

Chromosome Abnormalities in Malignant Lymphoma: Biologic and Clinical Correlations *

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A. Introduction

Among the hematologic malignancies, the clinical and biologic significance of chromosome abnormalities have been most extensively studied in the acute leukemias and chronic myelogenous leukemia (CML). Clonal chromosome abnormalities are now identified in essentially all cases of CML and in most cases of acute nonlymphoblastic leukemia (ANLL) and acute lymphoblastic leukemia (ALL). In the acute leukemias, specific chromosome abnormalities have now been correlated with morphology (ANLL), immunologic phenotype (ALL), and various clinical features. In both ANLL and ALL, they have been found to be clinically important as independent risk factors for predicting response to treatment, remission duration, and survival [1-4].

Limited data are available regarding chromosomal abnormalities in malignant lymphoma, other than Burkitt's. The few reported studies of banded chromosomes have generally included small numbers of cases, and primary tumor masses have rarely been the major source of tissue studied. Moreover, for a given lymph node, chromosome findings have rarely been correlated with histology and immunologic pheno-

type. Since July 1978, we have been prospectively studying chromosomes in lymph nodes from patients with lymphoma and correlating them with histology, immunologic phenotype, and clinical findings [5, 6]. This report briefly summarizes our findings in the first 115 patients.

B. Materials and Methods

Chromosomes from involved lymph nodes or other tumor masses from 115 patients (ages 8-85 years; median 55 years) with non-Hodgkin's malignant lymphoma were studied. In 73 patients, neoplastic tissue was analyzed at diagnosis prior to any treatment; in 42 patients, tissue was first studied at relapse. In all instances, the tumor was simultaneously studied for histology, immunologic markers, and G-banded chromosomes. Histologic classification was done using the International Working Formulation for Clinical Usage [7].

Immunologic phenotyping, as described previously, was based on study of both single cell suspensions and tissue frozen sections in all cases [8]. All cases were studied for surface (SIg) and cytoplasmic (CIg) immunoglobulin, and receptors for complement (C'), Fc, and unsensitized sheep erythrocytes (E); 79 cases were also studied with a panel of monoclonal antibodies including BA-1, BA-2, and BA-3 [9].

For cytogenetic studies, a portion of the same tumor mass biopsied for histology and immunologic phenotyping was obtained directly from the surgical pathology laboratory and processed within 1 h of bi-

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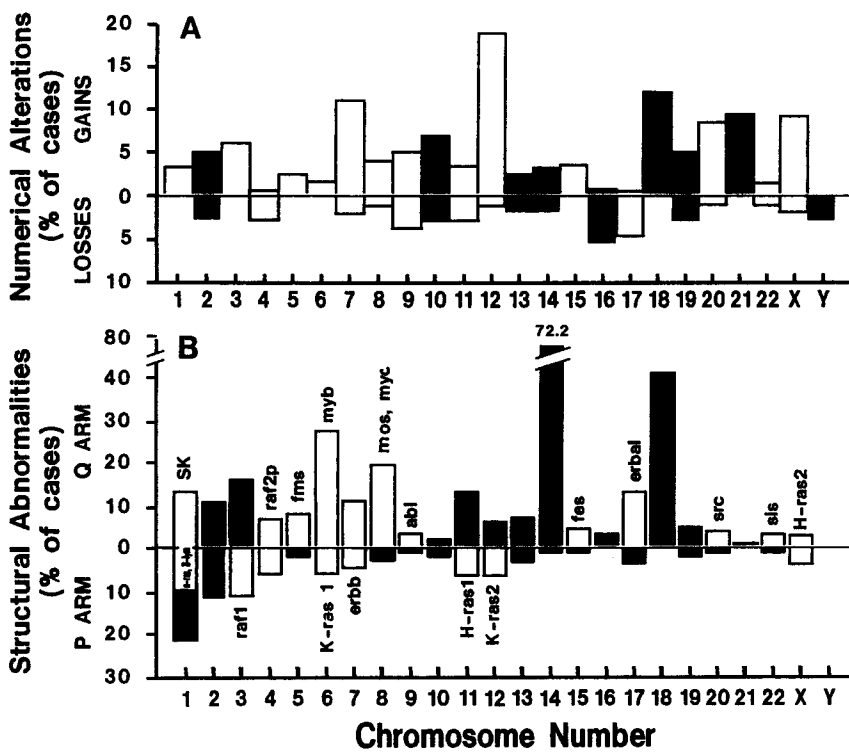


Fig. 1 A, B. Histogram of **A** gains and losses of whole chromosomes and **B** structural abnormalities involving each chromosome arm in 115 cases of lymphoma. Chromosomes **A** or the percentage of cases with rearranged chromosome arms **B** involving areas to which cellular oncogenes have been mapped are indicated by *open bars*

opsy. Metaphase chromosomes were harvested from direct preparations and unstimulated or methotrexate-synchronized short-term (24- and 48-h) cultures using methods described previously [6]. G-banding was done using the Wright's technique of Sanchez et al. [10]. Photographs of metaphases were taken on high contrast SO115 film, and multiple karyotypes were constructed in each case.

Chromosomes have been designated according to the ISCN (1978, 1981), and the karyotypes are expressed as recommended under this system [11, 12]. Chromosome abnormalities were designated as clonal if two or more metaphase cells had identical structural anomalies or extra chromosomes, or if three or more metaphase cells had identical missing chromosomes.

C. Results

Clonal chromosome abnormalities were identified in 96% of the 115 patients. Two clones were identified in 12% of patients. Most clones had multiple abnormalities. The abnormal karyotypes included gains of one or more whole chromosomes (without apparent structural abnormalities) in 58% of patients and losses of one or more whole chromosomes in 27%. Chromosomes most

commonly gained were 12 (19% of patients), 18 (12%), and 7 (11%). Chromosomes most frequently lost were 16 (5%) and 17 (4%).

Structural abnormalities were more frequent than numerical alterations, occurring in 90% of patients. All chromosomes were affected, but with considerable variation in frequency. The chromosome regions (by arm) most frequently rearranged were 14q (72% of patients), 18q (41%), 6q (28%), 1p (21%), 8q (19%), 3q (16%), 1q, 11q, and 17q (14% each), 2p, 2q, and 7q (11% each), and 3p (10%).

Translocations were the most common type of structural abnormality; 84 different translocations were identified, but only the t(14;18)(q32;q21) and t(8;14)(q24;q32) occurred in three or more different patients. Other common types of structural abnormalities included deletions, duplications, and isochromosomes. Specific chromosome regions most commonly rearranged were 14q32 (67% of patients), 18q21 (31%), 6q21 (15%), 8q24 (13%), 11q21-25 (11%), and 1p36 (10%).

An analysis was performed to determine if the chromosome abnormalities seen in lymphoma were preferentially in regions to which oncogenes have been mapped. In Fig. 1, the frequency of abnormalities involving individual chromosomes for nu-

Table 1. Karyotype findings which differed significantly among histologies^a

	International Working Formulation groups ^b									<i>P</i>
	A	B	C	D	F	G	H	I	J	
Cases	19	28	8	9	13	23	4	3	5	
	Median value									
Normal cells (%)	18	22	7	15	20	0	12	45	0	0.002
Modal number	46	47	48	49	47	49	48	46	46	<0.001
Chromosome or region altered	Frequency of specific abnormality in each histology (%)									
6q21	21	29	25	22	23	39	75	0	20	0.0085
+7	0	4	25	33	0	30	0	0	0	0.0071
+8	0	4	38	11	0	0	0	0	0	0.0018
8q	16	14	25	0	0	30	25	0	100	0.0003
8q24	16	0	0	0	0	30	0	0	100	0.0000
t(8;14)(q24;q32)	5	0	0	0	0	22	0	0	80	0.0000
13p13	0	0	38	0	0	4	0	0	0	0.0002
14q32	42	89	100	67	46	74	0	33	100	0.0001
17q21-25	0	0	0	44	8	9	0	0	0	0.0007
18q	16	68	63	44	15	52	0	0	20	0.0013
18q21	5	64	63	44	0	26	0	0	20	0.0000
t(14;18)(q32;q21)	0	61	63	44	0	26	0	0	20	0.0000

^a Only abnormalities with a $P < 0.009$ are listed. This conservative P value has been chosen because of the large number of possibilities tested

^b A small lymphocytic; B follicular, predominantly small cleaved; C follicular, mixed, small cleaved and large cell; D follicular, predominantly large cell; F diffuse, mixed small and large cell; G diffuse, large cell; H diffuse large cell immunoblastic; I lymphoblastic; J small noncleaved

merical alterations and each chromosome arm for structural abnormalities are indicated. Chromosomes or chromosome regions to which cellular oncogenes have so far been localized are indicated as open bars. As can be seen, many chromosome abnormalities involved regions to which oncogenes have been mapped. Chromosomes to which oncogenes have so far been mapped which were frequently involved in numerical changes were 12 (*K-ras2*), and 7 (*erbB*) (Fig. 1a). Among chromosomes commonly involved in numerical changes, only number 18 contains no known oncogene. Among structural abnormalities, the two most commonly involved regions (14q, 18q) are not ones to which oncogenes have been mapped; however, more than 10% of the patients had structural rearrangements in regions to which the following oncogenes have been mapped: *myb* (6q22-24), *myc* (8q24), *sk* (1q12→qter), *raf1* (3p23→pter), and *erbA1* (17p11→q22) (Fig. 1b). Overall,

91 (79%) of the 115 patients demonstrated clonal chromosome abnormalities involving regions to which oncogenes have been mapped. These data, of course, represent a high estimate of the possible number of patients in whom oncogenes may be rearranged since many oncogenes have not been precisely localized and patients with any abnormality of the broad area to which the oncogene might be mapped are included, as are patients with losses and gains to whole chromosomes.

The results of cytogenetic analysis were compared among histologic groups. Several features appeared to differ significantly in their distribution among histologies. These included the median percentage of normal cells, the median modal number of the clonal chromosome abnormalities, and the frequency of certain specific chromosome abnormalities (Table 1). No chromosome region or specific chromosome abnormality was restricted to a single histology, but sev-

Table 2. Chromosome abnormalities which differed significantly among B cell monoclonal groups

	Expression of BA-1, BA-2, BA-3						<i>P</i>	BA-1 ^a		
	+-	-	++	-+	+++	+-		+	-	<i>P</i>
Cases	28	5	13	5	6	4	<i>P</i>	51	12	<i>P</i>
Chromosome abnormalities in each immunologic group (%)										
1p abnl	4	40	46	20	0	50	0.0085			
5q abnl	0	60	0	40	0	0	0.0000	0	42	0.0000
6q15	0	0	0	40	17	0	0.0036			
6q21	18	60	8	60	0	0	0.0157	12	50	0.0086
+7	7	0	8	60	0	0	0.0065			
16q abnl	0	0	0	0	0	50	0.0000			

^a Two additional cases were studied for BA-1 which were not studied for BA-2 and BA-3

eral were frequent in only one or two groups. These included an extra 7 in follicular center cell lymphomas with large cells (groups C, D, and G), an extra 8 in the follicular lymphomas, mixed small cleaved and large cell (C), t(8;14)(q24;q32) in large and small noncleaved malignant lymphoma (G and J), 13p13 in C, 17q21-25 in D, and t(14;18)(q32;q21) in follicular lymphomas (B-D) and diffuse large (follicular center) cell lymphoma (G). Rearrangements of specific chromosome regions, but not specific abnormalities, were occasionally found in 100% of a given histologic group.

When the results of cytogenetic analysis were compared among broad immunologic groups (B, T, C', null), only abnormalities involving 18q differed significantly among the groups, occurring in all three cases of C' lymphoma, 44% of B lymphomas, but no T lymphomas ($P=0.003$). Since most cases studied were B cell (98), a detailed analysis of the distribution of chromosome abnormalities among lymphomas expressing different heavy and light chains was possible. No highly significant differences (all P values > 0.03) in distribution were found. However, when the B cell lymphomas were classified according to expression of the antigens identified by the monoclonal antibodies BA-1, BA-2, and BA-3, the distribution of certain chromosome abnormalities varied significantly (Table 2). Most interesting was the frequent absence of BA-1 expression in lymphomas with chromosome rearrangements involving 5q and 6q.

To determine if cytogenetic analysis had clinical use as a prognostic factor, the 73 patients studied at diagnosis prior to treatment were evaluated. Median follow-up of the surviving patients in this group is 32 months (minimum of 9 months). Various aspects of karyotype were correlated with response to treatment and survival. It was found that 65% of patients achieved a complete remission. No significant correlations with cytogenetic findings were identified. Length of survival varied significantly according to a number of aspects of karyotype analysis. In particular, patients whose lymphomas had more than 20% normal metaphases survived significantly longer than those with fewer than 20% normal cells (Fig. 2a). Similarly, patients whose lymphoma had a modal number of 46 survived longer than those with a modal number of ≥ 47 or ≤ 45 (Fig. 2b). Too few patients have been studied to determine if these various features of karyotype are independent prognostic factors.

D. Discussion

In this large study of G-banded chromosomes in lymphoma, clonal chromosome abnormalities have been found in 110 of 115 cases. Multiple recurring abnormalities were noted, some of which are associated with specific histologies or immunologic phenotypes. Certain aspects of cytogenetic analysis also seemed to correlate with patient survival. However, analysis is com-

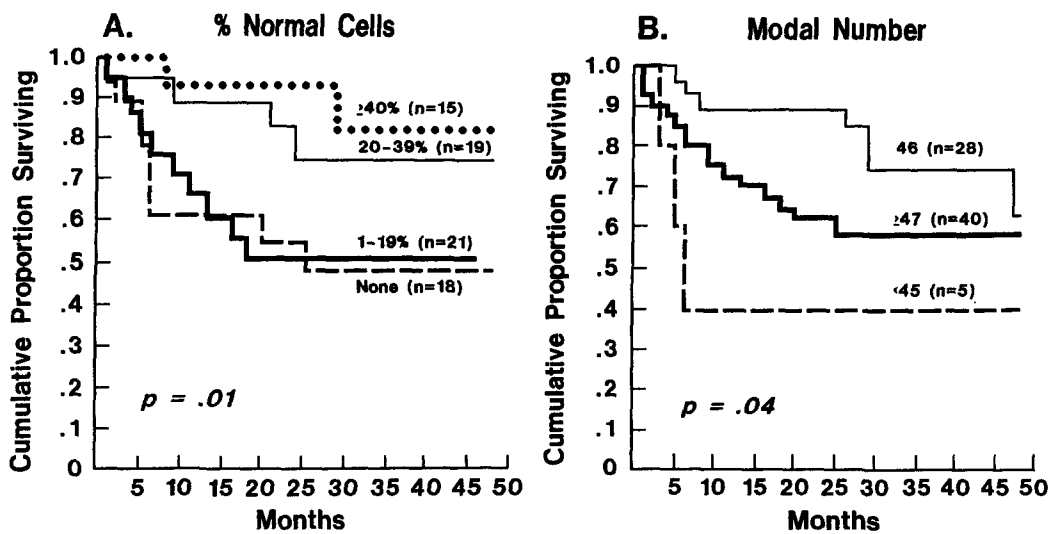


Fig. 2A, B. Survival of 73 patients with lymphoma according to **A** the frequency of normal metaphases identified cytogenetically in a neoplastic lymph node studied before treatment and **B** the modal number of the primary clonal chromosome abnormality identified in a pretreatment neoplastic lymph node

plicated by the multiple abnormalities found in most patients and the relatively small numbers of patients with each recurring abnormality studied. The clinical relevance of cytogenetic analysis for diagnosis, classification, and prognosis in non-Hodgkin's lymphoma obviously requires further study.

The biologic significance of these recurring clonal chromosome abnormalities is also unknown. However, that 79% of patients had numerical or structural rearrangements involving chromosomes or chromosome regions to which oncogenes have been mapped is of interest. Detailed study of the role of oncogenes in lymphoma would appear to be a profitable endeavor.

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