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ORIGINAL ARTICLE

Biallelic deletion 13q14.3 in patients with chronic lymphocytic leukemia: cytogenetic, FISH and clinical studies

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Abstract

Background and objective: Monoallelic deletion of 13q14.3 (13q14x1) is the most common abnormality in chronic lymphocytic leukemia (CLL). As a sole alteration, it predicts a favorable outcome. Biallelic 13q14.3 (13q14x2) deletion or concomitant 13q14x1/13q14x2 has been scarcely evaluated in the literature. We present the clinical, cytogenetic and fluorescence *in situ* hybridization (FISH) analysis of six CLL patients with normal karyotypes and 13q14x2 and their comparison to cases with 13q14x1 as a single abnormality. *Patients and methods:* A total of 103 CLL patients were studied. Cytogenetic and FISH analysis were performed on stimulated peripheral blood lymphocytes. Specific fluorescence DNA probes for CLL were used. *Results:* Six out of 103 (5.8%) patients showed normal karyotypes and 13q14x2. It was observed as a single alteration in one patient and combined with 13q14x1 in five cases. Biallelic clones were larger than monoallelic ones in 3/5 patients (60%). The comparison of clinical and hematological data between 13q14x1 and 13q14x2 groups showed progression of the disease in all 13q14x2 patients respect to 12/32 (37.5%) cases with 13q14x1 ($P = 0.008$), significant differences in the distribution by Rai stage ($P = 0.042$) and a tendency of a higher lactate dehydrogenase level in 13q14x2 patients ($P = 0.054$). Treatment free survival for 13q14x2 group was 28.5 months, shorter than those observed in patients with 13q14x1 alone (49 months). *Conclusions:* Our data would suggest that 13q14x2 could represent a more aggressive FISH anomaly than 13q14x1 alone, probably as a consequence of clonal evolution and/or due to the complete inactivation of this critical region by mean of more complex mechanisms.

Key words chronic lymphocytic leukemia; chromosomes; FISH; biallelic deletion 13q14.3

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Chronic lymphocytic leukemia (CLL) is the most frequent type of adult leukemia in the Western world, accounting for about 30% of all leukemic cases. It is characterized by a highly variable clinical course with survival times ranging from months to decades. Some patients have a more stable, indolent disease whereas others exhibit a progressive disease requiring early therapy (1).

The clinical staging systems, independently developed by Rai *et al.* (2) and Binet *et al.* (3), are of prognostic importance, but they are not accurate enough to predict the outcome of individual patients in early stages and to identify patients with poor prognosis. In recent decades, several prognostic markers, including the mutational status of the variable region of the immunoglobulin heavy chain (IGVH), CD38, ζ-chain-associated protein kinase (ZAP-70) and genomic abnormalities, have demonstrated promising ability to predict early onset of CLL progression (4–7).

Genomic abnormalities detected by interphase fluorescence *in situ* hybridization (FISH) are emerging as

an important prognostic indicator in CLL. They were observed in more than 80% of CLL patients and have been categorized into well-defined subgroups, associated with different clinical outcomes (5). Among them, monoallelic deletion of 13q14.3 (13q14x1) occurs in more than half of cases and constitutes the most frequent abnormality. Patients with 13q14x1 as a sole alteration have excellent prognosis. In contrast, those cases with deletion of 11q22 (10–15%), involving *ATM*, and 17p13 (5–10%), involving *TP53*, had the shortest median survival. Patients with trisomy 12 are associated with atypical morphology and immunophenotype, and correlate with intermediate prognosis. Unlike these exhaustively studied genomic aberrations, biallelic 13q14.3 deletion (13q14x2) has been scarcely evaluated in the literature (8, 9).

We here report the clinical, cytogenetic and FISH studies of six patients with diagnosis of CLL that showed 13q14x2 alone or concomitant 13q14x1/13q14x2. A more aggressive clinical outcome compared to cases with 13q14x1 as a single alteration was observed. A review of the literature was also performed.

Materials and methods

Patients

From 1997 to 2007, a total of 103 patients with diagnosis of CLL were cytogenetically and FISH studied in our Institution. The diagnosis of CLL was based on the National Cancer Institute Working Group (NCI-WG) guidelines (10) and according to flow cytometric criteria where tumor cells expressed CD5, CD19 and CD23, but showed a weak expression of immuglobulin as well as CD22 or CD79b. Stage was assessed according to the classification of Rai (2). Progressive disease was defined as any Rai stage and clinical criteria for progressive disease requiring therapy (10). As in previous studies (5, 11), patients were not selected by regimens of treatment. The study was approved by the Ethical Committee of our Institution. All patients provided their informed written consent.

Cytogenetic studies

Chromosome analyses were performed on peripheral blood lymphocytes, cultured for 72–96 h at 37°C in F-10 medium supplemented with 15% of fetal calf serum, stimulated with Pokeweed mitogen, Phytohemagglutinin A and Lipopolysaccharide. Slides were prepared by a conventional method. G-banded technique was used. Karyotypic abnormalities were described using the International System for Human Cytogenetic Nomenclature (12).

FISH analysis

FISH was performed on cells taken from the same samples that were used for cytogenetic analysis. The slides were hybridized with fluorescence DNA probes (CEP 12, LSI D13S319 and D13S25 loci at 13q14 band, LSI ATM at 11q22.3 and LSI p53 at 17p13 and LSI p53/ATM/13q14/13q34/CEP12, Vysis-Abbott, Downers Grove, IL, USA), according to manufacturer's protocol. Four hundred interphase nuclei were analyzed for each probe. The cut-off for positive values (mean of normal control + 3SD), determined from samples of ten cytogenetically normal persons, were: 3.02%, 10.2%, 7.6%, 7.7% and 5.1% for trisomy 12 and monosomies of D13S319, D13S25, ATM and p53, respectively.

Statistical analysis

Chi-squared and Fisher's exact tests were used to compare different subsets of patients defined by FISH anomalies. Treatment free survival (TFS) curves were estimated using Kaplan–Meier method. Groupwise comparison of the distributions of clinical and laboratory variables at the time of genetic study were performed with the Kruskal–Wallis test (for quantitative variables) and Fisher's exact test (for categorical variables). For all tests, $P < 0.05$ was regarded as statistically significant.

Results

Table 1 shows the distribution of our 103 CLL patients taking into account the hierarchical FISH risk categories. FISH was abnormal for 28/47 (59.6%) patients with stable disease and 49/56 (87.5%) cases with progressive disease ($P < 0.003$). The distribution according to the number of chromosome abnormalities showed progressive disease in 53% (27/51) of the cases with only one alteration, in 80% (16/20) of patients with two abnormalities ($P = 0.005$), and in all cases (6/6) with three anomalies ($P = 0.027$). Six out of 103 patients (5.8%) (three females; mean age 62.1 yr; range 47–77 yr) showed 13q14x2 by FISH alone or concomitant 13q14x1/13q14x2 (Fig. 1). Clinical characteristics and treatment details of them are shown in Table 2. Case 1 refused treatment. Case 2 developed hypogammaglobilinemia during treatment. Case 6 showed Richter's transformation. Two patients (cases 2 and 6) had received chemotherapy before cytogenetic and FISH studies. All cases presented kappa light chain restriction. All of them fulfilled five points of the score proposed by Matutes *et al.* (13). Lymphocyte doubling time below 12 months was also observed in all patients. ZAP-70 was evaluated in cases 2 and 6, being both negative. Mean time of follow up from

Table 1 Distribution of 103 patients with CLL according to FISH categories. Correlation with disease status and clinical stage

FISH	No. of cases	Disease status		Rai stage		
		Stable	Progression	0	I-II	III-IV
Normal	26	19	7[1]	11	10	5
Abnormal	77	28	49	24	33	20
13q14x1	32	20	12[2]	15	10	7
13q14x2	1	0	1	0	1	0
13q14x1/13q14x2	5	0	5	0	4	1
13q14x1/+12	7	2	5	2	4	1
13q14x2/+12	2	1	1	1	1	0
13q14x1/13q14x2/+12	1	0	1	0	0	1
+12	14	4	10	5	8	1
ATM/+12	1	0	1	0	1	0
ATM/13q14x1	2	1	1	1	1	0
ATM/13q14x1/13q14x2	1	0	1	0	1	0
ATM/13q14x1/+12	1	0	1	0	1	0
TP53/13q14x1	1	0	1	0	0	1
TP53/13q14x2	1	0	1	0	0	1
TP53/+12	4	0	4	0	1	3
TP53/13q14x1/+12	2	0	2	0	0	2
TP53/13q14x1/*ATM*	1	0	1	0	0	1
TP53/*ATM*	1	0	1	0	0	1
No of abnormalities						
1	47	24	23[3]	20	19	8
2	24	4	20	4	12	8
3	6	0	6	0	2	4

[1]Significant differences respect to abnormal: $P < 0.003$.
[2]Significant differences respect to patients with normal karyotype and 13q14x2 alone or concomitant 13q14x1/13q14x2: $P = 0.007$.
[3]Significant differences respect to two ($P = 0.005$) and three abnormalities ($P = 0.027$).

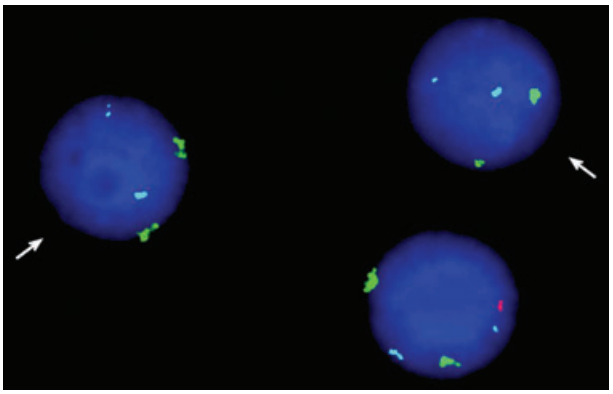

Figure 1 Interphase nuclei hybridized with LSI 13q14/13q34/CEP12 probes showing biallelic (arrows) and monoallelic deletion of 13q14 (green signals: CEP12; red signal: 13q14 locus; aqua signals: 13q34 locus).

diagnosis was 74 months (range: 20–186 months). Three patients have died at the moment of this analysis.

Table 3 shows the cytogenetic and FISH analysis of these CLL patients. All of them had normal karyotype. FISH showed 13q14x2 as a single alteration in one patient (Case 2; 58.5% of cells) and combined with 13q14x1 in the remaining five cases. Biallelic clones were larger than monoallelic ones in three patients (3/5 : 60%). Patients 3 and 5 were studied with D13S319 and D13S25 probes. Patient 3 showed mono and biallelic deletion of both loci, while case 5 showed monoallelic deletion of both probes and biallelic deletion of D13S25. In all cases, the chromosome 13 deletion was cryptic and a normal pattern of signals for CEP 12, LSI ATM and LSI p53 probes was observed.

Thirty-two cases (32/103; 31%) showed 13q14x1 as a sole alteration. The comparison of hematological and clinical data between patients with 13q14x1 and 13q14x2 was performed (Table 4). Significant differences in disease status and distribution by Rai clinical stage were observed. All patients with 13q14x2 showed progressive disease respect to 12/32 (37.5%) cases with 13q14x1 ($P = 0.007$). None patient with 13q14x2 had stage 0 compared to 46.9% of 13q14x1 cases ($P = 0.042$). A tendency of a higher lactate dehydrogenase (LDH) level in 13q14x2 patients was also observed ($P = 0.054$). No significant differences between 13q14x1 and 13q14x2 groups in: age, sex, WBC count, hemoglobin level, platelet count and β_2 microglobulin level were found. TFS for 13q14x2 group was 28.5 months, shorter than those observed in patients with 13q14x1 alone (49 months).

Discussion

Cytogenetic and FISH analysis play an important role as prognostic factors in CLL (5). Deletion 13q14 is the most frequent single genomic alteration associated to CLL. This anomaly is commonly a monoallelic deletion, but in a subset of patients a biallelic loss alone or a concomitant 13q13x1/13q14x2 can be detected. Table 5 summarizes the distribution of 13q14 deletions in the literature (8, 9, 11, 14, 15) and compares them with the present study. The percentage of total 13q14 deletion ranged from 30 to 50%, comparable with our series (36.8%). The present study showed more cases with 13q14x1 (84.2%) and a lower percentage of 13q14x2 alone (2.6%) than those reported. Our frequency of combined 13q14x1/13q14x2 (13.1%) was comparable with those previously described (12–18%), except for the study of Shanafelt *et al.* (15) that found 37.5% of cases with these abnormalities. In addition, our results showed larger biallelic clones in 60% of cases, contrary to those observed by Reddy (9) that found a greater number of patients with a larger monoallelic clone.

We did not have serial studies of our patients but, in 5/6 (83.3%) cases concomitant monoallelic and biallelic clones with different percentages of cells were found, indicating the presence of clonal evolution (12). This

Table 2 Clinical characteristics of patients with CLL and biallelic deletion of 13q14

Case	Age/sex	Clinical Stage	BMI (%)	Hb (g/dL)	WBC (Lymph)×10^9/L	Plt (×10^9/L)	LDH (UI/L)	β_2M (μg/mL)	Treatment	Resp	TFS/OS (m)
1	47/F	I	80	9.1	112 (92)	208	276	2.1	w/treatment		18
2[1]	60/M	I	20	14	35.8 (85)	241	287	1.6	CLB[2]	Null	19/54
									F[2]	PR	
									Fl + CLF + anti-CD20[3]	CR	
3[4]	70/F	I	85	13.1	33.0 (84)	189	410	5.5	CLB[3]	PR	18/56[5]
4	49/M	I	60	14.7	29.0 (70)	168	472	NA	Fl[3]	PR	3/23
5	70/M	II	40	13.8	62.9 (80)	142	319	7.5	CLB[3]	PR	61/107[5]
6[6]	77/F	III	60	10.4	31.4 (66)	138	286	2.5	CLB[2]	PR	39/186[5]
									Fl[2]	PR	
									Fl + CLF[2]	CR	
									anti-CD52[3]	PR	
									MP + anti-CD20[3]	Null?	

BMI, bone marrow infiltration; Hb, hemoglobin; WBC, white blood cells; Plt, plateles; LDH, lactate dehydrogenase; β_2M, β_2 microglobulin; Resp, response; TFS, treatment free survival; OS, overall survival; m, months; F, female; M, male; NA, not available; CLB, chlorambucil; Fl, fludarabine; CLF, cyclophosphamide; MP, methylprednisone; PR, partial response; CR, complete response.
[1]Hypogammaglobulinemia during treatment.
[2]Before cytogenetic study.
[3]After cytogenetic study.
[4]Lost to follow up.
[5]Dead patients.
[6]Richter's transformation.

Table 3 Cytogenetic and FISH results from patients with CLL and biallelic deletion of 13q14.3

Case	Karyotype [no. of analyzed cells]	Interphase FISH (%)				
		+12	13q14x1	13q14x2	17p-	11q-
1	46,XX [15]	1.4	**28.2**	**41.4**	2.7	1.5
2	46,XY [15]	0.8	9.5	**58.5**	2.7	2.2
3	46,XX [15]	1.2	**33.2**	**42.4**	4.3	1.6
			26.5[1]	**43.9**[1]		
4	46,XY [15]	0.7	**34.9**	**39.3**	0.4	1.1
5	46,XY [15]	0.5	**58.0**	2.6	4.3	1.0
			37.3[1]	**35.7**[1]		
6	46,XX [15]	0.7	**73.9**	**14.0**	1.7	2.0

Values in bold: percentage of clonal cells.
[1]D13S25 probe.

Table 4 Comparison of clinical and laboratory data among patients with 13q14x1 and 13q14x2

Variable	13q14x1	13q14x2	*P*-value
No. of patients	32	6	
Median age (yr)	64	65	0.680
Sex: Male/female	19/13	3/3	0.682
Median: WBC count, ×10^9/L	24.4	34.4	0.147
Disease status: stable/progression	20/12	0/6	0.007
Rai stage			
0	15	0	0.042
I-II	10	5	
III-IV	7	1	
Median hemoglobin level, g/dL	12.5	13.4	0.641
Median platelet count, ×10^9/L	180	175.5	0.872
Median lactate dehydrogenase level, IU/L	258	303	0.054
Median β_2 microglobulin level, mg/L	3.1	2.5	0.679

mechanism was previously suggested by Dewald *et al.* (8). They showed that 13q14x2 results from independent deletions on the maternal and paternal chromosomes 13 and suggested a different clinical behavior for this alteration. Recent reports showed acquired 13q14x2 in patients with clonal evolution that initially had monoallelic loss both, together with other anomalies (16) and as only alteration in IgV_H mutated cases (15). During long time, CLL was considered a genetically stable disease (17, 18). However, in the last decades, different studies showed clonal evolution in about 10–20% of patients, and their association to poor clinical outcome (15, 16, 19, 20). Interestingly, the comparison of clinical and hematological data between 13q14x1 and 13q14x2 groups showed progression of the disease in all our patients with 13q14x2 respect to 37.5% of those with 13q14x1, significant differences in the distribution by Rai stage and shorter time to treatment for 13q14x2, suggesting that this alteration could represent a more aggressive FISH anomaly than 13q14x1 alone. In agreement with our results, a significant faster lymphocyte growth kinetics in CLL patients with 13q14x2 than cases with a monoallelic deletion as a sole alteration has been recently described (21).

It is interesting to note that a recent study using high-density single nucleotide polymorphism (SNP) arrays found that, in a number of CLL cases (3/11), biallelic

Table 5 Deletion 13q14.3 in patients with CLL. Comparison with the literature

Reference	No. of cases	Probe	Patients with del(13)(q14.3) alone (%)			
			Total	Monoallelic	Biallelic	Combined
Dewald *et al.* (8)	113	D13S319	48/113 (42.5)	31/48 (64.5)	9/48 (18.8)	8/48 (16.7)
Aoun *et al.* (11)	72	D13S25	33/72 (45.8)	23/33 (69.7)	4/33 (12.1)	6/33 (18.2)
Reddy (9)	509	D13S319	160/509 (31.4)	122/160 (76.3)	18/160 (11.2)	20/160 (12.5)
Dicker *et al.* (14)	132	D13S25 D13S319	66/132 (50.0)	51/66 (77.3)	6/66 (9.1)	9/66 (13.6)
Shanafelt *et al.* (15)	152	D13S319	72/159 (45.3)	36/72 (50.0)	9/72 (12.5)	27/72 (37.5)
Present study	103	D13S319 D13S25	38/103 (36.8)	32/38 (84.2)	1/38 (2.6)	5/38 (13.2)

13q14 deletion has been originated by a complex mechanism leading to a uniparental disomy for the long arm of chromosome 13 and a homozygous loss of the deleted region (21). Case 2 of our series showed 13q14x2 as a single alteration, being impossible to discard the presence of uniparental disomy for this patient.

As known, deletions at 13q14.3 are frequently observed as a sole abnormality in CLL, indicating a pathogenic role for the deleted gene or genes. Recent studies have shown that microRNA genes *miR-15a* and *miR-16-1* form a miRNA cluster that maps at 13q14.3. The allelic loss in this region correlates with deletion and/or down-regulation of these genes in B cell CLL patients, suggesting that these two miRNAs can function as tumor suppressor genes in this pathology (22). A combination of loss of heterozygosity plus a germinal mutation in the pri-miR-16-1 affecting the level of expression of mature microRNAs was also found in two patients with CLL, supporting the Knudson model of tumor suppressor genes inactivation (23). In addition, the recently reported combined genetic and epigenetic pathomechanisms is also compatible with biallelic 13q14 loss that results in complete inactivation of the critical region (24). Complete loss of tumor suppressor function in 13q14 could be related with the more aggressive clinical course observed in our patients.

In conclusion, although our series do not have a large number of cases, our results would suggest that the acquisition of biallelic 13q14 deletion could represent a more aggressive FISH anomaly than 13q14x1 alone, probably as a consequence of clonal evolution and/or due to the complete inactivation of this critical region by mean of more complex mechanisms. More studies will be necessary to confirm this data.

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