Chromosomal Abnormalities in Hodgkin’s Disease

By Harry C. Schouten, Warren G. Sanger, Michael Duggan, Dennis D. Weisenburger, Ken A. MacLennan, and James O. Armitage

Numerous neoplastic states have associated or causal cytogenetic abnormalities. In some cancers, specific chromosomal abnormalities appear to correlate with the clinical characteristics and prognosis. Cytogenetic analysis of Hodgkin’s disease is thought to be technically difficult and only a small number of cases with evaluable results have been reported. We have attempted cytogenetic studies of lymph nodes from 37 patients with Hodgkin’s disease. In 29 of the 37 patients (78%), successful chromosomal analysis was accomplished. Chromosomal abnormalities were found in 13 patients (45%); five of these patients had been previously treated with chemotherapy. Numerical changes were found in all patients, most commonly involving chromosomes 5, 9, 15, 18, 22, X, and marker chromosomes.

In 1914, THEODOR BOVERI hypothesized that all cells of a malignant tumor had karyotypic alterations and that any event leading to such chromosomal abnormalities would result in a malignant tumor. Several diseases now have been found to have related cytogenetic abnormalities. At least 85% of patients with chronic myelogenous leukemia have a t(9;22) translocation, and most patients with Burkitt’s lymphoma have a t(8;14), t(2;8), or t(8;22). A t(14;18) is known to be correlated with follicular non-Hodgkin’s lymphomas. Certain cytogenetic abnormalities have been related to different treatment results in non-Hodgkin’s lymphoma. In acute leukemia some subtypes have been correlated with particular structural cytogenetic changes or numerical abnormalities.

In contrast to the non-Hodgkin’s lymphomas, only a few reports of cytogenetic abnormalities in patients with Hodgkin’s disease are present in the literature; these have been largely summarized by Kaplan and Sandberg and have consisted mostly of numerical abnormalities. Only a few studies have reported chromosomal analysis with QI-techniques of lymph nodes involved by Hodgkin’s disease. In contrast to the non-Hodgkin’s lymphomas, only a few reports of cytogenetic abnormalities in patients with Hodgkin’s disease are present in the literature; these have been largely summarized by Kaplan and Sandberg and have consisted mostly of numerical abnormalities. Only a few studies have reported chromosomal analysis with QI-techniques of lymph nodes involved by Hodgkin’s disease.

We performed chromosomal analysis of lymph nodes in 37 patients with Hodgkin’s disease to assess the types and the frequencies of chromosomal abnormalities, and their relationship to the histologic features and treatment outcome.

MATERIALS AND METHODS

Patient characteristics. Between November 1, 1982 and April 1, 1988, the lymph node biopsies from 37 patients with histologically confirmed Hodgkin’s disease were studied cytogenetically. All of the lymph nodes showed lymphoma. The characteristics of the patients are given in Table 1. A modified Rye histologic classification was used: nodular sclerosis subtype M1 (NS-M1), subtype M2 (NSM2), subtype mixed cellularity (NS-MC), subtype lymphocyte depleted (NS-LD), mixed cellularity (MC), lymphocyte depleted (LD), lymphocyte predominant subtype diffuse (LP-D), and subtype nodular (LP-N). Staging consisted of a complete history and physical examination, chest radiograph, computed tomography scan of the abdomen and chest, and bilateral bone marrow biopsies. If abnormal results of liver function tests were present, a liver biopsy was performed. A staging laparotomy was performed if it possibly could have resulted in a change of treatment. The patients were staged according to the Ann Arbor system: five patients were stage IA, one was stage IB, seven were stage II A, one was stage II AE, three were stage II B, six were stage III A, two were stage III B, one was stage IIIB, seven were stage IV A, and four were stage IV B (Table 1).

In 30 patients, the tumor was studied either at the time of primary diagnosis or at relapse after local radiation therapy, with a newly involved node outside the previously irradiated area. Seven other patients were studied in relapse. No selection criteria other than the availability of cytogenetic data was used. A piece of each tumor was submitted for histologic analysis.

Treatment. The choice of treatment depended upon the stage (Table 1). Patients with pathologically confirmed stage I disease were treated with radiation therapy. Among the patients with stage II and III disease, six were treated with radiation therapy and six with chemotherapy, while eight were treated with combined modality therapy. All patients with stage IV disease were treated with chemotherapy, and nine also received radiation therapy. The chemotherapy regimens consisted of CHLPP (chlorambucil, vincristine, procarbazine, and prednisone), MOPP (mechlorethamine, vincristine, procarbazine, and prednisone), alternating MOPP with ABVD (adriamycin, bleomycin, vinblastine and dacarbazine), or a combination of lomustine, vincristine, dexamethasone, procarbazine, and bleomycin.

A complete remission (CR) was defined as the absence of clinically demonstrable disease after the completion of the therapy.

From the Departments of Internal Medicine, Pediatrics, and Pathology and Microbiology, University of Nebraska Medical Center, Omaha; and Department of Pathology, University Hospital, Queen’s Medical Center, Nottingham, England.

Submitted July 20, 1988; accepted January 31, 1989.

Dr Schouten is a Netherlands Cancer Foundation Clinical Fellow.

Address reprint requests to Harry C. Schouten, MD, Department of Internal Medicine, Section Hematology-Oncology, University Hospital Maastricht, PO Box 1918, 6201 BX Maastricht, Netherlands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.
0006-4971/89/7308-0033$3.00/0
An abnormal clone microscopically analyzed, recorded, and photographed. plates were classified as inconclusive. The karyotypes were designated according to the classification of the International System for Human Cytogenetic Nomenclature (ISCN 1985). The cytogenetic abnormalities were mapped and their relative frequencies calculated. The results of the chromosome studies were correlated with the histologic diagnosis and clinical characteristics.

**RESULTS**

In 29 of the 37 patients (78%), successful cytogenetic studies were accomplished, and in eight patients the results were inconclusive. In 13 of the 29 (45%), the results were abnormal (Table 2). In 16 of 29 patients (55%), normal karyotypes were found. Patients with successful studies had the following histologic diagnosis: NS-M1 (n = 5); NS-M2 (n = 8); NS-MC (n = 2); NS-LD (n = 6); MC (n = 5); LD (n = 1); and LP-N (n = 2). The patients with inconclusive results had NS-M2 (n = 2); MC (n = 3); LP-D (n = 1); LP-N (n = 1); and LD (n = 1). Only one of the patients from the latter group was treated before the cytogenetic study. Of the patients with successful cultures, 21 had the cytogenetic studies at the time of diagnosis (Table 1, cases 1 to 6 and 14 to 28) and three patients (cases 7, 8, and 29) had the cytogenetic studies at the time of relapse. However, all of the latter patients had been treated with radiation therapy alone, and the lymph node that was analyzed had not been previ-
therapy and, therefore, therapy-induced abnormalities can-... to 13 were studied after treatment with combined modality ir-... as excluded.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Abnormal Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>15</td>
<td>7% 47,XY,+12, t(4;7)(q34;q11),/93% 61,XY,+3, +5,+9,+12,+14,+15,+16,+17,+del(11)(p12), +del(12)(q22), +del(11)(q13), t(4;7)(q34;?), +t(13;7)(q33;?), +t(22;1)(p11;?), +mar</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>6</td>
<td>50% 45,XY, -15/50% 45,XY, -18</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>2</td>
<td>46,X,-X,-del(2)(q23), dup(12)(q13q23), +del(11)(q13), t(18;7)(q23;7), t(20;7)(q13;7), +mar</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>2</td>
<td>47,XX,+15</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>1</td>
<td>70(3n+/-),XY,+X,+Y,+5, -13,-13,-15,-15,-20, +21, +del(6)(q24), +t(17q), t(18;7)(q23;7), t(20;7)(q13;7), +mar</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>6</td>
<td>57-69, further determination impossible because of poor morphology</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>3</td>
<td>46,X,-X,+5</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>5</td>
<td>47,XX,+18</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>3</td>
<td>78(4n+/-),X,-X,-X,-1,+2, -6,-7,-8,-9,-9,-10, -11,-13,-16,-16,-21, -21,i(8p), del(7)(q22), i(8q), 2t(17q), 2t(21)(p16;?), t(13;7)(p11;?), 2t(18;7)(p11;?), +2mar</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3</td>
<td>72,XY,+X,+3,+3,+4,+5,+6, +7,+8,+9,+9,-10,-12, -13,-13,+15,+17,+18, +19,+21,-22, -2der(11), +del(6)(q14), +3dup(12)(q13→q24), t(11)(q11;?), t(22;7)(p24;?), +t(6;7)(q24;7), +t(11;7)(q21;7), +t(22;13)(p13;7), +t(13;13)(p13;7), +2t(16;7)(p13;7), +3mar</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>7</td>
<td>29% 45,X,-Y/42% 45,XY, -18/29% 47,XY,+22</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>2</td>
<td>45,XX,-2,-6,-10,-16,-17, del(11)(p13), +t(22;8)(p12,p11), +mar</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>3</td>
<td>48,X,t(X;7)(p28;?), +9,+22, +der(19)(10;19)(q24;p13), +der(17)(17;22)(p13;11), t(4;7)(q32;7), t(5;7)(p16;?), t(8;7)(p23;7), t(15;7)(p11;?), t(22;7)(p11;?)</td>
</tr>
</tbody>
</table>

Table 2. Results of Cytogenetic Studies

In two patients, cases 1 and 10, only abnormal metaphases were obtained; all other patients had an admixture of normal cells. All abnormal cultures had numerical abnormalities. Cultures from eight patients were hyperdiploid (47 to 80 chromosomes) and three were hypodiploid (45 chromosomes). Most frequently, gains of chromosomes 3, 5, 9, and 15 were observed, but gains of chromosomes 4, 10, 12, 14, 17, 18, 19, 21, 22, and X were also seen in two patients each (Fig 1A). In five patients, marker chromosomes were present. Monosomes were also found, and occurred more than once in chromosomes 6, 13, 15, 18, 22, and X. Combining these numerical abnormalities, the chromosomes 5, 9, 15, 18, 22, and X were each involved in at least four patients. In addition to these abnormalities, structural abnormalities were also present in seven patients. Several of these structural abnormalities occurred only once. However, a translocation involving breakpoint 4q32-34 was found in two patients (cases 1 and 13), breakpoint 6q24 was involved in one translocation and one deletion (cases 5 and 10), and breakpoint 13p11-13 was involved in two patients (cases 9 and 10). Breakpoints 12q13 and 12q23-24 were each involved in two patients (cases 3 and 10), both as a part of a dup(12) (q13→q23-24). All other chromosomes except 9, 14, and Y were each involved in at least one patient; however, no consistent breakpoint was observed. The distribution of the structural chromosomal abnormalities is given in Figs 1B and C. There is no clear difference between the patients before or after treatment. Three patients (cases 1, 2, and 11) appeared to have two or more cytogenetically abnormal clones of malignant cells.

The clinical characteristics of the patients at the time of initial diagnosis, or at the time of relapse in those without previous radiotherapy on the involved node or chemotherapy (cases 1 to 8 and 14 to 29) were studied. There did not appear to be any significant differences between the cytogenetically normal and abnormal groups with regards to histology, B symptoms, stage, the presence of bulky or extra nodal disease, achievement of a CR, or relapse rate. The disease-free survivals for the patients with normal and abnormal karyotypes were calculated. The cytogenetically abnormal patients had a longer median disease-free survival (9 months) than those with normal results (3.5 months), but this difference was not significant using the chi-square test.

**DISCUSSION**

In contrast to the leukemias and non-Hodgkin's lymphomas, few cytogenetic studies in Hodgkin's disease have been reported due to the technical difficulties inherent to the disease. In a summary of the literature prior to 1980, Kaplan6 reported 100 cases of Hodgkin's disease and chromosomal abnormalities. The most striking findings were the presence of numerical abnormalities and marker chromosomes. These findings were confirmed by other reviews. Rowley14 also described 25 patients who had hyperdiploid cells and marker chromosomes.

Although numerical abnormalities have been reported frequently, studies dealing with structural chromosomal change in Hodgkin's disease are few. Only a few studies have used banding techniques to study lymph nodes involved with...
Hodgkin's disease. Fukahara and Rowley\(^{10}\) described one patient with a t(1;14)(q2;q32), along with other nonspecified rearrangements. Hansmann et al\(^{25}\) reported one patient with hyperploidy, a 6q- abnormality and several marker chromosomes. Fleischmann and Krizsa\(^{11}\) reported one patient with a del(9)(p11q11), whereas Reeves et al\(^{12,13}\) studied the nodes of five patients and found a variety of structural abnormalities involving the breakpoints 1q32, 2p13, 3p13, 3p21 or 23, 5q31, 6q13 or 15, 6q21, 6q23 or 25, 7q11, 9q11, 9q31, 10q26, 12q22 and 14q32, and inversions of 2q, 18p, and 18q. Lawler and Swansbury\(^{14}\) studied four patients and found paracentric inversion of 5q, 10q, 21q in one patient, in addition to gains of parts of long and short arms of chromosome 4, 10, and 11, not further specified. Slavutsky et al\(^{15}\) described one patient with a t(X;6)(q12;q13), and another patient with a del(5)(p15) in addition to numerical abnormalities. Cabanillas et al\(^{16}\) observed frequently occurring abnormalities located on 11q23, 14q32, 6q11-21, 8q22-24, and 11q13.

We report the chromosomal abnormalities of 13 of 37 patients in whom chromosomal analysis was performed. All 13 patients had numerical abnormalities and seven also had structural changes. Chromosomes frequently gained are 5, 9, 15, 18, 22, and X while less frequently gained chromosomes are 3, 10, 12, and 17 (each in at least three patients). The structural abnormalities that we found were more random than those found in the group with numerical changes. However, the breakpoints 4q32-34, 6q24, 12q13, 12q23-24, and 13p11-13 were each observed in more than one patient.

Numerical and structural abnormalities of chromosome 2 are considered to be very infrequent in malignancies.\(^{16}\) However, we found chromosome 2 involved in five patients, two with numerical and structural abnormalities, and three with only structural abnormalities. However, there was no consistent breakpoint. We also found an additional chromosome 5 and a translocation involving chromosome 6 in a higher than expected frequency in Hodgkin's disease. Also, we observed chromosome 22 involved in five of 13 patients with Hodgkin's disease, but the change was usually numerical rather than structural.

The known abnormalities of the non-Hodgkin's lymphomas as t(8;14), t(11;14), and t(14;18) did not occur in our series, in contrast to the findings of Cabanillas et al.\(^{16}\) However, our results and those from the literature suggest a large variety of chromosomal abnormalities involved.

From the cytogenetic studies of patients with non-Hodgkin's lymphoma, it is known that the chromosomal breakpoints correspond with the location of a variety of oncogenes.\(^{2}\) Activation of these oncogenes has been suggested as playing a role in the development of these malignancies.\(^{2,27}\) These correlations have not been described for Hodgkin's disease, although Cabanillas et al\(^{16}\) recently suggested correlations with c-ets, c-myb, c-myc, bcl-1 genes and the immunoglobulin heavy chain gene. The breakpoints that we observed to be involved in more than one patient are not known to be the sites of recognized oncogenes, except for the myb oncogene at 6q24.\(^{18}\)

In our study, the yield of successful metaphases, and especially abnormal metaphases, is higher than that reported in the literature.\(^{8,14-24}\) It is generally believed that the low mitotic rate of the neoplastic cell in Hodgkin's disease is responsible for this phenomenon,\(^{8,24}\) although a relationship between unsuccessful cytogenetic studies and the NS histol-
CHROMOSOMAL ABNORMALITIES IN HODGKIN'S DISEASE

An extensive analysis correlating clinical features such as the histologic type, presence of B symptoms, stage, presence of bulky disease, or extra nodular disease, ability to achieve a CR, and the relapse rate with the presence or absence of abnormal chromosomes did not reveal any significant correlations. Disease-free survival was not significantly different for the two groups. Further studies are needed to determine whether correlations between chromosomal abnormalities and disease characteristics in Hodgkin's disease exist.

In contrast to reports in the literature, we conclude that cytogenetic analysis in Hodgkin's disease is possible with current culture techniques, and reveals chromosomal abnormalities in a significant number of patients. However, cytogenetic techniques have to be further improved to fulfill the Boveri hypothesis. Because of the small number of patients in each subgroup, we were unable to demonstrate correlations between the chromosomal abnormalities and histological or clinical characteristics. However, our findings suggest that further studies should be performed on a large number of patients.

REFERENCES
