What Should Be the Morphologic Criteria for the Subdivision of Follicular Lymphomas?

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The members of the Pathology Panel for Lymphoma Clinical Studies undertook a collaborative study with the hope of resolving some of the controversies regarding the criteria and methods for the subclassification of follicular lymphomas (FLs). A group of 105 patients with FL were subclassified by seven hematopathologists according to two methods. In the first method, cases were subclassified according to the Rappaport, Lukes and Collins, and Working Formulation systems. In each of these systems, FLs are subclassified by estimation of the different cell populations, without actual counting of cells. In the second method, precise counts of different cells were made according to the standard and modified Berard methods. With this counting method, diagnoses were independently derived, based on counts provided by the seven pathologists, for large cleaved (LC), small noncleaved (SNC), and large noncleaved (LNC) cells. To ascertain what method and which criteria are most useful in predicting survival, we made clinicopathologic correlations. When the subjective (first method) diagnoses were rendered, and when the consensus diagnoses of the seven pathologists were used, there were no significant differences in survival among patients with the different subtypes. On the other hand, when we used the counting method of Berard (second method) and the cut-off points for the cell counts suggested by him for the subclassification, we were able to divide the patient population into prognostic subgroups. Because the cut-off points proposed by Berard are not derived objectively, we made statistical comparisons of survival curves to determine cut-off points (and thus to establish objective criteria). We found that the patient population could be separated into at least two prognostic groups, for SNC and/or LNC and for SNC + LNC + LC cells. The cut-off points which we derived differed with cell type, however. Until the usefulness of these new cut-off points is established, we recommend that the cut-off points and the counting method of Berard be used for the subclassification of FL. Because the choice of treatment for the different subtypes of FL is totally dependent on the histologic diagnosis, and because of the variability among the diagnoses of pathologists, treatment planning is difficult.

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FOLLICULAR (nodular) lymphomas (FLs) are subdivided morphologically into three cell types, small cleaved, large cell, and mixed (small cleaved and large cell). This subclassification, which usually is based on the subjective assessment of the percentages of small and large lymphoid cells (“histiocytes”), is important clinically, because major therapeutic decisions are based on it.‡ There is general agreement that nodular, poorly differentiated lymphocytic (NPDL) lymphoma is a low-grade malignant lymphoma,§ and that nodular “histiocytic” (NH) lymphoma is of an intermediate grade¶; however, there is disagreement regarding the clinical behavior of nodular mixed (NM) lymphoma.¶¶,¶¶¶ In a study by the National Cancer Institute, patients with NM had longer survival times than did patients with NPDL lymphoma.¶¶¶ Results obtained at Stanford University§ suggest that the NM type has a prognosis similar to that of NPDL, whereas the results of the Eastern Cooperative Oncology Group¶¶¶ indicate that the survival of patients with NM is significantly shorter than that observed in NPDL lymphoma. These conflicting data may be a result of disagreement among pathologists as to the number of large cells that are necessary for classification of a lymphoma as either the mixed cell type or the large lymphoid (“histiocytic”) type.

There are not only differences in the morphologic criteria for the subclassification of follicular lymphomas, but also in the methods used for determining the number of large cells. In the Rappaport,2 Lukes and Collins,14 and Working Formulation15 systems, the subclassification of FLs is subjective, based on estimates of the percentages of large cells within follicles and not on actual counting of cells. In the Berard method,15,16 on the other hand, actual counts of large noncleaved cells (LNC) are made and their number determine a subclassification. In the Working Formulation study, in which the Lukes and Collins classification was used, it was found that FLs of the small noncleaved (SNC) cell type had the most aggressive natural history among the FLs; they are placed in the high-grade category.16 These data suggest that increased numbers of SNC cells adversely affect survival.

In view of these considerations, we concluded that actual counting of large cleaved (LC), LNC, and SNC cells would be the optimal method of determining the precise criteria for the subclassification of FLs. Moreover, we believed that these criteria should not be based on preconceived ideas.

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regarding the cut-off points for the number of large cells, but that the cut-off points should be derived objectively on the basis of statistical comparisons of survival curves.

The current study, in which we set out to ascertain the precise criteria for subdivision of FLs, is based on a patient population taken from the Southwest Oncology Group (SWOG). All patients were treated uniformly and intensively with curative intent, and all were followed up for long periods (SWOG protocol numbers 7426 and 7713).17,18 The specific objectives of our study were: (a) to determine which parameters independently influenced survival, (b) to ascertain survival times based on subjective diagnosis (based on estimated counts), (c) to ascertain survival times based on actual counts by using two methods, and (d) to assess the relative value of the subjective and objective (counting) methods in predicting survival times.

MATERIALS AND METHODS

Histologic material from 105 patients with FL who had been treated previously in SWOG studies (nos. 7426 and 7713) was reviewed by seven hematopathologists. The details of patient selection have been described previously.19 In general, these patients were chosen for review because adequate histologic material was available, patient follow-up was >5 years, all had advanced disease (stage III or IV), and all had been treated uniformly with combination chemotherapy that included cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).17,18

The SWOG diagnosis of record is a refined diagnosis. The initial diagnosis is made by the local institution’s pathologist. A second review is made by the pathologists of the SWOG regional center, and a third review is made by the Repository Center for Lymphoma Clinical Studies.20,21 If the diagnoses by the regional center and by the Repository Center agree, they constitute the SWOG diagnosis of record. If the diagnoses disagree, the case is reviewed by the Pathology Panel, and its diagnosis is the SWOG diagnosis of record.19

A trial review session was held at the Repository Center at City of Hope National Medical Center on an 11× headed microscope. At this session, the design of the study and the morphologic criteria to be used were discussed. A series of cases were reviewed, and a consensus was obtained on the criteria for identification of small cleaved, LC, SNC, and LNC cells. The statistician for the study (G.E.M.), participated in this review to ensure that the research design was statistically adequate and that uniform standards were developed for the study. The published criteria of Lukes and Collins were circulated among the participants and, after extensive discussion, each pathologist was at liberty to use whatever criteria he or she felt were most useful for distinguishing the different cell types. All participants had an American Optics microscope with lenses having identical magnifications. Everyone had a WF10X eyepiece, and a 40× high-magnification lens. The field of view index was 18, and the diameter was 4.58 mm. The high-power field (HPF) for every pathologist was the same.

Rules and Criteria for Rendering of Diagnoses According to the Estimation Method

In the estimation method, FLs were subclassified according to cell type by estimation of different cell populations, without any attempt to count cells. Cases were diagnosed according to the Rappaport,2 Lukes and Collins,’4 and Working Formulation1 systems. To ensure consistency among the pathologists, we agreed on the following criteria for the rendering of diagnoses based on the three systems.

1. For the Rappaport classification,2 a case was categorized as NPDL when the estimated percentage of large cells was <25%. If the estimate for large cells was between 25% and 50%, the case was classified as NM; if the percentage of large cells was >50%, the case was classified as NH lymphoma. Rare cases were classified as undifferentiated when the majority of cells were interpreted as undifferentiated.

2. For the Lukes and Collins classification,14 a case was considered to be of the SC cell type when the predominant population was of the SC cell type. A case was classified as LC cell type when SC cells were mixed with LC and LNC cells; however, in this category, the estimate for LNC cells was always <25%. When the estimate for LNC cells was >25%, the case was classified as LNC cell type. A case was termed SNC cell type when SNC cells were the predominant cell population.

3. For the Working Formulation,1 a case was classified as small cleaved cell type when the estimated percentage of large cells was <25%. A case was classified as mixed-cell type when there was a mixture of small cleaved, LC, and LNC cells. If it was estimated that ≥50% of the cells were large cells, a case was classified as large-cell type. A case was considered to be of the SNC cell type when SNC cells predominated.

Rules and Criteria for Counting and Derivation of Diagnosis

Standard Berard counting method. The standard Berard13,14 method of deriving diagnoses is based on counts of LNC cells, with counts of 0 to 5 LNC cells per HPF giving a diagnosis of PDL; >5 to 15 LNC cells per HPF, NM; and >15 LNC cells per HPF, NH lymphoma.

Modified Berard method. In this study, we modified the Berard method19 in two ways: (a) Rather than counting LNC cells only, we applied the cut-off points of 5 and 15 cells to SNC cells only, to SNC + LNC cells combined, and to SNC + LNC + LC cells; and (b) we explored whether other numbers of cell counts provided better cut-off points with application to counts of LNC, SNC, SNC + LNC, and SNC + LNC + LC cells.

In addition, counts of the three cell types (SNC, LNC, LC) were made up to 50 cells. The pathologists did the counting only and did not translate the counts into diagnoses. The statistician (a) objectively derived diagnoses by using the 5-cell and 15-cell cut-off points, and (b) computed survival curves to obtain natural cut-off points.

Reasons for modifying the standard Berard method. 1. In the Rappaport classification, the nodular lymphomas are subdivided according to the subjectively determined relative number of large cells (histiocytes). Since the development of the Rappaport system, Berard,13,14 in his practice, has made precise counts of only LNC cells to subclassify follicular lymphomas, because he has found that counting of these cells is a more objective and reliable method than subjective estimation.

2. In the Lukes and Collins classification,14 on the other hand, FLs are subdivided based on the relative numbers of LC, SNC, and LNC cells; theirs is also a subjective method of estimation.

3. In the Working Formulation study,1 it was found that follicular lymphomas of the SNC cell type had the most aggressive natural history, and these lymphomas were placed in the high-grade category. The data from NCI studies suggest that increased numbers of SNC cells adversely affect survival. Therefore, we considered it important to include counts of these cells.

4. Rather than retaining current values of the cut-off point for the number of large cells required for the subclassification of follicular lymphomas, we decided that the criteria for cut-off points should be derived based on correlations of survival.

For each of the 105 cases, therefore, each of the seven pathologists
was required to count and record the number of LC, LNC, and SNC cells in a total of 10 HPF. The entire slide was screened, and only those follicles identified by the pathologist as showing a maximum of LC, SNC, and LNC cells were used for counting. The counts that are used in this study are the initial counts provided by the pathologists, and the results have been published. In 97 cases, at least four pathologists provided counts; therefore, in some cases, the average of 40 per HPF was used, whereas in others, the average of 70 per HPF was used.

To reach a diagnosis, we applied the standard and modified Berard criteria to the sum of the averages across 10 HPF of the three cell groups (LC, LNC, and SNC) for each case for each pathologist. Small noncleaved cells were included among the large cells because, with respect to nuclear size and the presence of distinct nucleoli, the SNC cells bore a greater resemblance to LNC cells than to small cleaved cells. In addition, the same determinations were made according to the standard Berard method, based only on counts of LNC cells.

**Statistical Methods**

Kaplan-Meier curves were computed and Mantel-Cox tests done for comparison of survival curves. Some of the comparisons had relatively low power for detecting a significant difference because of the small size of some subgroups and the small numbers of deaths in some of these subgroups. We used Cox regression models to examine the effect of potential prognostic variables on survival. Survival was calculated from the date when treatment was started until death or to the date of the last follow-up.

**RESULTS**

**Parameters Influencing Survival**

We derived Cox regression models for the diagnosis by each pathologist, for the consensus (the diagnosis agreed upon by four or more pathologists on initial evaluation or review), and for the SWOG diagnosis to determine which variables predicted survival times (Table 1). Variables considered in the step-up procedures were age, pathologic stage, sex, cell counts (LNC, SNC, and LC), and subjective diagnosis; those variables with a significance level <0.10 were included in the regression equation. When the Cox regression analysis was carried out for each pathologist, for the consensus, and for the SWOG diagnosis, patient age and pathologic stage were always important predictors of survival, with increased age and higher stage being associated with a poorer prognosis. As determined by univariate statistical analysis, the median survival of the 46 patients with stage III disease was significantly longer than that noted for the 59 patients with stage IV disease ($P = .049$). In stage III group, 38 patients (83%) obtained a complete remission (CR), whereas only 36 patients (61%) in stage IV group obtained a CR. The age range of our 105 patients was 20 to 85 years, with a mean of 54.1 years. Using a univariate analysis, patients $\geq$54 years of age had a shorter survival than those $\leq$54 years of age ($P = .03$). Patients $\geq$60 years of age had a shorter survival than those $<60$ years of age ($P = .01$); and patients $\geq$70 years of age had a shorter survival than did those $<70$ years of age ($P = .00001$). For three pathologists (B, C, and F), the number of SNC cells counted was also predictive; a greater number was associated with a poorer prognosis. For three pathologists (A, D, and G), the diagnosis of a "histiocytic" lymphoma was predictive of shorter survival. For pathologist E, neither counts nor subjective diagnosis contributed significantly to the prediction of survival. For the consensus diagnosis, the number of LNC cells was important, higher numbers being associated with a poorer prognosis. For the SWOG diagnosis, the presence of a mixed cell lymphoma was associated with shorter survival. (For the SWOG diagnosis, cell counts were not done.)

**Survival Based on Subjective Diagnosis**

Our previous study showed that there was great variability among the seven pathologists with respect to their subjective diagnosis and the actual cell counts. Therefore, it seemed appropriate to use only the consensus diagnosis and the overall averages of counts of the various cell types.

When we used the consensus Rappaport diagnosis to subclassify FLs into three groups, we obtained no significant difference in survival among these groups ($P = .33$). The results were similar with the Lukes and Collins classification.
culation ($P = .15$) and the Working Formulation ($P = .17$). In contrast, when we used the SWOG histologic diagnosis of record to generate survival curves, we found a highly significant difference (Fig 2) ($P = .001$).

**Correlation of Survival With Derived Diagnosis**

Using the standard Berard cut-off points of 0 to 5 LNC cells for PDL, >5 to 15 LNC cells for mixed, and >15 LNC cells for histiocytic lymphoma, we generated survival curves based on LNC cells (Fig 3). The cell count used was the average of the counts across 70 HPF (ie, 10 HPF for each pathologist for each case), which showed marginally significant differences among the cell types ($P = .07$). We applied these same cut-off points to SNC (Fig 4), to SNC + LNC (Fig 5), and to SNC + LNC + LC cells (Fig 6). Among these three, there was again a marginally significant difference for SNC + LNC cells ($P = .06$) (Fig 5); for the remaining two cell groups, the differences were not significant.

**Correlation of Cell Type and Cell Counts With Survival**

Table 2 shows the survival analysis for various cell types on the basis of increasing cell counts. The $P$ values listed are for pairs of survival curves at cell counts above and below sequential cut-off points. For LNC, the lowest $P$ value occurred at a cut-off point of four cells; the $P$ values then increased beyond the significant range. Based on these results, we could divide the patient population into only two groups ($P = .01$) (Fig 7).

For SNC, the $P$ values varied, with an initial lowest $P$ value at a cut-off point of three cells; the $P$ values then rose and began lowering again to the .01 range at seven cells and attaining their lowest level at 10 cells and then rising once more. Based on these results, we divided the patient population into three groups (0–3 cells, >3 to 10 cells, and >10 cells), and a statistical comparison of the survival curves for these three groups showed significant differences ($P = .009$) (Fig 8).

For LNC + SNC cells, the analysis illustrated in Table 2 suggested that there might be three patient groups, with a clear break at seven cells and possibly another at 16 cells. Although the difference among the groups was significant ($P = .015$) (Fig 9), it was due solely to the superior survival of the first group (0 to 7 cells), with no separation between the latter two groups (7 to 16 cells and >16 cells) (Fig 9).

For LNC + SNC + LC cells, the analysis for all large cells (LNC + SNC + LC) suggested that there were three patient groups (0 to 8 cells, >8 to 13 cells, and >13 cells) (Table 2). Statistical compari-

![Fig 2](image2) There were significant differences in survival times among the morphologic subtypes of follicular lymphomas when the SWOG diagnoses (subjective) were used ($P = .001$).

![Fig 3](image3) There were significant differences in survival times among the morphologic subtypes of follicular lymphomas when the standard Berard criteria (applied criteria to large noncleaved cells only) were used for subclassification of follicular lymphomas.
Fig 5. There were differences in survival times (P = .06) among the morphologic subtypes of follicular lymphomas when the Berard method was modified and applied to SNC plus INC.

son of these three patient groups revealed a significant difference among the groups, with that of 0 to 8 cells having the longest survival (P = .02) (Fig 10). A pairwise comparison between the group with >8 to 13 cells and the group with more >13 cells yielded a P value of only 0.40, however.

DISCUSSION

Parameters Influencing Survival

This attempt to establish definite and objective criteria for the subdivision of FLs was undertaken on a group of 105 patients, all of whom uniformly received aggressive combination chemotherapy[7][8] regardless of the histologic type. To ascertain whether histologic variables independently influence survival, we performed multivariate Cox regression analyses, taking into consideration clinical parameters, diagnosis, and cell counts (Table 1). The results of this analysis showed that the stage of the lymphoma and patient age were the most important parameters. In addition, when the diagnosis by each of the pathologists as well as that of the SWOG and the consensus diagnosis were used, it was clear that histologic characteristics were an independent parameter influencing survival. Specifically, we found that, for all except one pathologist, either the diagnosis or the cell counts were independent parameters influencing survival. Thus, based on these analyses, we considered it worthwhile to

Table 2. Paired Survival Analysis According to Objective Counts with Successive Cutoff Points (in Terms of Mantel-Cox P values)

<table>
<thead>
<tr>
<th>Cutoff Point* (No. of Cells)</th>
<th>LNC</th>
<th>SNC</th>
<th>LNC + SNC</th>
<th>LNC + SNC + LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>.149</td>
<td>.073</td>
<td>.172</td>
<td>.442</td>
</tr>
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<td>.809</td>
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<td>.033</td>
<td>.052</td>
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<td>.157</td>
</tr>
<tr>
<td>18</td>
<td>.777</td>
<td>.264</td>
<td>.176</td>
<td>.157</td>
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*Groups formed with cutoff point in lower group (eg. 0-2 v > 2).
Abbreviations: LNC, large noncleaved; SNC, small noncleaved; LC, large cleaved; □ designates the cutoff points (see also Table 3).
investigate the influence of subjective diagnosis and actual cell counts with survival.

Survival Based on Subjective Diagnosis

Because one of our purposes in this study was to find out whether the subjective diagnosis,4,9,14 or actual counting of cells15 (according to the standard and modified Berard methods) yields a meaningful separation of the survival curves, we first looked at the survival curves based on the subjective diagnosis.

When subjective diagnoses were rendered according to the Rappaport classification7 (Fig 1), Lukes and Collins14 classification, and the Working Formulation,1 based on the consensus diagnosis, there were no significant differences in the survival curves among the different subtypes. This result is in contrast with published reports showing that the FLs could be separated into meaningful prognostic subgroups.2,9,10 In most previous studies,2,9,10,25,26 FLs have generally been treated non-aggressively. It has been possible to separate the patient populations studied into at least two, if not three, prognostic groups. In the last 5 years, however, the histiocytic FLs have been treated aggressively, and a significant percentage of the patients have achieved a prolonged disease-free survival, which suggests a cure.9,10

For our 105 patients, the SWOG diagnosis of record showed that there were significant differences in survival times among the three subtypes of FL (Fig 2). Thus, in the standard process of arriving at the SWOG diagnoses of record, it appeared that histologic subclassification provided useful information; but this was not the case when the consensus diagnosis established in this study was used. Thus, in this study, even though some of the pathologists were able to make diagnoses that were predictive of survival, the consensus process used obscured these differences.

Survival Based on Actual Cell Counts

Use of standard Berard criteria for cut-off points. We used the cut-off points for LNC cells, as proposed by Berard, to ascertain whether this cell type was useful for the definition of prognostic subgroups. In this way, the patient population could be divided into two rather than three prognostic subgroups (Fig 3) (P = .07).

Use of the modified Berard method. We used the same cut-off points suggested by Berard also for SNC, LNC + SNC, LNC + SNC + LC cells, because (a) in the Lukes and Collins Classification and in the Working Formulation,
SNC is an important prognostic cell type; (b) it may be difficult to distinguish the LNC and SNC cells from one another; and (c) in the Rappaport system, the so-called "large" cells include the combination of the three cell types (LNC, SNC, LC). Although Figs 4 and 6 do not show any significant separation of survival curves, Fig 5 shows that, by using the cut-off points of Berard for combined SNC + LNC cells, one could obtain results (P = .06) similar to those noted for LNC alone (Fig 3; see previous paragraph). These data give credence to the Berard definition of LNC and to the cut-off points that they proposed.15

Derivation of new cut-off points. When we applied our data to the generation of survival curves by using sequential cut-off points (Table 2), we were able to obtain significant separations, not only for LNC (Fig 7), but also for SNC (Fig 8), LNC + SNC (Fig 9), and LNC + SNC + LC cells (Fig 10). We were able to derive three distinct prognostic subgroups only for the SNC counts, however (Fig 8). For each of the others, we were able to obtain only two prognostic subgroups. It becomes apparent that, by using cell counts, one can separate the patient population into at least two groups for each of the cell types. This clearly indicates the importance of counting (Figs 7–10). In contrast, when we used the subjective consensus diagnosis without counting (Fig 1), we found no separation of survival curves.

These results clearly show that, when cut-off points different from Berard's are used, both SNC and LNC cells are prognostically important. Thus, in the future, counting of cells may lead to a significant and more reproducible subclassification of FLs. This finding raises an important issue: What are the criteria for distinguishing SNC cells from LNC cells? Berard has stated that he refers to cells as LNC only when he can readily identify multiple, distinct nucleoli. The fact that different pathologists could identify either LNC or SNC cells, but not both, as having prognostic significance suggests that the criteria used in this study for the identification of these two cell types were not uniformly applied, difficult to apply, or invalid.

The criteria for distinguishing these two cell types are indeed difficult to apply. For example, the nucleoli of LNC cells are supposed to be larger than the nucleoli of SNC cells, according to the published criteria of Lukes and Collins. In actual practice, however, the nucleoli of the LNC cell appears to be relatively smaller because the nuclear and cell size are larger. On the other hand, in the SNC cell, since the nuclear and cell size are smaller, the nucleoli appear to be relatively larger. Moreover, if the LNC cell has a single nucleolus, it may be confused with a benign histiocyte.

Treatment Considerations Based on Morphology

Past studies have shown that there are significant differences in the natural history, response to therapy, and patient survival among the different subtypes of FLs. It is well known that patients with NPDL lymphoma may not require any treatment initially.25 When treatment is desired for relief of symptoms, there is no proven advantage of combination chemotherapy over the use of a single alkylating agent.26 On the other hand, the nodular "histiocytic" lymphomas are thought to be curable and are, therefore, treated intensive-ly.9,10 Our findings cast doubt on some of these generally accepted treatment practices and create a dilemma for both practicing oncologists and researchers.

The results of our study cause concern for the following reasons:

1. Because the choice of treatment for the different subtypes of FLs is totally dependent on the histologic diagnosis, it is imperative that the diagnosis rendered by the pathologist be reproducible and reliable. In our previous study, however, the diagnoses of seven "expert" pathologists had great variability, particularly when they were based on subjective evaluation, and major disagreement existed in 37% of the cases.19 A major disagreement was defined as two extreme diagnoses made on the same case (eg, one or more of the pathologists diagnosed NPDL and one or more of the other pathologists in the group diagnosed NH). These data suggest that treatment decisions based on histologic criteria are sometimes misdirected.

2. Moreover, this study showed that when we used the consensus subjective diagnosis, we found no significant differences in survival among patients with the different subtypes of FLs, indicating that survival is not necessarily predicted by the histologic findings as perceived by a consensus of "experts."

3. The results of our previous study showed that the greatest disagreement among the pathologists concerned the NM category.19 All seven pathologists could agree with the diagnosis of NM in only one of the 39 cases studied, and some of these were classified as NPDL, whereas others were categorized as NH.

These findings suggest that some patients with NPDL lymphoma may have a potentially curable form and that new techniques are needed for their identification. Routine administration of intensive drug regimens to all patients who have presumed NPDL lymphoma seems unreasonable, however, because most patients would experience unnecessary toxic effects.26

Debates regarding the appropriate management of NM lymphoma patients are moot because this disease category cannot be identified reproducibly. At institutions that have identified a single pathologist who has an established record of relating diagnoses to survival, treatment should be individualized. For most practicing oncologists, our results suggest that a diagnosis of NM lymphoma carries a substantial risk of being changed to NH lymphoma if a histologic review is conducted; consequently, patients should probably be given a trial of intensive chemotherapy designed for cure. In addition, investigators should be cautious to include the NM category in studies of either indolent or aggressive histologic types because the survival of these patients is not reproducibly predictable. As a possible solution to the problem, some investigators27 have suggested that immunologic methods may be useful in the identification of subgroups of NM. Immunologic interpretations are often subjective, however, and very little information is available regarding the reproducibility and the interobserver agreement on the interpretation when the results are based on immunologic techniques.28 Moreover, NM is a morphologic and not an immunologic diagnosis. It would be worthwhile to ascertain whether
growth fractions as determined by immunostaining with monoclonal antibody Ki-67, which distinguishes low-grade from high-grade lymphomas, is helpful in the subclassification of FL.

Some patients with a diagnosis of NHL lymphoma are probably not curable and are likely to have an indolent disease. Nevertheless, a high percentage of patients with this diagnosis have aggressive disease and, like those with the NM type, should receive a trial of intensive chemotherapy.

Can We Propose New Criteria (Cut-Off Points) for the Subclassification of FLs?

Based on the analyses illustrated in Table 2 and in Figs 7 through 10, we were able to generate cut-off points that might be useful for the subclassification of FLs (Table 3). These cut-off points would have to be verified independently on a different group of patients, however, and it would be necessary to ascertain whether they are indeed useful before they could be recommended for routine application.

Conclusions

Even though our previous study showed great variability among pathologists, certain conclusions can be drawn regarding the most appropriate method for subclassifying FLs. This could only be achieved because we analyzed the data in various ways (making a subjective diagnosis based on estimation of numbers of cells, counting cells and applying the standard and modified Berard methods to derive a diagnosis, and finally using data from these analyses to generate a new set of survival curves). We conclude the following:

1. Actual counting of cells is probably a method for arriving at a diagnosis that is superior to making a diagnosis based on subjective assessment of the percentages of different cells.
2. The cut-off points suggested by Berard for the subclassification of FLs based on the number of LNC cells appeared to be generally valid. This method divided the patients into only two groups with significantly different survival times. In the future, however, this method may be more reproducible and may also make it possible to select the group that requires aggressive therapy.
3. The cells that probably have a major influence on survival are those transformed lymphoid cells which contain multiple, distinct nucleoli. These cells were presumably identified by some investigators as SNC cells and by others as LNC cells.
4. Whether large transformed lymphoid cells with relatively small nucleoli (LNC) have a different biologic behavior than do transformed cells of intermediate size with multiple, relatively large nucleoli (SNC) cannot be established from our study.
5. It is difficult for "expert" hematopathologists to separate favorable from unfavorable FLs reproducibly by using subjective morphologic criteria alone.
6. If patients with NM lymphoma are identified by pathologists who do not have an established record for predicting survival based on morphologic subclassification, these patients should receive intensive combination chemotherapy designed for cure.
7. More objective and reproducible methods are needed for the separation of patients into favorable and unfavorable prognostic groups.

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