RAPID COMMUNICATION

Prognostic Value of Chromosomal Abnormalities in Follicular Lymphoma

By Hervé Tilly, Annick Rossi, Aspasia Stamatoullas, Bernard Lenormand, Claude Bigorgne, Antoine Kunlin, Mathieu Monconduit, and Christian Bastard

The t(14;18)(q32;q21) chromosomal translocation is observed in more than 75% of cases of follicular lymphoma. Several additional chromosomal abnormalities, which might contribute to tumor progression, have also been described. However, prognostic implications of cytogenetic features in follicular lymphoma have not been clearly established. In an attempt to correlate cytogenetic findings with clinical outcome, we have studied survival and risk of transformation into a more aggressive lymphoma in 66 follicular lymphoma patients from whom a lymph node had been karyotyped at the time of diagnosis. A t(14;18) was the most common abnormality, having been observed in 51 patients (77%), but this showed no correlation with clinical outcome. Seventeen other recurrent numerical or structural abnormalities were identified in more than 10% of the patients. A high percentage of cells (≥ 90%) with abnormal metaphases and a number of chromosomal breaks higher than 6 were associated with a poor survival (P < .01 each). Patients with an abnormality of chromosome region 1p21-22 (P < .01), of 6q23-26 (P < .001), or of the short arm of chromosome 17 (P < .001) had a significantly shorter survival in univariate analysis. Multivariate analysis identified a break at 6q23-26 (P = .01) and 17p (P = .01) as independent prognostic factors in this population. The risk of transformation into a diffuse large-cell lymphoma was significantly higher in patients with either a 6q23-26 (P < .001) or a 17p (P < .01) abnormality. Chromosomal analysis of follicular lymphoma at the time of diagnosis can thus provide important information about the risk of transformation and survival. Such analysis could help to define a subgroup of follicular lymphoma patients who might benefit from early introduction of intensive therapy.

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FOLLICULAR LYMPHOMA is defined as a nodular proliferation of B cells arising from the follicular center of the lymph node.1 According to the Working Formulation for clinical usage,2 three histologic subtypes can be characterized: those in which the neoplastic population comprises predominantly small cells or a mixture of small and large cells are considered low-grade lymphomas, whereas those composed of predominantly large cells are considered intermediate grade lymphomas. When histologic transformation, usually into a diffuse large-cell lymphoma, occurs during the course of the disease, it is associated with a worsening of the prognosis.3

Most patients with follicular lymphoma present with asymptomatic but advanced disease (stages III and IV).4 Although follicular lymphoma is usually sensitive to radiotherapy and chemotherapy, recurrence of the disease after an initial response is the rule and there is no evidence, to date, that patients with advanced disease can be cured by these treatments, regardless of how intensive they could be.3 These findings, together with the frequent indolent course of the disease, raise the question of whether initial therapy should be deferred in selected patients.5 Many factors that correlate with clinical outcome of patients with follicular lymphoma have already been described, including age, sex, B symptoms, Ann Arbor stage, serum lactate dehydrogenase (LDH) level, serum albumin level, bone marrow involvement, and the number of extranodal sites of the disease.6-10 Some of these variables have been incorporated into prognostic indexes.

The typical chromosomal abnormality in follicular lymphoma is the translocation t(14;18)(q32;q21),11 which is found in more than 75% of cases. At the molecular level, this translocation juxtaposes the bcl-2 proto-oncogene (band 18q21) with the Ig heavy chain gene (band 14q32), resulting in deregulation of bcl-2 gene expression and elevation of bcl-2 mRNA and protein.12-14 Deregulated BCL-2 protein is thought to inhibit the programmed cell death (apoptosis) and confer a survival advantage to the lymphoma cells.14,15 Besides the t(14;18), several other recurrent chromosomal defects are found; these defects have been correlated by Yunis et al16 with tumor histopathology and evolution. Nevertheless, prognostic significance of cytogenetic findings has not been as well documented in follicular lymphomas as in acute and chronic leukemias.17,18

MATERIALS AND METHODS

Patients. Cytogenetic analysis was performed on cells from lymph nodes or other sites of disease from 425 consecutive patients with non-Hodgkin's lymphoma admitted to the Centre Henri Becquerel (Rouen, France) between July 1984 and June 1992. Selection criteria for this study were (1) a diagnosis of follicular lymphoma all confirmed by the same pathologist; (2) absence of previous treatment for the disease; and (3) successful cytogenetic analysis, with interpretable abnormal metaphases, performed on the sample that has been analyzed by the pathologist. One hundred and five patients responded to the first step of selection, 72 to the second, and 66 fulfilled all three criteria.

From the Departments of Clinical Hematology, Pathology and Surgical Oncology, Centre Henri Becquerel, Rouen; the Department of Cytogenetics, Centre Régional de Transfusion Sanguine et de Génétique Humaine, Bois-Guillaume; and the Department of Biological Hematology and Cellular Immunology, Hôpital Charles Nicolle, Rouen, France.

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Address reprint request to Hervé Tilly, MD, Centre Henri Becquerel, Rue d'Amiens, 76038 Rouen Cédex, France.

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Staging and treatments. Staging was based on medical history, physical examination, and the results of a complete blood count; measurement of hepatic enzymes levels, serum albumin level, and LDH level; chest radiograph; computed tomographic scan of the chest, abdomen, and pelvis; and iliac bone marrow aspiration and biopsy. The clinical characteristics of the 66 selected patients are listed in Table 1.

The choice of initial treatment depended on age of the patient, stage of the disease, histologic subtype, and period of diagnosis. Eight patients with stage III or IV disease and an apparently indolent clinical course did not receive any treatment at the time of primary diagnosis. Six patients with stage I or II disease and no bulky disease were initially treated with radiotherapy alone. Before 1987, 16 patients with disseminated disease received first single-agent chlorambucil with or without radiotherapy. Subsequently, 22 patients aged less than 70 years with low-grade follicular lymphoma, disseminated disease, and a high lymphoma burden were included in a study from the Groupe d’Etude des Lymphomes de l’Adulte (GELA) and treated with a doxorubicin-containing regimen with or without randomly assigned α-interferon. Eleven patients with a predominantly large-cell follicular lymphoma and a high tumor burden were treated with an intensive GELA chemotherapy regimen (LNH-84 or LNH-87). Three patients were initially treated in a phase II study: 2 with α-interferon alone and 1 with fludarabine.

At the time of this analysis (September 1993), 35 patients were dead and the median follow-up was 68 months. Transformation into a diffuse large-cell lymphoma was documented, on histologic grounds, in 8 patients.

Tissue samples and cytogenetic analysis. Fresh lymph nodes were divided into two portions for morphologic and cytogenetic studies. All histologic material was reviewed by the same pathologist without knowledge of cytogenetic data and classified according to the Working Formulation for clinical usage. Methods for cytogenetic analysis have been previously described. R-banded metaphases were karyotyped and chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature. For each sample, at least 10 metaphases were karyotyped and the percentage of abnormal metaphases was estimated.

Statistical methods. Overall survival was chosen as major endpoint in this study. Time to transformation was also studied as a minor endpoint. Curves were plotted using the method of Kaplan and Meier and were compared using the log-rank test. Ninety-five percent confidence intervals were determined as described by Rothman. Univariate prognostic factor analysis was performed considering the following variables: age, sex, histology, performance status, presence of B symptoms, Ann Arbor stage, number of extranodal sites, bone marrow involvement, presence of a mass over 10 cm in diameter, LDH level, serum albumin level, percentage of abnormal metaphases, modal number of chromosomes, number of chromosomal breaks as defined by Offit et al, and all chromosomal abnormalities occurring in 7 patients or more. Because many statistical tests were undertaken, a P value of .01 was used as the criterion for statistical significance. Proportional hazards analysis was used to assess simultaneous effect of these variables on survival. Analyses were performed with use of JPSI statistical software (developed by F. Kwiatkowski, Centre Jean Perrin, Clermont-Ferrand, France).

RESULTS

Chromosomal findings. Clonal chromosomal abnormalities were found in all patients. The percentage of abnormal metaphases varied from 14% to 100%, with 22 patients having more than 90% abnormal metaphases. The t(14;18) (q32;q21) translocation, observed in 51 patients (77%), was the most common abnormality. It was the sole aberration in 5, 3 of whom had a small cell follicular lymphoma. Two patients had a t(2;18)(p12;q21) translocation, observed in 51 patients (77%), was the most common abnormality. It was the sole aberration in 5 patients, 3 of whom had a small cell follicular lymphoma. Two patients had a t(2;18)(p12;q21) translocation, which is a variant form of the classical t(14;18). Several other recurrent numerical and structural abnormalities were found, and those present in more than 6 patients are listed in Table 2. The number of chromosomal breaks ranged from 1 to 17 (median, 6).

Survival. As shown in Table 1, the serum albumin level was the only clinical variable significantly associated with a worse prognosis in this series (P < .001). Survival was not significantly influenced by treatment choice, but only 1 patient in the initially untreated group died (P < .03).

Patients with a high percentage of abnormal metaphases
Abnormalities in follicular lymphoma

Numerical abnormalities

- +X: 14 patients with +X had 90% abnormal metaphases. 13 patients with +X had less than 90% abnormal metaphases.
- +12: 7 patients with +12 had 90% abnormal metaphases. 29 patients with +12 had less than 90% abnormal metaphases.
- +18: 13 patients with +18 had 90% abnormal metaphases. 53 patients with +18 had less than 90% abnormal metaphases.

Structural abnormalities

- t(14;18): 51 patients with t(14;18) had 90% abnormal metaphases. 51 patients with t(14;18) had less than 90% abnormal metaphases.

A multivariate analysis was performed to study the relative influence of chromosomal parameters and clinical data found to significantly affect survival in univariate analysis. Three characteristics were associated with a poor survival: serum albumin level (P = .001), 1p abnormality (P = .01), and 6q23-26 abnormality (P = .01). Influence of the percentage of abnormal metaphases was of borderline significance (P = .05), whereas an elevated LDH level (P = .34), 1p21-22 abnormality (P = .4), and presence of more than 6 chromosomal breaks (P = .53) did not retain their negative influence on survival.

Transformation. Two chromosome abnormalities were significantly associated with a shorter time to transformation: break involving bands 6q23-26 (P < .001) and break at 17p (P < .01). There was an association of borderline significance between the presence of a mass larger than 10 cm and transformation (P < .03). The number of patients with documented transformation was too small to allow a proportional-hazards analysis of factors influencing this event.

Poor prognosis group. The presence at diagnosis of a chromosome break at 6q23-26 or 17p defined a subgroup of 16 patients with a very poor prognosis (P < .00001) (Fig 3) and a shorter time to transformation (P < .001) (Fig 4). Presence of at least one of these chromosomal abnormalities
The crucial importance of these indications prompted several investigators to examine this hypothesis in non-Hodgkin’s lymphomas. The present study is devoted to a group of 66 patients with follicular lymphoma, whose lymph nodes were studied at the time of primary diagnosis and with a median observation period of 68 months.

Our study confirms the findings of Levine et al. that a high percentage of abnormal metaphases in the lymph node of patients with follicular lymphoma correlates with a shorter survival. This measurement has also been shown, in multivariate analysis of a very large series, to be the most important cytogenetic feature influencing the prognosis of B-cell chronic lymphocytic leukemia. This parameter could reflect the proliferative ability of malignant cells as compared with that of normal cells, or even reactive T cells, in these low-grade B-cell malignancies.

The prognostic significance of the t(14;18) in follicular lymphoma is controversial. Yunis et al. reported 20 cases of follicular lymphoma in which the presence of this translocation was correlated with a poor response to treatment and a short survival. However, this correlation was not supported by the results of Levine et al. in 30 follicular lymphoma patients. More recently, Pezzella et al. studied bcl-2 rearrangement and BCL-2 protein expression in 70 patients with follicular lymphoma and showed the absence of correlation between molecular data and clinical behavior. In keeping with these findings, the present study did not identify t(14;18) as a prognostic factor. In the same way, it is noteworthy that break at 3q27, mostly involved in B-cell lymphoma of a large-cell type and involving the newly described zing-finger encoding gene Iaz3, did not seem to determine a different outcome in our patients.

Two structural abnormalities were found to dramatically influence the survival of patients with follicular lymphoma. Breaks in 6q23-26, involving interstitial deletions, are found in 10% to 40% of the patients with B-cell lymphoma, either of low- or high-grade histologic subtype, and they have been shown to be one of the most frequent additional chromosomal abnormalities in lymphomas bearing t(14;18). In these cases, it has been suspected that deletion 6q could be associated with a more aggressive histology. The present study demonstrates that the presence, at the time of diagnosis of follicular lymphoma, of a deletion involving bands 6q23-26 and with a short survival. It has been recently shown that these deletions involve three distinct regions at 6q21, 6q23, and 6q25 to 27. The observation of specific deletions in these regions suggests the presence of three tumor-suppressor genes.

A break on the short arm of chromosome 17 was also significantly associated with poor survival and with an increased risk of transformation. Abnormalities were isocho- mosome 17q, which leads to the loss of all the short arm; translocations involving band 17p12; and deletions at band 17p11 and 17p13. Abnormalities of chromosome 17 have been previously associated by Cabanillas et al. with a poor response to chemotherapy and short survival in patients with lymphomas of all histologic grade. Levine et al. have found aberrations of chromosome 17 to be the only structural change predictive of clinical outcome in a series of 30 follicular lymphomas. The possibility has been raised by these investigators that these aberrations involve the p53 tumor suppressor gene, which is located at band 17p13. More recently, it has been shown that p53 mutations are associated with a significant proportion of histologic transformations of follicular lymphomas and that they could be detected in the pretransformation samples. There is evidence that wild-type p53 induces apoptosis in cultured cells treated with agents that cause DNA damage, such as radiation or etoposide. Loss of p53 function could lead to malignant cells survival, tumor progression, and resistance to treatment with radiation and chemotherapy.

Despite a policy to repeat biopsy in patients with progressing follicular lymphoma, results concerning transformation should be interpreted with caution. It has been previously underlined that histologic transformation more likely occurred in extranodal, and particularly intraabdominal, sites. Because no autopsy data were available in our study, we cannot exclude that some patients whose death was attributed to follicular lymphoma progression or to treatment-related complications had indeed a site of transformation. However, our results suggest that the influence of chromosomal abnormality at 6q23-26 and 17p on survival is, at least in part, related to an increased risk of early histologic transformation.

In conclusion, this study shows that chromosomal findings...
### Table 3. Initial Characteristics and Outcome of the 16 Patients With Follicular Lymphoma and Abnormality in Regions 6q23-26 or 17p

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/ Sex</th>
<th>Histology</th>
<th>Stage</th>
<th>Initial Treatment</th>
<th>Time to Transformation (mo)</th>
<th>Surviv (mo)</th>
<th>% Abnormal metaphases</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>38/F</td>
<td>C IV</td>
<td>CHOP</td>
<td>No</td>
<td>38</td>
<td>100</td>
<td>Yes</td>
<td>49XX, +X1(1;1)q42(4q12),t(8;19)(q11;q13), +12, +1mar</td>
</tr>
<tr>
<td>48</td>
<td>63/F</td>
<td>C III</td>
<td>CHOP</td>
<td>No</td>
<td>27</td>
<td>72</td>
<td>Yes</td>
<td>47,XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
</tr>
<tr>
<td>88</td>
<td>80/F</td>
<td>D II</td>
<td>CHVP</td>
<td>No</td>
<td>30</td>
<td>100</td>
<td>Yes</td>
<td>64,XX,del(1)(p32),+6,del(9)(q62q63),+9,del(11)(q13(11q23)</td>
</tr>
<tr>
<td>100</td>
<td>59/F</td>
<td>C IV</td>
<td>Cb</td>
<td>Yes</td>
<td>7</td>
<td>12</td>
<td>100</td>
<td>43XX, +X1(1;1)q42(4q12),t(8;19)(q11;q13), +12, +1mar</td>
</tr>
<tr>
<td>117</td>
<td>36/F</td>
<td>B IV</td>
<td>Cb</td>
<td>Yes</td>
<td>10</td>
<td>19</td>
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<tr>
<td>121</td>
<td>61/M</td>
<td>C IV</td>
<td>Cb</td>
<td>Yes</td>
<td>41</td>
<td>45</td>
<td>80</td>
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<td>138</td>
<td>75/M</td>
<td>C IV</td>
<td>Cb</td>
<td>No</td>
<td>1</td>
<td>100</td>
<td>Yes</td>
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</tr>
<tr>
<td>187</td>
<td>35/M</td>
<td>D IV</td>
<td>ACVBP</td>
<td>No</td>
<td>22</td>
<td>35</td>
<td>Yes</td>
<td>43XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
</tr>
<tr>
<td>268</td>
<td>60/F</td>
<td>C III</td>
<td>ACVBP</td>
<td>No</td>
<td>47</td>
<td>62</td>
<td>Yes</td>
<td>43XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
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<tr>
<td>308</td>
<td>81/F</td>
<td>C IV</td>
<td>Cb</td>
<td>No</td>
<td>13</td>
<td>72</td>
<td>Yes</td>
<td>43XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
</tr>
<tr>
<td>349</td>
<td>68/M</td>
<td>B II</td>
<td>CHVP + Rx</td>
<td>No</td>
<td>33</td>
<td>100</td>
<td>Yes</td>
<td>43XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
</tr>
<tr>
<td>363</td>
<td>38/F</td>
<td>C III</td>
<td>CHVP</td>
<td>No</td>
<td>19</td>
<td>30</td>
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<tr>
<td>384</td>
<td>71/F</td>
<td>C IV</td>
<td>Cb</td>
<td>Yes</td>
<td>11</td>
<td>13</td>
<td>64</td>
<td>43XX,t(3;14)(p14;q32),+9,del(7)(q36),+11q13(11q23)</td>
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<tr>
<td>403</td>
<td>59/M</td>
<td>C IV</td>
<td>CHVP</td>
<td>No</td>
<td>19</td>
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<td>Yes</td>
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<tr>
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<td>56/F</td>
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<td>Interferon</td>
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<td>15</td>
<td>83</td>
<td>Yes</td>
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</tbody>
</table>

**Abbreviations:** UPN, unique patient number; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; Cb, chlorambucil; ACVBP, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; C, follicular, predominantly small cells; D, follicular, predominantly large cells; B, follicular, mixed small and large cells; Rx, irradiation; +, alive at last follow-up; B, follicular, predominantly small cells; C, follicular, mixed small and large cells; D, follicular, predominantly large cells.
have a strong influence on clinical behavior of follicular lymphoma. Further studies are now required to validate these findings in another population of patients with follicular lymphoma and to determine the molecular targets of these chromosomal aberrations. Such studies will ultimately help to define a high-risk group of patients who might benefit from early intensive treatment.

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