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Short Communication

Better method for detection of CD30: Immunohistochemistry or flow cytometry?

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We compared the two methods of assessing CD30 protein expression in DLBCL and TCL specimens routinely employed at our hospital, immunohistochemistry (IHC) and flow cytometry (FCM), using the same clone of the anti-CD30 antibody (Ber-H2) in 123 patients with DLBCL and 28 patients with TCL. FCM was more sensitive than IHC, especially in cases with low expression. In three cases of TCL and two cases of DLBCL, there was discordance between these two methods. Two of these TCL cases were ALCL and one was peripheral T-cell lymphoma, NOS, but ALCL was unable to be excluded. One of two cases of DLBCL was an anaplastic variant of DLBCL. The data suggested that CD30 was undetectable, though rare, by FMC in several cases. Based on this study, a combination of IHC and FCM is recommended for the reliable and quantitative detection of CD30.

Keywords: CD30, flow cytometry, immunohistochemistry, diffuse large B-cell lymphoma, T-cell lymphoma

INTRODCTION

CD30, initially termed Ki-1, was discovered by Stein *et al*. as a Hodgkin/Reed-Sternberg cell-specific marker of classical Hodgkin lymphoma (cHL).[1] Through the immunohistochemical analysis of CD30 in different types of lymphoma, anaplastic large cell lymphoma (ALCL) was recognized as a distinct entity of lymphoma.[2] As the expression of CD30 is restricted to the small population of activated hematopoietic cells in normal tissues, CD30 was hypothesized to be an effective therapeutic target of cHL and ALCL.[3-5] Brentuximab vedotin (BV), a CD30-directed antibody-conjugated drug,[6] demonstrated high efficacy after a single treatment with an objective response rate (ORR) of 72% in a phase II clinical trial of refractory or recurrent (R/R) cHL[7] and ORR of 86% in ALCL,[8] leading to its worldwide approval, including by the FDA (USA) and PMDA (Japan). Several clinical trials are currently ongoing, mainly aimed at its efficacy in combination with chemotherapy or immune checkpoint inhibitors.[9] Recently, the therapeutic applications of BV have expanded to other B-cell and T-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL)[10] and T-cell lymphoma (TCL).[11] In clinical trials of BV for DLBCL and TCL, patients with all ranges of CD30 expression on tumor cells responded.[10,11] This lack of correlation between CD30 expression and the efficacy of BV is not yet understood. One explanation is the limitation of immunohistochemistry (IHC) to detect low levels of CD30. Another explanation is the role of soluble CD30 (sCD30), as all patients in the DLBCL study had high sCD30 levels at baseline.[10] CD30 was reported to be transferred to bystander cells by trogocytosis[12] or via exosomes.[13] These bystander cells may release the free drug, thereby killing adjacent tumor cells. However, the expression of CD30 in lymphoma cells is essential to select patients for BV-containing therapies. For example, the CD30 gene was differentially expressed between those with cHL who achieved remission and those who did not respond to BV.[14] However, there have been no reports on which method is suitable for detecting CD30 expression in lymphoma specimens.

MATERIALS AND METHODS

We compared the two methods of assessing CD30 protein expression in DLBCL or TCL specimens routinely employed at our hospital, IHC and flow cytometry (FCM), using the same clone of the anti-CD30 antibody (Her-H2). We examined 123 patients with DLBCL and 28 patients with TCL

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diagnosed between March 2003 and November 2015 at Saitama Medical Center, Saitama Medical University. We selected the patients based on the availability of FCM data. The study was approved by the institutional review board (No. 1966-V). For IHC, 3-μm sections of formalin-fixed, paraffin-embedded tissues were deparaffinized with xylene and dehydrated in a graded series of alcohol. After heat-induced antigen retrieval (60 min. in ethylenediaminetetraacetic acid buffer, pH 8), the sections were incubated with an anti-CD30 antibody (Ber-H2, Roche Diagnostics Co., Ltd) for 32 min. at 37°C. For detection of the primary antibody, the OptiView DAB universal kit (Roche Diagnostics Co., Ltd) containing HQ linker was used. The CD30 IHC was assessed by visual estimation by two pathologists (J.K. and J.T.). For flow cytometry, single-cell suspensions were prepared immediately after surgical removal of the lymph node

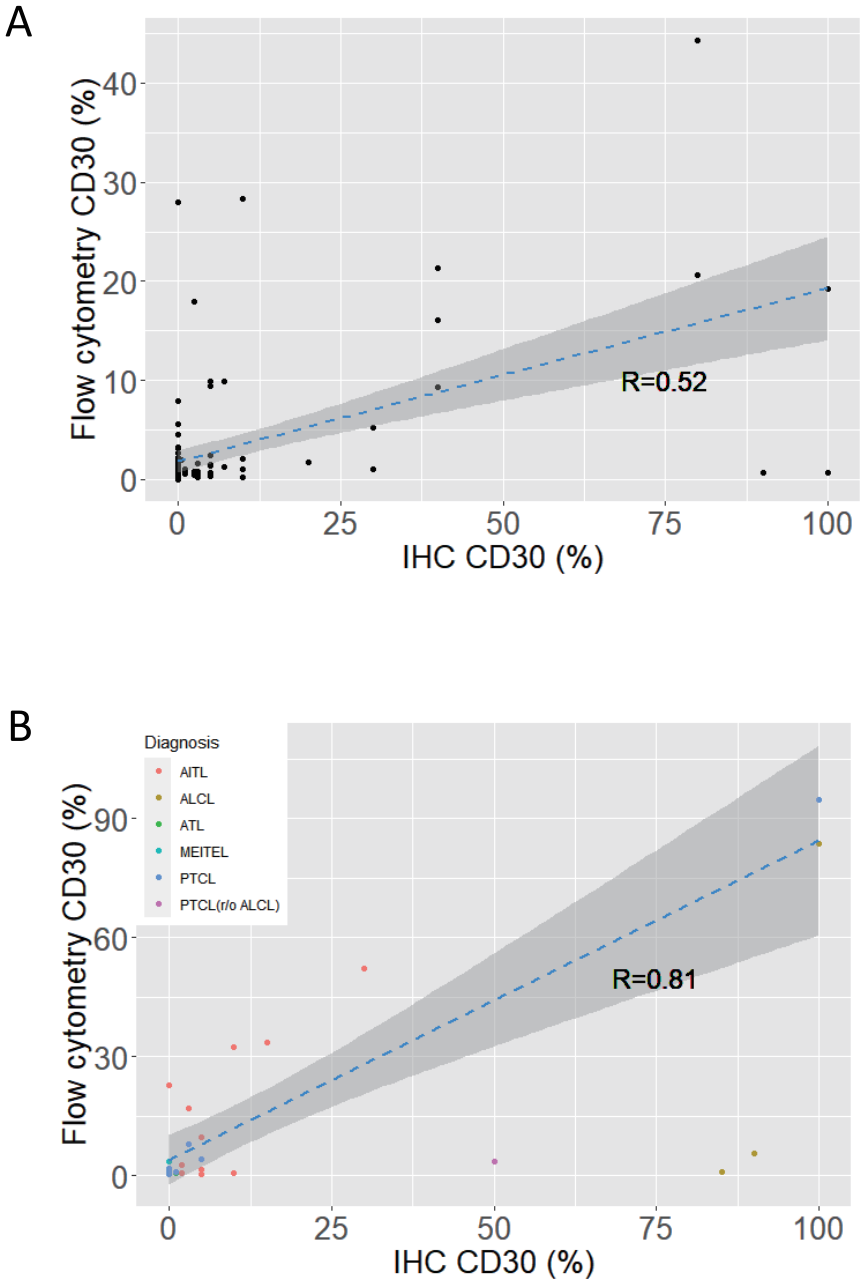

Fig. 1. Comparison of CD30 detection by immunohistochemistry and flow cytometry in diffuse large B-cell lymphoma (***A***) and T-cell lymphoma (TCL) (***B***). The dashed line indicates linear regression. The gray area represents the 95% confidence band. R indicates Pearson's correlation coefficient. The coefficient in TCL was calculated exclusive of anaplastic large cell lymphoma.
AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ATL, adult T-cell leukemia/lymphoma; MEITEL, monomorphic epitheliotropic intestinal T-cell lymphoma; PTCL, peripheral T-cell lymphoma

by gentle, repetitive scraping of the tissues with a sharp scalpel. Staining of cells and FCM analysis were conducted by a clinical laboratory company (SRL Inc., Tokyo, Japan) for routine diagnosis.

RESULTS AND DISCUSSION

We assumed CD45+, SSC-high cells to be lymphoma cells and analyzed the proportion of CD7-, CD30+ cells in this population for DLBCL. Ten of 123 patients (8.1%) were excluded from the following analysis because no lymphoma cells were present in FCM samples. For TCL, we first assessed the expression of CD7 and analyzed the proportion of CD7+, CD30+ cells among CD45+, SSC-high cells when CD7 expression was comparable with CD3 expression or the proportion of CD7-, CD30+ cells when CD7 expression was lower than CD3 expression.

Pearson's correlation coefficients between IHC and FCM were 0.52 and 0.81 in DLBCL and TCL, respectively. The coefficient in TCL was calculated exclusive of ALCL. The percentage of positive cells was slightly higher in IHC than in FCM. This may because positively stained cells in the IHC sample concealed the negative cells in the background or the CD30-positive bystander cells were mistaken for lymphoma cells. Flow cytometry was more sensitive than IHC, especially in cases with low expression. In three cases of TCL, there was discordance between FCM and IHC, with positive rates of less than 5% in FCM and greater than 50% in IHC. Two of these cases were ALCL and one was peripheral T-cell lymphoma, NOS, but ALCL was unable to be excluded. Two of three ALCL cases had discrepancies in positivity between IHC and FCM. A similar discrepancy was observed in two cases of DLBCL. One case was an anaplastic variant of DLBCL. As these cases expressed CD45, CD45 gating on FCM may not have excluded lymphoma cells. It is possible that lymphoma cells were not obtained in the FCM sample during processing of the lymph node because of the cohesiveness of lymphoma cells. Another explanation for the discrepancy is that lymphoma cells are surrounded by bystander cells and are difficult to isolate in the cell suspension. Singlet Hodgkin/Reed-Sternberg cells in classical Hodgkin lymphoma were reportedly difficult to obtain due to the rosetting of non-tumor T-cells.[15,16]

We demonstrated that FCM is more sensitive than IHC in detecting CD30 expression, but some pitfalls, such as preparation and gating, are present in FCM. Based on this study, a combination of IHC and FCM is recommended for the reliable and quantitative detection of CD30.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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