The Role of Flow Cytometry in the Diagnosis of Lymphoma: A Critical Analysis

EDWARD E. MORSE, M.D.,
HAROLD T. YAMASE, M.D.,
BERNARD R. GREENBERG, M.D.,
JONATHAN SPORN, M.D.,
SHARON A. HARSHAW, MT(ASCP),
THOMAS R. KIRALY, M.A.,
ROSEMARY A. ZIEMBA, B.S.,†
and MARGARET A. FALLON, M.D.†

The University of Connecticut Health Center,
Farmington, CT 06030
and
*University of Rochester, Strong Memorial Hospital,
Rochester, NY 14607

ABSTRACT

Flow cytometry, now used routinely to aid in the classification of leukemias, is increasingly being evaluated as a rapid technique for determination of surface antigens on the cells teased from lymph nodes and other masses with suspected lymphoma. The present study reviews biopsy specimens from patients examined during a two year period which were sent for flow cytometry with a diagnosis of suspected lymphoma. Sixteen of 25 samples (64 percent) produced cell suspensions of sufficient quantity and quality to be diagnostically helpful. Results showed that in 9/16 (56 percent) the diagnosis of lymphoma or cancer could be suspected by flow cytometry alone, while 4/16 were consistent with the final tissue diagnosis of normal or reactive hyperplasia. Three samples that came from patients who had morphologic evidence of malignant disease on biopsy (two Hodgkin's disease and one large cell lymphoma) had flow cytometry results that were interpreted as normal.

Flow cytometry is rapid and appears to be virtually diagnostic of non-Hodgkin's lymphoma when a majority of cells are B cells with an abnormal kappa/lambda ratio (>4.0 or <0.25). Nonhematologic malignancy can be suspected if less than 75 percent of the cells show CD45 (common leukocyte antigen). Hodgkin's disease cannot be detected by flow cytometry as it is currently used, and as many as 15 percent (1/6 in this study) of lymphomas may show normal results. It is extremely helpful when the biopsy sample actually contains the cells of interest in large proportion. Loss of architectural relationships in the course of processing specimens for flow cytometry is a major disadvantage when small foci of lymphoma or tumor cells exist together with large amounts of stroma or normal lymphocytes.
FLOW CYTOMETRY IN THE DIAGNOSIS OF LYMPHOMA

Introduction

Flow cytometry has been used successfully for the diagnostic classification of leukemias using such specific markers as CD10 (CALLA) for acute lymphocytic leukemia (ALL), CD5 and CD20 (TjBj) for chronic lymphocytic leukemia (CLL), CD2 (Tn) for T cell ALL and CD19 (B4) for B cell ALL, CD11b/CD14 (Mo^/Mo^) for monocytic leukemia and CD33/CD34 (My9/Myl0) for myeloid leukemia.1,6

Interest in defining the cells of lymphoid neoplastic diseases by flow cytometry has extended into study of lymphomas, and a number of investigators have expressed great enthusiasm for this technique.2,3,7 As experience has been acquired, it is clear that there are limitations which must be considered when cells are teased from lymph nodes and other masses to make a mononuclear cell suspension analogous to blood or bone marrow.4,8

Certain specific findings can be very helpful in classifying large undifferentiated cells, particularly in the B cell lymphoid lines, but caution must be used in the interpretation of numerical data such as T/B cell ratios, kappa/lambda light chain ratios, or common leukocyte antigen proportions, when one is dealing with tissues which may have normal lymphoid cells and stromal cells as part of the material being processed.5,8

In the present paper, the results are analyzed from patients studied by flow cytometry, each of whom had lymph nodes or other tissues sent for examination because they had large undifferentiated cells on initial frozen sections or a clinical picture that suggested lymphoma.

Methods

All patients for flow cytometry study of tissues with a preliminary diagnosis of lymphoma were reviewed. Sixteen of 25 patients were studied in detail. Each patient's chart, final pathologic diagnosis on permanent sections, flow cytometry results, electron microscopy, and special studies were reviewed and tabulated as to consistency with the final diagnosis.

Flow cytometry (FC) was performed on suspensions of cells teased from the fresh tissue of nodes or masses suspected of containing lymphoma. The minimum size of biopsies was about 0.5 cm x 0.5 cm x 0.5 cm. After suspension in Rosewell Park Memorial Institute (RPMI) culture medium, the red cells were lysed with ammonium chloride. The remaining cells were washed twice in phosphate buffered saline (PBS) and tagged with phycoerythrin or fluorescein-labelled antibodies CD2, 5, 10, 19, 20, kappa and lambda chains CD11b, 14, 33, 34*† (0.1 ml with 10^6 cells in PBS for 30 minutes. The tagged cells were washed twice in PBS and finally fixed with 2 percent paraformaldehyde to be analyzed on a Coulter Epics C flow cytometer.

Frozen section diagnosis was attempted in almost all cases. Frozen stored tissues were utilized for streptavidin immunoperoxidase staining.*

Tissue sections from similar tissues were also fixed in paraffin to slides, dehydrated, and stained with streptavidin immunoperoxidase-linked antibodies for tumor antigens AE-1, S100, CALLA, T and B antigens and light chains†.9 Electron microscopy (EM) using glutaraldehyde-fixed tissues and a Philips (Holland) 300 electron microscope was utilized selectively when uncertainty or discrepancy existed between the other methods. Tissue was sectioned into one mm-sized cubes and fixed in cacodylate buffered four percent glutaraldehyde,

* Coulter Immunology, 400 W. 20th Street, Hialeah, FL 33010.
† Becton Dickinson Immunocytometry, 2375 Garcia Avenue, Mountain View, CA 94039.
‡ Dako Corp., 6392 Via Real, Carpinteria, CA 93013.
 postfix in cacodylate buffered two percent osmium tetroxide, dehydrated and embedded in poly bed 812. Sections one μm thick were cut and stained with toluidine blue for light microscope evaluation, and areas were selected from thin sections. Final diagnosis was determined on thin sections fixed in formalin and stained with hematoxylin and eosin.

Results

Sixteen of 25 samples of lymph nodes or other tissues referred to the flow cytometry laboratory at the University of Connecticut Health Center with a preliminary diagnosis of lymphoma were of suitable quantity and quality for further analysis. The 16 samples included 11 lymph nodes, a nodule from the left pharynx, a scalp mass biopsy, a testicular biopsy, and two biopsies from mediastinal masses.

A final diagnosis of nonHodgkin’s lymphoma or lymphoblastic lymphoma was made in eight cases. A diagnosis of Hodgkin’s disease was made in two cases. A diagnosis of carcinoma of the lung was made in two cases (both biopsies of the mediastinum) and a diagnosis of benign or reactive hyperplasia was made in four cases (table 1). Of the four cases of lymphoma which failed to show a majority of B cells, one had a kappa/lambda ratio of 4.5 and two diagnosed as lymphoblastic lymphomas showed 36 percent and 75 percent CD10 (CALLA) positive cells. The one remaining patient with a final diagnosis of large cell lymphoma failed to show any abnormality on flow cytometric analysis.

Two patients with Hodgkin’s disease, whose studies showed patterns of B cell/T cell markers and kappa/lambda ratios similar to patients with lymph node hyperplasia, demonstrated that the proportion of B cells was less than 50 percent and the kappa/lambda ratio was less than 3.0.

Two patients, with a final diagnosis of carcinoma of the lung, whose mediastinal lymph nodes were studied, demonstrated less than 60 percent cells expressing CD45 (common leukocyte antigen) while all the patients with lymph node hyperplasia, lymphoma or Hodgkin’s disease demonstrated greater than 90 percent CD45 cells with one exception (79 percent).

Discussion

Several important lessons were learned during this study. Though it was initially thought that flow cytometry was potentially a major tool for the diagnosis of lymphoma, it became increasingly clear that there were disadvantages to this method if (a) the biopsy tissue were not uniformly involved, but represented a mixture including stromal elements and/or normal lymphocytes, (b) the antibodies used did not include antibodies to tumor antigens as well as leukocyte antigens, (c) deterioration occurred even after storage in tissue culture medium, and (d) technical difficulties were encountered obtaining sufficient cells from certain types of tissues.

Nonetheless, flow cytometry proved to be rapid (less than four hours) and was diagnostic in cases of lymphoma from which the tissue provided was heavily involved with B cells and had monoclonal kappa light chain excess.

In the present series, four patients demonstrated CD10 (CALLA) positivity in 36 to 81 percent of nodal cells, two in the absence of B cell excess or kappa/lambda ratio excess. One patient whose neck node morphologically demonstrated nonHodgkin’s lymphoma demonstrated “an admixture of T and B cells” and “no evidence of monotypic expression,” is considered a false negative.

It seems clear from this and other studies2,3 that a small proportion of morphologic lymphomas will not demonstrate B
cell markers, even when adequate material is available for flow cytometry. They usually represent lymphomas with more primitive cells or T cell differentiation. Some patients with equivocal findings may require examination of fixed tissue samples using peroxidase-labelled antibodies to confirm a suspected diagnosis.

Michie et al.\textsuperscript{4} studied a series of tissue biopsies containing large undifferentiated cells. They used three antibodies labelled with peroxidase to identify leukocyte common antigen (LCA), epithelial antigen (AE1), or S100 for melanoma cells. Of 120 cases, 17 showed reaction with none of the three. There were no tumors reacting with more than one antibody (sensitivity 86 percent, specificity 100 percent). There were 61 positive for LCA considered to be lymphoma (51 percent), and 17 positive for AE1 considered to be epithelial keratin positive neoplasms (14 percent).

The patients studied by the current authors showed a similar distribution of 50 percent (8/16) lymphoma, and 12 percent (2/16) epithelial tumors on final diagnosis. Flow cytometry results were “consistent” with the final diagnosis in 15/16 cases (94 percent). Flow cyto-

<table>
<thead>
<tr>
<th>Diagnosis &amp; Patient</th>
<th>% B Cells</th>
<th>% T Cells</th>
<th>Kappa/ Lambda</th>
<th>% CD45</th>
<th>% CALLA</th>
<th>% TdT</th>
<th>% CD34</th>
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</thead>
<tbody>
<tr>
<td><strong>Non Hodgkin’s lymphoma</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1. BET</td>
<td>66</td>
<td>34</td>
<td>69/4</td>
<td>99</td>
<td>57</td>
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<td>ND</td>
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<td>2. CAL</td>
<td>29</td>
<td>56</td>
<td>18/4</td>
<td>79</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>3. DEL</td>
<td>66</td>
<td>5</td>
<td>82/16</td>
<td>96</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>4. EAR</td>
<td>60</td>
<td>33</td>
<td>62/5</td>
<td>94</td>
<td>15</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>5. KAR</td>
<td>80</td>
<td>15</td>
<td>78/5</td>
<td>99</td>
<td>81</td>
<td>ND</td>
<td>ND</td>
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<td>6. DUN “Admixture of B &amp; T Cells”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No monotypic expression</td>
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<td>ND</td>
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<tr>
<td><strong>Lymphoblastic lymphoma</strong></td>
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<tr>
<td>7. HAR</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1/&lt;1</td>
<td>ND</td>
<td>75</td>
<td>56</td>
<td>66</td>
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<td>8. LAC</td>
<td>41</td>
<td>40</td>
<td>ND</td>
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<td>36</td>
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<td><strong>Hodgkin’s disease</strong></td>
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<td>9. BUC</td>
<td>11</td>
<td>88</td>
<td>8/4</td>
<td>95</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>10. ROD</td>
<td>14</td>
<td>85</td>
<td>10/5</td>
<td>91</td>
<td>&lt;1</td>
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<td>ND</td>
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<tr>
<td><strong>Normal or Hyperplastic/Reactive</strong></td>
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<td>11. QUI</td>
<td>28</td>
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<td>&lt;1</td>
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<td>ND</td>
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<td>12. ROM</td>
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<td>49</td>
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<td>&lt;1</td>
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<td>ND</td>
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<td>33</td>
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<td>28/23</td>
<td>92</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>72</td>
<td>20/7</td>
<td>99</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
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<td>15. MUL</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1/&lt;1</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>16. VER</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>56</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not done.
metry, as currently performed, does not appear to be useful in the diagnosis of Hodgkin's disease.

The present authors felt confident in the diagnosis of lymphoma only when the majority (>50 percent) of the cells in the processed tissue were B cells and kappa/lambda light chain ratios were sufficiently outside the normal range (3.0–0.25) to indicate a monoclonal population. Even the latter criteria must be viewed with caution, since in several series rare patients with reactive lymph node hyperplasia have shown abnormal ratios of light chain containing cells.2,3,7 Little et al2 reviewed 200 cases in which flow cytometry had been utilized during diagnostic evaluations. Over 85 percent showed lymphoma of B or T cell lineage while the remaining 15 percent were reactive nodes or Hodgkin's disease having no specific characteristics detectable by flow cytometry. Little and coworkers concluded cautiously "when combined with routine morphologic review and accompanied by other specialized diagnostic techniques when necessary, the use of flow cytometry represents a precise and reproducible method for rapidly and easily studying lymphoproliferative disorders in solid tissue." Those authors also had 13 cases of Hodgkin's disease of the nodular sclerosing type. Flow cytometry characteristics were nonspecific. One case with typical reactive follicular hyperplasia on morphologic examination showed 46 percent B cells and kappa/lambda ratio of greater than 5.0 (considered by the authors to be a false positive).

Picker et al7 found one patient with excess monoclonal light chains (>9 to 1 kappa) out of 25 patients with lymph node hyperplasia using the immunoperoxidase staining technique, but this patient showed no gene rearrangement on further study and was considered a false positive. The authors also considered the possibility of a staining artifact.

Demartini et al2 reviewed 271 cases of B cell lymphomas to determine the effectiveness of flow cytometry in characterizing these malignancies. Flow cytometry confirmed a majority of B cells in 92 percent and demonstrated monoclonal distribution of light chains in 79 percent (using a normal range of kappa/lambda 5.5 to 0.7 from Samoszuk8). They found 68 percent monoclonal by routine electronic gating around the lymphoid population, but another 12 percent could be detected by gating around a population of large lymphocytes, Polyclonal light chain cases (kappa/lambda ratio between 0.7 and 5.5) represented 15 percent of the cases and these tended to be high grade (23 percent), rather than low grade (10 percent) lymphomas. Relatively high percentages of T cells and both CD4 and CD8 subsets were observed in the polyclonal cases. Demartini et al concluded that "flow cytometry provides reliable information on neoplastic and nonneoplastic cells in lymph nodes involved by B-cell lymphomas."

Using lower ratios of kappa/lambda light chains (kappa/lambda down to 4.0) may be helpful (as in the present series) in defining monoclonal B cell populations for the diagnosis of lymphoma as long as B cells are in excess (>50 percent). In equivocal cases, electron microscopy, and/or immunoperoxidase staining can be useful.1,5

In conclusion, with proper preselection of patients for sampling when there is a strong clinical suspicion of lymphoma, flow cytometry is a rapid method to detect a monoclonal B cell population. It can fail to do so if the population of abnormal cells is small and mixed with normal lymphoid cells.

References


