MMA monoclonal antibody is a superior anti-CD15 reagent for the diagnosis of classical Hodgkin's lymphoma

CD15 is a useful immunohistochemical marker to identify Reed-Sternberg cells in classical Hodgkin's lymphoma (HL) and to distinguish it from HD-like neoplasms, but data from the literature concerning its expression in HL are quite variable. Using immunohistochemistry we compared the reactivity of three different anti-CD15 clones (MMA, C3D1 and BY87) and found that anti-CD15 MMA clone is a superior reagent in identifying atypical cells, detecting more numerous cells in 28.2%, and being the only positive marker in 12.8% of cases. We conclude that it is advisable to include this reagent in diagnostic immunohistochemical panels.

Haematologica 2007; 92:5:708-709

CD15 is a complex cluster of cell surface glycoproteins and glycolipids with a common trisaccharide structure, 3-fucosyl-N-acetyllactosamine (3-FL), which is also referred to as Lewis X (LeX) antigen.[1] CD15 is expressed in normal mature myeloid cells, but it has been predominantly used as an immunohistochemical marker to identify Reed-Sternberg cells (RSC) in Classical Hodgkin Lymphoma (CHL), useful to distinguish it from reactive lymphadenitis, lymphocyte-predominance Hodgkin lymphoma, non-Hodgkin lymphomas and HD-like neoplasms.[2,3] Data from the literature, however, indicate that CD15 expression in RSC is inconstant, ranging from 37% to 100% of reported cases.[4] This variability might be related to differences in clinical behavior of CHL, since it has been found that CD15-negative CHL are associated with worse prognosis.[4] However, as more than ninety clones referred to CD15 are available, it is also possible that the use of different reagents accounts for the divergent results obtained in various studies.[5] In order to verify this hypothesis, we tested the reactivity of three different commercially available anti-CD15 monoclonal antibodies (clone MMA, Cell Marque Corp., Hot Springs, U.S.A.; C3D1, Dako, Glostrup, Denmark; BY87, Novocastra Labs, Newcastle-U-Tyne, U.K.), in 80 cases of lymphoma, that included CHL (39 cases) and various neoplasms selected from those that more frequently entail in the differential diagnosis with CHL (Table 1). All antibodies were mouse IgM recognizing the non-sialylated form of CD15 and were applied at appropriate dilutions on formalin-fixed, paraffin-embedded tissue sections, using heat induced epitope retrieval in 1.0 mM EDTA (pH 8.0). MMA corresponds to the first original anti-CD15 clone developed[6] and recognized as to LeuM1, while BY87 and C3D1 have subsequently developed.[2,7] CD15 expression was evaluated semi-quantitatively and values of specificity and sensitivity were estimated using the Bayesian analysis model. Detailed results are reported in Table 1. In all cases, strong CD15 cytoplasmic expression on granulocytes was used as internal control. In 16/39 CHL cases, the reactivity of RSC was similar with the three Mabs; in 11 cases the number and intensity of CD15-positive RSC was obviously superior with MMA, and in 5 cases only MMA identified the atypical cells (Figure 1). With the exception of three cases (respectively one ALK-negative

Table 1. Cases studied and results of reactivity of the three anti-CD15 monoclonal antibodies.

Diagnosis (number of cases)	*CD15 positive*				*CD15 negative*
	A	*B*	*C*	*D*	
CHL (39)	16	11	5	1	6
LPHL (10)	0	0	0	0	10
ALCL (14)	0	1	0	0	13
T-NHL (9)	1	1	0	0	7
MDLBL (8)	0	0	0	0	8

CHL: classical Hodgkin lymphoma; LPHL: lymphocyte predominance Hodgkin lymphoma; ALCL: anaplastic large cell lymphoma; (4 cases ALK+; 10 cases ALK-); T-NHL: peripheral T-cell non-Hodgkin lymphoma; MDLBL: mediastinal diffuse large B-cell lymphoma. A: similar reactivity with the three antibodies (number and intensity on atypical cells); B: MMA+ cells more numerous and intensely stained than C3D1+ and BY87+ cells; C: only MMA+ cells found; D: MMA+ cells less numerous and intensely stained than C3D1+ and BY87+ cells.

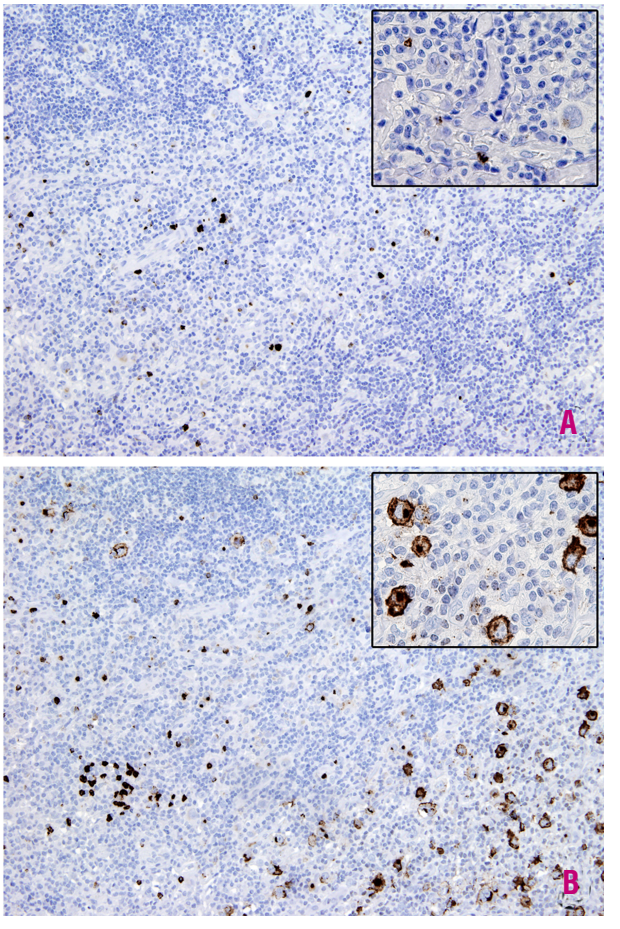

Figure 1. Serial sections from a case of Classical Hodgkin Lymphoma, stained for CD15 using monoclonal antibodies C3D1 (A) and MMA (B); the numerous RSC (*details in the insets*) are detected by MMA, while are largely negative for C3D1. Note that polymorphs are strongly stained by both antibodies.

anaplastic large cell lymphoma and two Peripheral T-cell Lymphomas) all other lymphoid neoplasms resulted negative for CD15.

Immunohistochemical results depend on variable elements, such as the kind of fixative, duration of fixation, and antigen retrieval system, but the choice of the primary reagent frequently represents a key factor. In consideration of the superior reactivity of the anti-CD15 MMA clone, it seems advisable to include this reagent in diagnostic immunohistochemical panels in hematopathology, as well as in studies aimed to evaluate the clinical behavior or the molecular features of hematolymphoid neoplasms based on their classification according to definite criteria.[8] Furthermore, this study confirms the usefulness of anti-CD15 in the differential diagnosis between CHL and mimickers, since it was found to be expressed in 84.6% of CHL cases, with high sensitivity (84,6%) and specificity (92,7%). As previously noticed, CD15 can be found in rare cases of peripheral T-cell lymphomas, thus justifying the use of broad panels of antibodies in lymphoma diagnosis.[9]

Wilma Pellegrini, Giuliana Bresciani, Anna De Zorzi, Daniela Marocolo, Marco Ungari, Fabio Facchetti

Department of Pathology, Spedali Civili, University of Brescia, Brescia, Italy

Key words: CD15, classical Hodgkin lymphoma, immunohistochemistry.

Correspondence: Fabio Facchetti, Department of Pathology, Spedali Civili Brescia, 25124 Brescia, Italy. E-mail: facchett@med.unibs.it

References

1. Ball ED. CD15 cluster workshop report. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, et al., eds. Leukocyte typing V. White cell differentiation antigens. Proceedings of the 5th International Workshop and Conference; Oxford University Press; 1995: 790-4.
2. Dorfman RF, Gatter KC, Pulford KAF, Mason DY. An evaluation of the utility of anti-granulocyte and anti-leucocyte monoclonal antibodies in the diagnosis of Hodgkin's disease. Am J Pathol 1986;123:508-19.
3. Vassallo J, Lamant L, Brugieres L, Gaillard F, Campo E, Brousset P, et al. ALK-positive anaplastic large cell lymphoma mimicking nodular sclerosis Hodgkin's lymphoma: report of 10 cases. Am J Surg Pathol 2006;30:223-9.
4. Von Wasielewsky R, Mengel M, Fischer R, Hansmann ML, Hubner K, Franklin J, et al. Classical Hodgkin's disease. Clinical impact of the immunophenotype. Am J Pathol 1997;151:1123-30.
5. Hall PA, D'Ardenne AJ. Value of CD15 immunostaining in diagnosing Hodgkin's disease: a review of published literature. J Clin Pathol 1987;40:1298-304.
6. Hanjan SNS, Keaney JF, Cooper MD. A monoclonal antibody (MMA) that identifies a differentiation antigen on human myelomonocytic cells. Clin Immunol Immunopath 1982;23:172-88.
7. Stein H, Hansmann ML, Lennert K, Brandtzaeg P, Gatter KC, Mason DY. Reed-Sternberg and Hodgkin cells in lymphocyte-predominant Hodgkin's disease of nodular subtype contain J chain. Am J Clin Pathol 1986;86:292-7.
8. Vanhentenrijk V, Vanden Bempt I, Dierickx D, Verhoef G, Wlodarska I, De Wolf-Peeters C. Relationship between classic Hodgkin lymphoma and overlapping large cell lymphoma investigated by comparative expressed sequence hybridization expression profiling. J Pathol 2006;210:155-62.
9. Barry TS, Jaffe ES, Sorbara L, Raffeld M, Pittaluga S. Peripheral T-cell lymphomas expressing CD30 and CD15. Am J Surg Pathol 2003;27:1513-22.