Malignant Lymphoma, Mixed Cell Type, Diffuse

By Bharat N. Nathwani, Gerald E. Metter, Richard A. Gams, Alfred A. Bartolucci, Robert J. Hartsock, Richard S. Neiman, Gerald E. Byrne, Jr., Maurice Barcos, Hun Kim, and Henry Rappaport

This retrospective study of diffuse mixed (DM) cell lymphoma was undertaken as a collaborative study between the Repository Center for Lymphoma Clinical Studies and four cooperative oncology groups (CALGB, ECOG, SECSG, SWOG), and was based on 82 patients from the files of the Repository Center. We wanted to ascertain whether there were any significant clinical differences among the various morphological subtypes of this lymphoma. All patients were treated according to different protocols of the Cooperative Oncology Groups sponsored by the National Cancer Institute. In 16 patients (26%), the malignant lymphoma (ML) had morphological features consistent with follicular center cell origin (FCC); in 34 patients (55%), the ML did not have features of follicular center cell type (non-FCC), but had morphology described for peripheral T-cell-derived ML. In 8 of the patients (13%), no agreement could be reached by the 7 histopathologists who participated in the study, and these were classified as unresolved; the remaining 4 (8%) were unclassifiable. We compared the survival times of the 16 patients having the morphological features of the FCC subtype with the survival times of the 34 patients with the non-FCC subtype and found that patients with FCC lived longer ($p = 0.07$). In the FCC group, all patients who had complete remissions (CR) were alive; however, their survival times were similar to those who had a partial or no response ($p = 0.32$). In contrast, in the non-FCC group, the median survival was 20 mo., and patients with a CR had a significantly longer survival than did noncomplete responders ($p = 0.003$). According to these results the non-FCC diffuse mixed cell lymphoma appears to be a high-grade malignant lymphoma, whereas the FCC type is not.

IN THE RAPPAPORT SYSTEM$^1$ of classification of non-Hodgkin's lymphomas, the diffuse mixed (DM) cell type is a heterogeneous category on morphologic and immunologic grounds.$^2$-7 Likewise, the recently proposed "Working Formulation on Non-Hodgkin's Lymphoma," which also has a category of diffuse mixed, small, and large cell,$^8$ includes at least two subtypes that are not specifically listed in this formulation but are elaborated upon in the text. Although both are composed of a mixture of small and large lymphoid cells, they can be morphologically separated by careful study of cellular and nuclear details. The two main types are: (1) lymphomas that have morphological features of follicular center cells (B cells),$^9$ and (2) lymphomas that have morphological features consistent with peripheral T-cell-derived lymphomas.$^10$

No clinical information is available comparing these two malignant lymphomas with respect to natural history, response to therapy, and survival. We undertook this study in an attempt to provide such information.

MATERIALS AND METHODS

One hundred sixty-one cases of diffuse mixed (DM) cell lymphoma submitted to the Repository Center for Lymphoma Clinical Studies$^1$ from April 1967 to July 1980 were reviewed. Sixty-two cases originated from the Eastern Cooperative Oncology Group; 49 cases from the Southwestern Oncology Group; 17 cases from Cancer and Acute Leukemia Group "B," and 33 cases from the Southeastern Cancer Study Group. In each case, the pathologist reviewed histologic material without knowledge of the clinical data. All 161 cases were reviewed by one of us (B.N.N.); of these, 99 cases were eliminated because (1) the technical quality of the sections was less than optimal for a precise classification of DM (42 cases); (2) the diagnosis was revised on review (41 cases); (3) the disease was limited to extranodal sites (14 cases); and (4) the quantity of material was insufficient (2 cases). The remaining 62 cases in which specimens were of adequate histologic quality were reviewed independently at the Repository Center by each of the 7 participating pathologists (R.J.H., R.S.N., G.E.B., M.B., H.K., B.N.N., and H.R.). The pathologists were considered as being in agreement when 5 or more concurred on a case.

All patients had been treated on a variety of different protocols over a 13-yr period. Most patients were untreated prior to their assignment to a protocol. The protocols tended to become more sophisticated with time and with the introduction of increasingly effective chemotherapeutic agents. Although the Ann Arbor staging system was used, initially the staging procedures were not uniform among the four cooperative groups. Gradually, however, the staging procedures and definitions became more rigorous and uniform.

Age, sex, race, date of diagnosis, initial symptoms and physical findings, response to therapy, status at last follow-up, and survival data were evaluated. The survival times were measured from the date when the biopsy first established the diagnosis of lymphoma, and survival curves were obtained by the product-limit method of Kaplan and Meier.$^{13}$ The log rank test was employed for evaluation of differences between survival curves.$^{13}$ A multivariate analysis was performed with Cox's regression model$^{14}$ and included only patients for whom we had complete data on all parameters of interest.
RESULTS

Morphological Observations

In 62 cases, the quality of the histologic material was adequate to allow a morphological subclassification. Table 1 shows that the majority (34) of these cases did not have the morphological features of FCC lymphoma, but instead were consistent with peripheral T-cell-derived lymphoma, also described under the terms T-immunoblastic, T-large cell lymphomas, and T-zone lymphoma. In this retrospective study, we did not perform immunologic studies to prove the T-cell nature of these lymphomas. Without immunologic confirmation, the designation of “T-cell” could not be totally justified; therefore, we preferred the term “nonfollicular center cell subtype” (non-FCC) for these morphologically interpreted peripheral T-cell lymphomas.

In 16 of the remaining 28 cases (Table 1), the morphology of the neoplastic cellular infiltrate was that of the FCC type. In 4 cases there were no distinctive features, and these were considered unclassifiable. The remaining 8 cases were classified as unresolved because no agreement could be reached among the 7 collaborating pathologists.

A decision on a case was considered as “agreement” when 5 or more pathologists concurred. Agreement was reached on 38 of the 62 cases (61%) (Table 2). For the remaining 24 (39%), no agreement was achieved initially. These 24 cases were reviewed again by all 7 pathologists simultaneously on a 9-headed microscope, and a consensus was reached in 16 of the cases (Table 2). Thus, there was agreement on a total of 54 cases (87%).

The follicular center cell (FCC) type of diffuse mixed cell lymphoma showed a mixture of small and large lymphoid cells in varying proportions without preponderance of either cell type. In places, however, small lymphoid cells predominated (Fig. 1). These small (“cleaved”) lymphoid cells ranged in size from 6 to 12 μ, had clumped chromatin without nucleoli, and showed variations in nuclear shape. These cells had small, imperceptible rims of cytoplasm. The large cells were cleaved and/or noncleaved (Fig. 2). The large cleaved cells varied in size from 13 to 30 μ, and most had vesicular nuclei, usually without perceptible nucleoli; when nucleoli were discernible, they were small and inconspicuous. The large noncleaved cells were more than 20 μ and had vesicular nuclei containing one to several small nucleoli, many of which were adjacent to nuclear membrane; the cytoplasm was scanty and pyroninophilic. Mitotic figures and inflammatory cells were present; epithelioid histiocytes were rarely found. Occasionally, both normal-appearing and atypical plasma cells were observed. Sclerosis and compartmentalization of tumor cells by fibrous tissue were noted in some cases.

The diffuse mixed cell lymphoma that did not have the features of the FCC subtype were histologically consistent with those described for peripheral T-cell-derived lymphoma by Waldron et al. and for T-immunoblastic lymphoma by Lukes and Parker and by other investigators. In this, as in the FCC subtype, a mixture of small and large lymphoid cells was present, without appreciable preponderance of either cell type (Fig. 3). The small lymphoid cells were not cleaved; they had round to slightly irregular nuclear contours, and they predominated in many areas. The small cells had a clumped chromatin structure and no perceptible nucleoli. The intermediate and the large cells were round and had vesicular nuclei with one to several nucleoli, which were either centrally or peripherally located. The striking finding in this subtype was the presence of a spectrum of cell sizes, with transitional forms being readily identifiable (Fig. 4). Many of the large cells had moderate to abundant quantities of pale to clear cytoplasm and either well or poorly defined cell borders, depending on the fixative employed. Mitotic figures were easily identified, and inflammatory cells were occasionally present. Epithelioid histiocytes were found in many cases; they were diffusely distributed throughout a node or they occurred in clusters (Fig. 5). In such instances, the morphology of the histiocytes was indistinguishable from that in non-Hodgkin’s lymphoma with a diffuse epithelioid histiocytic reaction.

Table 1. Morphological Subclassification of 62 Cases of Diffuse Mixed Cell Lymphoma

<table>
<thead>
<tr>
<th>Morphological Diagnosis</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular center cell type (FCC)</td>
<td>18 (26%)</td>
</tr>
<tr>
<td>Non-FCC</td>
<td>34 (55%)</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Unresolved</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Total</td>
<td>62 (100%)</td>
</tr>
</tbody>
</table>

Table 2. Diffuse Mixed Cell Lymphoma: Number of Cases According to Initial and Subsequent Review by Seven Pathologists

<table>
<thead>
<tr>
<th>Number of Pathologists Making Same Diagnosis</th>
<th>Initial Independent Review</th>
<th>Simultaneous Review by All 7 Pathologists on the 24 Disagreement Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreement</td>
<td>7</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Disagreement</td>
<td>4</td>
<td>17</td>
<td>24</td>
</tr>
</tbody>
</table>
Although typical and atypical plasma cells were present, no large cells with plasmacytoid features were evident. Sclerosis and compartmentalization of tumor cells by fibrous tissue were noted in some cases.

Clinical Correlations

For 3 of the 62 patients, no clinical information was available. The sex, age, stage, and symptoms of the remaining 59 patients, grouped according to subtypes, is shown in Table 3. Males were predominant in all subtypes. However, the highest male-to-female ratio was found in the non-FCC group. In the non-FCC subgroup, 85% of the patients were males; in the FCC subgroup, 60% of the patients were males ($p = 0.065$). In the elderly (>60 yr of age), the most common subtype was non-FCC. Thirteen patients (39%) morphologically classified as non-FCC were more than 60 yr of age, whereas only 2 patients (14%) had the FCC
subtype ($p = 0.086$). There were no significant differences in stage or symptoms among the subtypes of diffuse mixed cell lymphoma.

**Survival According to Clinical Parameters**

The survival times of the 59 patients on whom we had clinical information were as follows: 49% of the patients were dead after a median survival of 12 mo (range, 1–121+ mo).

The survival of patients who had a CR to therapy was significantly longer than that of patients who did not have a complete response (Fig. 6) ($p = 0.002$). The response to therapy was known for 37 patients; 20 of these (54%) achieved CR, ranging from 6 to 121+ mo, with a median not reached. In contrast, the duration of partial or no remissions ranged from 1 to 72+ mo, with a median of 12 mo.

A univariate statistical analysis, performed on the
other clinical parameters, revealed a significantly longer survival for asymptomatic than for symptomatic patients (Table 4) \( (p = 0.05) \). However, in a multivariate statistical analysis according to Cox’s model, which took into consideration 6 variables (age, sex, race, stage, symptoms, and histologic subtype), asymptomatic patients were found not to have longer survival times. Two other variables—sex and histologic subtype—influenced survival; the probability associated with the histologic subtype was at the level of \( p = 0.07 \) and for sex was \( p = 0.10 \).

**Survival According to Morphological Subtype**

In the FCC subtype, 11 patients (73%) were alive, whereas, in the non-FCC subtype, 15 (45%) were alive \( (p = 0.07) \). The survival curves (Fig. 7) show that a median survival for patients with the FCC subtype was not reached; whereas for the non-FCC subtype, the median was 20 mo \( (p = 0.16) \). Of the 3 patients considered unclassifiable, 2 were alive. Of the 8 patients classified as unresolved, 6 were dead, with a median survival of 7 mo.

**Survival According to Morphological Subtype and Response to Therapy**

To investigate further whether the histologic subtype influenced survival, we compared patients according to response to therapy categories. A comparison of complete responders (Table 5) showed that all patients (100%) with the FCC subtype were alive, and in the non-FCC group, most patients (67%) were alive \( (p = 0.26) \). The survival for the FCC subtype was slightly longer than that for the non-FCC subtype \( (p = 0.15) \) (Table 5).

A statistical comparison of non-CR (Table 5) revealed that 75% of the patients were alive in the FCC group, whereas in the non-FCC group, only 14% were alive \( (p = 0.09) \). There was also a trend suggesting a

<table>
<thead>
<tr>
<th>Table 3. Clinical Characteristics of 59 Patients With Diffuse Mixed Cell Lymphoma</th>
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<tbody>
<tr>
<td>Morphological Diagnoses</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>FCC</td>
</tr>
<tr>
<td>Non-FCC</td>
</tr>
<tr>
<td>Unclassifiable</td>
</tr>
<tr>
<td>Unresolved</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*Data available on 58 patients.
†Data available on 57 patients.
‡Data available on 40 patients.
longer survival for patients with the FCC subtype \((p = 0.13)\).

Within the FCC group of patients, no significant difference was found between those who achieved a CR and those who did not. The number of patients alive \((p = 0.50)\) and the median survivals \((p = 0.32)\) were also similar (Fig. 8 and Table 5).

In the non-FCC group of patients, those who achieved a CR had a significantly longer survival than that of patients who did not \((p = 0.003)\). The proportion of alive patients was also significantly higher in the CR group \((p = 0.03)\) (Table 5).

**DISCUSSION**

Since the diffuse histiocytic lymphomas are the most common types of diffuse lymphomas, it has been possible to subdivide them morphologically into various subtypes and ascertain their clinical significance. On the other hand, because of the rarity of the diffuse mixed cell lymphomas, no attempts have been made to compare the clinical features of these different subtypes. It was possible for the Repository Center and the Pathology Panel for Lymphoma Clinical Studies to undertake the present study because we could pool the cases from the four cooperative oncology groups. In spite of this collaboration, the Repository Center files had only 161 cases of diffuse mixed cell lymphoma in a total of 8,996 cases that were submitted from April 1967 till June 1980. This resulted in a study of a patient population treated over a 13-yr period by various protocols at different institutions. In spite of these shortcomings, many interesting findings became apparent.

The results of our study showed that the majority of diffuse mixed cell lymphomas had morphological features consistent with the peripheral T-cell lymphoma described by Waldron et al. and the T-immunoblastic lymphomas described by Lukes and Parker and other investigators (Figs. 3–5). However, in the absence of immunologic confirmation, we employed the term “non-FCC” for this subtype. These lymphomas characteristically showed a spectrum of cell sizes. The small lymphoid cells had round to irregular nuclear contours; however, “cleaved” nuclei were absent...
3–5). Some of the larger cells had moderate to abundant quantities of pale to clear cytoplasm. Clinically, most patients were elderly (median age 52 yr) white males and had a median survival of only 20 mo. Moreover, for patients who achieved a CR to therapy, survival times were significantly longer than those of patients with either partial or no response (Fig. 9) (p = 0.003).

On the other hand, the cells of diffuse FCC lymphomas had morphological features similar to those observed in follicles (cleaved and/or noncleaved cells) (Figs. 1 and 2). The natural history, response to therapy, and survival of patients with the FCC subtype appeared to differ from those observed for the non-FCC subtype. In the FCC subgroup, the median survival was not reached, and most of the patients were alive (Fig. 7) (p = 0.07). A statistical comparison of the FCC and non-FCC subtypes showed that the median survival of patients with the FCC subtype was longer; however, the difference was of borderline statistical significance because of the small number of patients in the FCC group and because the majority were still alive (Fig. 7). This trend, indicating a longer survival for the FCC subtype, gained support from the Cox regression analysis (p = 0.07).

When we compared survival times based on response to therapy among patients with FCC subtype, we found that patients who achieved a CR had survival times similar to those observed in patients who did not achieve a CR to therapy (Fig. 8) (p = 0.32). These data are similar to the results reported in most studies on nodular poorly differentiated22–34 and nodular mixed30,31,33–35 cell lymphoma, as well as in studies in which the Lukes and Collins classification was employed.16 However, more recent studies reported from the National Cancer Institute17,18 and the Eastern Cooperative Oncology Group19 suggest that it might be important to obtain a CR in patients with nodular

### Table 5. Response to Therapy and Survival According to Histologic Subtypes of Diffuse Mixed

<table>
<thead>
<tr>
<th>Morphological Type</th>
<th>Response to Therapy</th>
<th>Duration of Response (mo)</th>
<th>Number of Patients</th>
<th>Fisher’s Exact p Value</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Median</td>
<td>Total</td>
<td>Alive</td>
</tr>
<tr>
<td>FCC</td>
<td>CR</td>
<td>8–84</td>
<td>NR</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Non-FCC</td>
<td>CR</td>
<td>2–108</td>
<td>NR</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>FCC</td>
<td>Non-CR</td>
<td>6–72</td>
<td>NR</td>
<td>4</td>
<td>3</td>
</tr>
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<td>Non-FCC</td>
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<td>8–84</td>
<td>NR</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Non-FCC</td>
<td>Non-CR</td>
<td>6–72</td>
<td>NR</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

NR, not reached; CR, complete remission; Non-CR, partial and no remission.

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**Fig. 8.** Malignant lymphoma, diffuse mixed cell type. In patients diagnosed to have FCC type of diffuse mixed cell lymphoma, there was no significant difference in survival between CR and NCR (p = 0.32). However, it should be noted that the sample size available for statistical comparison was small, and the p value should be interpreted in the light of this fact.

**Fig. 9.** Malignant lymphoma, diffuse mixed cell type. In patients diagnosed as having non-FCC type of diffuse mixed cell lymphoma, the survival of patients who achieved a CR to therapy was significantly longer than that of patients who did not (p = 0.003).
mixed cell lymphomas because the survival of complete responders was longer than that observed in the non-CR.

Detailed histologic studies among the histopathologic categories of non-Hodgkin's lymphomas has revealed important clinicopathologic differences that seriously influence the choice of therapy as well as the design and conduct of clinical trials. The recognition that most nodular (follicular) lymphomas carry a good prognosis and may be influenced adversely by intensive therapy has altered the clinical approach to these disorders. At most centers, patients with nodular ("favorable") lymphomas, except for the "histiocytic" type, are currently managed with nonaggressive therapy. In contrast, patients with most diffuse lymphomas has altered the clinical approach to these patients perhaps would be similar to the basis of this study spanned a period of 13 yr, and these patients were treated by a multitude of protocols at several different institutions.

On the other hand, the non-FCC diffuse mixed cell lymphoma has an aggressive natural history (median survival 20 mo), which was significantly influenced by achievement of a CR to therapy. Therefore, the non-FCC subtype should be considered as a high grade malignant lymphoma, and patients should receive aggressive combination chemotherapy.

The importance of recognizing subtle differences within broad histologic categories of non-Hodgkin's lymphomas is nowhere more evident than in the design of randomized prospective clinical trials. Comparing therapeutic outcomes in heterogeneous groups of patients can certainly lead to erroneous conclusions. Consequently, it should be recognized that the diffuse mixed cell category of non-Hodgkin's lymphomas includes lymphomas that are either of FCC origin (presumably B) or not of FCC origin (presumably T). In view of the fact that the prognosis of the former is relatively favorable, and that of the latter unfavorable, they should be clearly separated when comparing results of clinical trials.

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Malignant lymphoma, mixed cell type, diffuse

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