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Original Research Article

Structural alterations in chronic lymphocytic leukaemia. Cytogenetic and FISH analysis†

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Abstract

In this study, we described cytogenetics and fluorescence *in situ* hybridization (FISH) analysis performed in chronic lymphocytic leukaemia (CLL) patients with structural alterations. Results were correlated with clinical characteristics. A total of 38 CLL patients: 16 cases with complex and 22 with simple karyotypes were studied. For comparison of clinical parameters, a control group of 78 CLL patients with normal karyotype and without FISH genomic alterations were also evaluated. We found 38 structural abnormalities not previously described in the literature, 28 (74%) of them were translocations. In cases with complex karyotypes, chromosomes 6, 8 and 13 were the most frequently involved in new alterations (nine each), followed by chromosomes 12, 14 and 15 (six each). Chromosome 8p was particularly involved in losses, being 8p21-pter the commonest region of overlap. Cases with simple karyotypes, showed del(6q) as the most frequent alteration (39%). Del(9)(q11) was recurrent in our series. Analysis of clinical parameters showed significant differences in white blood count ($p = 0.005$) and platelet count ($p = 0.015$) between patients with structural alterations and the control group. In addition, patients with structural alterations had a significantly shorter time to first treatment (TFT) (29 months) than the control group (69 months) ($p = 0.037$). Cases with complex karyotypes had a lower proportion of patients in Rai 0 clinical stage (15.4% vs 75%) ($p = 0.005$) and higher β_2 microglobulin levels (3.3 vs 2.5 μg/mL) ($p = 0.037$) than those with simple karyotypes. Furthermore, a shorter TFT (13 months) and overall survival (56 months) in the complex karyotypes group compared with controls (69 and 144 months, respectively) ($p = 0.015$ and $p = 0.005$, respectively) were also found. Our results support the importance of cytogenetic analysis for clinical outcome in CLL and suggest that the diversity of genomic alterations is much greater than previously appreciated.

Keywords: **chronic lymphocytic leukaemia; cytogenetics; FISH; complex karyotypes**

Introduction

Chronic lymphocytic leukaemia (CLL) is the most frequent type of adult leukaemia in Western World. The disease is characterized by a highly variable clinical course, with time to progression ranging from months to decades. Although some patients require therapy immediately after diagnosis, others have a more stable, indolent disease without needing treatment for decades [1]. Clinical staging systems established by Rai *et al* [2] and Binet *et al* [3] have been very useful in guiding disease management and treatment decisions; however, they are not accurate enough to predict the outcome of individual patients at early-stage disease or to identify patients with poor prognosis on an individual basis [4]. In the last decades, several prognostic markers including the mutational status of the immunoglobulin heavy chain variable genes, phenotypic expression of ζ-chain-associated protein kinase 70 (ZAP-70) and cluster differentiation 38(CD38), and genomic abnormalities are used for predicting survival [5–9].

Cytogenetic study is considered one of the major prognostic indicators in CLL. Analysis of genomic abnormalities using interphase fluorescence *in situ* hybridization (FISH) has resulted in detection of clonal aberrations in more than

80% of CLL patients making it possible to establish high-risk groups of prognostic value [7]. Among them, the most frequent chromosomal abnormalities are deletions on 13q14, 11q22 [involving Ataxia Teleangiectasia Mutated (ATM)], 17p13 [involving tumor protein 53 (TP53)] and trisomy 12. Each of these cytogenetic aberrations has significant prognostic implications. Monoallelic deletion of 13q14.3 (13q14×1) is the most frequent abnormality, occurring in approximately 50% of CLL patients, and is associated with good prognostic value when presented as a sole alteration. Deletions of 11q22 and 17p13 have the shortest median survival, whereas trisomy 12 correlate with intermediate prognosis.

In contrast with observations in other B-cell malignancies, which typically exhibit recurrent immunoglobulin locus-associated translocations, balanced translocations are rare in CLL, and their breakpoints affect regions recurrently involved [10,11]. However, in the last years, different studies suggest that the diversity of genomic alterations in CLL may be much greater than previously appreciated [12–14]. In this report, we describe a series of CLL patients with simple and complex karyotypes. Novel structural rearrangements affecting sites infrequently involved in CLL were observed. Cytogenetic results were correlated with clinical characteristics.

Materials and methods

Patients

Thirty-eight CLL patients were selected for inclusion in the present report on the basis of the presence of structural rearrangements in the karyotype. Among them, 16 cases showed complex karyotypes (12 males; median age: 65 years; range: 39–82 years), and 22 had simple karyotypes (15 males; median age: 63 years; range: 48–84 years). Patients were diagnosed according to the World Health Organization 2008 criteria [15]. Stage was assessed according to the classification of Rai [2]. For comparison of clinical parameters, a group of 78 CLL patients with normal karyotype and without genomic alterations by FISH analysis (42 males; mean age: 66 years; range: 40–89 years) was also evaluated. Cytogenetic and FISH analysis were performed at diagnosis in all patients. Only one patient (case 9) had a second study during the course of the disease. Four patients (11%) have died at the moment of this analysis (cases 3, 8, 13 and 15). The study was approved by the ethical committee of our institution. All patients provided their informed written consent.

Metaphase cytogenetic analysis

Chromosome analyses were performed on peripheral blood lymphocytes, cultured for 96 h at 37°C in F-12 medium supplemented with 15% of foetal calf serum, stimulated with pokeweed mitogen and lipopolysaccharide, or in presence of the immunostimulatory CpG-oligonucleotide (2 μM) and interleukin 2 (200 U/mL) for 72 h [16]. Slides were prepared by conventional method. G-banding technique was used. In case of translocations or complex aberrations, results of G-banding analysis were confirmed using biotin-labelled whole chromosome painting (WCP) probes for different chromosomes (CAMBIO, Cambridge, UK) and Spectra Vysion WCP probe (Vysis-Abbott Molecular, Downers Grove, IL, USA). In each case, a minimum of 10 informative metaphases were analysed. Image acquisition was performed using Cytovision 3.9 software (Applied Imaging Corporation, Santa Clara, CA, USA). In cases of unbalanced translocations resulting from gain as well as loss of chromosomal material, these unbalanced translocations were assessed as two aberrations. Karyotypic abnormalities were described using the International System for Human Cytogenetic Nomenclature [17].

Interphase cytogenetic analysis

For FISH analysis, slides were hybridized with LSI (Locus Specific Identifier) DNA (deoxyribonucleic acid) probes TP53/ATM/13q14/13q34/CEP12 (chromosome enumeration DNA probe 12) (Vysis-Abbott Molecular, Downers Grove, IL, USA), according to manufacturer's protocol. Two hundred interphase nuclei were analysed for each probe. The cut-off for positive values (mean of normal control + 3 standard deviations), determined from samples of 10 cytogenetically normal donors, were: 3.02%, 10.2%, 7.7% and 5.1% for trisomy 12, monosomies of D13S319 (13q14), ATM and TP53, respectively.

Statistical analysis

Groupwise comparison of the distributions of clinical and laboratory variables were performed with the Student *t*-test (for quantitative variables) and the χ^2 or Fisher exact test (for categorical variables). Time to first treatment (TFT) was calculated as the interval between diagnosis and the start of first-line treatment. Overall survival (OS) was calculated from the date of diagnosis until death of any cause or until the last patient follow-up [18]. Survival curves were plotted by the Kaplan–Meier method and compared by the log-rank test. For all tests, $p < 0.05$ was considered statistically significant.

Results

Age, sex, stage at diagnosis and clinical characteristics of patients with structural alterations and the control group are summarized in Table 1. Cytogenetics and FISH analysis of 38 patients with structural aberrations: 16 with complex karyotypes and 22 with simple karyotypes are shown in Tables 2 and 3. Except for case 17 and the second study of case 9 that had exclusively abnormal metaphases, all patients

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Table 1. Clinical characteristics of patients with chromosomal alterations and the control group

	Control group	Chromosomal alterations group	*p*
Number of patients (*n*)	78	38	
Sex F/M	36/42	11/27	0.591
Mean age (years) (range)	66 (40–89)	63.8 (39–84)	0.323
Clinical stages (%)			
Rai 0	33.3	48.3	0.242
Rai I–II	45	31	0.288
Rai III–IV	21.7	20.7	0.873
Mean WBC count ($\times 10^9$/L) (range)	30 (8.3–400)	63.9 (8.9–354)	0.005
Mean lymphocyte percentage (range)	76 (40–99)	75.2 (54–97)	0.778
Mean Plt count ($\times 10^9$/L) (range)	169 (15–440)	207.8 (47–400)	0.015
Mean Hb (g/dL) (range)	12.9 (6.8–16)	13.1 (8.2–15.8)	0.552
Mean LDH (UI/L) (range)	349.2 (149–626)	326.9 (125–526)	0.358
Mean β2M (μg/mL) (range)	2.9 (1.1–6)	2.8 (1.2–5.9)	0.408
Mean *TFT* (months)	69	29	0.037
Mean OS (months)	144	69	0.214

F, female; M, male; WBC, white blood cells; Plt, platelets; Hb, haemoglobin; LDH, lactate dehidrogenase; β_2M, $\beta_2$microglobulin; TFT, time to first treatment; OS, overall survival.

showed normal and abnormal cells. The analysis of chromosome alterations showed the presence of 38 structural abnormalities not previously described in the literature [19]: 28 translocations (18 unbalanced and 1 complex), 6 deletions (one recurrent), 1 isochromosome, 1 duplication and 2 dicentric chromosomes. Chromosome 16 and 21 were not involved in structural rearrangements in our series.

When complex karyotypes were analysed, chromosomes 6, 8 and 13 were the most frequently involved in structural alterations showing a total of nine anomalies each, followed by chromosomes 12, 14 and 15 with six alterations each. Figure 1(a) shows the distribution of abnormalities by chromosome in this group of patients. New alterations are highlighted in Table 2. Interestingly, our series showed several novel alterations of chromosomes 8, 12 and 15, chromosome pairs scarcely involved in structural abnormalities in CLL. Particularly, chromosome 8 showed eight translocations (four unbalanced and one complex) not previously described in the literature. Unbalanced translocations determined losses involving its short arm, being 8p21-pter the commonest region of overlap. Only one patient (case 2) showed also gain of 8q22–qter region (Figure 2). In reference to chromosome 15, all translocations (five unbalanced) involving this chromosome were novel, and three of them were observed in the same patient (case 9; Figure 3). Chromosome 15 imbalances are shown in Figure 2. As for chromosome 12, novel aberrations (five translocations and three unbalanced) determined gains of part of its long arm, with a region of overlap located between 12q13-qter bands. Chromosomes 6, 13 and 14 are commonly involved in rearrangements in CLL. In our cohort, six out of nine alterations of chromosome 13 were novel, and they included five translocations, three of them with chromosome 8 and the psu dic(13;3)(q34;p21). Chromosome 14 also showed new translocations with partner chromosomes 2, 8 and 10, and chromosome 6 had only recurrent deletions characteristics of lymphoid malignancies [17]. In addition, we found three alterations involving sex chromosomes, which are very rare events in this pathology. The analysis of all genomic imbalances showed chromosomal losses (72%) more frequently than gains (28%). A total of 105 breakpoints were found, being the most frequent: 6q21 and 14q32 (3.8% each) followed by 6q25, 7q22, 8p21, 8q22, 11q23, 12p13, 13q14 and 13q22 (2.9% each). In this group of patients, a mean chromosome aberrations number of 9.4 was observed, with a higher value (14.4) for patients with loss of 17p. Interphase FISH was performed in 12/16 patients showing alterations not detected by conventional cytogenetics in 31% of cases (Table 2).

Twenty-two patients showed simple karyotypes, being deletion 6q the most frequent anomaly (39%), followed by deletions 13q and 14q (14% each), 11q (7%), 8p, 10q and 17p (3.5% each) (Table 3). In these group of patients, we found two new alterations (cases 17, 30 and 31), one of them recurrent in our series: del(9)(q11). Figure 1(b) shows the distribution of all chromosome abnormalities observed in these cases. A total of 38 breakpoints were found, being the most common 6q25 (15.8%), followed by 6q15, 13q14 (10.5% each) and 11q21 (7.9%). FISH analysis was performed in 15/22 patients. The comparison of FISH analysis and metaphase cytogenetics showed discordant results in 53% of cases where genomic deletions could be detected by FISH but not by chromosome banding.

Deletion of 6q was the most frequent structural alteration leading to loss of genetic material in both groups of patients with simple and complex karyotypes. In addition, it is interesting to point out that we found two deletions that were observed only once in the literature and appear to be recurrent in CLL from our data: del(3)(p11) [20] and del(10)(q22) [21] (cases 4 and 31, respectively).

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Table 2. Cytogenetics and FISH results in chronic lymphocytic leukaemia patients with complex karyotypes

Case	Age/sex	Combined karyotype (G-banding and FISH)	FISH (%)			
			del13q14	+12	del11q22	del17p13
Complex karyotypes						
1	62/M	45~47,Y,**der(X)t(X;5)(q22;q13)**,del(1)(q23q25),**i(2)(q10)**, del(3)(p13),+del(3)(q21),del(6)(q25),+i(6)(p10), del(7)(q22),**t(8;12)(q13;p13)**,+10,del(11)(q23),+**der(12)t(12;15)(p13;q11)**,-16,-17,-18,+22,+r[cp11] /46,XY[2]	0.5	***71***	0	***58***
2	79/F	46~47,XX,**der(8)t(8;8)(p21;q22)**,+12, t(14;18)(q32;q21), der(22)t(22;?)(q11;?),+r [cp12]/46,XX [6]	1.1	***35.4***	0.7	2.1
3	55/M	45~46, XY,+6,**t(11;13)(p15;q22)**,add(17)(q25)[cp9]/46, XY[1]	ND	ND	ND	ND
4	66/M	46,XX,**der(14)t(10;14)(q11;p11)**,+del(14)(q24),+r [cp5]/76~122,XXYY,+del(3)(p11), del(6)(q21), −11,+**der(14)t(10;14)(q11;p11)**,+del(14)(q24),-16,+20, +21,+22,+r [cp4]/46,XY[9]	ND	ND	ND	ND
5	72/F	46,XX,**dup(X)(q22q26)**,del(7)(q32),**der(8)t(8;12)(p21;q13)**[17]/46,XX [2]	2.3	1.6	4.8	2.3
6	71/M	48,XXY,**der(4)t(4;5)(q35;q13)**,+**del(9)(q13q22)**, der(14)t(10;14)(q22;q32)[6]/46,XY[9]	ND	ND	ND	ND
7	61/M	46,XY,del(6)(q25),**t(7;7)(q22;p22)**[4]/46,XY,+21, -22[3]/46,XY,**t(12;13)(q24;q14)**[2]/46,XY[12]	***x1:79.6 /x2: 4.9***	0	0	1.3
8	39/M	46,XY,**t(2;14)(q31;q32)**,**t(2;20)(p13;p13)**,**t(8;13)(q22;q22)**, del(11)(q21)[8]/46,XY[20]	0	0	***8.6***	0.9
9	72/M	43-44,X,-Y,del(7)(q22q32),**der(8)t(8;13)(p11.2;q11)**, **der(15)t(Y;15)(q11;p11)**,del(17)(p11),**der(17) t(15;17)(q15;q25)**,**del(18)(q11q21)**,**der(19)t(10;19)(q24;p13)**, **der(19)t(15;19)(q15;q13)** [cp11] /46,XY [8]	5.3	0	0	***44.3***
	73/M	44,XY,-2,del(6)(q21),del(7)(q22q32),**der(8)t(8;13)(p11.2;q11)**, add(12)(p13),**psu dic(13;3)(q34;p21)**, del(17)(p11),**der(17)t(15;17)(q15;q25)**,**del(18)(q11q21)**, **der(19)t(15;19)(q15;q13)** [cp6]/ 44,X,-Y,del(4) (p12), del(6)(q21),del(7)(q22q32),**der(8)t(8;13)(p11.2;q11)**, add(12)(p13),**psu dic(13;3)(q34;p21)**, **der(15)t(Y;15)(q11;p11)**, **der(17)t(15;17)(q15;q25)**,**del(18)(q11q21)** [cp4]	***12.7***	0	0	***72***
10	52/M	51,XY,**der(1)t(1;4)(q32;q21)**,+12,+18,+19,+22, +mar [15]/46,XY[10]	***x1:44 /x2:22***	***56.4***	0	0
11	82/M	41~44,XY,**t(1;8)(p34;p21)**,del(6)(q13q21),-8, add(13)(p11),-13,-16,-19,-22[8]/46,XY[22]	ND	ND	ND	ND
12	61/F	81~83,XXX,-1,-2,-3,del(6)(q15),-8,-9,-10, **der(12)t(12;13)(q24;q22)**, **der(14)t(8;14)(q22;q32)**,-14,-16, **del(17)(q11)***x*2, **der(19)t(4;19)(q21;q13)**,-20 [cp8]/46,XX [2]	***98.6***	0	4	***97***
13	40/M	46,XY,del(6)(q13q21),del(7)(q22q34),-8,add(9)(q34), del(9)(q22),**der(11)t(1;11)(q21;q23)**,-17,+r,+dm [cp14]/ 46,XY[3]	1.8	1.3	***67.6***	***76.3***
14	80/M	46,X,-Y,der(8)t(8;13)(p12;q14),+12, del(13)(q14)*x*2,-21 [6]/46,XY [1]	***82***	***71***	1.2	0
15	81/F	44,XX,**der(2)t(2;15)(p21;q12)**,-8,**t(8;11;15)(q10;q24;q10)**,-15,-17, **der(17;20)(p10;q10)**,+dm [3]/46,XX [5]	1.7	0	0	***46***
16	59/M	46,XY,del(6)(q25),+10,+18,-19 [cp 9]/46,XY[10]	1.4	0.5	***10.2***	0

F, female; M, male; bold, new structural alterations; bold italics, percentage of abnormal clone, *x*1, monoallelic deletion of 13q14; *x*2, biallelic deletion of 13q14; ND, not done; FISH, fluorescence *in situ* hybridization.

For the analysis of clinical parameters, a control group of 78 patients previously described were used. Significant differences in white blood count ($p = 0.005$) and platelet count ($p = 0.015$) between patients with structural alterations and the control group were observed (Table 1). In addition, patients with structural alterations had significantly shorter TFT (29 months) with respect to the control group (69 months) ($p = 0.037$). When we compared CLL cases with complex and simple karyotypes, the former showed a lower proportion of patients in Rai 0 clinical stage (15.4% vs 75%) ($p = 0.005$) and higher β_2 microglobulin levels (3.3 vs 2.5 μg/mL) ($p = 0.037$) with respect to those with simple karyotypes. Furthermore, shorter TFT (13 months) and OS (56 months) in the group of complex

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Table 3. Cytogenetics and FISH results in chronic lymphocytic leukaemia patients with simple karyotypes

Case	Age/sex	Combined karyotype (G-banding and FISH)	FISH (%)			
			del13q14	+12	del11q22	del17p13
Simple karyotypes						
17	84/F	45,XX,-3,**der(3)t(3;6)(q11;p23)**,del(17)(p11) [20]	***73***	0	0	***62***
18	56/M	46,XY,del(6)(q13q15),del(11)(q21) [cp6]/ 46,XY [14]	ND	ND	ND	ND
19	48/M	46,XY,del(6)(q13q15) [6]/46,XY [6]	1.5	***14.1***	3.5	***8.7***
20	67/F	46,XX,del(6)(q15) [5] / 46,XX [6]	ND	ND	ND	ND
21	48/M	46,XY,del(6)(q15q21),del(14)(q22) [cp4]/46,XY[10]	0	0.5	0	***9***
22	63/M	46,XY,del(6)(q21q23) [4] / 46,XY [20]	***49***	0	0	0
23	78/F	46,XX,del(6)(q23q25),del(13)(q14q22)[3]/46,XX[16]	***x1:27 / x2:26***	***3.4***	0.9	1.8
24	63/F	46, XX,del(6)(q25) [4]/46,XX [9]	ND	ND	ND	ND
25	67/M	46, XY,del(6)(q25) [5]/46,XY[10]	ND	ND	ND	ND
26	71/M	46,XY,del(6)(q25) [8]/46,XY[4]	ND	ND	ND	ND
27	63/F	46,XX,del(6)(q25)[10]/46,XX[14]	5.9	0	0.5	***6.3***
28	67/F	47,XX,del(6)(q25),del(11)(q21) [6]/46,XX [14]	ND	ND	ND	ND
29	64/F	46,XX,del(8)(p11) [14] / 46,XX [5]	***x1: 71.2 / x2: 8.4***	0	***13.6***	1.3
30	53/M	46,XY,**del(9)(q11)**[5]/46,XY[27]	2.8	0.5	0	0
31	63/M	45-47,XY,+**del(9)(q11)**,del(10)(q22)[cp4]/46,XY[12]	ND	ND	ND	ND
32	61/M	46,XY,del(11)(q21) [7] / 46,XY [10]	***16.4***	0	***32.2***	0
33	59/M	46,XY,del(13)(q12q14) [6]/ 46,XY [14]	***62***	0	1	1,6
34	58/M	47,XY,del(13)(q12q14),+12 [3]/ 46,XY [18]	***21.5***	***24.4***	0.5	0.8
35	73/M	46,XY,del(13)(q14q22) [5]/46, XY [9]	***28.7***	0.5	0.5	0.4
36	58/M	46,XY,del(14)(q22) [3]/46,XY [32]	0	2.9	3.8	0.6
37	70/M	46,XY,del(14)(q24) [4]/46,XY [8]	0.1	2.1	2.1	2.9
38	62/M	46,XY,del(14)(q24) [5]/46,XY [14]	2.3	0.8	1.7	2.1

F, female; M, male; bold, new structural alterations; bold italics, percentage of abnormal clone; *x*1, monoallelic deletion of 13q14; *x*2, biallelic deletion of 13q14; ND, not done; FISH, fluorescence *in situ* hybridization.

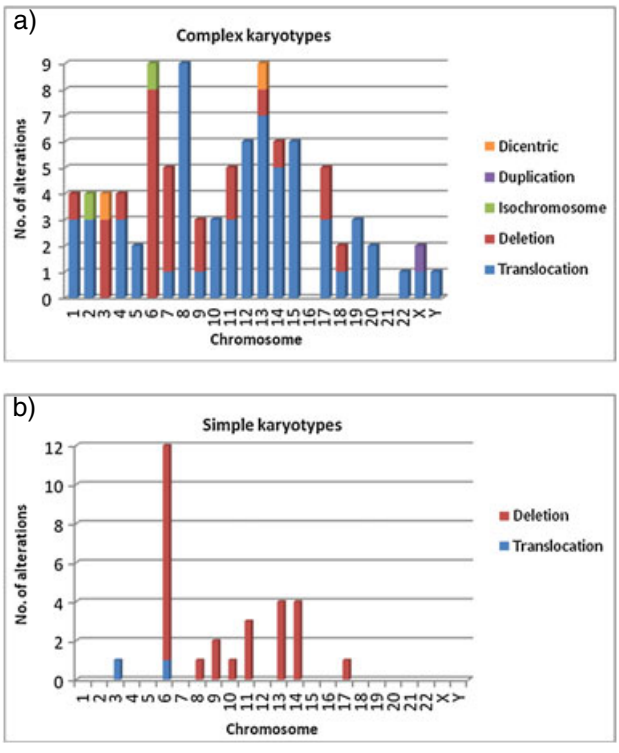

Figure 1. a) Histogram showing the distribution of chromosome rearrangements in chronic lymphocytic leukaemia patients with complex karyotypes; and b) Histogram showing the distribution of chromosome rearrangements in patients with simple karyotypes

karyotypes compared with controls (69 and 144 months, respectively) ($p = 0.015$ and $p = 0.005$, respectively) (Figure 4) were also found.

Discussion

Cytogenetic studies in lymphoid malignancies have proved to be an important tool in the biologic characterization of these pathologies. In this study, novel chromosome alterations and new recurrent rearrangements were described.

Chromosome 8 was the most frequently involved in new alterations, with a total of nine anomalies not previously described in the literature [19]. All five unbalanced translocations led to losses of 8p, particularly involving 8p21-pter region. Comparative Genomic Hybridization (CGH) studies have shown 8p losses in 5–7% of CLL cases [12,14], with an increased frequency in patients with Richter transformation (44%) [12]. The literature refers different regions of 8p deletions. By cytogenetic analysis, Haferlach *et al* [11] found recurrent losses at 8p11–pter, whereas CGH and microarray studies found deletions at 8p21.2–p12 [22], 8p11.2–p23.3 [12,13,23], 8p12 and 8p23.1–p23.3 [13]. In a large cytogenetic study [11], losses of 8p were found associated to complex karyotypes and immunoglobulin heavy chain locus (*IGH@*) translocations. In our series, this alteration was also observed associated to complex karyotypes, but only one case showed

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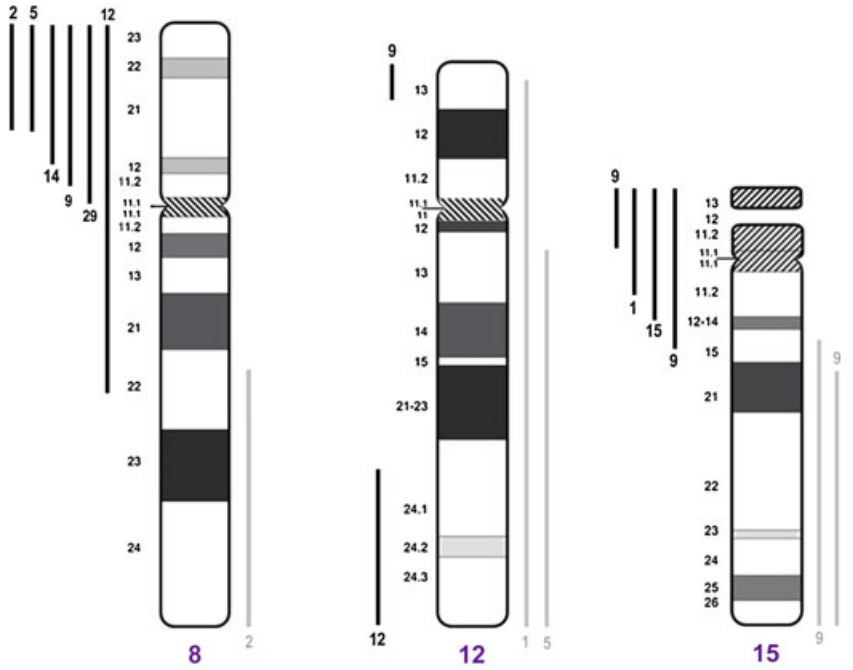

Figure 2. Genomic imbalances observed in structural rearrangements of chromosomes 8, 12 and 15. Numbers on the bars indicate the cases with chromosome gains/losses

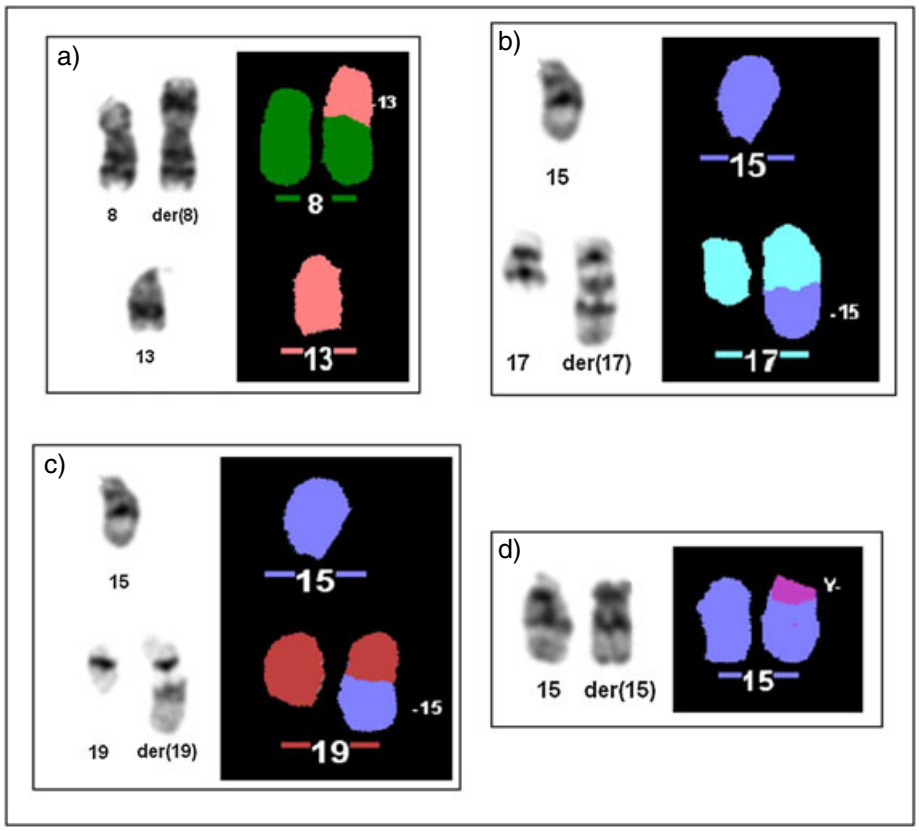

Figure 3. Partial karyotypes with G-banding and M-FISH techniques of case 9 showing: a) normal chromosomes 8 and 13 and der(8)t (8;13)(p11.2;q11); b) normal chromosomes 15 and 17 and der(17)t(15;17)(q15;q25); c) normal chromosomes 15 and 19 and der(19)t (15;19)(q15;q13); and d) normal chromosome 15 and der(15)t(Y;15) (q11;p11)

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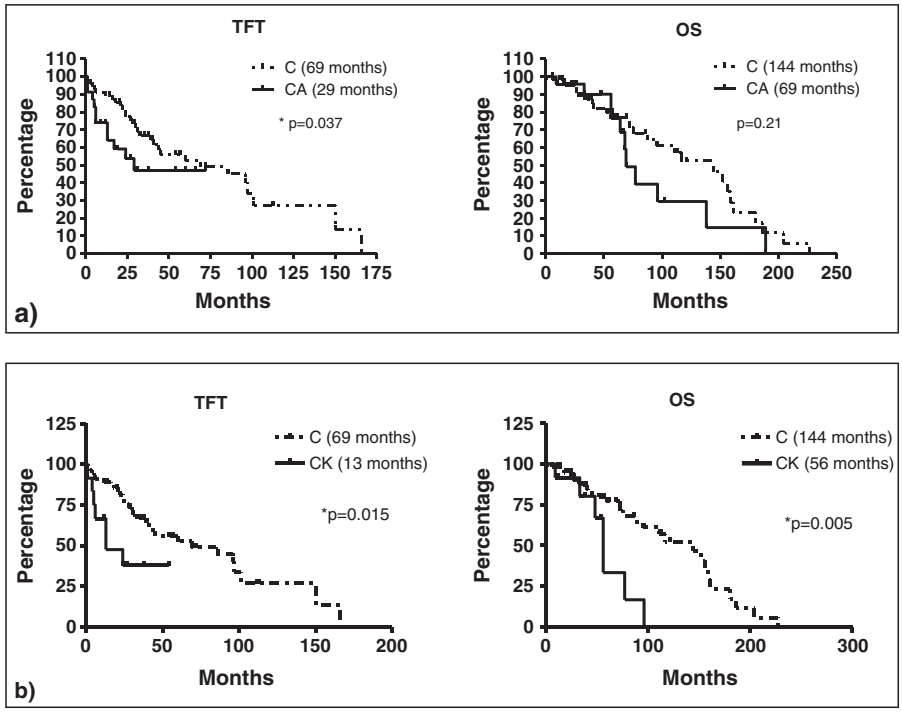

Figure 4. a) Time to first treatment (TFT) and overall survival (OS) from chronic lymphocytic leukemia patients with chromosomal alterations (CA) and the control group (C); and b) TFT and OS from chronic lymphocytic leukemia patients with complex karyotypes (CK) and C

simultaneously an *IGH@* translocation (case 2). Interestingly, we found in our cohort the second case with del(8)(p11) as a single alteration [24]. Chromosome 8p is a genetically unstable region with low copy repeats and submicroscopic genomic polymorphism that would predispose to translocations by non-allelic homologous recombination [25]. Losses of 8p include tripartite motif containing 35 (*TRIM35*) (8p21.2) and Tumor Necrosis Factor Receptor Superfamily, member 10a/b (*TNFRSF10A/B*) [Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor *(TRAIL-R1/2)*] (8p21.3) genes, whose deletion in B-cell malignancies was associated with impaired apoptosis and aggressive transformation [26,27]. In concordance, Forconi *et al* [13] found significantly shorter treatment free survival and OS in patients with 8p losses, suggesting a clinical significance for this alteration.

One important benefit of cytogenetic analysis is that the prognostic subgroups defined by interphase FISH can be further subdivided if translocation status is included. Deletion 13q14 is the most common genomic alteration in CLL patients, being associated to favourable outcome when presented as a single anomaly [7]. On the contrary, structural rearrangements of this chromosome are not frequent, but its presence in the karyotype changes the prognosis of the disease. Our series showed six novel structural alterations involving chromosome 13, all as a part of complex karyotypes, and some of them producing large 13q deletions that have been associated with disease progression [28]. One of these alterations was a pseudodicentric chromosome, a rare event in CLL [19], usually related with genetic instability. In concordance, most of our cases with 13q translocations showed a very short *TFT*.

Recurrent gains were uncommon in our series and involved part of the long arms of chromosomes 12 and 15 at bands 12q13-qter and 15q15–qter, respectively. Gains of 12q were observed in 5% of CLL cases with chromosome aberrations [29]; meanwhile, Dicker *et al* [16] found 3.2% of patients with chromosome 15 translocations. Previous data from our group suggest association between chromosome 12 structural abnormalities and poor clinical outcome [20]. Interestingly, our series also showed novel alterations of chromosomes X and Y. As well known, the median age for diagnosis of CLL is 65 to 70 years; thus, cytogenetic losses of sex chromosomes are common events. However, structural aberrations of these chromosomes are very infrequent, particularly those of chromosome Y [19].

Deletion 9q11 was recurrent in our series. Losses of 9q21.33-q22.2 and 9q11–q32 were previously reported with low incidence in CLL patients [13,30]. This region includes FANCC (Fanconi Anaemia Complementation Group C) and XPA (Xeroderma Pigmentosum Complementation Group A) (9q22) and RAD23B (RAD 23 homolog B (S. cerevisiae)) (9q31.2) (9q32) genes involved in deoxyribonucleic acid repair and maintenance of chromosome stability and was related to aggressive clinical behaviour [30].

Ten patients of our series showed deletion 17p, alteration related to poor prognosis [7]. However, three cases had

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coexistence with deletion 13q, association that may attenuate the short survival related to 17p- [31], and other three patients had less than 25% deleted nuclei, percentage associated with a more favourable clinical outcome for patients with this abnormality [32]. Thus, despite a multivariate analysis was not performed, the significantly shorter TFT observed in our patients with structural aberrations compared with the control group, and the differences in TFT and OS found in cases with complex karyotypes with respect to controls, would confirm previous studies that show the adverse clinical impact of structural abnormalities and complex karyotypes in CLL [10,11,16,18,29,33].

In conclusion, the present study identified 38 new chromosome abnormalities, one of them recurrent in our series, and detected two deletions reported only once in the literature that became recurrent from our data. Chromosome 8p was particularly involved, with recurrent losses at 8p21-pter, suggesting it as an important site of rearrangements in CLL. In addition, our results support that cytogenetic analysis provides additional information for clinical outcome and showed that the diversity of genomic alterations in CLL is much greater than previously appreciated.

Conflict of interest

The authors reported no potential conflicts of interest.

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Authorship

AT, IS and MRC contributed to the study design, collection and analysis of the data and prepared the first draft of this manuscript. LP and AA helped with the acquisition and interpretation of data. AR and RB were responsible for the clinical management of patients. MRC and IS were responsible for a final revision of the manuscript. All the authors gave the final approval for its submission.

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