

Geographic Variance in the Frequency of the t(14;18) Translocation in Follicular Lymphoma: An Israeli Series Compared to the World Literature

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ABSTRACT: It has been suggested that differences in the frequency of the t(14;18) translocation in follicular lymphoma might explain ethno-geographic variation in the incidence of these tumors. We tested Israeli follicular lymphoma patients for the frequency of the t(14;18) translocation, and reviewed the published literature, comparing the frequency in our series with data from different parts of the world.

Tissue specimens from 36 Israeli follicular lymphoma patients were tested for presence of the translocation by PCR amplification of the MBR breakpoint. Twenty-two of the 36 patients (61%) tested positive.

A systematic search of the literature yielded 35 papers reporting the frequency of the t(14;18) translocation in follicular lymphoma. We analyzed cytogenetic data and molecular data separately. For each method, data were pooled from all studies within each of three geographical regions - USA, East Asia and Europe. Pooled data from cytogenetic studies show a low frequency of the translocation in the Far East (38%) compared to the USA (71%), with an intermediate frequency found in Europe (61%). Molecular studies show a similar frequency of the translocation in the Far East and Europe, significantly lower than the frequency in pooled data from American studies. The frequency in our Israeli series is relatively high, comparable to that detected in the USA.

We suggest that the apparent geographical differences we describe are unlikely to be caused by a difference in the biology of the tumor, and are more likely due to technical and methodological factors. We conclude that it is unlikely that differences in the frequency of the t(14;18) translocation explain the difference in the epidemiology of lymphoma between East and West.

Keywords: follicular lymphoma, epidemiology, t(14;18), Israel, Europe, Asia, United States

INTRODUCTION

Ethnic and geographic variation in the incidence and clinical behavior of non-Hodgkin's

lymphomas (NHL) is well described. In particular, the majority of lymphomas in Western countries are of B-cell origin, whereas in the Far East, T-cell lymphomas are more prevalent. In the

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USA, about one third of NHL are follicular B-cell tumors. By comparison, only 3 - 13% of lymphomas in East Asian patients are follicular (1). The explanation of this phenomenon may shed light on the pathogenesis of these malignancies. An analogy can be drawn from Burkitt's lymphoma. In this tumor, the African "Endemic" type is clinically and epidemiologically distinct from the Western "Sporadic" type. Burkitt's lymphoma has been shown to be associated with the t(8;14) chromosomal translocation. Molecular analysis has revealed that each of the two types of Burkitt's lymphoma is associated with distinctive breakpoints on both chromosomes 8 and 14 (2).

Follicular lymphoma is associated with the t(14;18) translocation, reported to be present in 19-89% of these tumors (3,4). The translocation adjoins the proto-oncogene *bcl-2* on chromosome 18 with the immunoglobulin heavy chain locus on chromosome 14, placing *bcl-2* downstream to the JH enhancer. *Bcl-2* has been shown to prevent programmed cell death (apoptosis). The change in the molecular environment of *bcl-2* results in overexpression of the gene, thus immortalizing the cell. This is hypothesized to be an early event in the pathogenesis of the lymphoma, in that it enables other oncogenic mutations to accumulate (5).

About 60% of the t(14;18) translocation breakpoints are clustered within a 150 bp region on chromosome 18 designated the Major Breakpoint Region (MBR) (6). An additional 30% are found in a region 30 kb downstream of MBR, designated the Minor Cluster Region (MCR) (7). Analysis of the t(14;18) translocation in follicular lymphoma is likely to shed light on the ethno-geographic variation in the incidence of these tumors, as did the analysis of the t(8;14) translocation in Burkitt's lymphoma. A number of studies have been published comparing the frequency of the t(14;18) translocation among follicular lymphoma patients from different parts of the world. Both breakpoint regions have been shown to be present in patients from different parts of the world. However, some studies found

a lower overall frequency of the translocation in Japan compared to the USA. We tested Israeli follicular lymphoma patients for the frequency of the t(14;18) translocation at the MBR using PCR amplification of the breakpoint, and reviewed the published literature, comparing the frequency in our series with data from different parts of the world.

MATERIALS AND METHODS

Patients and Samples

Cases of follicular lymphoma were identified in three hospitals - the Shaare Zedek Medical Center and the Hadassah University Hospital in Jerusalem, and the Sheba Medical Center in Tel HaShomer. Most cases were found by searching the records of the pathology archives. Archival biopsy tissues were retrieved, and diagnoses were reviewed by a qualified pathologist (G.S., S.G.S.). In cases in which the diagnosis of follicular lymphoma was confirmed, 2-3 microtome sections were collected to be used for molecular analysis. Additional patients were drawn from the files of the Laboratory for Molecular Diagnosis of Malignancies in Hadassah Hospital. Patients with follicular lymphoma were selected from these records. Presence of malignant cells in the various tissue samples was verified either by a histopathological examination that revealed malignant involvement, or molecular analysis showing rearrangement of the immunoglobulin JH heavy chain locus by Southern blot hybridization.

Extraction of DNA

DNA was extracted from fresh tissues and peripheral blood using standard techniques (8). Extraction of DNA from paraffin embedded archival tissues was performed by dissolving the paraffin in xylene, rinsing in ethanol, and then digesting the tissue pellets with proteinase K for 8-12 hours at 50C, in 100 μ l of a lysis buffer containing 50 mM Tris-HCl pH 8.1, 1 mM

EDTA, 0.5% Tween 20 (Mazer Chemicals, Gurnee, IL, USA) with a final enzyme concentration of 0.2 mg/ml. The proteinase and tissue enzymes were then inactivated by boiling samples for 8 minutes. Suitability of the resulting sample for PCR amplification was ascertained by preliminary amplification of a 459 bp fragment of the beta-globin gene, using 1-10 µl of the sample as template. In cases in which this initial amplification failed, a second attempt was performed after further purification of the sample using the Wizard Minipreps DNA Purification System (Promega, Madison, WI, USA), according to the instructions provided by the manufacturer.

Polymerase Chain Reaction of the MBR

PCR for the MBR of the t(14;18) translocation was performed using a primer (5'-ACC-TGA-GGA-GAC-GGT-GAC-CAG-GGT-3') representing a consensus sequence found at the 3' region of all six known JH segments of the immunoglobulin heavy chain locus on chromosome 14 (9). The primer for chromosome 18 (5'-TTA-GAG-AGT-TGC-TTT-ACG-TG-3') represents a sequence immediately upstream of the MBR (10). Reaction mixes of 50 µl were prepared using commercially supplied Taq DNA polymerase and reaction buffer (Oncor, Gaithersburg, MD, USA). The quantity of template used was identical to that used for successful amplification of the globin gene fragment. Samples were overlaid with 50 µl of light mineral oil. Amplification conditions were initial denaturation at 94C for 5 minutes, followed by 40 cycles of denaturation at 94C for 1 minute, annealing at 51-55C for 1.5 minutes and extension at 72C for 1-1.5 minutes, with a final extension of 10 minutes at 72C. PCR products were demonstrated by electrophoresis on a high-resolution 2% 3:1 NuSieve-agarose gel stained with ethidium-bromide. Specificity of amplification was demonstrated by Southern blot hybridization with an internal probe for the MBR (5'-GCC-TGT-TTC-AAC-ACA-GAC-CC-3'),

using Hybond-N nylon membranes (Amersham, UK) and standard techniques. Stringent precautions were employed in all procedures in order to avoid cross-contamination of samples (11). Variability in size of the amplification product, stemming from different N-sequences (12), confirmed that no contamination had occurred, as no two patients had products of exactly the same size.

Review of Literature

A computerized search of the English language literature from 1966 to 1995 was performed using SilverPlatter 3.11 MEDLINE EXPRESS and the following search terms: MeSH: Lymphoma, non-Hodgkin's (all subheadings) AND (Follicular OR Nodular) AND (Bcl-2 OR MeSH: Translocation genetics (all subheadings)). The automated search was supplemented by a thorough manual search of papers cited in the retrieved articles.

RESULTS

Israeli Samples

70 paraffin embedded tissue specimens of follicular lymphoma were tested. Of these, DNA extraction was successful in 29 specimens from 30 patients, demonstrated by PCR amplification of the 459 bp beta-globin gene fragment. In addition, 7 samples tested in the Laboratory for Molecular Diagnosis fulfilled the inclusion criteria detailed in the Methods section (tissues from patients diagnosed as follicular lymphoma, in which presence of malignant cells in the specimen could be demonstrated conclusively). We were thus able to analyze a total of 37 samples from 36 patients.

Of the 36 patients included in the study, 11 were Ashkenazi Jews, 11 were Jews of Oriental origin, 10 were Jews whose ethnic origin was unknown, and 4 were Arabs.

Histopathologically, 30 of our samples were from purely follicular lymphomas (15

predominantly small cleaved cells, 10 mixed, 1 predominantly large cells and 4 of unspecified cell type). The remaining 6 samples were from mixed follicular and diffuse lymphomas (2 predominantly small cleaved cells, 2 mixed, and 2 predominantly large cells).

The results of our analysis of this series of Israeli follicular lymphoma patients are shown in Table 1. PCR amplification of the MBR was demonstrated in 22 of the 36 patients, or 61%.

Table 1. Data from Israeli Follicular Lymphoma Patients (Current Study)

Patient	Age at Diagnosis	Gender	Ethnic Group	Pathology	MBR
GC	58	m	J	FL-M	+
MA	62	f	JA	FL	-
FP	61	f	JS	FL-M	-
AR	70	m	JA	FL-SC	-
AH	74	f	Ar	F&D-M	+
ASI	77	m	Ar	FL-SC	+
DS	70	f	JS	FL-SC	+
OR	65	f	JA	FL-SC	+
MS	47	m	JS	FL-SC	+
YB	65	m	JS	FL-M	+
DC	31	f	JS	FL-SC	+
MM	41	m	JS	FL-M	-
LM	35	f	JA	FL-SC	+
YK	49	m	JA	FL	+
YO	38	m	J	FL-M	+
YN	83	m	JA	F&D - SC	-
ZI	80	m	Ar	FL-SC	-
SGO	91	m	JS	FL-SC	+
YH	47	f	JS	FL-SC	+
SB	nd	f	J	SC-FL	-
EY	54	m	J	M-F&D	+
MOD	nd	m	JA	SC-F&D	-
YG	41	m	J	F&D-SC	-
OS	nd	f	J	F&D-L	-
VS	nd	f	J	FL-M	-
SGA	57	f	JS	FL-SC	+
YD	80	m	JS	FL-SC	+
GK	36	m	JA	FL-M	-
BS	60	m	Ar	FL-SC	+
BAR	nd	m	JA	FL	+
EH	nd	f	J	F&D-L	-
YP	53	m	J	FL-M	-
MW	nd	m	JA	FL	+
BAL	nd	m	JA	FL-SC	+
MD	43	m	JS	FL-M	+
NM	nd	f	J	FL-M	+

Abbreviations: **m**, male; **f**, female; **J**, Jewish, origin unknown; **JA**, Jewish Ashkenazi; **JS**, Jewish Sephardic; **Ar**, Arab; **M**, mixed cellularity; **SC**, predominantly small cleaved cells; **L**, predominantly large cells; **FL**, follicular lymphoma; **F&D**, follicular and diffuse; **MBR**, major breakpoint region.

Table 2. Cytogenetic Studies of the Frequency of t(14;18) in Follicular Lymphoma

Study	Country	Frequency of t(14;18)
Yunis et al (3)	USA	60/71 (84%)
Fukuhara et al (13)	Japan	4/9 (44%)
Bloomfield et al (14)	USA	18/33 (54%)
5th Workshop (15)	Worldwide	37/64 (58%)
Levine et al (16)	USA	29/48 (60%)
Koduru et al (17)	USA	14/20 (70%)
Ross et al (18)	UK	13/17 (76%)
Zelenetz et al (19)	USA	25/34 (73%)
Clark et al (20)	UK	18/28 (64%)
Lee et al (21)	USA	8/10 (80%)
Benitez et al (22)	Spain	3/5 (60%)
Takechi et al (23)	Japan	6/15 (40%)
Seite et al (24)	France	11/24 (46%)
Maseki et al (25)	Japan	3/10 (30%)
Pooled (6 studies)	USA	154/216 (71%)
Pooled (4 studies)	Europe	45/74 (61%)
Pooled (3 studies)	Far East	13/34 (38%)

Table 3. Molecular Studies of the Frequency of t(14;18) in Follicular Lymphoma in the USA

Study	Country	Method of Detection	Frequency at MBR	Frequency at MCR	Overall Frequency
Zelenetz et al (19)	USA	PCR	22/40 (55%)	6/40 (15%)	28/40 (70%)
		Southern	25/40 (63%)	7/40 (17%)	32/40 (80%)
		PFGE	-	-	25/29 (86%)
Lee et al (21)	USA	Southern	18/26 (69%)	-	-
Aisenberg et al (26)	USA	Southern	9/35 (26%)	-	-
Weiss et al (27)	USA	Southern	21/36 (58%)	11/36 (31%)	32/36 (89%)
Tsujimoto et al (28)	USA	Southern	11/18 (61%)	-	-
Lipford et al (29)	USA	Southern	11/17 (65%)	-	-
Liu et al (30)	USA	PCR	24/48 (50%)	5/48 (10%)	29/48 (60%)
Shibata et al (31)	USA	PCR	24/51 (47%)	-	-
Gribben et al (32)	USA	PCR	56/88 (64%)	18/88 (20%)	74/88 (84%)
Said et al (33)	USA	PCR	-	-	12/20 (60%)
Gulley et al (34)	USA	PCR	4/8 (50%)	1/8 (12%)	5/8 (62%)
		Southern	3/8 (37%)	-	-
Pooled		Southern	98/180	18/76	64/76
		PCR	130/235	30/184	148/204
		PCR or Southern	203/367	42/220	184/240

Table 4. Molecular Studies of the Frequency of t(14;18) in Follicular Lymphoma in the Far East

Study	Country	Method of Detection	Frequency at MBR	Frequency at MCR	Overall Frequency
Osada et al (4)	Japan	Southern	2/16 (12%)	1/16 (6%)	3/16 (19%)
Takechi et al (23)	Japan	Southern	5/11 (45%)	0/11 (0%)	5/11 (45%)
Amakawa et al (35)	Japan	Southern	8/30 (27%)	2/30 (7%)	10/30 (33%)
Loke et al (36)	Hong Kong Chinese	Southern	8/16 (50%)	1/16 (6%)	9/16 (56%)
Chen et al (37)	Taiwan Chinese	Southern	8/17 (47%)	1/17 (6%)	9/17 (53%)
Liang et al (38)	Hong Kong Chinese	Southern	2/8 (25%)	0/8 (0%)	2/8 (25%)
Mitani et al (39)	Japan	PCR	12/41 (29%)	1/41 (2%)	13/41 (32%)
Pooled		Southern	33/98	5/98	38/98
		PCR	12/41	1/41	13/41
		PCR or Southern	45/139	6/139	51/139

Table 5. Molecular Studies of the Frequency of t(14;18) in Follicular Lymphoma in Europe

Study	Country	Method of Detection	Frequency at MBR	Frequency at MCR	Overall Frequency
Clark et al (20)	UK	Southern	25/49 (51%)	2/49 (4%)	27/49 (55%)
Benitez et al (22)	Spain	PCR	3/5 (60%)	0/5 (0%)	3/5 (60%)
		Southern	3/5 (60%)	0/5 (0%)	3/5 (60%)
Seite et al (24)	France	PCR	30/64 (47%)	-	-
		Southern	12/64 (19%)	9/64 (14%)	21/64 (33%)
De Jong et al (40)	Holland	Southern	-	-	14/19 (74%)
Pezzella et al (41)	UK,	PCR only	15/51 (29%)	2/51 (4%)	17/51 (33%)
	Denmark	Southern and/or PCR	18/51 (35%)	3/51 (6%)	21/51 (41%)
Lee et al (42)	UK	PCR	7/20 (35%)	1/20 (5%)	8/20 (40%)
		Southern	6/20 (30%)	2/20 (10%)	8/20 (40%)
Kneba et al (43)	Germany	PCR	13/33 (39%)	-	-
Lambrechts et al (44)	Holland	PCR	14/28 (50%)	1/28 (4%)	15/28 (54%)
		Southern	10/19 (53%)	1/19 (5%)	11/19 (58%)
Corbally et al (45)	Ireland	PCR	8/14 (57%)	-	-
Pooled		Southern	56/157	14/157	84/176
		PCR	90/125	4/104	43/104
		PCR or Southern	118/264	17/217	109/236

Table 6. Frequency of t(14;18) in Follicular Lymphoma by Geographical Region: Pooled Data

		Israel (95% CI)	Far East (95% CI)	Europe (95% CI)	USA (95% CI)
Cytogenetics		-	38% (23-56%)	61% (49-72%)	71% (65-77%)
Southern	MBR	-	34% (25-44%)	36% (28-44%)	54% (46-61%)
	MCR	-	5% (2-12%)	9% (5-15%)	24% (15-35%)
	total	-	39% (29-49%)	48% (40-55%)	84% (74-91%)
PCR	MBR	61% (43-76%)	29% (17-46%)	42% (35-49%)	55% (49-62%)
	MCR	-	2% (0.1-14%)	4% (1-10%)	16% (11-23%)
	total	-	32% (19-48%)	41% (32-51%)	72% (66-78%)
PCR or Southern	MBR	61% (43-76%)	32% (25-41%)	45% (39-51%)	55% (50-60%)
	MCR	-	4% (2-10%)	8% (5-12%)	19% (14-25%)
total		-	37% (29-45%)	46% (40-53%)	77% (71-82%)

Comparison with the World Literature

Our search of the literature yielded 35 papers reporting the frequency of the t(14;18) translocation in follicular lymphoma. Eight studies used cytogenetic methods only. 27 studies used molecular methods, and six of these compared the molecular data with cytogenetic analysis. Cytogenetic data are shown in Table 2. Pooled frequencies from each geographical region are shown in Figure 1: 38% in the Far East (95% confidence interval: 23-56%), 61% in Europe (95% CI: 49-72%) and 71% in the USA (95% CI: 65-77%). This data indicates a low frequency of the translocation in the Far East compared to the USA, with an intermediate frequency found in Europe. Whereas the 95% confidence intervals for pooled data frequencies from Europe and USA overlap, as do those of Europe and the Far East, the difference between the Far East and USA is statistically significant.

Eleven molecular studies performed on American patients reported frequencies ranging from 26% to 89% (Table 3). Seven studies were from East Asia, reporting frequencies ranging from 19% to 56% (Table 4). The remaining nine studies were from Europe, and the range of reported frequencies was 33% to 74% (Table 5). Pooled data from molecular studies are shown in Figure 2. Figs. 2a and 2b show data regarding the MBR and MCR breakpoints respectively. Figure 2c shows data on total frequencies. Data from

studies using Southern blot analysis and PCR amplification are shown both separately and together. The trend is similar to that seen in the cytogenetic studies. However, 95% confidence intervals (Table 6) suggest that the pooled frequencies of the translocation in Europe and the Far East are very similar. The frequency in American studies is distinctly higher. This pattern is preserved in both breakpoint regions, regardless of whether PCR or Southern blotting were used as the method of detection of the translocation. The frequency of the translocation in our series of Israeli patients (61%) is relatively high, comparable to that detected in American series.

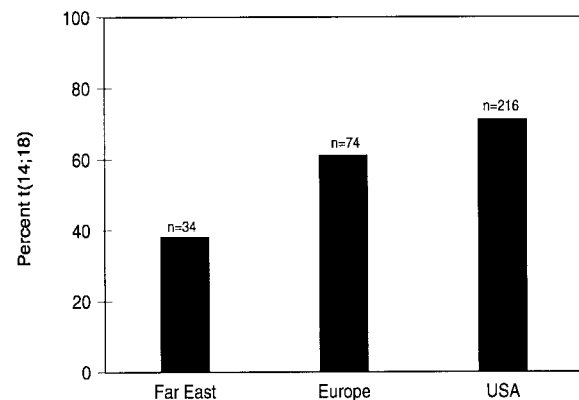


Figure 1. Pooled frequencies of the translocation by geographical region in cytogenetic studies. The number of subjects (n) is noted above bars.

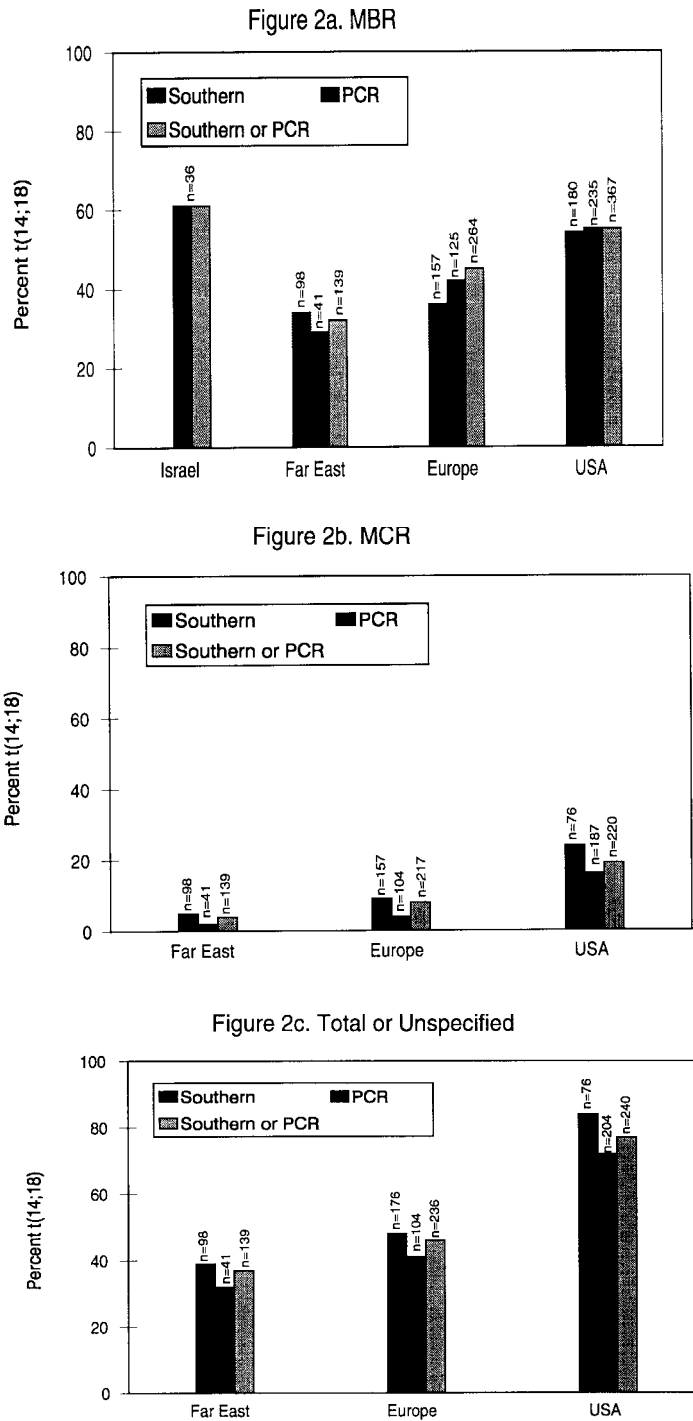


Figure 2. Pooled frequencies of the translocation within each geographical region in studies using molecular methods, compared to data from current Israeli series. The number of subjects (n) is noted above each bar. Data from studies using Southern blot analysis and from those using PCR amplification of the breakpoint are shown both separately and combined. Figure 2a shows the data from studies of the MBR, Figure 2b studies of the MCR, and Figure 2c the overall frequencies. Note that the latter is not an arithmetic sum of the data from the studies of the MBR and MCR because some studies studied only one of the breakpoints and were therefore excluded from the analysis of the total frequency; others reported only a total frequency and therefore do not appear in the analysis by breakpoint; and finally some studies detected the translocation at breakpoints located in neither the MBR nor the MCR.

DISCUSSION

Our data from a series of Israeli follicular lymphoma patients shows a frequency of the t(14;18) translocation at the MBR similar to that found in American patients, in studies using similar techniques. This frequency is somewhat higher than that found in Europe and the Far East.

In our study we performed PCR amplification of the breakpoint region using DNA that was extracted mainly from archival tissues. Attempts to use previously described protocols for parallel amplification of the MCR breakpoint (46) were unsuccessful in our experience. Unlike MBR, in which individual breakpoints are limited to a region of 150 bp, the MCR is much more diffuse, spanning approximately 800 bp. Successful PCR amplification of large fragments is difficult, and the quality of DNA obtained from our archival specimens did not permit amplification of such a large segment. Moreover, using these same protocols on DNA extracted from fresh tissue we found no MCR positive samples in a consecutive series of 36 samples routinely analyzed for the presence of t(14;18). There is no reason to suspect that geographical patterns of the frequency of the MCR differ significantly from those of the MBR. Of note, our finding that 61% of our samples were positive for the translocation at the MBR demonstrates that Israeli patients are no exception to the universal finding that the majority of t(14;18) breakpoints fall within the MBR. Therefore we believe that our data on the prevalence at the MBR are representative of the overall frequency of the t(14;18) translocation.

It has been hypothesized that a lower frequency of the t(14;18) translocation in follicular lymphomas might explain the lower incidence of these tumors in the Far East compared to Western countries, the rationale being that the lower frequency of the translocation reflects a difference in the biology of the tumor (4,35). Our analysis of the published literature shows that studies performed in the USA indeed demonstrate a higher frequency of the translocation than that found in studies of patients from the Far East. However, we question

the significance of this observation. We suggest alternative explanations for the observed differences in frequency.

Cytogenetic studies from Europe show a frequency of the translocation intermediate between the low frequency found in Asian patients and the higher frequency detected in American studies. Molecular studies show a frequency of the translocation similar to that found in the Far East, and significantly lower than that found in the USA. This contrasts with the fact that the epidemiology of follicular lymphoma in Europe is similar to that in the USA. Namely, a similar proportion (about 25%) of NHL in the USA and Europe are of follicular pattern (47), compared to the much lower proportion in the Far East (1). Furthermore, the clinical characteristics of follicular lymphoma are fairly uniform worldwide. Thus there is a discrepancy between the phenotypic similarity of the tumor in different parts of the World, and the apparent genotypic differences we have described.

There is considerable variability in the results of studies within the same geographical region, even when similar techniques were used. Results of molecular assays are heavily dependant on the details of the protocol used, and even on the individual laboratory. The use of different systems of pathological classification in different parts of the world (eg the use of the Working Formulation in the USA vs. the predominance of the Kiel Classification in Europe) adds a further element of confusion, as this might influence the selection of samples chosen for analysis. The fact that the majority of studies are performed in hospital based case series consisting of haphazard samples, rather than consecutive, population based series, is another source of possible bias.

We conclude that there is little to support significant ethno-geographical differences in the frequency of the translocation among follicular lymphoma patients. To the extent that differences exist, they do not seem to have a biologic basis or clinical effect, and are most likely explained by methodological and technical factors.

It seems clear that the t(14;18) translocation is present in many, but not all, follicular

lymphomas worldwide. Much has been published recently on the molecular biology of the translocation, strongly supporting the theory that the translocation has pathogenetic importance (5). There have also been studies showing that the translocation can be demonstrated in the tissues of some healthy individuals (48,49,50), and even that the frequency of the translocation in the population at large can be shown to rise with age, as does the incidence of follicular lymphoma (51). It is yet unknown whether populations with an unusually high frequency of the translocation among healthy individuals are at higher risk for the development of lymphomas. A difference in the prevalence of the translocation in healthy Asian and Western populations may help to explain the observed differences in the incidence of follicular lymphoma. Aster et al (49) found no difference in the frequency that the t(14;18) translocation was detected in hyperplastic (benign) lymphoid tissues from American and Japanese patients, but this small study (25 patients) is the only of its kind published to date. It might be possible to establish a link between environmental or other risk factors for the development of lymphoma, and an increased incidence of the translocation in healthy individuals. Such associations would contribute to the understanding of the pathogenesis of the lymphoma, and might even justify molecular surveillance of high-risk groups for early detection.

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