Lymphoid-rich effusions frequently are encountered in the clinical practice of cytology. The majority are benign and associated with various conditions. Rarely is the specific cause of a benign effusion identified by cytomorphologic review of the lymphoid cells (eg, lupus erythematosus cells). Although lymphomatous effusions will develop in as many as 45% of all patients with lymphoma during the course of the disease,1 malignant lymphoid effusions constitute the initial manifestation of neoplasm in only 0.5% of these patients.2 Benign lymphoid-rich effusions also can develop in patients with disseminated lymphoma in response to therapy or infection.

Although most benign lymphocytes are T-cell proliferations, the majority of lymphomatous effusions are composed of B cells.3 Immunostaining for immunoglobulin light chains,4-6 and more recently gene rearrangement studies,7,8 have been useful to classify lymphoid-rich effusions as benign or malignant. Making this distinction may be difficult using morphologic and clinical criteria. These studies however, are expensive, labor intensive, and often associated with technical drawbacks.9

We previously described the application of a microcomputer-based video system, computerized interactive morphometry (CIM), and a simple rule-based expert system for the diagnosis of malignant lymphoma and benign lymphocytosis in lymphoid-rich effusions.9-10 The analysis was based on the distribution of lymphoid nuclear profile areas (NPA), and required manual tracing of real-time images of nuclear profiles visualized on a video monitor. This method was associated with high predictive values for the diagnosis of malignant lymphoma (96.5%) and benign lymphocytosis (94.0%), but did not distinguish chronic lymphocytic leukemia (CLL) from benign lymphocytosis. Technical drawbacks of this methodology included the arbitrary selection of nuclear profiles by the trained observer rather than by the instrument, the time and manual skill required to trace the nuclear profiles, and the proprietary nature of the software.

The present study also uses a relatively inexpensive microcomputer-based video system, CIM, and the simple rule-based expert system to classify lymphoid-rich effusions. The methodology differs from that in the previous study in that (1) all hardware and software components are commercially available, (2) NPAs are measured automatically, (3) lymphoid nuclear profile integrated optical densities (NPIODs) are measured concurrently and automatically. Our goals were to (1) reevaluate the predictive value of the previously used rule-based expert system for the diagnosis of malignant lymphoma and benign lymphocytosis in lymphoid-rich effusions based on NPA data obtained automatically and (2) assess the application of NPIOD data for these diagnoses.

**MATERIALS AND METHODS**

Papanicolaou-fixed and stained cytospin smears from lymphoid-rich body cavity fluids were retrieved from the files of the Cytopathology Laboratory at Cedars-Sinai Medical Center. All of the smears had been prepared and stained between 1984 and 1990 using a Shandon cytocentrifuge (Shan-
Computerized Morphometry and Lymphoid-Rich Effusion Diagnosis

**Data Collection**

All measurements were performed by one trained observer (R.M.) to whom the diagnoses were not known. This observer viewed representative microscopic fields on the video monitor. At the beginning of each procedure, the observer calibrated the instrument for photometric measurements by determining incident light and background conditions. The observer used the mouse to select a threshold between the background illumination and the nuclei for each field. This threshold allowed the "intelligent cursor" facility of the system to identify and echo ("trace") most discrete nuclear profiles in each microscopic field. The trained observer deleted all nonlymphoid and overlapping images from the microscopic field using the editing function of the software. Lymphoid nuclear profiles that "touched" each other were "cut" into individual profiles using the editing facility. All adequate traced lymphoid nuclear profiles that remained in the field after editing were measured automatically by the system (Fig. 2). For each case the following parameters were measured from 200 consecutive lymphoid nuclear profiles: NPA (determined by a trapezoidal method), nuclear profile circularity (defined as $4 \times \text{area/periimeter}^2$), and NPIOD (defined as $\log_{10} \frac{\text{pixel intensity}}{\text{incident intensity}}$). The software automatically sorted the NPA data into 11 bins ranging from 0–109.9 $\mu m^2$ and the NPIOD data into 20 bins ranging from 40.0 to 239.9 arbitrary units of optical density.

**Rule-Based Expert System**

The simple rule-based expert system was employed as previously described. The classification of effusions as benign lymphocytoses or malignant lymphomas was affected after the

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**Computerized Interactive Morphometry System**

The CIM system is based on a Compaq 386/25e microcomputer (Compaq, Houston TX) with a PC Vision Plus video board (Imaging Technology, Woburn, MA). A Microsoft mouse (Microsoft, Seattle WA) and a Sony color video monitor (Sony, Fujisawa, Japan) constitute the interactive peripheral equipment. The real-time image of each cytologic preparation is acquired by a monochrome video camera (Cohu Corporation, San Diego CA) mounted on a Zeiss light microscope (Carl Zeiss, Germany) with a 40X objective (Fig. 1). The software, the IM5200 module created by Microscience (Phoenix Technology, Seattle WA), permits visualization of the real-time microscopic images in pseudocolor. The measurements are performed in a monochrome mode, however, using a green filter.
FIG. 2. Editing function of the software allows observer to (A) delete nonlymphoid and overlapping nuclei (X) and (B) cut (separate) touching lymphoid nuclei (cross-bar) within the window to be measured (highlighted right half of screen). Notice that the dark nuclei and those touching edges of the defined window are automatically excluded from data collection.
**Computerized Morphometry and Lymphoid-Rich Effusion Diagnosis**

**TABLE 1. RULE-BASED EXPERT SYSTEM FOR CLASSIFICATION OF LYMPHOID-RICH EFFUSIONS**

<table>
<thead>
<tr>
<th>Diagnosis (No.)</th>
<th>Expert System Diagnosis</th>
<th>Immunostaining Performed</th>
<th>Immunostaining Not Ordered</th>
<th>Immunostaining Result</th>
<th>Immunostaining Uninterpretable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lymphocytosis (86)</td>
<td>Benign lymphocytosis (84)</td>
<td>42</td>
<td>42</td>
<td>Polyclonal (34)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Lymphoma (2)</td>
<td>2</td>
<td>0</td>
<td>Polyclonal (2)</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma (26)</td>
<td>Lymphoma (18)</td>
<td>13</td>
<td>5</td>
<td>Monoclonal (9)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Benign lymphocytosis (8)</td>
<td>8</td>
<td>0</td>
<td>Monoclonal (7)</td>
<td>1</td>
</tr>
<tr>
<td>CLL (10)</td>
<td>Benign lymphocytosis (10)</td>
<td>6</td>
<td>4</td>
<td>Monoclonal (4)</td>
<td>1</td>
</tr>
</tbody>
</table>

**RESULTS**

All effusions were diagnosed as malignant lymphoma, benign lymphocytosis, or CLL based on clonality, gene rearrangement studies, and/or clinical follow-up. As shown in Table 2, if the CLL cases are excluded, the expert system based on the distribution of NPA classified 69.2% (18 of 26) of the malignant and 97.6% (84 of 86) of the benign effusions correctly (specificity 97.7%, sensitivity 66.7%). All 10 effusions from patients with histories of CLL were classified as benign lymphocytoses by the expert system. At least four of these effusions were malignant (ie, monoclonality of light chains was demonstrated), but the diagnosis of the remaining six is uncertain.

Immunostaining had been performed on 80.8% (21 of 26) of the lymphomatous and 51.1% (44 of 86) of the benign effusions in the study. Results were uninterpretable in 19.7% (14 of 71 cases) representing 18.1% of the benign, 23.3% of the lymphomatous, and 16.6% of the CLL cases for which immunostaining had been performed (Table 2). All of the benign effusions and four of the five lymphoma effusions with uninterpretable immunostaining were classified correctly by the rule-based expert system. The latter group of effusions included one that required gene rearrangement studies to establish the diagnosis of lymphoma. Insufficient numbers of lymphoid and/or abnormal lymphoid cells and/or poorly preserved cells with altered antigenic sites accounted for the uninterpretable immunostaining.

Discriminant analysis showed no difference in the mean values of nuclear profile circularity factors in the benign, malignant, and CLL groups. The mean and standard deviation of NPAs were 23.98 ± 3.52 μm² (benign effusions), 35.75 ± 11.80 μm² (lymphoma effusions), and 21.32 ± 1.97 μm² (CLL effusions).

The distribution of NPAs for the benign, lymphoma, and CLL effusions is shown in Figure 4. Only two distinct populations are apparent: the distribution of NPAs of benign effusions is almost identical to that of the effusions from CLL patients. The lymphoma effusions are characterized by a broader distribution of NPA and a greater proportion of larger nuclei when compared with the benign effusions. Statistical analysis of these data with discriminant classificatory analysis (DISCRIM) resulted in correct classification of 24 of the 26 (92.3%) lymphomas (posterior probability = 0.78–1.00). One half (43 of 86) of the benign effusions were classified by the analysis as CLL consistent with the similar distribution of NPAs in these two groups.

The distribution of NPIODs for the benign, lymphoma, and CLL effusions is shown in Figure 4. The lymphoma effusions are characterized by the widest distribution curve and the largest mean value of NPIOD. The CLL group is characterized by the narrowest distribution of NPIODs. The majority of nuclei in this group have optical densities that are lower than those in the benign effusion group. The mean and standard deviation of NPIOD were 103.5 ± 5.67 (benign effusions), 126.5 ± 30.62 (lymphoma effusions), and 136.7 ± 151.27 (CLL effusions). Statistical analysis of these data with discriminant classifica-
DISCUSSION

This study is a continuation of our investigation into the application of CIM to cytodiagnosis.9 We applied the simple rule-based expert system developed previously to data collected by an improved CIM technology from a new mix of unselected effusions. Advantages offered by the improved CIM equipment include (1) commercial availability of every component; (2) elimination of manual tracing of nuclear profile images, which decreased the time required to measure each case while providing more objective measurements (data were collected from the actual image rather than from a tracing of the image); (3) a more objective selection of cells (the system automatically measures all distinct lymphoid cells in chosen field while permitting the observer to exclude individual cells; (4) automatic measurement of NPIODs without additional observer time; (5) less time required for data collection; and (6) easier statistical analysis of data.

Our results confirm the value as well as the limitations of CIM in the classification of lymphoid-rich effusions as previously described.9 Briefly, the simple rule-based expert system based on NPAs provided accurate classification of 84 of 86 effusions as benign and of 18 of 26 lymphomas as malignant. The resulting predictive value of a diagnosis of lymphoma was 88.9% and that of benign lymphocytosis was 91.3%. These values are slightly lower than those in our previous study because the present study includes more small-cell lymphomas; hence a higher percentage were classified as benign by the expert system. This is also manifest when the distribution curves for NPA of lymphomas in the present (Fig. 3) and previous study9 are compared. On review, smears from all eight lymphomatous effusions incorrectly diagnosed by morphometry as benign (ie, false-negatives) were either composed of small to medium-sized lymphoid cells or contained few large abnormal lymphoid cells in a background of small lymphoid elements, confirming the unsuitability of the expert system and CIM to diagnose lymphoma in these situations based on NPA. Applications and limitations of the rule-based expert system are being evaluated. At this time, we believe that it is premature to use this system for routine diagnosis of lymphoid-rich effusions, and we are not revising diagnoses that were based on standard morphologic and/or immunocytochemical studies. Our observations require confirmation by other laboratories, and we hope that this study using relatively inexpensive commercially available methodology will stimulate other laboratories toward this end.

Our results suggest that NPIOD data provide a reliable method by which to distinguish most benign and lymphomatous effusions, and that such data also may be useful in situations that are unsuitable to the expert system and NPA determinations. We did not incorporate the NPIOD data into the previously developed rule-based expert system because we wanted to test the reproducibility of that system with the new instrumentation. We hope to develop a refined rule-based expert system that will combine NPA and NPIOD data, and by so doing achieve greater sensitivity and specificity of effusion diagnosis.

Our results based on morphometric analysis compare favorably with the uninterpretable rate (19.7%) of immunostaining among our cases. All cases with uninterpretable immunostaining were correctly classified by the CIM system, again suggesting that immunostaining might be reserved for effusions (1) with a small percentage of large abnormal lymphoid cells suspected to be malignant, (2) with a predominance of small but abnormal lymphoid cells, and (3) from CLL patients. Immunostaining is not an efficient, cost-effective means by which to evaluate all lymphoid-rich effusions. It requires fresh material and fresh reagents, is labor intensive, and is associated with a relatively high rate of uninterpretable results. Our morphometric methods are proposed as an efficient and accurate adjunct to the diagnosis of routinely prepared Papanicolaou-stained cytospin effusion smears. In patients with known lymphoma or with smears that are clinically suggestive of lymphomatous effusions, this methodology can confirm the clinical impression inexpensively. Moreover, it can be applied to standard preparations of lymphoid effusions that might otherwise not be submitted for immunocytochemical staining because of cost considerations, a low index of clinical suspicion of lymphoma, lack of fresh cellular material, or unavailability of an immunocytochemistry laboratory.
REFERENCES