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13q14 deletion in non-Hodgkin's lymphoma: correlation with clinicopathologic features

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ABSTRACT

Background and Objective. **13q14 deletion frequently occurs as a single anomaly in chronic lymphocytic leukemia (CLL) with favorable prognosis. This study was performed to assess the distribution of 13q14 deletion in non-Hodgkin's lymphoma (NHL) and to analyze its correlation with salient clinicopathologic features.**

Design and Methods. **One hundred and twenty-five NHL were analyzed by cytogenetics and by interphase fluorescence *in situ* hybridization (FISH), using a 13q14 cosmid probe recognizing DNA sequences between the Rb gene and the D13S25 marker. Clinical records all patients were surveyed.**

Results. **A 13q14 rearrangement was present in the stemline in 10 patients; 15 additional cases were shown by FISH to carry 13q14 deletion in 55-90% of the interphase cells, giving a 20% overall incidence for this anomaly. Six of 44 patients had a low-grade NHL, 14/28 had mantle cell lymphoma (MCL), 5/42 had a high grade NHL ($p<0.0001$). There was not correlation between 13q, karyotype status and complexity. A statistically significant association was found between 13q-, presence of splenomegaly and PB involvement, lower probability of attaining complete remission (CR) and shorter survival. These findings were not simply a function of the association of 13q- with MCL. In multivariate analysis, a complex karyotype had prognostic importance ($p=0.0078$), along with age ($p=0.01$), histology ($p=0.001$), LDH ($p=0.03$), PS ($p=0.001$), sex ($p=0.03$) and splenomegaly ($p=0.02$).**

Interpretation and Conclusions. **13q14 deletion represented an early chromosome change and showed a preferential association with MCL, though it was found in virtually all principal histologic subtypes, irrespective of clinical stage, karyotype status and complexity. Patients with 13q14 deletions had a low CR rate, suggesting that genes relevant to lymphomagenesis are located in this chromosome segment that warrants molecular cytogenetic investigation.**

Key words: FISH, 13q14 deletion, cytogenetics, non Hodgkin's lymphomas

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Deletions of chromosome 13 involving the q14 band frequently occur in B-cell chronic lymphocytic leukemia (CLL), having been detected in approximately 8-10% of cases by conventional chromosome analysis (CCA)[1,2] and in up to 40-50% of cases by fluorescence *in situ* hybridization (FISH), using probes targeting a few hundred kb region,[3-5] where a candidate tumor suppressor gene is probably located.

Liu *et al.* described loss of 13q14 material in non-Hodgkin's lymphomas;[6] more recently, the association of 13q14 deletions with chronic lymphoproliferative disorders carrying the t(11;14)(q13;q32) and with multiple myeloma was documented,[7-10] suggesting that imbalances involving this chromosome region may be implicated in the genesis of distinct subtypes of lymphoid neoplasms.

To define the incidence and significance of 13q14 deletion in NHL better, we studied a series of 125 NHL by interphase FISH using a cosmid probe recognizing DNA sequences comprised between the Rb gene and the D13S25 marker, and subsequently analyzed the correlation of 13q- with cytogenetic and clinicopathologic features.

Design and Methods

Patients and clinical parameters

CCA was successfully performed in 125 cases of NHL, seen at the Institute of Hematology, University of Ferrara. These cases represented approximately 40% of all NHLs seen at our center over the study period. The remaining cases were not studied because no material was sent to the cytogenetic laboratory or, rarely, because an insufficient mitotic yield was obtained.

The histologic diagnoses were made according to the REAL classification[11] on lymph node material (112 cases), on surgically removed spleen (4 cases), or on bone biopsy section (9 cases).

Staging procedures included physical examination, a routine laboratory profile, a bone biopsy, a chest X-ray film and abdomen ultrasonography. When indicated, barium contrast radiography was performed. CT scan was performed for staging purpos-

es in 115 cases. PB involvement was studied by light microscopy examination of smears stained by the May-Grünwald-Giemsa method. Clinical records of all cases were surveyed and the parameters outlined in Table 3 were collected.

CR was defined as the resolution of all abnormal symptoms, signs and imaging attributable to the lymphoma for at least 3 months after the completion of treatment. Treatment was heterogeneous, depending on histologic type, age and time of diagnosis.

Conventional chromosome analysis

Cytogenetic investigations were performed at diagnosis. Single cell suspensions were prepared, as previously described,[12] after collection of a portion of surgically removed lymph node or spleen (112 and 4 cases, respectively). In 9 cases with leukemic presentation BM and PB samples were studied. The cells obtained from BM, lymph node and spleen specimens were separated over a 1,077 density gradient. In all cases cell suspensions were cultured for 24-72 hours, with and without the following mitogens: phorbol miristate acetate (50 ng/mL) and lipopolysaccharide from *E. Coli* (100 mg/mL). Whenever possible, 20 metaphases were studied and karyotypes described according to the ISCN.[13] A complex karyotype was arbitrarily defined as the presence of 5 or more clonal abnormalities.

Interphase cytogenetics

FISH studies were performed on cells taken from the same samples as those used for cytogenetic analysis, using the 13q14 C21 cosmid, recognizing DNA sequences between the Rb gene and the D13S25 marker, that was isolated as previously described.[7] To reduce the rate of false positive results due to inefficient hybridization, dual color FISH was performed, by simultaneous hybridization with a commercially available control probe recognizing DNA sequences at the telomeres of chromosome 13q (Oncor, Gaithersburg, MD, USA). The conditions for hybridization and signal amplification have been described previously.[7] Signal screening was performed on those slides with high hybridization efficiency, as indicated by the presene of >80% cells showing the expected 2 signals of the 13q telomere.

Statistical analysis

Chi-square test was applied for categorical variables. Patient survival was estimated by the Kaplan-Meier method from the date of diagnosis until death due to any cause or until the last patient follow-up. The survival curves were statistically compared by the log-rank test. Because many statistical tests were undertaken in the evaluation of prognosis a p value of 0.02 was used as criterion for statistical significance. Proportional hazards regression analysis was used to identify the most significant independent prognostic variables on survival. p values less than 0.05 were considered statistically significant.

Results

Cytogenetic features

Clonal abnormalities were detected in 119/125 patients, 42 of whom had a complex karyotype. The correlation of histologic subtypes and recurrent chromosome anomalies defining the stemline (i.e. those aberrations present in all abnormal cells in a given patient) is shown in Table 1.

A chromosome 13 rearrangement involving the q14 band was seen by CCA in 10 patients (7 deletions, 3 translocations): in all 10 cases this anomaly was present in the stemline (in 9 cases additional aberrations were also present). FISH detected a hemizygous 13q14 deletion (one fluorescent spot in 55-90% interphase cells) in these 10 cases and in 12 additional cases having 45-90% interphase cells with 1 signal. No 13q14 signal was detected in 3 additional patients as a consequence of a biallelic deletion involving this chromosome segment. Thus, a total of 25 out of 125 (20%) patients in this series carried monoallelic or biallelic 13q14 deletion. The correlation of 13q14 deletion and salient cytogenetic features is shown in Table 2.

Correlation of 13q14 deletion and clinicopathologic features

The male to female ratio was 70/55. Sixty-four patients died after 2-120 months (median 16); the median follow-up for 61 surviving patients was 28 months, range 4-120.

Seven patients (2 with 13q-) had small lymphocytic lymphoma (SLL), 28 (14 with 13q-) had mantle cell lymphoma (MCL), 8 (1 with 13q-) had marginal zone B-cell lymphoma (MZBCL), 33 (3 with 13q-) had follicle center cell lymphoma (FCCL), 45 (4 with 13q-) had diffuse large cell lymphoma (DLCL), 4 (1 with 13q-) had a T-cell NHL.

Table 1. Recurrent chromosome anomalies in the stemline in 125 NHL patients.*

	No. of cases						
	SLL	*MCL*	*MZBL*	*FCCL*	*B-DLCL*	*T-NHL*	*Total*
t(14;18)(q32;q21)				24	2		26
t(11;14)(q13;q32)		21					21
t(3;V)(q27;V)		1	1		4		6
t(8;14)(q24;q32)					3		3
+12	2	5	3	3	4		17
+3		2	2	2	1		7
+11				1		3	4
+7		1		2		2	5
del(6q)		2	1	2	1		6
1q23 abn					3		3
13q14 abn	1	4	1	1	3		10
1p22 abn	1	2		1			4

**Anomalies found in at least 5 cases are included; some aberrations were associated in the same patient; consequently the sum in each column may exceed the number of patients that were observed in each histologic group.*

Table 2. Correlation of 13q14 deletion and cytogenetic findings in 125 cases of NHL.

Cytogenetic patterns					
	0-4 anomalies	*5 or + anomalies*	*NN*	*AN*	*AA*
13q-/total	15/83	10/42	1/6	8/59	16/60
	p=n.s.			*p*=n.s.	

Specific chromosome changes*						
	t(11;14)	*others*	*t(14;18)*	*others*	*+12*	*others*
13q/total	12/21	13/104	3/26	22/99	3/17	21/108
	p<0.001		*p*=n.s.		*p*=n.s.	

*NN: normal metaphases; AN: a mixture of normal and abnormal metaphases; AA: only abnormal metaphases; *only those anomalies seen in at least 10 cases were included.*

Table 3. Clinical and laboratory data according to the presence or absence of 13q14 deletion in 125 cases of NHL.

Parameter	*13q14 deletion** *(25 pts)*	*no 13q14 deletion** *(100 pts)*	*p value*
Age			
<60	9	39	0.783
>60	16	61	
stage			
I-II	3	17	0.53
III-IV	22	83	
Histology			
MCL	14	14	<0.001
other low	6	44	
other high	5	42	
Involved sites			
BM	17/25	47/100	0.06
PB	11/25	16/100	0.002
spleen	13/25	29/100	0.03
extranodal	1/25	16/100	0.03
CR	7/25	53/100	0.02
5-yr survival (%)	9.5	53.3	0.02
(standard error %)	(8.7)	(5.9)	

**No. of cases/total.*

The salient clinicopathologic features in our patients, in correlation with the 13q- anomaly are summarized in Table 3, showing that a statistically significant association existed between 13q-, histologic subtype (MCL), presence of splenomegaly and PB involvement, lower probability of attaining CR and shorter survival. These findings were not simply a function of the association of 13q- with MCL since, among the above reported variables, only PB involvement (p=0.0001) and shorter survival (p=0.002) were more frequently found in MCL with respect to other histologic subtypes.

Table 4. Influence on survival of hematologic and cytogenetic parameters.

Parameter	*No. of patients*	*5-yr survival (%)*	*Standard error (%)*	*p*
All patients	125	44.7	5.5	
Age				
<60	48	65.8	7.7	0.015
>60	77	36.7	6.6	
Sex				
male	70	31.6	6.6	0.001
female	55	62.2	8.6	
Histology				
low grade	50	63.1	8.9	0.002
mantle	28	13	8.1	
high grade	47	49.1	8.1	
ECOG:				
0-1	88	57.8	6.2	0.004
2-4	37	21.6	8.0	
Stage:				
I-II	20	52.2	11.7	0.81
III-IV	105	44.4	6.0	
LDH				
normal	56	58.3	9.2	0.006
abnormal	69	34.2	6.6	
Extranodal	17	31.8	14.7	0.12
Nodal	108	46.0	6.0	
PB involvement				
yes	27	18.1	10.5	0.057
no	98	52.0	5.9	
BM involvement				
yes	64	46.1	7.8	0.64
no	61	43.8	7.7	
Splenomegaly				
yes	42	23.9	8.8	0.004
no	83	53.7	6.6	
CR	60	76.6	6.7	<0.0001
no CR	65	15.5	5.9	
Cytogenetics				
Complex				
yes	42	24.0	8.0	0.01
no	83	55.8	6.8	
del(13q14)				
yes	25	9.5	8.7	0.02
no	100	53.3	5.9	
t(11;14)				
yes	21	17.1	10.5	0.11
no	104	51.5	5.9	
t(14;18)				
yes	26	43.8	13.3	0.47
no	99	44.6	6.1	
+12				
yes	17	29.0	11.9	0.03
no	108	46.9	6.1	

Other cytogenetic and hematologic factors having an impact on the 5-year survival rate, are shown in Table 4.

In multivariate analysis, complex karyotype maintained its prognostic importance (p=0.0078), along with age (p=0.01), histology (p=0.001), LDH (p=0.03), PS (p=0.001), sex (p=0.03) and splenomegaly (p=0.02).

Discussion

Besides typical B-CLL,[14] 13q14 deletions were described in a wide spectrum of hemopoietic neoplasms of lymphoid lineage, including atypical CLL, lymphoid neoplasias carrying the 11;14 translocation and multiple myeloma where, unlike B-CLL, they have recently been shown to represent an adverse prognostic factor.[3,6,7,10,15] Uncertainty exists as to the frequency and significance of 13q- in NHL.

The global incidence of 13q14 deletion in this study was 20%, with a significant association with CD5/CD19+ NHL (45.7% in MCL plus SLL) and an approximate 10% incidence in other low-grade or high grade NHL. No correlation was found between 13q- and some important clinical parametrs, such as age and stage at presentation, BM involvement and presence of extranodal disease. These data must be considered with the understanding that our cases did not represent the whole population of NHL seen during the study period and that the sample size was relatively small. In the study by Liu *et al.*,[6] 4 out of 16 (25%) follicle center cell NHL and 1/15 (6.6%) T-cell lines carried a 13q deletion. While confirming a previously reported association with MCL,[8,16] these data would suggest that, though 13q14 deletion is not a necessary event for the genesis of the majority of NHL, it may be present in virtually all principal histologic subtypes, irrespective of the histologic grade and tumor dissemination at diagnosis.

Although this chromosome lesion was not an isolated anomaly, it affected the majority of interphase cells. Moreover, no case was found in which a cytogenetically detectable 13q14 rearrangement was present as an additional change, suggesting that alterations at this locus occurred early in the transformation process. Interestingly, no association was found between 13q14 deletion, karyotype status and complexity, the only significant correlation with other cytogenetic parameters being its association with the t(11;14).

The correlation of cytogenetic features and prognosis in NHL[15] was investigated in several studies, showing that karyotype complexity and some specific chromosome aberrations, such as 1q21-23 rearrangements, del(6q), del (17p) and total or partial trisomy 12[17,18,19,20] may have an adverse impact on outcome.

13q14 deletion in this study was associated with a lower probability of attaining CR, a finding that was independent of its relationship with MCL. The biologic reasons accounting for this finding are unclear. It is apparent from our data that deletion of this locus did not affect karyotype stability and did not influence the *in vitro* mitotic rate of the neoplastic clone, since no correlation was found with karyotype complexity or percentage of abnormal cells. It is tempting to speculate that, unlike typical B-CLL, in which an isolated 13q14 deletion may specifically involve a small DNA region distal to the Rb-gene,[3] the disruption of other genes on 13q may justify the observation of a lower CR rate in these patients. This argument is supported by the demonstration that total or partial monosomy 13q was found to be an independent factor with an unfavorable impact on prognosis in multiple myeloma.[9,10] Interestingly, the study by La Starza *et al.*[21] documented that rather a large extent of DNA loss may occur around 13q13.1-14.3 bands in myeloid malignancies, suggesting that more than one gene may be involved in hematopoietic neoplasms carrying these chromosomal rearrangements. Indeed, because this anomaly represents an early chromosome change in NHL, it is reasonable to assume that pathogenetically important genes may be located in this chromosome region that warrants detailed molecular cytogenetic studies aimed at the delineation of the commonly deleted segment(s).

Contributions and Acknowledgments

AC, RB and GLC designed the study and prepared the manuscript; RB, DC, FB and CC were responsible for the FISH experiments; GMR and RM analyzed the clinical data and performed the statistical analyses; MGR, CM, PA and AB performed the cytogenetic studies; all authors reviewed the manuscript for important intellectual content.

The order of the names reflects the importance of the contribution of each author to the work and the hierarchical position in the Institution.

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Disclosures

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