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Correlation of Secondary Cytogenetic Abnormalities With Histologic Appearance in Non-Hodgkin's Lymphomas Bearing t(14;18)(q32;q21)^{1,2}

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Successful cytogenetic studies were performed on 69 biopsies from 64 patients with non-Hodgkin's lymphoma bearing a t(14;18)(q32;q21) translocation. This translocation appears to be a primary abnormality associated with the development of certain B-cell non-Hodgkin's lymphomas. We correlated the occurrence of secondary abnormalities, in addition to the t(14;18)(q32;q21), with histologic subtype to test the hypothesis that secondary abnormalities correlate with more aggressive histologic appearance. A large number of secondary abnormalities were identified, the most frequent being additional copies of chromosomes 7 (30%), 12 (22%), 18 (22%), 20 (16%), or 21 (14%), deletion of a portion of the long arm of chromosome 6 (17%), and either an additional chromosome 17 or an isochromosome for the long arm of chromosome 17 (13%). An extra chromosome 7 was highly associated with a diffuse histologic pattern; it was present in 52% of patients with a diffuse pattern and in only 15% of those with a follicular pattern ($P = .002$). A weaker association with a diffuse growth pattern was found for the ad-

dition of chromosome 17 or an i(17q); it was found in 24% of patients with a diffuse pattern and only 5% of those with a follicular pattern ($P = .05$). No other significant correlations between secondary chromosome abnormalities and histologic subtype were identified. Although the explanation for this association is not clear, it appears that patients with B-cell non-Hodgkin's lymphomas bearing the t(14;18)(q32;q21) translocation which also have an additional chromosome 7 are likely to exhibit a diffuse growth pattern. [*J Natl Cancer Inst* 1988;80:576-580]

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Since the discovery by Manolov and Manolova (1) of an abnormal chromosome 14 in Burkitt's lymphoma, it has become increasingly apparent that recurrent, consistent chromosomal abnormalities are associated with non-Hodgkin's lymphomas (2-7). These include the t(8;14), t(2;8), and t(8;22) translocations in the small noncleaved lymphomas of the Burkitt's type, the t(14;18)(q32;q21) translocation in a variety of B-cell non-Hodgkin's lymphomas, and the t(11;14) translocation in the small lymphocytic and diffuse intermediate non-Hodgkin's lymphomas. The t(14;18)(q32;q21) has been particularly associated with follicular lymphomas. However, this translocation is also found in diffuse, aggressive non-Hodgkin's lymphomas, and it is present in 20%-30% of diffuse large cell lymphomas in some series (6,8).

Changes in the microscopic appearance and behavior of tumors have been proposed to be related to clonal evolution secondary to sequential genetic changes most easily recognized by cytogenetics (9). Recent reports from the University of Minnesota and Memorial Sloan-Kettering Cancer Center have suggested that patients with diffuse, aggressive lymphomas bearing the t(14;18)(q32;q21) might represent examples of transformation from more indolent non-Hodgkin's lymphomas (10,11). In addition, these authors have proposed that the transformation might be associated with specific secondary chromosomal abnormalities. We have examined our patients with the t(14;18)(q32;q21) and have correlated the occurrence of secondary abnormalities with histologic and clinical variables and treatment outcome to see if we could identify any significant associations.

Materials and Methods

Between July 1982 and August 1987, 245 successful chromosome karyotypes were obtained for patients whose histologic diagnosis from the same biopsy was non-Hodgkin's lymphoma. A clonal chromosomal abnormality was identified in 201 (82%) of these biopsies. The presence of a t(14;18)(q32;q21) was discovered in 71 (35%) of the karyotypes containing a chromosomal abnormality. No clinical information was available on two of these patients and they are not considered further. The remaining biopsy specimens were obtained from patients who were being studied and treated by the Nebraska Lymphoma Study Group.

All biopsies were processed according to a standard protocol for histologic, immunologic, and cytogenetic studies. Tissue specimens involved by a lymphoma were received and processed by the cytogenetics laboratory within 1 hour after the biopsy procedures, or the specimens were similarly received, cultured, and processed through the fixation stage by the outside hospital laboratory before shipment. The tissue was placed directly into RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 20% fetal bovine serum and gentamicin. The tissue was mechanically minced and placed in culture without mitogens at 37°C for 24 hours. If sufficient tissue was available, a 48-hour culture was also performed. Two hours prior to the initiation of harvest, the cells were exposed to colcemid (0.05 µg/mL). The cells were then resuspended in 0.074 M of potassium chloride for 10 minutes

and fixed with a methanol/glacial acetic acid solution (3:1). Fixation was repeated 3 times and slide preparations were made. The slides were aged for a minimum of 24 hours and G-banded with Wright's stain. All metaphase plates were microscopically analyzed, recorded, and photographed. Karyotypes were arranged and reported according to the Paris Conference criteria (12). An abnormal clone was defined as ≥ 2 cells with the same structural abnormality or the same extra chromosome or the presence of ≥ 3 cells with the same missing chromosome. If mitoses could not be confidently analyzed, the mitotic cell was not included in the data.

Portions of each biopsy specimen were fixed in B5 and Formalin for routine histologic processing. Patients were classified using the Working Formulation (13) with the addition of a category for diffuse intermediate lymphoma (14). Representative portions of fresh tissue were also prepared for frozen-section immunohistochemical analysis. Briefly, a 2.0-mm section of tissue was placed in OCT embedding medium (Miles Laboratories, Naperville, IL) and snap frozen at -150°C in isopentane quenched in liquid nitrogen. The tissue was then stored at -70°C until sectioned. Cryostat sections were air-dried and fixed in 4°C acetone for 10 minutes. In situ immunologic phenotyping was performed with a three-stage immunoperoxidase technique employing unconjugated mouse antibody as the first stage, followed by biotin-conjugated goat anti-mouse F(ab')₂ antibody, and then avidin-conjugated horseradish peroxidase (Vector Laboratories, Inc., Burlingame, CA). Primary mouse antibodies to immunoglobulins (G, A, M, D, kappa, and lambda; DAKO Corporation, Santa Barbara, CA) were used.

The significance of differences between groups was determined using the chi-square test with the Yates correction. The significance of differences between survival curves was determined using the log-rank test.

Results

A total of 69 biopsy specimens from 64 patients with the t(14;18)(q32;q21) form the basis for this report. The relationship between the t(14;18)(q32;q21) and histologic subtype is presented in table 1. The occurrence of the t(14;18)(q32;q21) was highly associated with a follicular pattern and was present in 63% of the biopsies with a follicular pattern and

Table 1. Cytogenetic results by histologic type

Histology*	No. of biopsies with abnormal karyotypes	Biopsies showing t(14;18) (%)
Small noncleaved	15	0 (0)
Follicular small cleaved	13	9 (69)
Follicular mixed	28	15 (54)
Follicular large cell	23	16 (70)
Diffuse small cleaved and diffuse intermediate	21	0 (0)
Diffuse mixed	17	4 (24)
Diffuse large cell	39	17 (44)
Immunoblastic	33	8 (24)
Lymphoblastic	7	0 (0)
Small noncleaved	6	0 (0)
Not otherwise specified	1	0 (0)

*Classified by the Working Formulation + diffuse intermediate lymphoma and not otherwise specified.

Table 2. Clinical characteristics of 64 patients with non-Hodgkin's lymphoma bearing the t(14;18) (q32;q21)

Characteristic	% of patients
Median age in yr (range): 61 (27-89)	
Male/female: 35/29	
Stage	
I	12
II	12
III	21
IV	54
B symptoms	20
Bone marrow involvement	30
Previously untreated (% of total karyotypes)	52
B-cell immunophenotype	100

in 21% of the biopsies with a diffuse pattern ($P < .001$). However, patients with diffuse mixed cell, diffuse large cell, and diffuse immunoblastic lymphomas had this translocation from 24% to 44% of the time. Thirty-five percent of the tumors that previously would have been classified as diffuse histiocytic lymphoma in the Rappaport classification (i.e., diffuse large cell and immunoblastic in the Working Formulation) had the t(14;18)(q32;q21).

Clinical characteristics of the 64 patients with the t(14;18)(q32;q21) are presented in table 2. The patients had a median age at diagnosis of 61 years. Fifty-five percent of the patients were male. Most of the patients had disseminated disease at diagnosis but only 20% had B symptoms. The tumors of all 64 patients had a B-cell immunophenotype. The survival from diagnosis for all 64 patients is presented in figure 1. The median survival was 69 months, with 38 currently alive. One patient had 3 biopsies studied and 3 patients had cytogenetic studies on 2 biopsies at different points in time. Two patients had no change in karyotype, 1 patient underwent a histologic change from follicular small cleaved to follicular large cell and acquired a new t(1;1)(q23;q24), and 1 patient underwent a histologic change from follicular small cleaved to diffuse large cell and acquired an additional chromosome 7.

A very large variety of secondary chromosomal abnormalities occurred in these patients. The most frequent secondary abnormalities (i.e., those that occurred in at least 6 biopsies) are presented in table 3. The most frequently occurring abnormalities were additions of chromosomes, with the most

Table 3. Frequently occurring secondary abnormalities in 69 biopsies bearing the t(14;18) (q32;q21)

Abnormality	Frequency (%)
+7	21 (30)
+12	15 (22)
+18	15 (22)
del (6q)	12 (17)
+20	11 (16)
+21	10 (14)
+17, i(17q)	9 (13)
+9	7 (10)
+8	7 (10)
+3	7 (10)
Abnormalities at 1p21-22	6 (9)
Abnormalities at 3q21-27	6 (9)

frequent being an additional chromosome 7 in 30% of the patients. Deletions of the long arm of chromosome 6 and deletions or translocations involving the short arm of chromosome 1 and the long arm of chromosome 3 were the only structural abnormalities that occurred with this frequency. These secondary abnormalities were tested for their association with histologic growth pattern (i.e., follicular or diffuse), cell type (i.e., large cell vs. small cell), and specific histologic subtype. The only significant abnormalities that emerged are presented in table 4. The addition of an extra chromosome 7 was highly associated with a diffuse growth pattern since it was present in 52% of 29 biopsies with a diffuse growth pattern and in only 15% of 40 biopsies with a follicular growth pattern ($P = .002$). There was also an association of borderline significance between the presence of an addition of genetic material from the long arm of chromosome 17 with a diffuse histologic pattern (24% vs. 5%, $P = .05$). The occurrence of these two secondary abnormalities by histologic subtype is presented in table 5. The possibility was considered that the addition of chromosome 7 might be related to preceding chemotherapy. However, an additional chromosome 7 was found in 22% of biopsies performed after the patient had received chemotherapy and in 39% of biopsies from patients who had never been treated.

None of the secondary abnormalities, including +7, +17, or i(17q), were significantly associated with response to therapy or survival. In previously untreated patients, patients who had a +7 in addition to the t(14;18)(q32;q21) achieved complete remission 55% of the time in contrast to a 65% complete remission rate in patients without this abnormality. The survival of patients with both the +7 and +17q was slightly, but not significantly, shorter from the time of biopsy than that of patients without these abnormalities.

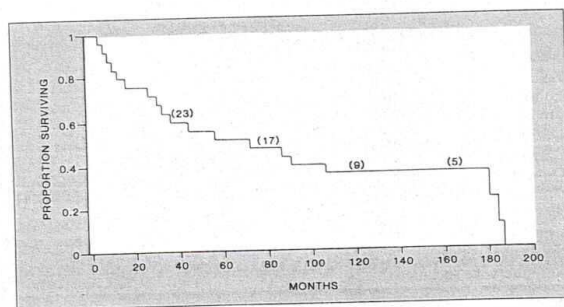


Figure 1. Survival from diagnosis for all 64 patients with t(14;18) (q32;q21). Nos. in parentheses = patients surviving to that interval.

Table 4. Secondary abnormalities significantly associated with a diffuse histologic pattern

Karyotypic abnormalities	No. (%) of biopsies with histologic pattern		Significance
	Follicular (n = 40)	Diffuse (n = 29)	
+7	6 (15%)	15 (52%)	$P = .002$
+17, +17q	2 (5%)	7 (24%)	$P = .05$

Association of secondary karyotypic abnormality and histologic subtype

Pathology*	Total No. of biopsies with t(14;18)	Frequency (%)	
		+7	+17, +i(17q)
Small cleaved	9	0 (0)	2 (11)
Fixed	15	2 (13)	0 (0)
Large cell	16	4 (25)	1 (6)
Diffuse	4	4 (100)	2 (50)
Large cell	17	7 (41)	3 (18)
Diffuse	8	4 (50)	2 (25)

ed by the Working Formulation.

Discussion

t(14;18)(q32;q21) is the most commonly occurring somal translocation discovered in patients with non-Hodgkin's lymphoma in the United States (3,6). Our findings of association of the t(14;18)(q32;q21) with follicular pattern and B-cell immunophenotype are similar to reports (7,10,15). This translocation appears to be a primary chromosomal abnormality in the development of B-cell non-Hodgkin's lymphomas. Although the t(14;18)(q32;q21) is more common in patients with B-cell lymphoma that has a follicular growth pattern, it is also seen in patients with the diffuse aggressive histologic growth pattern had this translocation and it was present in 35% of the patients with diffuse large cell and immunoblastic subtypes. Most of these patients had not undergone clinically apparent histologic transformation, but presented with the diffuse lymphoma. Recently, Yunis et al. (10) and Richardson et al. (11) proposed that lymphomas bearing the t(14;18)(q32;q21) with a diffuse aggressive histologic pattern might have undergone progression from a more indolent process—frequently clinically observed—associated with the development of secondary chromosomal abnormalities. Both studies identified an additional chromosome 7 as one of the abnormalities possibly associated with this change. Rowley and Fukuhara (2) found an association between an additional chromosome 7 and the diagnosis of diffuse histiocytic lymphoma. Kristofferson et al. (16) found a shorter survival in patients with +7 compared to patients with normal karyotypes. At the Fifth International Workshop on Chromosomes in Leukemia-Lymphoma (7) it was reported that a numerical chromosome 7 abnormality (i.e., trisomy, monosomy, or isochromosome) was found in 32% of patients with the t(14;18). The association of an additional chromosome 7 and a diffuse growth pattern, regardless of the presence of t(14;18)(q32;q21), has been suggested by several authors (2,4,6,7). However, the explanation for this association is not clear. The epidermal growth factor receptor gene is located on chromosome 7 (17). Also, a number of other malignancies have been reported to have abnormalities of chromosome 7 (18–22). How this relates to a more aggressive histologic appearance in non-Hodgkin's lymphomas remains, at best, speculative.

The relationship we found between the +17 or +i(17q) and a diffuse growth pattern was statistically much weaker than for the +7. Since a large number of secondary abnormalities were evaluated, it is possible this association occurred only by chance. However, Cabanillas et al. (23) have previously suggested that this abnormality is associated with a poorer outlook in certain non-Hodgkin's lymphoma. Levine et al. (8) found that patients with structural abnormalities of chromosome 17 had a shorter survival than patients without these abnormalities. Further studies will be necessary to be certain of the relation of chromosome 17 abnormalities with histologic pattern.

The relevance of an additional chromosome 7 to treatment outcome remains uncertain. There were too few patients in this series studied at the time of diagnosis and observed after uniform therapy to be confident of identifying any difference in treatment outcome. However, in other hematologic malignancies such as acute leukemia, the occurrence of certain chromosomal abnormalities is highly associated with response to therapy (24). It seems likely that further studies of large numbers of patients with non-Hodgkin's lymphomas will identify similarly important prognostic subgroups. The use of molecular biologic techniques (25,26) to identify genetic abnormalities in addition to traditional cytogenetics should increase the number of patients who can be studied and increase the likelihood of identifying clinically relevant genetic abnormalities.

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Nutrients in Diet and Plasma and Risk of In Situ Cervical Cancer^{1,2}

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Both plasma and dietary measures of vitamin A status were investigated along with previously established risk factors (number of sexual partners, age at first intercourse, smoking, and oral contraceptive use) in a study of 117 in situ cervical cancer patients and 196 matched community controls in Sydney, Australia. Neither total calories nor retinol from foods was related to cancer risk, nor was plasma retinol. When plasma and dietary indexes were considered together, vitamin C, fruit juices, and plasma beta-carotene showed protective effects. Plasma beta-carotene reduced risk from top to bottom quartile by 80%, vitamin C by 60%, and fruit juices by 50%. Thus the evidence suggests that cancer risk is associated with some aspect of diet that is reflected in the effect of plasma beta-carotene. There is no clear effect of any one nutrient but fruit juices appear protective. Thus vitamin C and beta-carotene are likely candidates. [*J Natl Cancer Inst* 1988;80:580-585]

Extensive cellular (1), animal (2), and human evidence (3) links vitamin A with cancer prevention. Retinol and related compounds have been implicated experimentally in animals as potential human antineoplastic agents (4) and are thought to inhibit specifically the later stages of carcinogenesis. Epidemiological investigations of past dietary intake of carotenoids and blood measures of carotene and beta-carotene in both case-control and cohort settings support these speculations (5-11).

Of recent interest is the relationship of vitamin A to the risk of cervical squamous neoplasms. Several cohort and case-

control studies have investigated the role of either dietary or blood measures of retinol and/or carotene in the etiology of invasive cervical cancer (12-16). The majority reveal no effect of retinol but a protective effect of beta-carotene. Other studies investigating preinvasive cervical neoplasms have followed the same pattern in general, although sample sizes were usually small and often dysplasia and in situ cancer were analyzed together (17-19). Two studies found

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