ABSTRACT: Chromosome analyses were performed on leukemic cells from 102 patients with B-CLL, of whom 84 were untreated. B-cell mitogen-induced CLL cells yielded suitable metaphases in 85 patients, and 55 showed clonal chromosomal aberrations. Trisomy 12 was found in 28 patients. In nine patients the +12 was a single aberration. A 14q+ chromosome or deletions of the long arm of chromosomes 6, 11, or 13 were other recurrent aberrations. Patients with Rai stage I or more had more frequently clonal aberrations than patients with stage 0 disease (p < .02). Patients with clonal aberrations had poorer 5-year survival than those with a normal karyotype (p < .05). Patients with a high percentage of abnormal metaphases in the sample had poorer prognosis than patients with high admixture of normal metaphases (p < .01). Of the specific clonal aberrations those with 14q+ or trisomy 12 tended to have slightly poorer and those with 6q− or structural aberrations involving the long arm of chromosome 13 tended to have better prognosis than patients with other chromosomal aberrations. A complex karyotype tended to be an adverse prognostic sign. Clonal evolution is rare: complex karyotypes are found at diagnosis and clones with single aberrations did not acquire additional chromosome aberrations despite progressive disease and treatment. Nine hundred and seventy-nine published cases are reviewed, and pathogenetic mechanisms, such as oncogenes and gene dosage, are discussed.

INTRODUCTION

Specific chromosome aberrations have been found to give clinical, prognostic, and pathogenetic information in hematological malignancies [1]. The history started in 1960 with the finding of a consistent minute chromosome in chronic myelocytic leukemia (CML) [2], known as the Philadelphia chromosome, which following the advent of the chromosome banding [3] was identified to be a chromosome 22 with the long arm deleted at band q11 [4].

In chronic lymphocytic leukemia the initial chromosome studies yielded poor results [5, 6]. The reason for this was, firstly, the low mitotic index of CLL cells, and secondly, that the mitogen that was used, phytohemagglutinin [7], activates T-cells, whereas CLL in 97% of the cases is a B-cell disease. It is likely that the cells from CLL patients studied in the early and mid 1970’s were normal T-cells [8, 9], with a higher rate of mitosis than the leukemia cells. This difficulty delayed chromosome studies in CLL compared with those in many other hematological malignancies. Then in 1978

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the polyclonal B-cell activating substances were found to induce mitosis in CLL cells [10], and this made it possible to perform chromosome analysis on relevant cells. Clonal chromosomal aberrations were found [11, 12], and it is noteworthy that the specific abnormalities that later were found to be among the most common in CLL, the trisomy 12 and the t(11;14), [13–15] were present also in these early cases.

During the 1980’s the number of studied patients have reached a thousand. However, there are still many question marks. What is the nature of B-cell mitogen-activated CLL cells with normal chromosomes? What is the mechanism behind the occurrence of trisomy 12? Why is trisomy 12 associated with CLL? Is the pathogenesis of CLL purely a matter of gene dosage, and which genes are relevant in the malignant transformation? Are the chromosome aberrations markers for the evolution of the disease with increasing derangements of the genotype, or are they more like “phenotypic markers” of the individual leukemic clone? Are there correlations between the karyotype and the phenotype, or with stage and other clinical features? It is generally agreed that chromosome changes give prognostic information in CLL as well as in CML and in acute leukemias. Patients without clonal aberrations do better than patients in which chromosome changes are found [15–18]. However, the prognostic impact of the trisomy 12 is a matter of discussion [15–28].

Some of these problems have been elucidated by recent studies, and they will be discussed in the following. An updated review of the literature and of 102 patients studied by us will be presented.

PATIENTS AND METHODS

Patients

One hundred and two consecutive patients with B-CLL were studied. Sixty-eight patients were males and 34 were females, and their ages at diagnosis ranged from 35 to 89 years (median 67 years). In this report patients with prolymphocytic leukemia [29] and leukemic states of follicle center cell derived lymphomas [30] were excluded. Diagnosis was confirmed with immunological markers on blood lymphocytes, and lymph node biopsy was performed in almost every case with lymphadenopathy. Sampling for chromosome analysis was performed within 2 months from first elevated blood lymphocyte count in 30 patients, and within 1 year in 63 patients. Median time from first blood count with lymphocytosis to sampling for cytogenetic analysis was 7.4 months. Sixteen patients had received cytostatic treatment and two were splenec-tomized before sampling for cytogenetic analysis, whereas 84 patients were untreated. Patients were treated only if progressive disease with anemia, thrombocytopenia, generalized lymphadenopathy, or clear B-symptoms were found. Initial treatment according to protocols of the Lymphoma Group of Central Sweden consisted of chlorambucil—prednisone, or doxorubicin, cyclophosphamide, vincristine and prednisone, or splenectomy. Patients in the different treatment arms have as yet similar survival. Survival analyses were performed by the Log rank test [31] from the date of diagnosis. Subgroup analyses were also performed excluding patients that were treated before cytogenetic analysis and patients older than 80 years at diagnosis, leaving 64 of 85 cytogenetically evaluable patients for analysis.

Cells

Cells were obtained from heparinized blood and also from heparinized bone marrow aspirates, freshly minced lymph nodes, spleens, and pleural effusions. Lymphocytes were isolated, cultured, and activated by Epstein-Barr virus, lipopolysaccharide from

Clinical Implication

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<td>Clonal aberr:</td>
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E. coli, te [17]. The chromosomes

RESULTS

Chromosome Aberrations

Adequate patients, the cases, aberrations cytogenetic 10 eval shown in nine pat one pat 14 with sing in 14 fragment number t(11;14), chromosomal patients, 6 were i
Clinical Implications of Chromosomes in B-CLL

Table 1  Chromosome findings in 102 patients

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Total number</th>
<th>Percent of evaluable patients</th>
<th>Percent of abnormal karyotypes</th>
</tr>
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<tbody>
<tr>
<td>All</td>
<td>102</td>
<td></td>
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</tr>
<tr>
<td>Evaluable</td>
<td>85</td>
<td>35</td>
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<tr>
<td>Normal karyotype</td>
<td>30</td>
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</tr>
<tr>
<td>Clonal aberration</td>
<td>55</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Trisomy 12a</td>
<td>27</td>
<td>32</td>
<td>49</td>
</tr>
<tr>
<td>Non-Trisomy 12</td>
<td>28</td>
<td>33</td>
<td>51</td>
</tr>
<tr>
<td>Single aberration</td>
<td>25</td>
<td>29</td>
<td>45</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>9</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Non-Trisomy 12</td>
<td>18</td>
<td>19</td>
<td>29</td>
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<tr>
<td>Two aberrations</td>
<td>16</td>
<td>19</td>
<td>29</td>
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<tr>
<td>Trisomy 12b</td>
<td>11</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Non-Trisomy 12</td>
<td>5</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>≥ Three aberrations</td>
<td>14</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>7</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Non-Trisomy 12</td>
<td>7</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>11q (4 single)</td>
<td>9</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>6q (4 single)</td>
<td>7</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>14q+ (1 single)</td>
<td>7</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>13q (3 single)</td>
<td>5</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

a  [PLL and leukemic follicle center cell derived lymphomas excluded].

b  Including one patient with dup(12)

E. coli, tetradecanoyl-phorbol-acetate, and cytochalasin B as previously described [17]. The Q-banding technique was utilized for chromosome analysis [3]. A clonal chromosomal abnormality was defined by conventional criteria [32].

RESULTS

Chromosome Aberrations

Adequate numbers of metaphases evaluable for chromosomes were obtained in 85 patients, whereas in 17 patients we failed to induce metaphases despite, in most of the cases, repeated attempts with different modes of activation. Clonal chromosomal aberrations were found in cells from 55 patients. Thirty patients were considered cytogenetically normal, since no clonal chromosomal aberration was found in at least 10 evaluable metaphases. The cytogenetic pattern and correlation to clinical stage are shown in Tables 1 and 2. Extra chromosome 12 was found as the only aberration in nine patients and together with other aberrations in another 18 patients, including one patient with a dup(12) [33]. Translocations most commonly involved chromosome 14 with a breakpoint at q32 (i.e., the immunoglobulin heavy chain gene locus) resulting in 14q+ chromosomes. The donor chromosome for the additional chromosome fragment on 14q was number 11 in three patients, unidentified in one patient, and number 2, 7, and 12 in three patients, respectively. Of the three patients with a t(11;14), two had a breakpoint at 11q13, and one had a breakpoint at 11q15. Deleted chromosomes 11 were found in five patients, with breakpoints at 11q21 in two patients, 11q22 in two patients, and at 11q14 in one patient. Deletions on chromosome 6 were found in five patients, and translocations involving chromosome 6 in two
Table 2  Chromosome findings according to Rai stage [66] at first karyotype study. In treated patients highest Rai stage during course of the disease prior to first karyotype study is indicated.

<table>
<thead>
<tr>
<th>Rai stage</th>
<th>All patients n = 102</th>
<th>All evaluable n = 89</th>
<th>Normal karyotype n = 30</th>
<th>Abnormal karyotype, non +12 1</th>
<th>Abnormal with +12 1 2 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% of evaluable</td>
<td>% of evaluable</td>
<td>% of evaluable</td>
<td>% of evaluable</td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>38%</td>
<td>14%</td>
<td>11%</td>
<td>7%</td>
</tr>
<tr>
<td>I</td>
<td>37</td>
<td>35%</td>
<td>23%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>22%</td>
<td>22%</td>
<td>8%</td>
<td>25%</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>100%</td>
<td>20%</td>
<td>40%</td>
<td>10%</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>20%</td>
<td>20%</td>
<td>40%</td>
<td>10%</td>
</tr>
</tbody>
</table>

* Number of clonal aberrations: 1 = single aberration, n = two or more aberrations. Abnormal karyotypes were significantly more common in Rai stage I or more than in stage 0 (p < .01).

patients. The breakpoints were 6q15 and 6q23 in two patients each, and 6q12, 6q21, and 6q24 in one patient each. Five patients had structural abnormalities of chromosome 13, involving band 13q13, 13q21, and band 13q34 in two patients each, and band 13q22 and 13q31 in two patients, respectively.

Prognosis

The 5-year survival of patients with clonal chromosomal aberrations was poorer than that of patients with a normal karyotype (p < 0.05), although the overall survival curves were not significantly different (Fig. 1). Patients with clonal abnormalities in

Figure 1  Survival according to karyotype. Patients with clonal chromosomal aberrations versus patients without clonal aberrations in at least ten studied metaphases. Difference is significant at 5 years (p < .05) but overall survival is not significantly different. Figures on curves indicate number of patients at risk.

DISCUSSION

Incidence of Ti

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Clinical Implications of Chromosomes in B-CLL.

![Graph showing survival rate over years with normal and abnormal chromosome sets]

Figure 2: Survival according to percentage of metaphases with clonal chromosomal abnormalities. Patients with more than 90% abnormal metaphases versus patients with clonal aberrations in less than 90% of studied metaphases. Difference is significant (p < .01). Figures on curves indicate number of patients at risk.

all or nearly all metaphases had a poorer survival than patients with admixture of normal metaphases to the aberrant cells (p < 0.01, Fig. 2). The survival curves of patients with complex karyotypes (three or more aberrations within the clone) and those with non-complex abnormalities (one or two aberrations) diverged, but the difference was not significant (Fig. 3). Patients with 14q+ marker chromosomes or with trisomy 12 tended to have a slightly poorer prognosis than patients with other types of chromosomal aberrations (Fig. 4). When comparing only patients with single abnormalities, patients with trisomy 12 tended to have poorer survival than patients with single aberrations other than +12 (Fig. 5). Patients with deletions of the long arm of chromosome 6 or structural aberrations involving the long arm of chromosome 13 tended to have a better prognosis than patients with other types of clonal aberrations (Fig. 6). Patients with 11q− were also analysed separately, and their survival curve overlaid that of all patients with clonal aberrations. The survival curves of specific subgroups were similar when only untreated patients diagnosed before the age of 80 years were analysed as compared to those including all patients.

DISCUSSION

Incidence of Trisomy 12

Trisomy 12 is found in more than one third of CLL patients with clonal chromosomal aberrations (Table 3). However, it is also found in other B-cell malignancies, such as lymphocytic lymphoma [34], hairy cell leukemia [35], and prolymphocytic leukemia [36, 37], but only rarely in non-B-cell malignancies. Recent studies on CLL-cells using the restriction fragment length polymorphism (RFLP) of genes located on the long arm of chromosome 12 show that the trisomy consists of a duplication of one of the chromosomes 12 [38–40]. In none of our studied patients with +12 did we find a
Figure 3  Survival according to karyotype. Patients with one or two specific aberrations versus patients with three or more abnormalities. Difference not significant. Figures on curves indicate number of patients at risk.

Figure 4  Survival according to karyotype. Patients with trisomy 12 with or without other aberrations including one patient with dupt(12) and patients with 14q+ chromosomes versus patients with clonal abnormalities except trisomy 12 and 14q+. Two patients are included in both trisomy 12 and 14q+ groups. Differences are not significant. Figures on curves indicate number of patients at risk.
Figure 5  Survival according to karyotype. Patients with trisomy 12 as the sole abnormality versus patients with single abnormalities other than trisomy 12. Difference not significant. Figures on curves indicate number of patients at risk.

Figure 6  Survival according to karyotype. Patients with deletion of 6q, and patients with structural aberrations involving 13q versus patients with clonal abnormalities excluding 6q and 13q aberrations. One patient is in both 6q- and 13q groups. Differences are not significant. Figures on curves indicate number of patients at risk.
Table 3 Published chromosome data: Number of patients with involved chromosomal abnormality (% of all) (% of abnormal)

<table>
<thead>
<tr>
<th>Source (Reference)</th>
<th>All Abnormal</th>
<th>+12</th>
<th>11q</th>
<th>13q</th>
<th>14q</th>
</tr>
</thead>
<tbody>
<tr>
<td>IWCLL[15]</td>
<td>427</td>
<td>214 (50)</td>
<td>67 (31)</td>
<td>37 (17)</td>
<td>50 (23)</td>
</tr>
<tr>
<td>Bournemouth[26]</td>
<td>141</td>
<td>75 (53)</td>
<td>22 (29)</td>
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<tr>
<td>Huddinge[Table 1]</td>
<td>102</td>
<td>55 (54)</td>
<td>27 (49)</td>
<td>9 (16)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Edinburgh[28]</td>
<td>138</td>
<td>54 (39)</td>
<td>15 (26)</td>
<td>10 (19)</td>
<td>14 (26)</td>
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<tr>
<td>Buffalo[27]</td>
<td>98</td>
<td>39 (40)</td>
<td>24 (62)</td>
<td></td>
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<tr>
<td>London[20]</td>
<td>63</td>
<td>33 (52)</td>
<td>7 (21)</td>
<td>4 (12)</td>
<td>11 (33)</td>
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<tr>
<td>Copenhagen[77]</td>
<td>110</td>
<td>29 (26)</td>
<td>6 (27)</td>
<td>4 (18)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Montpellier[85]</td>
<td>22 (21)</td>
<td>10 (9)</td>
<td></td>
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<tr>
<td>Stockholm[83]</td>
<td>24</td>
<td>16 (67)</td>
<td>1 (6)</td>
<td>5 (21)</td>
<td>3 (13)</td>
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<tr>
<td>Chicago[40]</td>
<td>38</td>
<td>15 (39)</td>
<td>7 (47)</td>
<td>4 (27)</td>
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<tr>
<td>Ferrara[86]</td>
<td>23</td>
<td>18 (65)</td>
<td>10 (67)</td>
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<tr>
<td>Philadelphia[87]</td>
<td>40</td>
<td>14 (35)</td>
<td>4 (29)</td>
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<tr>
<td>Helsinki[12]</td>
<td>14</td>
<td>8 (57)</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Christchurch[88]</td>
<td>8</td>
<td>7 (80)</td>
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<tr>
<td>Helsinki[89]</td>
<td>66</td>
<td>5 (8)</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Woodville[90]</td>
<td>6</td>
<td>5 (83)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Rochester[91]</td>
<td>7</td>
<td>2 (40)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>979</td>
<td>394 (40)</td>
<td>142 (36)</td>
<td>41 (10)</td>
<td>62 (16)</td>
</tr>
</tbody>
</table>

* IWCLL, International Working Party on Chromosomes in CLL[15]. First report contains patients from Bournemouth, Edinburgh, Huddinge, Ferrara and Helsinki. IWCLL excluded in “Total” since most patients are reported from the individual institution. Figures in “Total” might be too low because of missing data.

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Pathogenetic Mechanisms of Trisomy 12

Malignant transformation may occur through an alteration of a specific gene or by an abnormal gene dosage. The high incidence of +12 in B-CLL implicates some role of this abnormality in the pathogenesis of the disease. Why then trisomy 12, and is the duplicated chromosome normal? If so, one explanation would be that B cells randomly acquire an extra chromosome 12 copy, and the increased amount of genes localized on chromosome 12 renders the malignant characteristics to the affected B cell. Another cause might be that the malignant transformation hits and alters a specific gene on chromosome 12 that induces or facilitates a nondisjunction leading to trisomy 12. A third possible explanation is a genetic change anywhere in the genome giving rise to CLL cells, which subsequently becomes prone to nondisjunctive mitoses with trisomy 12 occurring within a subclone.

All possibilities suggest a responsible specific gene product, produced either by the increased gene dosage alone or by gene alteration. However, there are no valid suggestions of a specific CLL gene. The bcl-1[42] and bcl-2[43,44] genes have been identified through their localization at sites of specific recurrent chromosome
Clinical Implications of Chromosomes in B-CLL.

translocations to the immunoglobulin heavy chain genes on chromosome 14. Structural aberrations involving chromosome 12 are not common and not very consistent in CLL. One of our patients had a duplication of 12(q13→q22), i.e., a triplication of the genes in this segment [33], and Ross (personal communication) found a patient with dup(12)(q13→q22). Another patient of ours had trisomy 12 with a subclone having a deletion of the third chromosome 12 at q22 [17]. A fourth patient had initially two clones, one with trisomy 12 and monosomy 17, and one with two normal chromosomes 12, one chromosome 17, and a marker chromosome consisting of 12q material distal to q13 translocated to a chromosome 17 deleted at p13. This patient thus had a trisomy of chromosome 12 genes from q13 to qter [46]. This specific t(12;17)(q13;p13) marker was also found in a single CLL cell by Ross (personal communication). Furthermore, two similar clonal markers, i.e., a + del(12)(q22) and a t(12;17)(q21;q11) were recently reported by Bird and coworkers [46]. Also, Mecucci and coworkers observed a patient with a duplication of a marker chromosome consisting of the short arm of chromosome 11 and the long arm of chromosome 12 [47]. Together, these karyotypes may indicate that the most important genes are located on the long arm of chromosome 12 at band q13 to q22, and that the centromeric region of chromosome 17 also may be of interest. Of defined oncogenes, two are located on chromosome 12, i.e., k-ras-2 [48] and int-1 [49]. However, no data have accumulated supporting a role for these oncogenes in CLL [50, 51].

The third explanation might be supported by the finding of normal metaphases in most cell samples containing CLL cells with trisomy 12. However, our RFLP data show interestingly that polymorphic genes on 12q have a clear 2:1 ratio in all samples containing some metaphases with trisomy 12 [40], indicating that the frequency of mitotic cells with normal karyotype are not equally represented in the nondividing cell population, but showing that almost all cells in such samples have trisomy 12. This supports the view of Knuttila and coworkers, who found that cells with a normal karyotype among cells with clonal aberrations are contaminating normal T cells [8, 9]. Furthermore, if explanation three was true one would expect to commonly find the development of trisomy 12 during the course of the disease. However, trisomy 12 is frequently found in very early disease [17, 45], and rarely superimposing during disease progression [45, 52]. Thus, it seems likely that the important transforming event induces changes by quality or quantity of the product of a specific gene localized on chromosome 12. However, since more than half of the patients never show changes of chromosome 12 there ought to be additional pathogenetic mechanisms, eventually using parts of the previously discussed transforming pathway. Alternatively, the trisomy 12 aberration might be a common but not obligatory result of a transforming genetic change that by itself is not possible to detect by conventional cytogenetics.

Oncogenes

Two oncogenes, designated bcl-1 and bcl-2 from B-cell leukemia/lymphoma, are found to be involved in rare cases of CLL. The specific chromosomal localizations are 11q13 [52] and 14q21 [43, 44], respectively, and the recurrent chromosomal abnormalities are translocations of the oncogenes to the immunoglobulin heavy chain gene on 14q32, forming t(11;14) and t(14;18), respectively.

Bcl-1 was cloned from CLL cells [42] but the t(11;14) is more commonly seen in lymphocytic lymphomas [34] and prolymphocytic leukemia [36, 45, 53]. In the compiled European data on 427 patients, the t(11;14) was found in 11 of 214 patients with clonal chromosomal aberrations [15]. Molecular analyses on 3 patients from Huddings showed the classic localization of the bcl-1 gene within the major translocation cluster (MTC) [42] in one CLL patient prior to PLL transformation, whereas
the bcl-1 was localized 63 kb telomeric of the MTC in one patient with primary prolymphocytic leukemia [34], and the bcl-1 gene was not detected with any of these two probes in the third patient with stage 0 CLL. No bcl-1 involvement was found in 38 patients studied by Rechavi and coworkers [55].

bcl-2 is involved in most patients with follicular lymphoma [34], but also in diffuse B cell lymphomas [56]. The bcl-2 gene products are p22-p26 proteins [57, 58] that are present in low amount in nonmalignant lymphoid tissue and abundant in (t(14;18)-
carrying lymphomas and cell lines [59]. The proteins have shown to be oncogenic in gene transfer assays [58]. Bcl-2 involvement is rare in CLL; among our patients, we have found three cases of 50 by Southern blotting [60], whereas Rechavi did not find any CLL-cell clone with bcl-2 involvement in 38 studied cases [55].

A putative bcl-3 oncogene is also suggested from three CLL-patients with a t(14;19)(q32;q13) [62], and the gene at 19q13 has been cloned [62], but its role remains to be established.

Chromosomal Changes During Progressive and Indolent Disease

We and others have previously suggested from incidence and survival analyses that trisomy 12 is the primary genetic change in CLL [19], whereas other aberrations are secondary [16]. However, clonal evolution is rare in CLL [40, 45, 52]. We have analysed most patients close to diagnosis and before any treatment, and we performed sequential analysis on 41 patients with different karyotypes in indolent and in progressive states [63]. In most cases the karyotype was unchanged [45], with some patients having complex karyotypes at diagnosis and others continuously showing normal karyotype or single aberrations during long-term observation. No CLL cell clone with a single trisomy 12 aberration developed additional changes. The same finding was made by Nowell and coworkers [52]. Thus, the complexity of the karyotypic aberrations is mostly established early in the clinical disease, and disease progression is usually not accompanied by further derangement of the karyotype. This is thus in contrast to the case in CML [64] and follicular lymphoma [65, 66], and it makes it possible to use chromosomes for prognosis prediction in CLL at any time during the course of the disease.

Chromosomes and Clinical Stage

We and others have found that Rai [67] stage 0 patients less commonly have clonal abnormalities than patients with more advanced stages (Table 2, p < .02). Single abnormalities are common in stage I-II, whereas complex karyotypes seem to be more common in stage III and IV (Table 2) [18, 68]. However, the proportion between +12 and non +12 karyotypes do not differ between the Rai stages.

In the studies from Buffalo [69, 70] and Chicago [46], the incidence of chromosomal abnormalities differed between untreated and treated patients. However, our longitudinal studies [45] discussed above, show that the karyotype does not change during therapy. With a random time for cytogenetic sampling and a greater likelihood of a progressive disease in patients with clonal abnormalities, patients with clonal aberrations would have a greater risk of having developed a therapy-demanding disease before sampling than patients with a normal karyotype. Thus, the karyotypic changes are not caused by treatment [45], but the individual karyotype marks the progression rate of the tumor [17].

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Clinical Implications of Chromosomes in B-CLL

Chromosomes and Phenotype

Surface membrane immunoglobulin phenotype and other cell surface markers, such as CD5, CD19, CD20 were not associated to the chromosome results. Likewise, there was no correlation of the chromosomes to the lymphocyte count or the percentage of T cells in the peripheral blood.

Chromosomes, Prolymphocytic Leukemia and Monoclonal Proteins

Chromosome aberrations are more frequently found in prolymphocytic leukemia [27, 30, 37, 45], a disease with activated tumor cells [71], and in CLL transforming into PLL [27, 45, 49] than in CLL ‘proper’. In Pittman’s PLL-study, 14q+ chromosomes were the most frequent abnormalities [36], whereas Sedamori et al. found a specific translocation, t(6;12)(q15;p13), in all of 3 PLL cases [37]. This translocation has not been reported in other PLL studies. We have found clonal aberrations in 3 of 3 primary PLLs and in 3 of 3 PLL transformations of CLL. Two PLL cases had +del(3)(p13) together with other aberrations [72]. One patient with PLL and one CLL/PLL had t(11;14), one CLL/PLL had 6q – and one had +12 [45].

Furthermore, clonal chromosomal aberrations are reported to be more frequent in CLL with monoclonal serum protein bands [73]. We also found that all our seven patients with M components had clonal abnormalities, with the same distribution ratios 12 and of single and complex abnormalities as the total material. Immunoglobulin-secreting cells have a distinct lymphoplasmacytic appearance, and we have subdivided our CLLs by their lymph node and bone marrow cell morphology into CLL ‘proper’ and immunocytomas (IC) according to the Kiel classification [30]. CLL cells have a homogeneous lymphocytic appearance, whereas ICs have cells with a lymphoplasmacytoid differentiation within the malignant clone, which however is dominated by the small lymphocytes. Lymph nodes from patients with M components mostly had IC morphology, but only a small proportion of the ICS had M proteins detectable with conventional electrophoresis.

Clonal chromosomal aberrations were slightly more common in the IC group (62%) than in the CLL ‘proper’ group (48%) (difference not significant). One explanation might be that immunoglobulin-secreting CLL cells, due to their higher metabolic activity are more easily activated by mitogens in vitro. In fact, the mitogen-induced thymidine uptake of the malignant cells in in-vitro cultures is higher in PLL and IC than in CLL patients [25, 74]. If a greater success rate in activating and karyotyping PLL and IC cells would be the cause of the higher incidence of chromosomal aberrations, one might expect to find unrevealed karyotypic abnormalities in CLL with ‘normal’ karyotype. However, our RFLP studies showed no trisomy 12 in CLL cells from patients that did not yield evaluable metaphases or from those who had what we designated a ‘normal’ karyotype [40]. Thus, there might be a true correlation between gross cytogenetic abnormalities and the immunoglobulin secretion of the CLL cells. Since the pattern of the specific chromosomal aberrations is similar in IC and CLL ‘proper’ it seems not likely that any specific aberration is closely associated to the immunoglobulin secreting phenotype.

Chromosomes and Prognosis—Abnormal versus Normal Karyotype

Patients with clonal aberrations have been found to have poor prognosis compared to patients with a normal karyotype [15–18]. There is an early need for therapy [17, 19, 24], and, in the study of Han et al., the survival was significantly shorter [16, 27]. In our study, the overall survival curves were not significantly different, but at 5 years the survival was significantly poorer for patients with clonal aberrations (p < .05). In
this context it is again relevant to discuss the nature of the CLL cells with "normal"
karyotype. Such a finding in chromosome analyses might frequently be the result of
a low proliferation rate of the malignant cells compared to that of the normal lymphoid
cells [8], and low-proliferative CLL cells are associated with good prognosis [24, 74–76].

Chromosomes and Prognosis—Single versus Multiple Aberrations
We have shown that the therapy-free survival is very significantly shorter in patient
groups with increasing number of chromosomal aberrations within the leukemic
clone [17, 24]. Also, we demonstrated that the survival curves of patients with single
abnormalities and complex karyotypes are separated [17, 74], and this has also been
shown in studies from other groups [16, 77]. However, in studies of about 100 patients,
the difference in survival has not been significant. The constant appearance of the
curves in different studies although makes it likely that the poorer prognosis of
complex karyotypes compared to single aberrations is true, and might be significant
in larger studies, such as in future analyses of the IWCCLL [15]. This finding also
corresponds well with recent studies of chromosomes and prognosis in lymphomas
[78].

Chromosomes and Prognosis—Percentage Abnormal Metaphases
Most samples for cytogenetic analysis, with or without clonal abnormalities, contains
normal metaphases. We first showed a significant association between high percentage
of clonal aberrations and poor survival [24, 79], statistically analysed with Log rank
test [31], as well as Cox multivariate analysis [80]. This finding has also been confirmed
by Han et al. [81], and in the present study (Fig. 2), and is concordant with previous
findings in acute leukemia [82]. However, the interpretation is not quite obvious.
Our RFLP data [40] discussed above indicate that the normal metaphases are not
representative for the cell sample since almost all cells in a sample with a trisomy 12
close contain trisomy 12 irrespective of a low percentage of abnormal cells with the
cytogenetic technique. Thus, we believe that the finding of normal metaphases mixed
with the malignant clone indicates a mitotic activity of the residual nonmalignant
cells, which is of benefit for the patient.

Chromosomes and Prognosis—Trisomy 12
Trisomy 12 is the most common aberration in CLL, and it is therefore the abnormality
that has been the most suitable one for prognostic analyses. It was soon found by our
group [19] that patients with trisomy 12 deteriorated faster and had an earlier need
for treatment than patients with normal karyotype. The same observation was made
by Sedanor et al. [70], and it was strongly confirmed by us with different statistical
methods [21, 24, 25, 74]. With respect to survival there has been no statistical differ-
ence between all patients with trisomy 12 and patients with other abnormalities [16,
17, 20, 22, 26, 28, 77]. However, when low-risk patients (Rai stage 0 through II) were
analysed separately, patients with trisomy 12 had a significantly poorer survival than
patients with other aberrations [23]. Because of our finding of a prognostic implication
of the complexity of the karyotype [17, 21], we also compared patients with trisomy
12 as the sole abnormality versus single abnormalities other than trisomy 12, showing
a close to significant difference in survival (Figure 5) [18]. Such analyses have not
been presented by other groups, probably because of small numbers of patients.
The largest study available today is the IWCCLL compilation of 427 patients, and a
Clinical Implications of Chromosomes in B-CLL

Preliminary analyses show poorer survival for trisomy 12 patients with or without additional aberrations [15]. More detailed analyses will be of greater interest.

Chromosomes and Prognosis—14q+ Chromosomes

Pittman and Catovsky observed a poorer survival for patients with 14q+ marker chromosomes [20]. We have analysed our patients in this respect, and the result supports this finding, although the number of patients with 14q+ chromosomes is very small (Fig. 4). The 14q+ group comprises tumors with oncogene-involvements, such as the bcl-1 in the t(11;14) commonly found in PLL, the bcl-2 in follicle center cell-derived lymphomas, and the c-myc in the (8;14)-translocation of Burkitt-like lymphomas and leukemias. CLL with such clonal aberrations might have achieved some of the distinctive character from these mostly aggressive diseases.

Chromosomes and Prognosis—Structural Aberrations on 13q and 6q

In the studies from Bournemouth [26], Edinburgh [28], and Stockholm [83] it was found that structural aberrations involving the long arm of chromosome 13 was, next to trisomy 12, the most common chromosomal abnormality. In the IWCCLL study [15] it was found that this group of abnormalities was associated with a better prognosis than other clonal aberrations. Figure 6 shows survival curves of patients from Hudginge with 13q aberrations, and although the number of patients is very small the shape of the curve might be indicative of a better survival of patients with 13q aberrations than of patients with other chromosomal abnormalities.

Aberrations involving 13q, like trisomy 12, frequently occur as a single aberration, possibly indicating a greater pathogenetic role than aberrations that mostly are accompanied by other aberrations. The pathogenetic discussion of 14q aberrations is highly interesting, since they frequently result in the loss of an oncogene, the retinoblastoma gene at 13q14 [84]. Future studies will tell if a deficiency of the retinoblastoma protein is involved in the pathogenesis of CLL.

Deletions of the long arm of chromosome 6 is another recurrent finding in CLL cells, and the survival of these patients also seemed to be superior to that of patients with other aberrations (Fig. 6). There is no obvious interpretation of this finding, which must be confirmed in other studies.

These data might predict that in CLL, like in acute leukemia [1], different karyotypic abnormalities will be found to be associated with different clinical behavior, eventually requiring different management of the disease.

DISCUSSION

Repeated analyses of patients from a number of different institutions seem to confirm the findings of clinical implications of the karyotype in CLL. Chromosomal aberrations are associated with poor prognosis compared to normal karyotype, and complex karyotypes and high percentage of abnormal metaphases are adverse prognostic factors. Trisomy 12 is the most common abnormality, and it is associated with an earlier need for treatment, and possibly poorer survival, as may be the case also with the 14q+ marker chromosome. On the other hand, structural aberration involving 13q, and possible 6q− chromosomes, might be associated with a better prognosis than other clonal aberrations. Chromosome analyses in CLL are laborious, even more than in other tumors since they require mitogenic activation and tumor cell culture. Thus only limited progress in the present knowledge is likely to be achieved from single institutions. Further studies with compiled data are necessary, and they might result in the definition of karyotypic subgroups with distinct clinical implications. Further
studies are also needed in the search for specific genes involved in the malignant transformation elucidating the pathogenetic mechanisms of C.L.L.

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ABSTRACT: A cyt... skin fibroblasts. There were... rearrangements. There was... between... different... in the ang... high rates... affecting a... in TSC by

INTRODUCTION

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