Diagnostic Usefulness of CD23 and FMC-7 Antigen Expression Patterns in B-Cell Lymphoma Classification

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Abstract

CD23 and FMC-7 are normal B-cell antigens used during diagnostic immunophenotyping of suspected lymphoproliferative disorders, but the diagnostic usefulness of antigenic expression patterns of simultaneous 2-color staining and flow cytometric analysis has not been reported. We evaluated the FMC-7 and CD23 expression pattern in 201 cases of B-cell lymphoma from tissue biopsy specimens by multiparameter flow cytometry. The CD23-/FMC-7+ pattern was the most common pattern in large cell, mantle cell, and marginal zone lymphomas. The CD23 and FMC-7 antigen, along with the CD5 coexpression pattern, permitted accurate classification of all 71 cases of small lymphocytic, mantle cell, and marginal zone types of lymphoma. The widest variation of patterns was with follicular cell lymphoma, although most cases expressed the CD23±/FMC-7+ pattern (±, partial or minor subset expression). The CD23 and FMC-7 antigen expression pattern was predictive of subtypes in more than 95% of lymphoma cases and could narrow the differential diagnosis in the remaining cases. We conclude the flow cytometric CD23/FMC-7 expression pattern achieved by dual staining facilitates accurate and reproducible classification of B-cell lymphomas and has diagnostic usefulness.

The recent World Health Organization classification for lymphomas has made immunophenotyping by flow cytometry or immunohistochemistry an integral part of the diagnostic evaluations of lymphomas and lymphoproliferative disorders leading to the selection of the most appropriate therapy.¹,² Flow cytometry has the unique capability of being a relatively rapid, highly sensitive, and reproducible quantitative assay, substantially contributing to potential medical care cost savings by reducing the time to initiating appropriate therapy and increasing diagnostic accuracy and reproducibility.³ While the immunophenotypic characteristics of the different lymphomas have been defined, some immunophenotypic markers are not used routinely, and their patterns of expression in the different types of lymphomas are not well characterized. In particular, the patterns of antigenic coexpression having diagnostic and subclassification usefulness have received little attention with the exception of CD5 coexpression on B-cell lymphoproliferative disorders as being indicative of either chronic lymphocytic leukemia (CLL) or mantle cell lymphoma (MCL). We have found that the patterns of expression of CD23 and the FMC-7 antigen obtained by simultaneous immunophenotyping staining on low- and intermediate-grade lymphoproliferative disorders have diagnostic usefulness that could further improve the accuracy and reproducibility in lymphoma classification.

CD23 is a low-affinity IgE receptor belonging to the superfamily of type II integral membrane proteins displaying the lectin-binding motif.⁴ It is expressed on the surface of IgM- and IgD-expressing B cells. CD23 also is found on monocytes, minor subsets of T cells, platelets, eosinophils, Langerhans cells, and follicular dendritic cells.⁵ The antibody FMC-7 detects an antigen on certain subgroups of neoplastic and normal B cells that have arisen from cells in later stages of
B-cell maturation. Although antibodies to these antigens have been used in the immunophenotypic study of B-cell disorders, the flow cytometric patterns of CD23 and FMC-7 expression have not been specifically and systematically studied and reported in the literature. We studied the usefulness of flow cytometric analysis of simultaneous CD23 and FMC-7 expression patterns for distinguishing different types of B-cell lymphomas in lymph node and tissue biopsy specimens.

Materials and Methods

Patient Samples

A total of 201 cases of clonal B-cell disorders in tissue specimens other than blood and bone marrow from the files of the Analytical Cytometry Laboratory, William Beaumont Hospital, Royal Oak, MI, from January 1996 to June 1999 were reviewed. These included 123 lymph nodes, 19 gastrointestinal biopsy specimens, and 59 samples from various tissue sites, including spleen, salivary glands, mediastinum, bone, eye, central nervous system, and breast. Plasmacytoma (multiple myeloma) cases were excluded from the study. The study was performed in accord with the ethical standards established by the institutional review board and with the Helsinki Declaration of 1975.

Flow Cytometric Analysis

Tissue samples transported in tissue culture media were minced and teased in 0.1% bovine serum albumin/phosphate-buffered saline (BSA/PBS) solution to release the mononuclear cells. The preparation was washed twice with 0.1% BSA/PBS in an automatic cell washer (DACII, Baxter Scientific, Chicago, IL) and filtered through a nylon mesh. The cells were resuspended in 0.1% BSA/PBS, and the cell concentration was adjusted to a final desired concentration of 5 to 7 × 10⁶ cells per milliliter as determined by an automated cell counter (Sysmex K-1000, Baxter Diagnostics, McGaw Park, IL). The cells were stained with 7-AAD (Molecular Probes, Eugene, OR) to determine cellular viability for 3-color immunophenotyping, along with 2 monoclonal antibody reagents to specific lymphocyte-associated antigens. Fluorochrome-conjugated monoclonal antibodies used as a panel for lymphoma were run in pairs on 10 aliquots of the sample. The antibodies used included CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD19, CD20, CD22, CD23, CD38, FMC-7, CD45, and kappa and lambda light chains. The combination CD23-PE (phycoerythrin) (Becton Dickinson Immunocytometry System, San Jose, CA) and FMC-7–FITC (fluorescein isothiocyanate) (Immunotech, a Beckman Coulter Company, Marseilles, France) was 1 panel combination for flow cytometric analysis. Specimens were analyzed on a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA) equipped with Cellquest software or on an Ortho Cytoron Absolute (Ortho Diagnostic Systems, Raritan, NJ) with Ortho Research software, acquiring a minimum of 10,000 cellular events. List-mode files were collected for all specimens and analyzed using WinList software (Verity Software House, Topsham, ME). Expression of the antigen was interpreted as follows: positive, +; negative, −; and partial or minor subset expression, ±, which was arbitrarily defined as 5% to 20% of the cells expressing either CD23 or FMC-7 that also expressed the antigen being scored.

Histologic Evaluation and Classification

The corresponding archived histologic sections were obtained from the files in the Department of Anatomic Pathology at William Beaumont Hospital (Royal Oak and Troy, MI). Histologic review of H&E-stained sections and immunohistochemical stains when available was performed by 2 authors (D.P.G. and M.T.R.). Classification was based on the Revised European-American classification of lymphoid neoplasms (REAL) and used all available clinical and other laboratory data to achieve the most accurate diagnosis. We included cases diagnosed as splenic marginal zone lymphoma (MZL; n = 4) and splenic lymphoma with villous lymphocytes (n = 1) in the MZL group. Although mucosa-associated lymphoid tissue (MALT) lymphomas are considered MZLs in the REAL classification, we elected to separate the MALT lymphomas from the nodal and splenic MZLs to determine whether the anatomic site affected the findings of antigenic expression. The morphologic distribution of the study data set was as follows: small lymphocytic lymphoma (SLL)/CLL, 27; follicular center lymphoma (FCL) grade I, 30; FCL grade II, 15; FCL grade III, 17; mantle cell lymphoma (MCL), 13; MZL, 14; MALT lymphoma, 17; diffuse large B-cell lymphoma (DLCL), 64; lymphoplasmacytoid lymphoma, 2; acute lymphocytic leukemia, 1; and Burkitt lymphoma, 1.

Results

The results of the study are summarized in Table 1. Histograms of the major CD23 and FMC-7 coexpression patterns are shown in Figure 1A, Figure 2A, and Figure 3A.

Small Lymphocytic Lymphoma/Chronic Lymphocytic Leukemia

All 27 diagnosed cases of SLL/CLL expressed both CD5 and CD23 (Figures 1A and 1B). Of these 27 cases, 16 cases (59%) were CD23+, but they did not coexpress FMC-7. In the remaining 11 cases (41%), only a minor subset of B lymphocytes coexpressed FMC-7, and these cases showed a
greater degree of “prolymphocytoid” morphologic features and/or prominent growth zones on histologic sections (data not quantified).

**Follicle Center Lymphoma**

The largest variation of expression patterns was seen in this group (Figures 2A-2D). Of 62 cases, 61 (98%) were either FMC-7+ or FMC-7±. Only 1 case, a grade III FCL, did not express either FMC-7 or CD23. Nine cases (14%) coexpressed CD23 and FMC-7 (Figure 2D). The variation in immunophenotypic patterns was due mainly to variable expression of CD23, ranging from negative to moderate intensity. Neither histologic grade nor pattern of involvement seemed to be associated with the expression of CD23, as the variations were randomly distributed among the 3 histologic grades and in both diffuse and follicular patterns of involvement. However, the presence of large cells in FCL showed a relationship to the expression of FMC-7 and the lack of CD23 expression, although not in a manner permitting reliable distinction of grades I through III (results not

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<th>SLL/CLL (n = 27)</th>
<th>FCL (n = 62)</th>
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<th>MZL (n = 14)</th>
<th>MALT (n = 17)</th>
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ALL, acute lymphoblastic leukemia; BL, Burkitt lymphoma; DLCL, diffuse large B-cell lymphoma; FCL, follicle center lymphoma; LPL, lymphoplasmacytoid lymphoma; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; +, positive; –, negative; ±, dim or partial expression.

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**Figure 1** Most common expression patterns in small lymphocytic lymphoma/chronic lymphocytic leukemia. CD23+/FMC-7– pattern (A) and CD23+/FMC-7± pattern, which correlated with prolymphocytoid morphologic features in a subset of lymphocytes (B). All histograms are on a gated lymphocyte population based on CD45 and side-scatter properties. +, positive; –, negative; ±, dim or partial expression.
shown). CD10 expression was observed in approximately 75% of these FCL cases and did not correlate with the expression of either CD23 or FMC-7. Interestingly, the CD23 and FMC-7 pattern of FCL is similar to that seen in lymph nodes showing follicular hyperplasia, in which coexpression typically is seen, although there are anatomic differences as with oropharyngeal regions in particular showing a higher level of CD23 expression (results not shown).
Mantle Cell Lymphoma

All cases of MCL were CD5+ and CD23–. FMC-7 was expressed substantially by 12 of 13 (92%) cases (Figure 3A). The only case that was FMC-7± was a blastoid variant of MCL.

Marginal Zone Lymphoma

Of 13 cases, 10 (77%) were CD23–/FMC-7+. Two cases were CD23±/FMC-7+, and the remaining case was CD23–/FMC-7+. In this group, we included splenic MZL (n = 4), all of which were among the 10 cases that were CD23–/FMC-7+. The only case of splenic lymphoma with villous lymphocytes was CD23±/FMC-7+. None of these cases expressed CD10 or CD5.

MALT Lymphoma

All 17 cases of MALT lymphoma were CD23–. Of these, 14 (82%) were positive for FMC-7 and 3 (18%) were FMC-7± (Figure 3A). The expression level of FMC-7 was similar to the cases of MZL that were not mucosal associated.

Diffuse Large B-Cell Lymphoma

The second widest variation of patterns was seen in this group. Unlike that found in the SLL/CLL group, the pattern spread was due to variability in the expression of both CD23 and FMC-7. However, the predominant pattern was CD23–/FMC-7+, which was observed in 46 (72%) of 64 cases (Figure 3A). No correlation between the CD23 and FMC-7 expression pattern and the morphologic features of the malignant large cells was observed.

Acute Lymphoblastic Leukemia, Lymphoplasmacytoid Lymphoma, and Burkitt Lymphoma

The only case of acute lymphocytic leukemia in the study was CD23–/FMC-7– (Figure 3B). This was from a femoral lesion in a patient suspected of having lymphoma. Two cases of lymphoplasmacytoid lymphoma and 1 case of Burkitt lymphoma included in our study were CD23–/FMC-7+ (Figure 3A). The number of samples of these types of lymphoma included in our study, however, is not sufficient for statistical analysis, but we report them because the antigen patterns observed were similar to other such cases sampled from blood or bone marrow.

Discussion

The morphologic distribution of our cases is similar to previously reported observations, with DLCLs and FCLs...
being the most common, together accounting for 63% of cases in the present study. Since the present study was limited to lymphomas and lymphoproliferative disorders manifesting in tissue sites, other tissue sampling sites and types of lymphoid neoplasms are not represented (eg, prolymphocytic leukemia, hairy cell leukemia, Waldenström macroglobulinemia, and pre-B-cell lymphoblastic leukemia/lymphoma). CD23 and FMC-7 expression patterns in these entities, although similarly helpful in diagnosis and classification as seen in the present study, have been studied and reported separately.

A review of the English literature for the past 15 years did not reveal studies conducted specifically on the simultaneous staining expression patterns of CD23 and FMC-7 in B-cell lymphomas and related disorders, although these monoclonal antibodies have been used in flow cytometric immunophenotyping panels for many years. CD23 has been used frequently to aid in discriminating between SLL and MCL, diseases that may be difficult to differentiate by morphologic examination using routine staining techniques alone. The monoclonal antibody FMC-7, developed at the Flinders Medical Center (Australia) by somatic hybridization against the human B-cell line HRIK, seems to define a subset of normal human B lymphocytes and aids in distinguishing more mature B-cell leukemias from immature variants. Some studies, however, indicate that the use of FMC-7 antibody in immunophenotypic studies of lymphoid neoplasms does not contribute additional information or diagnostic reliability. These studies paired the staining of FMC-7 expression with a B-cell antigen (CD20 or CD19), which may introduce the potential of energy transfer and/or fluorescence quenching of the FMC-7-related fluorescence intensity. In addition, our findings suggest the diagnostic usefulness of FMC-7 is inherent in the coexpression pattern with CD23, similar to the diagnostic information afforded by the pairing of CD5 and a B-cell lineage marker to confirm the histologic impression of SLL/CLL or MCL.

The usefulness of the CD23/FMC-7 combination is best demonstrated by its ability to distinguish between SLL/CLL, MZL, and MCL. SLL/CLL usually is CD23+/FMC-7–, while MZL and MCL are consistently CD23–/FMC-7+. In the study by Ahmad et al7 of blood and bone marrow samples, this pattern of expression was useful for detecting prolymphocytic leukemia and prolymphocytic transformation in CLL (CLL/PL) as well; most of these cases were CD23–/FMC-7+. This finding is similar to previously published observations on prolymphocytic leukemia and prolymphocytic transformation in CLL. The pattern of CD23+/-FMC-7+ also may be expressed by a subgroup of CLLs with trisomy 12, which are at greater risk for a more aggressive course. In a study of 540 cases of B-cell CLL, high FMC-7 and low CD23 expression was associated with a short survival.

While MCL can resemble SLL/CLL or diffuse FCL morphologically, a more aggressive clinical course and unfavorable prognosis for MCL is well documented. It is, therefore, important to distinguish these entities from classic SLL/CLL for purposes of prognostication and medical decision making. Our findings indicate that the CD23/FMC-7 coexpression phenotype by flow cytometry is an objective diagnostic method of achieving this goal and is more useful than other antigen expression, such as CD10 and CD38, that cannot consistently assist in this differential diagnosis. The MALT lymphomas are categorized as MZL in the REAL classification, yet we elected in the present study to separate the 2 categories to permit the separate study of the CD23/FMC-7 antigen expression pattern. As shown in Table 1, we observed a similar predominant CD23–/FMC-7+ pattern in the 2 groups, thus supporting the similarity of these forms of lymphoma despite the differences in the anatomic manifestations.

Despite the advent of major advances in ancillary immunologic and molecular techniques, some problems remain in diagnosing diffuse low-grade B-cell lymphomas with certainty. Diffuse MZL or MALT lymphomas may morphologically resemble SLL/CLLs, diffuse MCLs, or some FCLs with areas of diffuse histology. The CD23/FMC-7 patterns in these entities may be similar, although FCL is more likely to have the CD23+/FMC-7+ pattern. The additional finding of CD10 and CD38 expression and a nodular or follicular histologic pattern makes the diagnosis of FCL even more likely. We found no correlation in FCL between either CD10 or CD38 expression and the CD23/FMC-7 expression pattern; thus, information provided by CD23 and FMC-7 staining is adjunctive and additionally helpful. In cases of MZL vs MCL, both of which are CD23+/FMC-7+, the additional finding of CD5 positivity would be indicative of MCL.

The variation of patterns observed in both FCLs and DLCLs most likely reflects the heterogeneity of these entities. FCLs may be composed of centrocytes and/or centroblasts in varying proportions, and the size of the centrocytes may vary among cases. Immunophenotypically, FCLs express a variety of patterns, with more than 50% of cases expressing CD10 and fewer than 50% of cases expressing CD23. However, as found in our experience, FCLs are commonly FMC-7+. Similarly, DLCLs showed a wide variation of CD23/FMC-7 staining patterns, with most cases expressing the CD23–/FMC-7+ phenotype. Of note is the fact that this group of lymphomas includes a wide spectrum of large lymphocytic neoplasms that were assigned various diagnostic names in past classifications. Proponents of the REAL classification acknowledge the difficulty subclassifying this group of lymphomas on routine sections. The treatment is similar for all types of DLCL, and, therefore,
subclassification is not clinically important. In our study, we found that of the 14 cases that were purely of the CD23+/FMC-7+ pattern, 9 (64%) were FCL and 5 (36%) were DLCL. Interestingly, of the 9 FCL cases that coexpressed CD23 and FMC-7, 6 were FCL grade I, and 3 were FCL grade II; none of them were grade III FCL. In the present study, looking solely at CD23 expression in the FCL group showed that only 2 (7%) of 30 cases of grade I FCL were CD23−. Among the 15 cases of grade II FCL, 4 (27%) did not express CD23. Of 17 cases of grade III FCL, 9 (53%) cases were CD23−. Hence this pattern of CD23/FMC-7 expression may be of greater diagnostic and prognostic usefulness than that of CD10 and CD38 in FCL. Previously published reports show that increased CD23 expression is associated with a favorable prognosis, and low expression is a feature of more advanced disease in CLL. Whether this can be extrapolated to the subsets of FCLs and DLCLs that express CD23 is not known but is an issue worthy of future studies.

We found that simultaneous staining of B-cell neoplasms with CD23 and FMC-7 is a useful tool to assist in the reproducible subclassification of lymphoma, particularly when it is clinically relevant for prognostic and therapeutic purposes. The CD23/FMC-7− pattern of expression, when used in combination with other monoclonal antibodies, has a similar discriminatory usefulness as the paired CD5/CD20 expression pattern. When CD23, FMC-7, and CD5 expression are studied in B-cell populations with monoclonal light chain expression, a diagnostic algorithm can be used as shown in Figure 4, which, in our experience, will result in the accurate classification of more than 95% of tissue biopsy specimens with lymphoma. We conclude that the CD23/FMC-7 coexpression pattern, as defined by flow cytometric immunophenotyping in conjunction with other antibodies, is a valuable contribution toward accurate and reproducible classification of B-cell lymphomas. The question of prognostic significance of the CD23 and FMC-7 expression pattern in lymphoma requires more extensive study, but it may be worthwhile, especially in FCL, in which there is a heterogeneity of CD23 and FMC-7 antigen coexpression patterns.

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Acknowledgments: We are grateful to Katharine A. Steel, Cindy Sounart-Miscovitch, and Carole A. Ceckowski for their expert technical assistance with flow cytometric analysis. Kenneth Ault, MD, provided valuable editorial comments on the manuscript.

References


![Figure 4](https://example.com/figure4.png)

**Figure 4** Antigenic algorithm for classification of lymphoproliferative disorders. Given the finding of a monoclonal B-cell population, the above approach to antigenic separation of various subtypes of lymphoma along with morphologic and other immunophenotypic data can allow for the ready and reproducible categorization of at least 95% of cases. ALL, acute lymphoblastic leukemia; DLCL, diffuse large B-cell lymphoma; FCL, follicle center lymphoma; LPL, lymphoplasmacytoid lymphoma; MALT, mucosa-associated lymphoid tissue lymphoma; PLL, prolymphocytic leukemia; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; +, positive; −, negative; ±, dim or partial expression.