positive, similar to that of the previous T-cell lymphoma at the onset (data not shown). The diagnosis was made as the relapse and infiltration of CD4-positive T-cell lymphoma in the liver. MVCP (mitoxantrone, etoposide, carboplatin, and prednisone) chemotherapy was administered as salvage.

Around six months after the relapse in the liver, the patient was referred to the hospital again for the examination of the swollen inguinal lymph node. The HE image of the biopsy of the lymph node showed diffuse proliferation of medium-sized atypical lymphoid cells, which appeared almost the same as that of CD4-positive T-cell lymphoma in the lymph node at the onset around one year before (**Figure 3A**). We suspected the relapse of the CD4-positive T-cell lymphoma. However, surprisingly, immunohistochemical examination of the inguinal lymph node at this time showed that the lymphoma cells were negative for CD4, which was totally unexpected (**Figure 3B**). The flow cytometry of the lymph node specimen at this time also showed infiltration of medium-sized CD3-positve lymphoid cells containing only 0.2% of CD4-positive cells, suggesting infiltration of CD4-negative T-cell lymphoma, which was consistent with the CD4-immunonegativity at the second relapse. We reasoned that the CD4-immunonegativity at the second relapse was specific. We explored the source of the discrepancy of the CD4 immunophenotype of the lymphoma cells between the onset around one year ago and the presumed second relapse at this time. We noticed that CD4 antibodies from different suppliers were used for immunohistochemistry at the onset and at the second relapse: CD4 antibody purchased from Leica (catalogue number: #NCL-L-CD4-368) was used at the onset and the first relapse in the liver, while that purchased from Roche (catalogue number: #790-4423) at the second relapse in the lymph node. In all occasions, we used Ventana BenchMark GX autostainer in immunohistochemistry, which is a qualified and

recommended autostainer for the use of antibodies supplied by Roche. Herein, Leica is designated as X and Roche/Ventana as Y for simplicity. Then, it turned out that at the onset and the first relapse, we used CD4 antibody supplied by X in combination with the autostainer supplied by Y (herein designated as X-Y combination) (**Figure 3D**), while at the second relapse we used the Y-Y combination in CD4 immunohistochemistry. The T-cell lymphoma cells at the second relapse were CD4-negative in the Y-Y combination (**Figure 3B**). The CD4-immunoreactivity of the lymphoma cells at the onset was reexamined by using either X-Y or Y-Y combination. The comparison revealed that the lymphoma cells at the onset were "CD4-positive" by the X-Y combination (**Figure 3D**), while they were CD4-negative by the Y-Y combination (**Figure 3E**). Since the lymphoma cells at the second relapse were CD4-negative by the Y-Y combination (**Figure 3B**) as well as the flow cytometry, we concluded that CD4 immunoreactivity by the X-Y combination was false positive. We further examined the CD4-immunophenotypes of the T-cell lymphomas by the X-X combination, that is, CD4 antibody supplied by Leica and an autostainer supplied by Leica microsystems (BOND-MAX) (**Figure 3C** and **3F**). The T-cell lymphoma cells were CD4-negative by the X-X combination, both at the onset (**Figure 3F**) and at the second relapse (**Figure 3C**). These results suggested that CD4 immunohistochemistry by either X-X or Y-Y combination was specific, while that by the X-Y combination yielded false positive.

Taken together, the final diagnosis was established as nodal relapse of CD4-negative T-cell lymphoma.

Discussion

In this paper, we report a case of T-cell lymphoma whose histopathological diagnosis was confounded by an immunohistochemical pitfall: a false positive caused by incompatibility between an antibody and an autostainer. In this case, based on CD4 immunohistochemistry of the affected lymph nodes, the T-cell lymphoma was diagnosed as CD4-positive at the onset, while it appeared to be CD4-negative at the second relapse. We noticed that CD4 antibodies and autostainers of different suppliers (designated as suppliers X and Y) were used in an unqualified combination in immunohistochemistry at the onset: that is, the combination of an antibody supplied by X and an autostainer supplied by Y (designated as X-Y combination) was used at the onset. On the other hand, the Y-Y combination was at the second relapse. Based on flow cytometry data of the affected lymph node at the presumed second relapse, we hypothesized that CD4 immunonegativity obtained by the Y-Y combination at the second relapse was specific, while CD4 immunopositivity by the X-Y combination at the onset was false positive. This was confirmed by reexamination of the lymph nodes at the onset and at the second relapse by the X-X as well as Y-Y combinations. The lymphoma cells were negative for CD56, granzyme B, perforin, and TIA-1 in immunohistochemistry (data not shown). *In situ* hybridization of Epstein-Barr virus-encoded small RNAs (EBER) was also negative (data not shown), excluding the possibility of extranodal NK/T-cell lymphoma, nasal type.

CD4 staining of macrophages, histiocytes, monocytes, or tumors derived from them, are often encountered in routine practice of histopathology [2-7]. Most of them are thought to be either non-specific, false positive, or aberrant. In the present case, the tumor cells were immunohistochemically negative for CD68, CD56, CD34, MPO, and KIT, presumably ruling out these possibilities including myeloid sarcoma (data not shown). Together with these data, immunohistochemical false positive by the X-Y combination at the onset led us to erroneously conclude that the T-cell lymphoma of this case was CD4-"positive".

Our case illustrates an immunohistochemical false positive caused by incompatibility between an antibody and an autostainer. In our case, the compatible combination of X-X and Y-Y yielded specific results, while the incompatible combination of X-Y yielded false positives. To our knowledge, this is the first report on this type of immunohistohemical false positive.

Besides the specificity, the results of the Y-Y combination exhibited a slightly stronger signal than that of the X-X combination. For example, the staining in non-tumor cells of the Y-Y combination at the onset (**Figure 3E**) was slightly stronger than that of the X-X combination (**Figure 3F**). In addition, the staining in non-tumor cells of the Y-Y combination at the second relapse (**Figure 3B**) was slightly stronger

than that of the X-X combination (**Figure 3C**). According to the supplier's catalogues, CD4 antibody supplied by Leica (X) is a mouse monoclonal antibody, while that supplied by Roche (Y) is a rabbit monoclonal antibody. Rossi S et al. reported that some rabbit monoclonal antibodies showed better sensitivity without loss of specificity than mouse monoclonal antibodies [8]. For example, a key to pathological diagnosis of mantle cell lymphoma is an immunohistochemical surrogate for the hallmark t(11;14)(q13;q32) translocation leading to overexpression of cyclin D1 protein. For this purpose, mouse monoclonal antibodies against cyclin D1 had been used, but the low sensitivity and specificity had been problematic for long. However, the introduction of rabbit monoclonal antibodies against cyclin D1 has greatly improved the sensitivity and specificity of immunohistochemical detection of cyclin D1 in mantle cell lymphomas [9, 10]. Presumably, this implies that the difference of staining intensity between the X-X and Y-Y combination in the present case may partly be due to the difference of species, that is, rabbit versus mouse, of the CD4 monoclonal antibodies.

This case illustrates that not only knowledge in immunology but also recognition of practical pitfalls is essential for correct interpretation of the immunohistochemical results. The case highlights the importance of using compatible combinations of antibodies and autostainers in diagnostic immunohistochemistry. Furthermore, in this case, correlation of the flow cytometry and CD4 immunohistochemistry at the second relapse was a hint to solve immunohistochemical discrepancy between the onset and the second relapse. Paying attention to such a clinicopathological correlation should not be overemphasized in the histopathological diagnosis of lymphomas in general.

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Disclosure of conflict of interest

None.

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