ORIGINAL ARTICLE

Diagnosis of lymphoproliferative disorders: experience of a single institution in the long-term follow-up of discordant cases

Irene Sadek, MD
Wenda Greer, PhD
Annette Foyle, MD

Drs. Sadek and Foyle are Assistant Professors and Dr. Greer is Associate Professor in the Department of Pathology, Dalhousie University, Halifax, NS

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Abstract

Objective: To evaluate the usefulness of morphologic diagnosis, immunophenotyping and immunoglobulin (Ig) and T-cell receptor (TcR) gene rearrangement studies in the diagnosis of lymphoproliferative disorder.

Design: A retrospective cohort study with clinical follow-up of controversial cases.

Setting: Single institution, tertiary care centre.

Patients: All 273 patients whose lymphoid tissue samples were sent for molecular analysis by Southern blotting over a 4-year period.

Interventions: Patient reports were retrieved from the laboratory data system. Repeat assessment and clinical follow-up was done for discordant cases.

Outcome measures: Correlation between morphologic features and the results of immunophenotype and gene rearrangement studies of the samples. Value of the different tests in discordant cases.

Results: The 273 samples included 130 of non-Hodgkin’s lymphoma (NHL), 23 of Hodgkin’s disease, 80 of benign lymphoid hyperplasia, 16 of atypical lymphoid hyperplasia and other diagnoses. Of the 130 NHL cases diagnosed by morphologic study, 22 (17%) did not show gene rearrangement. Of the 80 morphologically benign samples, 4 (5%) showed gene rearrangement, and malignant disease developed later in those patients. Five (31%) of the 16 cases of atypical lymphoid hyperplasia showed gene rearrangement. Six of the remaining 11 cases had no detectable gene rearrangement, but hematologic malignant disease developed. No gene rearrangement was detected in Hodgkin’s disease samples. One carcinoma showed gene rearrangement. Of the NHL group, 86% of the B cells and 50% of the T cells showed gene rearrangement. Seven samples showed both Ig and TcR gene rearrangement.

Conclusions: Gene rearrangement analyses correlate highly with conventional morphologic diagnosis and phenotyping. The detection of gene rearrangement in lymphoid tissue has a high specificity (99%) and a reasonable sensitivity (83%) to the development of a lymphoproliferative disorder.
Introduction

The diagnosis and classification of lymphoproliferative disorders have been traditionally based on morphologic evaluation of tissue biopsies by an experienced hematopathologist. This approach can give rise to considerable difficulty, particularly with respect to the distinction between benign and malignant diseases and the determination of cell lineage. Immunophenotyping and immunogenotyping techniques have emerged as important tools for an accurate diagnosis of controversial cases. These techniques may become even more important as treatment is tailored to specific subsets of lymphoma.

The development of immunophenotyping techniques has helped in the diagnostic laboratory, and provides a tool to recognize B- or T-cell lineage of proliferating cells. Also, in a significant number of cases, the detection of uniform expression of an antigenic marker such as immunoglobulin light-chain restriction supports the identification of a monoclonal process. However, immunophenotyping methods have often fallen short of detecting cell lineage and monoclonality, for a number of technical reasons. For instance, malignant B cells may be intermixed with residual polytypic (κ and λ) cells, resulting in an inability to detect light-chain restriction. Furthermore, there is no available immunohistochemical marker that can be used to detect monoclonality in T-cell proliferation.

The ability of the immune system to recognize a variety of foreign antigens is achieved by the diversity of immunoglobulins (Ig) and T-cell receptors (TcRs) expressed on B and T cells respectively. This structural diversity, which is inherent in and necessary to immune responsiveness, creates a situation in which each rearranged lymphocyte carries within its DNA a unique molecular fingerprint. It has been well documented that non-Hodgkin’s lymphomas (NHLs) represent a monoclonal growth of neoplastic lymphoid cells. Nearly all B- and T-cell malignant lymphomas have one or more rearranged antigen receptor genes that provide a diagnostic marker for monoclonality and cell lineage. The use of DNA hybridization to detect rearrangements of Ig or TcR genes obviates some of the difficulties encountered with immunophenotyping.
and remains the only conventionally applied marker for T-cell clonality. For this reason, the demonstration of clonality by Ig or TcR gene rearrangement analysis has emerged as a major diagnostic approach in hematopathology.\textsuperscript{10} However, it is important to note that a number of clonal growths, such as monoclonal gammopathy of uncertain significance and lymphomatoid papulosis, have benign clinical outcomes.\textsuperscript{11–14} Published reports have established the usefulness of gene rearrangement in diagnosing lymphoma in conjunction with morphologic assessment and immunophenotyping.\textsuperscript{1,10,15} Because of the complexity involved in diagnosing lymphoproliferative disorders, it is important to emphasize that the interpretation of any single test result has to be considered in the light of all other findings. The question remains, how to integrate all the information obtained from several different diagnostic tests to give the most accurate diagnosis.

In this retrospective study, we evaluated not only the role and contribution of morphology, immunophenotyping and gene rearrangement studies in the diagnosis of lymphoid disorders but also correlated these diagnostic findings with relevant patient clinical information and outcomes.

**Materials and methods**

**Tissue samples**

All 273 tissue samples analyzed in the molecular diagnostic laboratory over a 4-year period were reviewed. Case selection was based on availability of fresh tissue and not on diagnostic difficulty as all fresh lymphoid tissue sent to our laboratory for morphologic diagnosis was also analyzed in the molecular diagnostic laboratory for Ig and TcR gene rearrangement. The tissue samples included biopsies from 203 lymph nodes, 19 skin samples, 24 soft-tissue samples and 27 from other miscellaneous sources (e.g., spleen, liver, gastrointestinal tract, bladder, bone, breast, pleural fluid).

**Morphologic and immunophenotyping studies**

Morphologic assessment was done by an expert hematopathologist, and classification was according to the working formulation. Immunophenotypic analysis was done by immunoperoxidase staining for leukocyte common antigen, B-cell marker (L26) and T-cell marker (UCHL-1). Morphology and immunoperoxidase reports were retrieved from our laboratory computerized data system.

**DNA analysis**

High-molecular-weight DNA was extracted from fresh frozen tissue and digested with 3 restriction enzymes, Eco RI, Hind III or Bam HI. Restriction fragments were electrophoretically size-separated then transferred to nylon membranes according to the method of Southern.\textsuperscript{16} The fragments of interest were identified using phosphorus-32-labelled DNA probes specific for Ig and TcR genes. The 3 Ig gene probes used in this study included genomic fragments of the human immunoglobulin heavy chain joining region (J\textsubscript{H}), the \kappa constant region (C\textsubscript{\kappa}), and the \lambda constant region (C\textsubscript{\lambda}). Two TcR probes were used, including cDNA fragments of the \beta chain constant region (C\textsubscript{\beta}) and \delta chain constant region (C\textsubscript{\delta}). The diagnosis of clonality required that clonal rearrangements were seen with at least 2 enzymes or 2 rearranged bands observed with 1 single enzyme (2 rearranged alleles) and was reviewed by an expert molecular biologist. This rule reduces the risk of a false-positive interpretation resulting from cross-hybridization or partial digestion that can result in multiple bands.\textsuperscript{10} Gene rearrangement reports were retrieved from patient files.

**Follow-up of discordant cases**

The cases in which the diagnostic conclusions from morphologic, immunoperoxidase and DNA studies were not in agreement were reviewed in detail. Tissue sections, immunoperoxidase slides and Southern blots were reviewed by 2 observers. These discordant cases were followed up for a minimum of 12 months. Clinical information and outcome results were obtained from the patient’s health record charts.

**Results**

**Morphologic classification**

Of the 273 samples, 80 showed benign lymphoid...
proliferation, 130 were diagnosed as NHL (31 high grade, 45 intermediate grade, 45 low grade, 4 mycosis fungoides, 1 angiocentric lymphoma of the skin, 2 cutaneous T-cell lymphoma and 2 unclassifiable), 23 as Hodgkin’s lymphoma, 14 as metastatic carcinoma, 5 as metastatic sarcoma, 16 as atypical lymphoid hyperplasia and 1 as plasmacytoma; 4 specimens were insufficient for morphologic diagnosis (Table 1).

Immunophenotyping

Immunophenotyping was done on only 110 of the 130 NHL samples. Seventy-one (64%) were positive for B-cell markers, and of these only 19 showed light-chain restriction (7 κ and 12 λ). Twelve (11%) were positive for T-cell markers. In 27 (25%), immunostaining gave inconclusive results (Table 2).

Gene rearrangement analysis

Ig and TcR gene rearrangement analyses were inconclusive in 13 (4.8%) of the 273 samples (Table 1), based on apparent clonality with use of only 1 of 3 restriction enzymes. Of the remaining samples, the diagnosis was made in 80 by morphologic analysis as benign lymphoid proliferation; 4 (5%) of these showed clonality by gene rearrangement. Twenty-two (17%) samples of the NHL cases did not show a clonal Ig or TcR gene rearrangement. A clonal gene rearrangement was detected in 1 of the metastatic carcinomas and the plasmacytoma. None of the Hodgkin’s lymphomas or the samples insufficient for morphologic diagnosis showed any evidence of clonal gene rearrangement. Of the 16 samples of atypical lymphoid hyperplasia, 5 (31%) showed monoclonality by this technique.

Of the 102 monoclonal NHL samples, 91 had Ig, 4 had TcR and 7 had both Ig and TcR clonal gene rearrangements.

Correlation between immunophenotyping and gene rearrangement studies

In the NHL samples in which immunophenotyping studies were inconclusive (27 cases), 16 (59%) had a clonal Ig gene rearrangement, 1 (4%) showed TcR clonality, and 9 (33%) showed no evidence of monoclonality; in 1 the molecular result was inconclusive. Of the 71 samples identified as B cells by immunophenotyping, 55 (76%) had a clonal Ig gene rearrangement, 7 (10%) showed both Ig and TcR clonality, none showed only TcR rearrangements, 6 (8%) showed germline DNA with no evidence of monoclonality and 3 had inconclusive results. Of the 12 samples phenotyped as T cells, 3 (25%) showed clonal TcR rearrangement, 3 (25%) showed clonal Ig gene rearrangement and 6 (50%) showed germline DNA with no evidence of monoclonality (Table 2). When monoclonality was detected by light-chain restriction (19 samples), all samples showed clonal gene rearrangements except 2, which had inconclusive DNA analysis.

Discordant cases

When morphologic analysis showed benign proliferation and gene rearrangement analysis indicated clonality or, conversely, the morphologic diagnosis was NHL and no clonal gene rearrangement was detected, the samples were designated discordant and were studied in more detail.

Twenty-two samples were discordant NHL (diagnosed by morphologic analysis) but no showed
clonal gene rearrangement (Table 3); 4 were cutaneous lymphoma; 2 were diffuse mixed cell NHL, T-cell type; 2 were anaplastic large cell lymphoma, one with T-cell phenotype and the other with inconclusive immunophenotyping results. One case of large cell lymphoma was thought to be a true histiocytic lymphoma. On review, 6 samples showed focal involvement of the diagnostic tissue, pointing to a sampling error. These included 5 lymph nodes (3 diffuse large cell non-cleaved, 1 diffuse large cell immunoblastic and 1 diffuse mixed cell) and 1 sample of jejunum (diffuse, large immunoblastic cell). Two samples were positive for clonal immunoglobulin gene rearrangement by the polymerase chain reaction. The 5 remaining cases are presented in detail in Table 4. All samples that were diagnosed by morphologic study as malignant lymphoma were treated accordingly regardless of the gene rearrangement results.

Four samples were discordant benign cases (benign morphologic features but gene rearrangement). These were followed up for at least 2 years.

In all cases malignant disease developed after a variable length of time (Table 5).

Cases

The first case was of a 35-year-old woman having a diagnosis 1 year earlier of stage IV NHL, diffuse large cleaved cell type. She had received chemotherapy and her disease was in remission. A small left inguinal lymph node, measuring $1.2 \times 1.2 \times 0.9$ cm, was submitted for assessment. Morphologic study showed reactive lymphoid follicles with preservation of sinusoidal architecture. Southern blotting showed $Ig$ gene rearrangement, which raised the possibility of involvement by a low-grade NHL. A repeat biopsy 5 months later consisted of a somewhat larger lymph node that was diagnosed as NHL, follicular small cleaved cell type. Three years later, the patient’s disease progressed to NHL, diffuse large cell type, and she died 2 years later.

The second case was a 78-year-old man who had a 2-year history of splenomegaly. A bone marrow study showed lymphocytosis with B lymphocytes double positive for CD19 and CD5. Two months after diagnosis, he underwent splenectomy. Microscopic analysis of the spleen showed a mild lymphocytic infiltrate. $Ig$ gene rearrangement was detected by Southern blotting. A diagnosis of small lymphocytic lymphoma/chronic lymphocytic leukemia was made. He received no treatment.

The third patient was a 64-year-old man with multiple abdominal lymphadenopathy. Three consecutive lymph-node biopsy specimens were obtained 2 weeks apart. The first specimen from a retroperitoneal mass showed an inflammatory reaction and a faint clonal rearrangement band of the $Ig$ heavy chain gene. Two weeks later, microscopic assessment of 2 resected para-aortic lymph nodes showed reactive follicular hyperplasia. A clonal rearrangement of $Ig$ heavy chain gene was detected. A third biopsy from the retroperitoneal mass showed NHL, diffuse mixed cell type.

The last patient, a 60-year-old woman, presented with arthritis and general lymphadenopathy. She had undergone a left hip replacement. Bone and tissue from the left hip showed severe osteoarthritis and chronic active synovitis. Sections of a lymph node

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**Table 3: Discordant non-Hodgkin’s lymphoma samples (n = 22)**

<table>
<thead>
<tr>
<th>Morphologic features</th>
<th>Phenotype</th>
<th>ND/Inc</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular, mixed cell</td>
<td>B = 1 T = 0 Inc = 0</td>
<td></td>
<td>Described in Table 4</td>
</tr>
<tr>
<td>Follicular, large non-cleaved cell</td>
<td>B = 1 T = 0 Inc = 0</td>
<td></td>
<td>PCR positive</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse, mixed cell</td>
<td>B = 0 T = 3 Inc = 0</td>
<td></td>
<td>1 sampling error</td>
</tr>
<tr>
<td>Diffuse, large non-cleaved cell</td>
<td>B = 2 T = 0 Inc = 2</td>
<td></td>
<td>3 sampling errors, 1 PCR positive</td>
</tr>
<tr>
<td>Diffuse, large cell</td>
<td>B = 0 T = 0 Inc = 1</td>
<td></td>
<td>Described in Table 4</td>
</tr>
<tr>
<td>High grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse, large cell</td>
<td>B = 2 T = 1 Inc = 1</td>
<td></td>
<td>2 described in Table 4</td>
</tr>
<tr>
<td>Diffuse, large cell anaplastic</td>
<td>B = 0 T = 1 Inc = 2</td>
<td></td>
<td>2 sampling errors</td>
</tr>
<tr>
<td>Diffuse, lymphoblastic</td>
<td>B = 0 T = 0 Inc = 1</td>
<td></td>
<td>Described in Table 4</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin, mycosis fungoides</td>
<td>B = 0 T = 1 Inc = 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin, angiocentric lymphoma</td>
<td>B = 0 T = 0 Inc = 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>True histiocytic lymphoma</td>
<td>B = 0 T = 0 Inc = 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>B = 6 T = 6 Inc = 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* B = B cell, T = T cell, ND = not done, Inc = inconclusive, PCR = polymerase chain reaction.
from the left axilla showed benign reactive hyperplasia. Ig gene rearrangement was detected in the same sample. Nine months later, tissue from the right femoral head showed plasma cell malignant disease.

**Atypical morphology**

A diagnosis of atypical lymphoid hyperplasia was made when the morphologic features were ambiguous and not definitive for a benign or a malignant disorder. The 5 samples positive for gene rearrangement were treated as NHL (Table 6). In 4 of the 11 samples diagnosed as atypical lymphoid hyperplasia, which were negative for gene rearrangement, hematologic malignant disease developed subsequently (Table 7).

**Discussion**

The data presented here represent the experience of a single institution in diagnosing lymphoproliferative disorders by histopathology, immunophenotyping and gene rearrangement analysis. The role of molecular pathology in the diagnosis of lymphoproliferative disorders has already been established. However, reports of cases showing discrepancy between morphologic assessment and gene rearrangement results are scarce. Furthermore, none of the previous studies have reported long-term follow-up of these discordant cases.

**Correlation between morphology and gene rearrangement**

The high concordance rate (more than 85%) between the morphologic diagnosis and the detection of gene rearrangement in this study confirms previous reports that described a gene rearrangement detection rate ranging from 90% to 100% in B-cell NHL.1,10,15 The gene rearrangement detection rate in T-cell NHL ranges from 60% to 100% and is affected by many variables.1,10,11,15 In this study, the gene rearrangement detection rate was higher in B-cell NHL (92%) than T-cell NHL (50%).

None of the Hodgkin’s lymphomas showed gene rearrangement and only 1 case of carcinoma showed gene rearrangement, similar to what has been reported previously.10

**Correlation between immunophenotyping and gene rearrangement**

Gene rearrangement studies were helpful in some polymorphous lymphoid proliferations when im-

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**Table 4: Discordant non-Hodgkin’s lymphoma (NHL) samples having malignant characteristics but no gene rearrangement**

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Morphologic diagnosis</th>
<th>Follow-up</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal LN</td>
<td>NHL, large cell, immunoblastic in CLL</td>
<td>Treated. Splenectomy for small lymphocytic lymphoma</td>
<td>CLL diagnosed by cell surface marker of PB</td>
</tr>
<tr>
<td>Paratracheal LN</td>
<td>NHL, diffuse, lymphoblastic</td>
<td>Treated</td>
<td>Ig-R in bone marrow</td>
</tr>
<tr>
<td>Paraduodenal LN</td>
<td>NHL, large cell, NOS</td>
<td>Treated for 3 yr</td>
<td>NHL after HD. Relapse 7 yr later</td>
</tr>
<tr>
<td>Axillary LN</td>
<td>NHL, large cell, immunoblastic B cell</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td>Axillary LN</td>
<td>NHL, follicular mixed cell type</td>
<td>Treated</td>
<td></td>
</tr>
</tbody>
</table>


**Table 5: Discordant samples having benign morphologic features and gene rearrangement**

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Morphologic diagnosis</th>
<th>Gene rearrangement</th>
<th>Time to final diagnosis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal LN</td>
<td>None</td>
<td>R R G</td>
<td>5 mo</td>
<td>Inguinal LN: NHL, FSCC. Died 5 yr later</td>
</tr>
<tr>
<td>Spleen</td>
<td>Mild lymphocyte infiltrate</td>
<td>R G G</td>
<td>Same time*</td>
<td>Has CLL and SLL. No treatment</td>
</tr>
<tr>
<td>Para-aortic LN</td>
<td>Reactive follicular hyperplasia</td>
<td>R R G</td>
<td>2 wk</td>
<td>Soft tissue, retroperitoneal, NHL, diffuse mixed cell</td>
</tr>
<tr>
<td>Axillary LN</td>
<td>Benign reactive hyperplasia</td>
<td>R G G</td>
<td>9 mo</td>
<td>Tissue, femoral head, plasma cell malignant disease</td>
</tr>
</tbody>
</table>

*Diagnosis reconsidered on the same tissue.

JH = immunoglobulin heavy chain-joining region, JK = light chain-joining region, CB = T-cell receptor β gene constant region, LN = lymph node, R = rearranged, G = germline DNA, no rearrangement, NHL = non-Hodgkin’s lymphoma, FSCC = follicular small cleaved cell, CLL = chronic lymphocytic leukemia, SLL = small lymphocytic lymphoma.
munophenotyping failed to show monoclonality or lineage specificity.\textsuperscript{1,2} Our data show that immunophenotyping was inconclusive in 25\% of the cases (27 out of 110 studied), but gene rearrangement studies showed monoclonality in 64\% of these. Gene rearrangement results were inconclusive in only 5\% of all cases (6 of 130).

Rearrangement of both Ig and TcR genes was noted in 7 cases (10\%) of B-cell NHL. Previous reports describe similar observations in a subset of tumours.\textsuperscript{18,19} The reason for out-of-lineage gene rearrangement is not fully understood, but it is speculated that common recombinase enzymatic pathways catalyse both Ig and TcR rearrangement. Rearrangement of Ig and TcR are also seen in rare cases of acute myeloid leukemia.\textsuperscript{20} In 3 cases in which immunophenotyping showed a T-cell proliferation, only Ig gene rearrangement was detected. These tumours could represent T-cell tumours with mixed genotype\textsuperscript{8,18–20} or a B-cell tumour masked by a polymorphous T-cell proliferation.\textsuperscript{2,18} These cases were eventually classified as undefined lineage.

Reports of gene rearrangement detection rate in T-cell lymphoma vary from 60\% to 100\%.\textsuperscript{8,15,17} Of the 12 T-cell lymphoma cases described here, 6 showed only germline bands. Two samples showed focal involvement of the tissue, pointing to a sampling error. Two samples were a cutaneous lymphoma and an anaplastic lymphoma, which are not expected to consistently show rearrangement.\textsuperscript{10,21–23} The remaining 2 samples were from the same patient a few months apart.

Weiss and colleagues\textsuperscript{24} reported 7 cases of peripheral T-cell NHL that lacked TcR gene rearrangement. They hypothesized 3 possible explanations for the lack of gene rearrangement: the process of gene rearrangement did not occur; rearrangement of the TcR occurred, but the gene later underwent deletion; or multiple rearrangements occurred in a polyclonal fashion after malignant transformation and proliferation of a cell that lacked rearrangement.

**Discordant cases**

NHL with no gene rearrangement

Twenty-two samples were diagnosed as NHL, and no gene rearrangement was detected in the tissue submitted for DNA analysis. In the multi-institutional study of Cossman and associates,\textsuperscript{10} gene rearrangement was also not detected in some lymphoma cases. The majority were accounted for on

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Morphologic diagnosis</th>
<th>Gene rearrangement</th>
<th>Time to final diagnosis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Intense small lymphocytic infiltrate</td>
<td>R G G</td>
<td>3 mo</td>
<td>Bladder: NHL, small lymphocytic lymphoma. Treated</td>
</tr>
<tr>
<td>Soft tissue, arm</td>
<td>Atypical lymphoid hyperplasia</td>
<td>R R G</td>
<td>5 mo</td>
<td>Soft tissue, arm: NHL, NOS</td>
</tr>
<tr>
<td>Inguinal LN</td>
<td>Atypical lymphoid hyperplasia</td>
<td>R G G</td>
<td>Same time*</td>
<td>Treated as low-grade NHL</td>
</tr>
<tr>
<td>Cervical LN</td>
<td>Atypical immunoblastic proliferation</td>
<td>G G R</td>
<td>1 mo</td>
<td>Positive for EBV</td>
</tr>
<tr>
<td>Cervical LN</td>
<td>Atypical lymphoid proliferation</td>
<td>R G R</td>
<td>Same time*</td>
<td>Positive for EBV. Treated</td>
</tr>
</tbody>
</table>

\*Diagnosis reconsidered on the same tissue.

\textsuperscript{JH} = immunoglobulin heavy chain-joining region, \textsuperscript{JK} = light chain-joining region, \textsuperscript{CB} = T-cell receptor \text{\textbeta} gene constant region, \text{\textR} = rearranged, \text{\textG} = germline DNA, no rearrangement, \text{\textNHL} = non-Hodgkin’s lymphoma, \text{\textNOS} = not otherwise specified, \text{\textLN} = lymph node, \text{\textEBV} = Epstein-Barr virus.

**Table 6: Samples with atypical morphologic features and gene rearrangement**

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Morphologic diagnosis</th>
<th>Gene rearrangement</th>
<th>Time to final diagnosis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin, arm</td>
<td>Mild lymphocytic infiltrate</td>
<td>R</td>
<td>1 wk</td>
<td>Known mycosis fungoides</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 wk</td>
<td>Axillary LN, HD</td>
</tr>
<tr>
<td>LN NS</td>
<td>Atypical lymphoid hyperplasia</td>
<td>2 mo</td>
<td>Axillary LN, HD</td>
<td>Paraduodenal LN, NHL large cell</td>
</tr>
<tr>
<td>Axillary LN</td>
<td>Atypical hyperplasia</td>
<td>Same time*</td>
<td>Ig-R in peripheral blood</td>
<td></td>
</tr>
<tr>
<td>Iliac LN</td>
<td>Atypical reactive</td>
<td>11 mo</td>
<td>Autopsy LN, BM, anaplastic myeloma</td>
<td></td>
</tr>
</tbody>
</table>

\* Diagnosis reconsidered on the same tissue.

\textsubscript{LN} = lymph node, HD = Hodgkin’s disease, NS = not specified, \text{\textNHL} = non-Hodgkin’s lymphoma, Ig-R = rearranged immunoglobulin, BM = bone marrow.
the basis of partial or minor involvement of the tissues. On second review of the discordant cases in this study, only 6 of the samples had partial or minor involvement of tissues; 4 were B-cell NHL and 2 T-cell NHL. Four others of the 22 discordant samples were cutaneous lymphomas. The diagnosis of cutaneous lymphoma is problematic, and many reports have studied the role of TcR rearrangement as an adjunct to the histopathologic diagnosis.21–23 Four of 8 cases of cutaneous lymphoma in this study did not show TcR β gene rearrangement. The rearrangement of the TcR γ gene, which seems to be detected more often in cutaneous lymphoma, especially in mycosis fungoides,24 was not studied. Therefore, gene rearrangement may not have been detected for a variety of reasons. Two of the remaining samples were T-cell lymphoma (2 samples taken from the same patient a few months apart) and 2 were anaplastic large cell lymphoma. Both T-cell and anaplastic large cell lymphoma, as previously reported, may not show rearrangement.8,10,25 One sample was a true histiocytic lymphoma where no gene rearrangement is expected to occur. In 2 final samples, where gene rearrangement was not detected by the Southern blotting technique, gene rearrangement was observed with use of the polymerase chain reaction, which is a more sensitive technique.

The samples that did not show partial involvement of the tissue and did not fall into the categories of cutaneous lymphoma, T-cell lymphoma, anaplastic large cell lymphoma or true histiocytic lymphoma are described here in more detail.

Two of the 5 samples showed monoclonality at another site, one by immunophenotyping and another by gene rearrangement analysis. In the first case there was progression of chronic lymphocytic leukemia, and the presence of a monoclonal population of lymphocytes in the peripheral blood was detected by immunophenotyping (flow cytometry). Gene rearrangement was detected in the bone marrow but not in the lymph-node tissue in the second case, a sample with diffuse lymphoblastic lymphoma (Table 4).

Three samples did not show evidence of monoclonality. The findings were reviewed, and the diagnosis of a malignant lymphoma was reconfirmed. One was a very interesting case of NHL after treatment of Hodgkin’s lymphoma. The patient whose tissue sample this was responded to treatment and suffered relapse 7 years later. Ig gene rearrangement was detected in the tissue at relapse.

Benign morphologic features with gene rearrangement

Although it is clear that clonality does not always mean malignancy,11,14 in this study, the 4 patients whose tissue samples that were diagnosed by morphologic assessment as benign hyperplasia and had a detectable gene rearrangement all had malignant disease at a later date (Table 5). This indicates that the presence of a clonal gene rearrangement might represent an early tumour and close follow-up is needed.

Atypical morphologic findings

Williams and colleagues26 reported 11 patients with abnormal lymphoid hyperplasia. In all of 6 patients who had gene rearrangement, lymphoma developed later, and 2 of 5 patients with no gene rearrangement also had a lymphoma. Our findings are similar. Five samples showed atypical lymphoid hyperplasia and gene rearrangement, and lymphoma was found in 3 of the patients a few months later. Although the remaining 2 samples belong to the same patient, TcR rearrangement was detected in one sample and both Ig and TcR rearrangement in the other. This patient was also positive for Epstein–Barr virus and was treated for lymphoma.

Of the 11 patients having atypical lymphoid hyperplasia and no gene rearrangement, only 4 had hematologic malignant disease later. Three patients subsequently had tumours that usually lack the detection of gene rearrangement (as already discussed): 1 cutaneous lymphoma, 1 Hodgkin’s lymphoma and 1 anaplastic myeloma. Only 1 (9%) patient out of 11 had an NHL.

Conclusions

This report summarizes a single institution experience with the diagnosis of lymphoproliferative disorders after the establishment of a molecular diagnostic laboratory. It is the first report to present long-term follow-up on controversial cases.
The results of this study indicate that patients showing adenopathy and gene rearrangement with either benign or atypical morphology are at high risk for progression to clinically evident NHL. All patients with gene rearrangement had NHL or NHL developed within a few months, indicating a very high specificity of gene rearrangement studies. On the other hand, when the diagnosis of NHL was established by morphologic assessment, the lack of detection of gene rearrangement did not necessarily change the diagnosis. The lack of detectable gene rearrangement could be attributed to a variety of reasons: the disease itself might lack a detectable gene rearrangement; there may have been a sampling error in cases with minor or partial involvement; in rare cases, gene rearrangement could be missed owing to inability of the probes to bind to a specific rearrangement.

The data reported here suggest that in cases in which the morphologic diagnosis of B-cell NHL is well established, gene rearrangement studies provide little added value. Molecular studies for other markers such as bcl-2 and bcl-1 might be more valuable in the classification and prognosis of the lymphoma. Gene rearrangement studies in T-cell NHL are valuable in ruling out the possibility of a clonal B-cell proliferation in a predominant polymorphous T-cell population. Furthermore, TcR rearrangement is the only marker for clonality in T-cell lymphomas.

In specific situations, gene rearrangement studies provide added value that influences diagnosis and treatment. This was evident in patients who presented with lymphadenopathy and splenomegaly, and their morphologic features were either benign or atypical but not diagnostic. In these cases, the presence of gene rearrangement almost always indicated a progression to NHL.

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References


Reprint requests to: Dr. Irene Sadek, Queen Elizabeth II Health Sciences Centre, VG Site, Hematology Laboratory, Rm. 223A, Mackenzie Building, 5788 University Ave., Halifax NS B3H 1V8; fax 902 473-4113, plmis@qe2-hsc.ns.ca