Cytogenetic and histopathologic data were correlated with clinical parameters from 423 patients with non-Hodgkin’s lymphoma (NHL). Clinical correlations were performed on subgroups of 149 patients with low-grade lymphoma (LG) and 205 patients with diffuse lymphoma with a large cell component (DLLC). Correlations were made between clinical outcome and individual recurring cytogenetic aberrations, each of which was noted in >5% of cases belonging to LG NHL and DLLC, and derived measures of karyotypic complexity, comprising modal chromosome number, number of marker chromosomes, and number of translocation breakpoints. No correlations with survival were noted in LG NHL, although median follow-up was only 2 years. Seven patients with t(8;14) LG NHL had an indolent course. Among 104 patients with DLLC and abnormal karyotypes at diagnosis, breaks at 1q21-23 or more than 4 marker chromosomes was associated with a shortened median survival. Using these variables we constructed a proportional hazards model with a good fit to observed data. Breaks at 6q21-25 predicted a decreased probability of achieving remission. Patients with DLLC and breaks at 1q21-23 or 1p32-36 had a shorter duration of complete remission. Of 41 DLLC studied at relapse, the only long-term survivors had t(14;18).

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rately. Triploid or tetraploid clones were reflected in the measure of modal number; structural abnormalities (if present) were measured as number of breaks or marker chromosomes. For analysis of correlations between karyotype and histologic subtype, the method of inference from proportions based on a chi-square analysis of two-way tables was used.\(^2^\) Means were compared with two-sample \(t\) tests.

Survival analysis was performed on subsets of patients with differing cytogenetic characteristics using the method of Kaplan and Meier.\(^2^\) Cytogenetic subsets were composed of cases with any breakpoint or numerical chromosomal abnormality observed with greater than 5% incidence in this series, cases with a recurring reciprocal translocation, or cases partitioned according to measures of karyotypic complexity. Actuarial survival curves were compared using the log-rank test. Detailed survival analysis was limited to patients with cytogenetics sampled at time of diagnosis and two histologic subsets: (a) low-grade (LG) NHL, consisting of the Working Formulation diagnoses of small lymphocytic, small cleaved cell follicular, and mixed follicular; and (b) diffuse lymphoma with a large cell component (DLC), including diffuse mixed, large cleaved, large noncleaved, and immunoblastic subtypes. These groups were analyzed separately because these subgroups of NHL have been shown to have different natural histories and responses to therapy. Multivariate analysis was restricted to the largest subset of DLC patients. The Cox proportional hazards regression model\(^2^\) was used to identify a subset of variables that had significant impact on survival. Diagnostic hazards plots were performed whenever feasible to ascertain the appropriateness of the proportional hazards assumption. Multiple logistic regression was used in the multivariate analysis of factors prognostic for achieving a complete response.

RESULTS

Correlation between cytogenetic status and clinical characteristics. Of 423 patients from whom specimens for cytogenetic analysis were obtained, the median age at diagnosis was 54 years (mean 51.4 years, range 6 to 82 years). There were 231 males and 192 females in the series. There was no difference in age, stage, or LDH between patients with normal metaphases or those with failed cytogenetic analyses.

Lymphomas of LG histology. Of 149 specimens of LG NHL, 86 demonstrated an abnormal karyotype. The cytogenetic features of these cases were reported separately.\(^3^\) The most common cytogenetic aberrations were, in decreasing order of frequency, chromosomes with translocation breaks at 14q32 (66.3% of cases), 18q21 (55.8%), 1q21-23 (17.4%), 1p32-36 (14%), 6q21-25 (12.8%), trisomy 3 (12.8%), trisomy 18 (9.3%), 8q24 (8.1%), trisomy 12 (8.1%), trisomy 7 (7.0%), breaks at 1p32-22 (7.0%), trisomy 21 (5.8%), trisomy 11 (5.8%), and trisomy 5 (5.8% of cases). Other aberrations were noted in less than 5% of cases. Of the reciprocal translocations, t(14;18)(q32;q21) was noted in 47 (54.6%) specimens, t(8;14)(q24;q32) in 7 (8.1%) specimens, and t(11;14)(q13;q32) in 3 (3.5%) specimens.

Cytogenetic analysis was performed before treatment on 99 specimens, of which 53 specimens, derived from 53 patients, showed abnormal karyotypes. The median age at diagnosis of the 53 cases was 59 years (mean 56 years, range 23 to 81 years). Of these, 28 patients were stage IV, 11 were stage III, 7 were stage II, and 7 were stage I at diagnosis. Patients were managed with a variety of treatment strategies: 15 patients received doxorubicin-containing combination chemotherapy, 12 patients received alkylating agents with or without radiation therapy, 9 patients were treated by a Memorial Hospital protocol using “intermediate intensity” combination chemotherapy,\(^2^\) 6 patients received radiotherapy only, 6 patients received no therapy, 2 patients had surgery only, and 3 patients had biologic response modifiers or other therapies. Of the 53 patients studied before treatment, 47 were alive at a median follow-up of 2 years.

When the analysis was restricted to patients studied at the time of diagnosis there were no correlations between survival duration and type of treatment, presence or absence of normal metaphases, modal number, number of translocation breaks, or number of marker chromosomes. Univariate analysis was performed on survival of subsets of patients whose tumors demonstrated individual recurring cytogenetic aberrations each observed in > 5% of LG NHL. There were no differences between subsets of patients with or without t(14;18) (29 patients). Seven patients had LG tumors with t(8;14); the median age of five of these patients whose tumors were studied before treatment was 59.4 years as compared with 55.7 for the non-t(8;14) LG NHL. There was no difference in survival of the t(8;14) subgroup as compared with patients without this translocation; all five patients were alive at median follow-up of 14.6 months from diagnosis. One patient (patient 59) with stage IV mixed follicular lymphoma and t(8;14) was alive at 36 months, never having received treatment.

Univariate analysis of survival of subsets of cases with other chromosomal aberrations observed in more than 5% of LG NHL did not show any significant correlations; this analysis was limited by the small numbers of pretreatment samples and the excellent overall survival of the entire group. Fourteen patients demonstrated either trisomy 7, trisomy 12, or breaks at 1p32-36; the median survival of these patients was 56 months from diagnosis, as compared with a median not reached for patients with LG NHL did not show any significant correlations; this analysis was limited by the small numbers of pretreatment samples and the excellent overall survival of the entire group. Fourteen patients demonstrated either trisomy 7, trisomy 12, or breaks at 1p32-36; the median survival of these patients was 56 months from diagnosis, as compared with a median not reached for patients with abnormal karyotypes without these abnormalities. The survival curves for these groups were significantly different (\(P = .028\)) (Fig 1).

Thirty-three specimens derived from 31 patients with LG NHL were studied at the time of relapse; 22 were alive at a median follow-up of 74 months from diagnosis and 15 months from cytogenetic analysis. There were no correlations between survival from time of relapse and subsets of patients with individual recurring aberrations, each observed in more than 5% of LG NHL.

DLLC. Of 285 specimens of intermediate-grade and high-grade NHL ascertained, 205 (71.9%) met the criteria of DLLC; of these, 145 demonstrated abnormal karyotypes. The cytogenetic features of these cases were reported separately.\(^3^\) The most frequently observed aberrations in this group were chromosomes with breaks at 14q32 (28.6% of cases), 18q21 (17.2%), 1q21-23 (16.6%), trisomy 7 (16.6%), 8q24 (15.2%), 6q21-25 (14.5%), trisomy 12 (13.8%), breaks at 1p32-36 (11.7%), trisomy 5 (9.7%), trisomy 3 (9%), trisomy 18 (9%), breaks at 1p22 (7.6%),
breaks at 7q32 (7.0%), trisomy 11 (6.2%), and aberrations of 17p,q, or cen (6.2% of cases). The most frequent recurring translocations were t(14;18) (17.2% of cases), t(8;14) (14.5%), and t(3;22)(q27;q11) (4.1% of cases).

Abnormal karyotypes were detected at the time of diagnosis in 106 specimens derived from 104 patients; the median age of the 104 patients was 55 years (mean 53.3 years, range 12 to 76 years), 5 were stage I, 26 were stage II, 28 were stage III, and 42 were stage IV. The median LDH at the time of diagnosis for the 76 patients on whom data were available was 272 (mean 441, range 102 to 3,240).

Of the five stage I patients, all had solitary extranodal lesions that were resected and treated with adjuvant chemotherapy. Thirty-two patients received treatment with first-generation combination chemotherapy (NHL-5,25 CHOP, or BACOP), 21 with second-generation chemotherapy (NHL-7,26 m-BACOD), and 41 with third-generation chemotherapy (MACOP-B, NHL-9L17/20)]; 8 received radiotherapy plus nonanthracycline-containing chemotherapy, and 2 died before treatment could be given (one death was a suicide). The median survival of the 104 patients was not reached; actuarial survival for the group was 53% at 5 years.

There was no difference in survival between patients with abnormal karyotypes as compared with patients with normal karyotypes or those who were cytogenetic failures. Univariate analysis showed no correlation between survival and age greater than the median, A v B symptoms, bulk of disease, histologic subtype of DLLC, or generation of chemotherapy treatment. Of the 76 patients with LDH data at diagnosis, 19 patients with values greater than 500 had a diminished survival (P = .02); of the 101 patients on whom staging data were available, those with stage IV disease had diminished survival (P = .01).

Of 16 patients with t(8;14) or variants, the mean age of the t(8;14) patients was 10 years younger than DLLC with abnormal karyotypes lacking t(8;14); the mean LDH and survival of this cohort, however, was no different from that of other DLLC patients (Table 1). Fourteen cases of DLLC demonstrated t(14;18)(q32;q21) in biopsies obtained before cytotoxic therapy. The mean age of the t(14;18) DLLC patients was 11.3 years older than for the non-t(14;18) DLLC; there was no difference in mean LDH or proportion stage IV, although there was a trend for decreased survival of the t(14;18) subset of patients (P = .07) (Table 1). Two patients had a history of transformation of histologic grade. One had a previous history of chronic lymphocytic leukemia, and another had a history of follicular small cleaved lymphoma. Neither patient had received cytotoxic chemotherapy at the time of cytogenetic analysis, both showed a t(14;18) with multiple additional abnormalities, and both died within 2 months of transformation.

Among the individual recurring chromosomal aberrations observed in more than 5% of DLLC, significant correlations with survival were noted for subsets of patients with breaks on the long arms of chromosomes 1 or 6. Eighteen patients whose tumors demonstrated a translocation break at 1q21-23 and 12 patients with breaks at 6q21-25 had median survivals of less than 1 year; their survival curves are shown in Figs 2 and 3 (P < .001). The shortened survival of patients with DLLC demonstrating breaks at 1q21-23 or 6q21-25 remained significant in the smaller subset of 41 patients receiving “third-generation” combination chemotherapy (P < .001, P < .001, respectively). Partitioning of the survival analysis according to

![Fig 1. Actuarial survival from time of diagnosis of 14 patients with LG lymphoma and abnormal karyotypes at diagnosis with trisomy 7, trisomy 12, or breaks at 1p32-36 as compared with 39 patients without these abnormalities.](image)

### Table 1. Survival of Clinical and Cytogenetic Subsets of DLLC Patients With Karyotypic Analysis Performed at Time of Diagnosis

<table>
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<tr>
<th>Subgroup</th>
<th>n</th>
<th>Mean Age</th>
<th>Mean LDH</th>
<th>CR (%)</th>
<th>Median Survival (mo)</th>
<th>Value*</th>
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<tr>
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<td>63</td>
<td>NRY</td>
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<td>50</td>
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<td>—</td>
<td>37t</td>
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<td>.02</td>
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<td>44.2t</td>
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<td>NRY</td>
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<td>&gt; 4 Markers</td>
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<td>48</td>
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<td>.01</td>
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The cytogenic groups are defined by the presence of listed abnormalities, numbers of marker chromosomes, or chromosome breaks as defined in the text; 1q21-23, 1p32-36, and 6q21-25 indicate tumors with rearrangements involving these sites appearing as nonrandom abnormalities, and str17 denotes tumors with any clonal rearrangements affecting chromosome 17 (p,q, or cen).

Abbreviations: NRY, not yet reached; LDH, lactate dehydrogenase; DLLC, diffuse lymphoma with large cell component; CR, complete remission (survival in months).

*P value for survival differences by logrank test comparison of Kaplan-Meier survival curves.

†Significantly different mean value from subset without this aberration.
level of LDH did not diminish the prognostic value of the two cytogenetic variables; 13 patients with either a break at 1q21-23 or 6q21-25 and LDH < 441 had a shorter survival as compared with 44 patients without these aberrations and LDH < 441 (P = .01). Similarly, 19 patients with LDH > 441, five patients with breaks at 1q or 6q had a decreased survival (P = .01). There was no difference in the mean age or LDH of patients with breakpoints at 6q21-25 or 1q21-23 as compared with patients without such breaks (Table 1).

In 3 of the 18 patients with 1q21-23 breaks, the tumors had a reciprocal translocation involving both 1q and 6q breaks; the survival of these patients from diagnosis was 6, 8, and 10 months, despite intensive combination chemotherapy. In none of these patients was the t(1;6) accompanied by a t(14;18), t(8;14), or t(11;14). In all of these patients, the break on chromosome 1 was at band q21; the break on chromosome 6q could not be unequivocally resolved in one patient, it was 6q21 in one patient, and 6q25 in another.

Among the other cytogenetic subsets analyzed by univariate analysis there were no correlations with patient survival; there was a trend for diminished survival for 19 patients with trisomy 7 (P = .07). The median survival was only 20 months for five patients with structural abnormalities of chromosome 17. The mere presence or absence of unidentified marker chromosomes was not a prognostic factor for survival (P = .27). Significant correlations were noted between survival and derived measures of karyotypic complexity. The cohorts of 34 patients with more than four marker chromosomes (P < .001) and 27 patients with more than 4 breakpoints (P = .01) had decreased survival. There was limited coincidence of these measures of chromosomal complexity and other cytogenetic aberrations of prognostic utility; of the 18 patients with 1q21-23 breaks, 10 (56%) had more than five markers; of the 12 patients with 6q21-25 breaks, 6 (50%) had more than five markers. Of the five patients with structural abnormalities of chromosome 17, all had complex karyotypes with more than four marker chromosomes and four to nine breaks.

Cox regression analysis was used to identify a subset of variables which, in combination, significantly predicted survival. However, multivariate analysis was limited both by overall sample size and by our ability to assess the assumption of proportional hazards in the multivariate setting. Two variables, number of marker chromosomes greater than 4 and breaks at 1q21-23, where chosen in this analysis because of their individual contribution to prediction in the bivariate setting (Fig 4). Breaks at 6q21-25 appeared to contribute additional prognostic power to the multivariate model; however, this variable was not included because of difficulties in validation of the more complex model. The other clinical and cytogenetic variables were outweighed by these cytogenetic variables in the multivariate analysis.

With respect to the remission status, univariate analysis identified one cytogenetic variable for study in the multivariate model; breaks at 6q21-25 correlated with a diminished probability of achieving complete remission in a stepwise logistic regression model (P = .005).

Among the 66 patients who achieved complete remission, there was no correlation between duration of the remission and age, stage, or LDH at diagnosis. Among the cytogenetic variables tested, duration of complete remission was also shorter in nine patients with breaks at 1q21-23 and seven patients with breaks at 1p32-36; the actuarial curves differed from those for patients without such aberrations (P = .02, P = .0002, respectively).

In 41 specimens derived from 41 patients with DLLC, cytogenetics was studied at the time of relapse. As expected, the overall outcome for these patients was dismal, with a median survival of only 14 months. Patients with t(14;18) had a median survival not yet reached from time of posttreatment cytogenetic sampling, as compared with 12.8 months in patients without this translocation (P = .07) (Fig
tumors who died, 3 had previous LG lymphomas. All 10 of the t(14;18) patients sampled at relapse had multiple additional secondary abnormalities, but small numbers in cytogenetic subsets precluded correlation with median survival after relapse. No other primary or secondary cytogenetic abnormality was of prognostic value in the cohort of 41 previously treated patients; homogeneously staining regions (HSRs) were detected in 2 patients, both of whom died within 1 year of their detection.

DISCUSSION

Correlations between nonrandom cytogenetic aberrations and histologic and immunophenotypic subsets of NHL have been established and confirmed in large series. Recently, several studies identified cytogenetic subsets of NHL with differing response to therapy, survival, and clinical behavior. These studies were limited by small numbers of patients in the cytogenetic subsets, mixing together of subsets of patients with different histologies and grades of NHL, inclusion of samples studied after cytotoxic treatment, and lack of breakpoint-specific chromosomal analysis.

In the current study, sufficient numbers of patients were available to permit analysis by histologic subgroup. Although different therapies have yet to demonstrate a significant effect on survival of patients with LG lymphomas in single-institution trials, analysis of this cohort in the current study was limited by different treatment approaches and short median follow-up. The survival of patients showing trisomy 7 or trisomy 12 or breaks at 1p32-36 was shorter than that of patients without these abnormalities; however, the relatively long median survival of this cohort (> 4 years) limits the clinical utility of this observation. Other cytogenetic features reported to correlate with a differing clinical behavior in LG lymphomas, including the presence of normal metaphases, trisomy 2, or deletions of 2p, did not correlate with clinical outcome; other aberrations, eg, chromosome 17 abnormalities and breaks at 13q32, were observed too infrequently to analyze.

Most surprising in the LG lymphomas was the observation of t(8;14)(q24;q32) in seven patients, five of whom had not yet been treated. The indolent clinical behavior of the disease in these patients (one patient remained untreated > 2 years) suggests a biology of disease different from that of high-grade NHL bearing the identical translocation.

Analysis of the DLLC-containing histologies did confirm a trend for decreased survival of t(14;18) bearing DLLC, although not at the level of significance of another series. This translocation, usually associated with follicular NHL, was also associated with an older age at diagnosis and a trend for prolonged survival at the time of relapse, suggesting a resemblance to the natural history of follicular lymphoma. Continued follow-up of this cohort will, however, show continued lymphoma-related mortality as patients die of the complications of recurrent disease. Although observed in 15% of DLLC tumors, the clinical behavior and clinical features at presentation of patients with t(8;14)-bearing DLLC were no different from that of other DLLC.

Multivariate analysis showed that two cytogenetic vari-
The variable treatment regimens and short median follow-up in this study suggest the need for large-scale prospective trials using uniform treatment approaches. Although such trials may help resolve some of the reported differences in cytogenetic-clinical correlations in DLLC, this approach will not necessarily be definitive. First, in DLLC it is unclear that any anthracycline-containing treatment regimen is superior to another.
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Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: clinical correlations

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