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Abnormalities on 1q and 7q are associated with poor outcome in sporadic Burkitt's lymphoma. A cytogenetic and comparative genomic hybridization study

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Comparative genomic hybridization (CGH) studies have demonstrated a high incidence of chromosomal imbalances in non-Hodgkin's lymphoma. However, the information on the genomic imbalances in Burkitt's Lymphoma (BL) is scanty. Conventional cytogenetics was performed in 34 cases, and long-distance PCR for t(8;14) was performed in 18 cases. A total of 170 changes were present with a median of four changes per case (range 1–22). Gains of chromosomal material (143) were more frequent than amplifications (5) or losses (22). The most frequent aberrations were gains on chromosomes 12q (26%), Xq (22%), 22q (20%), 20q (17%) and 9q (15%). Losses predominantly involved chromosomes 13q (17%) and 4q (9%). High-level amplifications were present in the regions 1q23–31 (three cases), 6p12–p25 and 8p22–p23. Upon comparing BL *vs* Burkitt's cell leukemia (BCL), the latter had more changes (mean 4.3 ± 2.2) than BL (mean 2.7 ± 3.2). In addition, BCL cases showed more frequently gains on 8q, 9q, 14q, 20q, and 20q, 9q, 8q and 14q, as well as losses on 13q and 4q. Concerning outcome, the presence of abnormalities on 1q (ascertained either by cytogenetics or by CGH), and imbalances on 7q ($P = 0.01$) were associated with a short survival.
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Introduction

Burkitt's lymphomas (BLs) comprise a heterogeneous group of highly aggressive B-cell malignancies.[1] Recently, the World Health Organization (WHO) classification has included the L3 variant of acute lymphoblastic leukemia (ALL) as equivalent to BL in leukemic phase or Burkitt's cell leukemia (BCL).[2] All cases of BL share a common genetic lesion, characterized by a reciprocal chromosomal translocation involving the c-*MYC* gene on chromosome 8q24 and one of the immunoglobulin (Ig) genes located on chromosome 14q32 (Ig heavy chain, IgH), chromosome 2p12 (Ig kappa, Igκ) or chromosome 22q11 (Ig lambda, Igλ). All 8q24 breaks lead to a deregulation of the c-*MYC* proto-oncogene. Over the last 10 years, several cytogenetic reports have suggested that so-called 'secondary' chromosome changes could influence the clinical picture of lymphoid tumors.[3,4] Most of the secondary chromosome changes are unbalanced rearrangements, leading to DNA gains or losses.[5] The development of molecular cytogenetics techniques, allowed for a refinement of the cytogenetic profile of NHL. Thus, comparative genomic hybridization (CGH), provides in a single experiment a general view of genomic imbalances, including partial or complete trisomies, monosomies or amplifications within the tumor genome. This technique can be used to identify previously unexpected genetic abnormalities. Several groups have studied chromosomal changes by CGH in B-cell NHL, such as mantle cell lymphoma, follicular lymphoma, marginal lymphoma and diffuse large-cell lymphoma.[6–11] However, little is known about the genomic imbalances in BL. The present study was designed to screen DNA copy changes by CGH in a series of 46 patients diagnosed as BL or BCL and to correlate the results obtained from CGH with the most relevant clinical, biological and cytogenetic characteristics, including disease outcome.

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Materials and methods

Patients

A total of 46 patients with histologically and morphologic confirmed diagnosis of BL were analyzed by CGH. Histopathologic evaluation was performed based on morphologic and immunohistochemical criteria according to the WHO classification of malignant lymphoma by two expert hematopathologists (TF and MAP).[2] The median age was 35 years (range 5–77 years). Most of them were male (74%). In each patient, the following initial data were recorded and evaluated for prognosis: age, gender, performance status according to the Eastern Cooperative Oncology Group (ECOG) scale, serum lactate dehydrogenase (LDH) level, tumor extension, extranodal involved sites and bone marrow (BM) infiltration. Patients were treated with chemotherapy-based protocols as BFM, CODOX-M-IVAC and LSA2-L2 with slight modifications. All samples were analyzed at diagnosis of the patients.

Cytogenetics

Cytogenetic studies were carried out in 34 patients. Chromosome analysis was performed in BM (15 cases), peripheral blood (six cases), lymph nodes (eight cases) and material with neoplastic cells (spleen, fluids and tumoral mass) in the remaining five patients. Cytogenetics was performed after direct or 24 h culture without stimulating agent according to the methodology previously reported.[12]

Comparative genomic hybridization

DNA was extracted from fresh frozen tissue (21 cases), from archival cells fixed in Carnoy (eight cases), from BM slides (nine cases) or from paraffin-embedded tissues in the remaining eight patients. The phenol–chloroform method was used for DNA

extraction according to standard procedure. In cases with a low yield of extracted DNA, a universal amplification of genomic DNA with degenerate oligonucleotide primed PCR (DOP-PCR) was used.[13] CGH analysis was performed according to the method described by Lichter *et al*.[14] Briefly, tumor DNA (test DNA) was labeled with biotin-16–dUTP (Boehringer Mannheim, Mannheim, Germany) and normal DNA (reference DNA) was labeled with digoxigenin-11–dUTP (Boehringer Mannheim) by a standard nick-translation reaction. The sizes of the nick-translated fragments range from 300 to 1000 bp. Equal amounts (1 μg) of labeled tumor and normal DNAs, and 70 μg of unlabeled human Cot-1 DNA (GIBCO/BRL, Gaithersburg, MD, USA) were cohybridized to slides with human metaphase chromosome spread prepared from phytohemagglutinin-stimulated lymphocytes from normal individuals. After hybridization for 1–2 days in a moist chamber at 37°C, posthybridization washes were performed to a stringency of 0.1 × SSC at 42°C. Tumor and normal DNA were detected by avidin–fluorescein isothiocyanate (FITC) and rhodamine-conjugated antidigoxigenin, respectively. The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with an antifade solution. Image acquisition was performed with an epifluorescence microscope (Nikon MDR1000) equipped with a cooled charge-coupled device (CCD) camera. The images were analyzed with Quips CGH software (QUIPS, Vysis, Downers Grove, IL, USA). Calculation of the tumor DNA to normal DNA fluorescent ratios along the length of each chromosome was performed by means of an automated CGH software package (Quips). Ratio values above 1.25 and below 0.75 were considered to represent chromosomal gains and losses, respectively. A high-level amplification was considered when the fluorescence ratio values exceed 1.5. Ratio values obtained from at least 10 metaphase cells for each case were averaged. Ratio values above 1.25 and below 0.75 were considered to represent chromosomal gain and loss, respectively. Over-representations were defined as high-level amplifications when the profiles exceeded the cutoff value of 1.5. Chromosomal gains exceeding 1.5 involving the whole chromosome or large areas of a chromosomal arm were not considered as high-level DNA amplification.

Negative control experiments were performed using differentially labeled male *vs* male DNA, and female *vs* female DNA. Additional control experiments included the interchange of the digoxigenin–dUTP and biotin-dUTP labels between normal and tumor. According to previous reports, changes on terminal 1p and chromosome 19 were excluded from the final calculation because they are known to be critical in CGH.[15]

Genomic DNA preparation and long distance-PCR amplification

High-molecular weight DNA was isolated from fresh or frozen adenopathies and/or bone marrow samples by standard proteinase-K digestion, phenol–chloroform extraction and ethanol precipitation. Based on the structure of the genes involved in the t(8;14)(q24;q32) translocation and previous data[16,17] (Figure 1), we used one MYC-specific primer, recognizing a region in exon 2, three primers for the constant IgH regions Cμ, Cγ and Cα and one for the JH regions. The specificity of this assay was established by the lack of t(8;14)(q24;q32) specific PCR product in genomic DNA from healthy individuals (Figure 2).[17] LD-PCR was performed in a 9700 thermal cycler (Aplied Biosystems, Foster City, CA, USA) using the Expand Long Template PCR System Kit (Boehringer Mannheim, Mannheim, Germany) in 50 μl total volume following the manufacture's instructions. For each tube 250 ng genomic DNA was added, together with 60 pmol of each of the corresponding primer. The quality of DNA was tested with the tPA control primer set (Boehringer Mannheim, Mannheim, Germany) for long amplification. PCR products were analyzed by agarose gel electrophoresis (0.8% agarose) and ethidium bromide staining.

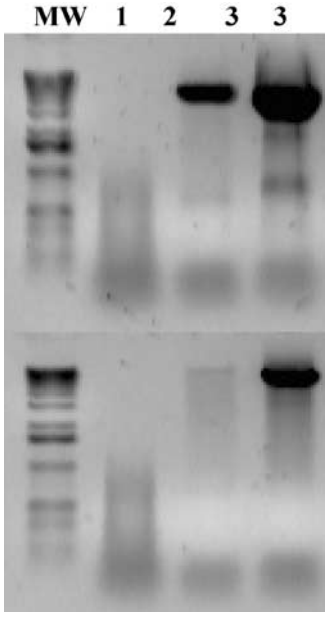

Figure 1 LD-PCR analysis of BL/BCL. Amplification of the tPA control gene. In lane 1 (case 3) there is no amplification of neither of the two fragments. Lane 2 (case 6) represents a relatively high-quality DNA sample as we could amplify the 4.5 kb fragment of the tPA gene. In lane (case 7), we found amplification of both 4.5 and 9 kb fragments of the tPA gene.

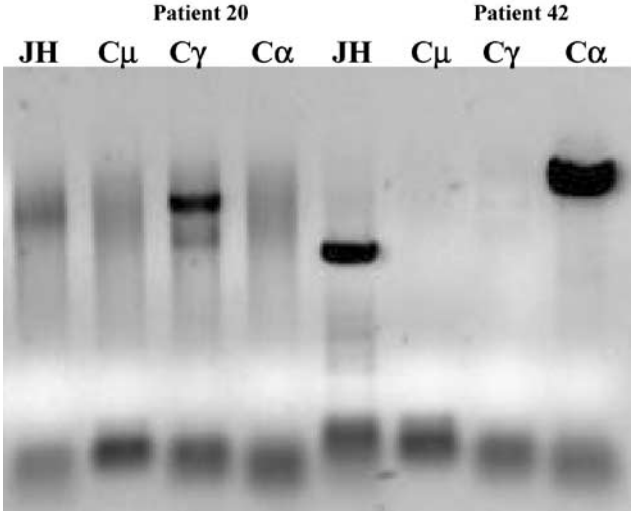

Figure 2 LD-PCR amplification of t(8;14). Patient 20 shows amplification of the translocation involving c-myc and Cγ constant region. In patient 42, we found amplification of c-myc–JH together with c-myc–Cα.

Statistical methods

Differences between groups were compared by the Fischer's exact test (two-tailed) and the Student's test. The actuarial survival analysis was performed according to the method described by Kaplan and Meier[18] and the curves were compared by log-rank test. The univariate analysis was performed for each of the parameters mentioned earlier. *P*-values less than 0.05 were considered to indicate statistical significance. Data were analyzed with SPSS statistical software (SPSS Inc., Chicago, IL, USA). The incidence of clinical features among subgroups was analyzed with the Pearson χ^2 test.

Results

Clinical characteristics

The most relevant demographic and clinical characteristics are shown in Table 1. A high proportion of patients had extranodal involvement (74%), and approximately half of them showed bulky disease (44%) high serum LDH levels, more than 1000 IU (46%) or advanced performance status (50%). The overall survival of the whole series was 7 ± 7 months. At the time of the analysis 27 patients (59%) died. The median survival for patients with diagnosis of BL was 25 months (range: 52–136), while the patients with BCL had a median survival of only 6 months (range: 0–12 months).

Cytogenetic analysis

Cytogenetic results are shown in Table 2. In 85% of the cases, chromosomal abnormalities were found and, most of them (24 out of 26) showed pseudodiploid karyotypes. Five cases (case nos. 2, 20, 22, 34 and 40) had a composite karyotype (more than two chromosomal abnormalities). A t(8;14) translocation was present in 76% of patients, while the variant translocations t(8,22) and t(2;8) affected 21 and 3% of cases, respectively. The most frequent secondary cytogenetic abnormalities were changes in 1q (29%), partial trisomies of chromosome 1 (10%), changes in 13q (7%) and deletion of 9p (10%), 13q (7%), 6q and 17p (3% each) (Table 2).

Table 1 Clinical Characteristics of 46 Patients with BL/BCL

	No.	*%*
Age (years)		
Median	36	
Range	(5–77)	
<14 years	10	21
Sex		
Male	34	74
Female	12	26
Involvement		
Lymph node (BL)	19	41
Bone marrow+lymph node (BCL)	16	35
Bone marrow (BCL)	11	24
Extranodal involvement[a]	34	74
Increased LDH level >1000 IU[b]	21	46
Performance status (ECOG >2)	23	50
Bulky disease	20	44
t(8;14)[c]		
Present	32	86
Not present	5	13

[a]In 26% cases, more than one region was involved.
[b]All patients showed an increased amount of LDH.
[c]Translocation was analyzed by cytogenetics or LD-PCR in 37 cases.

LD-PCR amplification of t(8;14)(q24;q32)

Tumor specimens obtained from 18 unselected BL/BCL were studied with the four combinations of *MYC* and *IGH* primers. We were able to detect the t(8;14)(q24;q32) in nine out of 18 patients analyzed (50%). Nevertheless, in seven out of the nine patients in which no LD-PCR product could be detected, we also failed in the amplification of the tPA control gene, indicating that poor-quality DNA was obtained. Therefore, t(8;14)(q24;q32) was detected in nine out of the 11 valuable patients (81%). In six out of 11 patients (60%) with a cytogenetically detectable t(8;14)(q24;q32), an LD-PCR translocation product was seen (cases nos: 20, 21, 38, 40, 42 and 45). Moreover, we could confirm the t(8;14)(q24;q32) by LD-PCR in another three samples (case nos. 6, 7, and 23, Table 2) in which cytogenetic data were not available. In three of the 11 cases showing t(8;14) positive samples (case nos. 7, 21, 42) two primer combinations showed an LD-PCR product (Table 2).

Comparative genomic hybridization

The distribution of genomic imbalances are shown in Figure 3 and in Table 2. Out of the 46 patients with BL/BCL (76%), 35 showed DNA changes by CGH. A total of 170 DNA copy number changes were detected with a median of four abnormalities per case (range: 1–22): 143 gains, 22 losses and five high-level amplifications (Table 3). Most cases showed more than one chromosomal imbalance. However, single alterations were identified in some cases, including one case with a high-level amplification on 1q (case 20), one patient with loss on 17p (case 43) as well as cases with gains on 4p, 13q, 7p, Xq and 2p (case nos. 12, 13, 32, 35 and 36, respectively). Irrespective of the morphology or clinical characteristics, the most frequent findings (present in $>5\%$ of cases) were gains involving regions at 12q (26%), Xq (22%), 22q (20%), 20q (17%), 9q and 8q (15% each), 1q, 4p, 7q, 13q, 10q (13% each) and 6q and 14q (11% each). The gains of whole chromosome involved chromosomes 12, 16 and 20 (11% each), as well as 11 and 21 (9%) (Figure 3). The losses were detected on chromosomes 13q (17%), 4q (9%) 6q and 17p (4% each). The commonly affected regions were delineated to 13q14–q31, 4q21–q28 6q21–q23 and 17p13. In five cases, high-level DNA amplifications were found that mapped on chromosomes band 1q23 to 1q31 (three cases), 6p12–p25 and 8p22–p23 (Figure 3). Details of the GCH are listed in Table 2, and complete data are illustrated in Figure 3.

Associations among cytogenetics, histopathologic and clinical baseline parameters

The comparison between the genomic changes observed in BL and BCL is summarized in Table 3. Overall, 15 out of the 19 cases of BL (79%) and 20 out of the 27 BCL (74%) had genomic imbalances. The mean number of chromosome imbalances per case was more frequent in BCL (mean of 4.3 ± 2 per case) than in BL (mean of 2.7 ± 3 per case), but the differences did not reach statistical significance. The chromosomal imbalances detected

Table 2 Cytogenetics, CGH and LD-PCR results in BL/BCL

Ref	Histology	Cytogenetics		CGH			LD-PCR	
		Primary	Secondary	Gains	Losses	Amplification	LD-PCR result	Region Involved
1	BL	t(8;14)		4q26–q28, Xq28–qter	0	0	ND	
2	BL	t(8;22)	dup(1)(q25q32), add(9)(p23)	10q22–q25	12p11–13	1q22–q25	–	–
3	BL	ND		8q24–qter,9q34–qter, +10,+12,13q32–q34	0	8p11-p23	–	–
4	BL	t(8;14)		Xq11-q22	0	0	ND	
5	BL	t(8;22)	+7	3q26-q29,7q32-q36, 20q11-q13	0	0	–	–
6	BL	NG		7p15-p22,Xp21-p22	0	0	+	Cα
7	BL	ND		+2,7q22-q36,+12,+21,+22	0	1q21-q25	+	cα+cγ
8	BL	ND		11p14-p15, Xq12-q21	13q13-q21	0	–	–
9	BL	NG		4p15-p16,12q22-q24, 17p11-p13	0	0	ND	
10	BL	ND		1q22-q24,6q25-q27,7q34–36	Xp11-p22	0	ND	
11	BL	ND		0	0	0	ND	
12	BL	NG		4p15-p16	0	0	ND	
13	BL	ND		13q21-q22	0	0	ND	
14	BL	ND		0	0	0	ND	
15	BL	ND		1p11–p22;2q21–q33, 13q21–q22, Xq13–q22	0	0	ND	
16	BL	NG		0	0	0	ND	
17	BL	ND		12q22–q24,20,+22	4q21–q27, 13q14–q22	0	ND	
18	BL	ND		2q14–q32,4q22–q35,5q13–q21,6q14–q27,3q22–q33,8q22–q23	1p32–p36, 17p11–p13, 20q11–q13	0	+	–
19	BL	NG		0	0	0	ND	
20	BCL	t(8;14)	add(1)(q41), +del(1)(p21p32)	0	0	1q21–q25	+	Cγ
21	BCL	t(8;14)		10p11–p13,Xq13–q21	0	0	+	cμ+cγ
22	BCL	t(8;14)	+del(1)(p13p35), del(6)(q15q2), +15,+16,der(19), t(7;19)(q12;q13)	3q21–q27,6q23–q27,8q24–qter,12,+15,Xp11–p22	0	0	ND	
23	BCL	ND		1q31–q44,5p13–p15,7q31–q36,12q22–q24,Xq26–q28	0	6p12–p25	+	cγ
24	BCL	t(8;22)		0	0	0	ND	
25	BCL	t(8;14)	+i(1)(q)	+21,+22	13q21–31	0	ND	
26	BCL	t(8;14)		8q24–qter, xq26–q28	0	0	ND	
27	BCL	t(2;8)		Xp11–p22	13q21–q31	0	ND	
28	BCL	t(8;14)		9q22–q34, 12q22–q24, 14q24–q32, 15q22–q26, 17q23–q25, +20, +22	13q21–q31		ND	
29	BCL	t(8;22)		22q11–q13, Xq26- q28	0	0	ND	

Table 2 Continued

Ref	*Histology*	*Cytogenetics*		*CGH*			*LD-PCR*	
		Primary	*Secondary*	*Gains*	*Losses*	*Amplification*	*LD-PCR result*	*Region Involved*
30	BCL	t(8;22)	i(1)(q10)	0	0	0	ND	
31	BCL	t(8;14)		0	0	0	–	
32	BCL	t(8;14)		7p14–p21	0	0	ND	
33	BCL	t(8;14)	dup(1)(q31q42),	0	0	0	ND	
34	BCL	t(8;14)	del(6)(q2?1q23), del(10)(q22q26), del(9)(p21p23)	9q22–q34,12p12–p13,+22	6q15–q25, 13q14–q22	0	ND	
35	BCL	t(8;22)	del(13)(q14q34)	Xq24–q26	0	0	ND	
36	BCL	ND		2q32–q37	0	0	ND	
37	BCL	t(8;14)		0	0	0	ND	
38	BCL	t(8;14)		9q33–q34, 12q22–q24, +16,+17,20q11–q13, +21	4q21–q27, 13q14–q22		+	cμ
39	BCL	t(8;14)		7q34–q36, 10q23–q25, +16,17p11–p13, 20q11–q13, +22	0	0	+	–
40	BCL	t(8;14)	add(13)(q34), add(16)(p13)	4p15–p16, 5q32–q34, 6q24–q27,8q23–q24,+11, 12q22–q24,14q32,+16, +17,20, +22	4q13–q32,13q14–q22	0	+	cγ
41	BCL	t(8;14)	add(17)(q25)	0	0	0	–	–
42	BCL	t(8;14)	del(9)(p13)	0	0	0	+	JH+cμ
43	BCL	t(8;14)	del(17)(p11)	0	17p11–p13	0	–	–
44	BCL	t(8;14)	del(3)(p24)	2p21–p25, 2q35–q37,4p15–p16, 5p13–p15, 5q32–q35 6p21–p25, 6q24–q27, 7q32–q36,8q23–q24, 9q33–q34, 10q25–q26,+11,+12, 13q32–q34,14q31–q32, 15q24–q26, +16, +17, +20, +22	0	0	ND	
45	BCL	t(8;14)	dup(1)(q22q31)	1p22–p32, 3p21–p25 4p13–p16, 4q21–q31, 7p13–p21, +8, 9q21–q31, +10, +11, 12q14–q22, 13q13–q21, 14q21–q31, Xq12–q28	0	0	+	cμ
46	BCL	t(8;14)	der(4)t(1;4)(q21;q32)	+1, 2q33–q37, 4p15–p16,5p13–p15, 8q23–q24, 9q32–q34,+10, +11, +12, 13q31–q34, 14q31–q32, 15q22–q24,+16, +17, 20,+21,+22	4q12–q35, 5q12–q21, 6q12–q21, 7q21–q31		ND	

BL, Burkitt's lymphoma; BCL, Burkitt's cell leukemia; CGH, comparative genomic hybridization; LD-PCR, long-distance polymerase chain reaction; Cγ,Cμ, Cα and JH refer to the regions of the IgH locus (C, constant; J, joining) recognized by the primer that obtained the PCR product; ND, not done; NG, not growth.

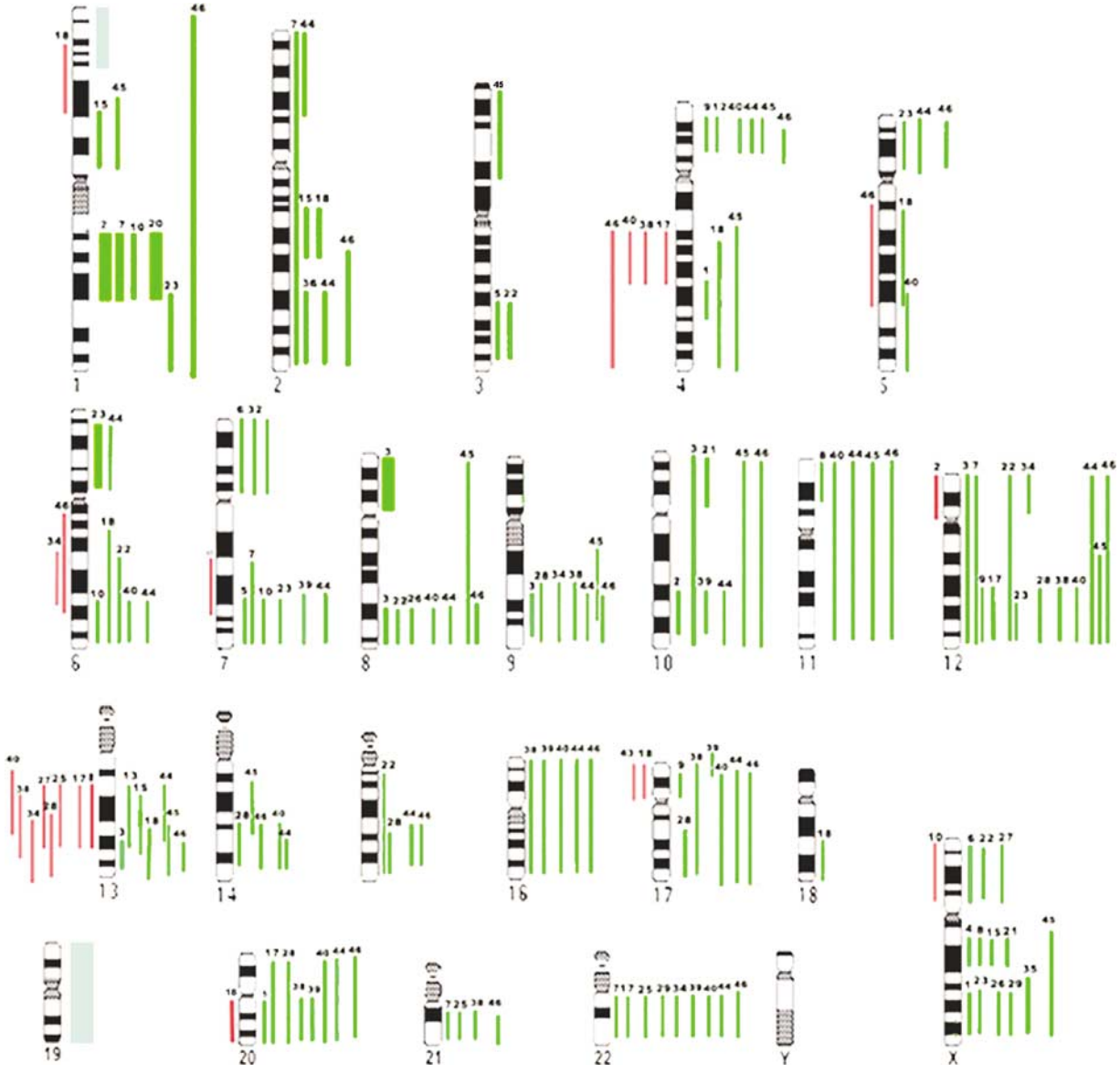

Figure 3 Summary of the genomic imbalances in 46 patients with BL/BCL. Lines on the left-hand side of the ideogram indicate loss of chromosomal material; lines on the right-hand side indicate gain of chromosomal material. High-level DNA amplifications are represented as solid squares. Each line represents a gained or lost region in a single tumor. The numbers on top of each line refer to the patient analyzed (see Table 2): Cases 1–19 had a diagnosis of BL; cases 20–46 had a diagnosis of BCL.

Table 3 Comparison between BL and BCL in relation to the genomic imbalances

	No (%) cases with changes	*Number of changes*	*Median of changes per case*	*Gains*	*Losses*	*Amplifications*
BL ($n=19$)	15 (79)	51	2.7±3	40	8	3
BCL ($n=27$)	20 (74)	119	4.3±2	103	14	2
Total BL/BCL ($n=46$)	35 (76)	170	4.1±5.1	143	22	5

BL, Burkitt's lymphoma; BCL, Burkitt's cell leukemia.

in BL and BCL are shown in Figure 4. BCL cases had a total of 103 chromosome gains (mean 3.7±2 per case) and 14 losses (mean 1±1.2 per case). By contrast, BL patients only showed 40 gains (mean 2.2 ± 2 per case) and eight losses. Patients with BCL showed more gains in 22q, 20q, 9q, 8q, 14q, 15q and 16 than BL cases (Figure 3). Chromosome losses at 13q and 4q were also more frequent in BCL than in BL patients (Figure 3), but the differences were not statistically significant. Regarding outcome, abnormalities on 1q either by CGH or cytogenetics were associated with a significantly shorter survival in the whole series ($P=0.03$) (Figure 4a). In addition, gains on 7q were associated with a poor outcome (10.5 *vs* 4.5 months without abnormalities on 7q, $P=0.01$). By contrast, no correlation between the overall number of genomic imbalances assessed by

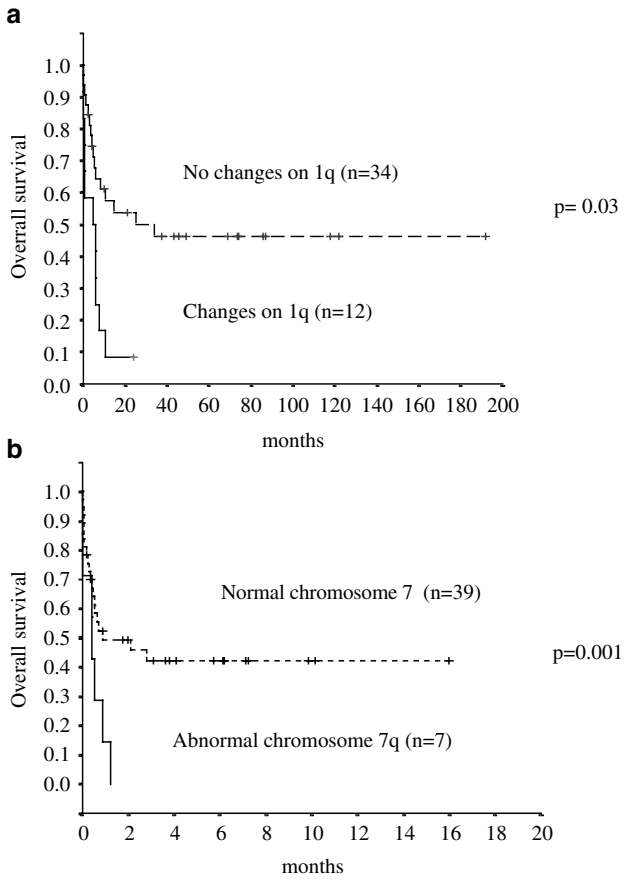

Figure 4 Survival curves of patients with BL. (a) Survival curve of the whole series according to the presence of changes on 1q assessed either by cytogenetics or by CGH (normal *vs* changes; $P=0.03$). (b) Survival curve of the whole series according to the presence of genomic imbalances by CGH on 7q ($P=0.001$). ------, no changes on 1q; ——, changes on 1q.

CGH and survival was found. More than one extranodal manifestation ($P=0.002$) and ECOG score >1 ($P=0.02$) were shown to be associated with a poor outcome.

Discussion

The increasing number of studies applying CGH to different entities illustrates the potential of this approach to detect chromosomal gains and losses in tumor genome. Several studies have been performed to assess the genomic imbalances in NHL.[19] However, few data concerning CGH in BL have been reported: to our knowledge, only three BL cell lines and two patients with BL have been previously reported.[20–22]

In the present study, 46 BL/BCL patients have been studied and we have identified chromosomal imbalances by CGH in 76% of them. This incidence of changes in BL/BCL is similar to the overall frequency reported in follicular lymphoma (75%),[8,22–26] but higher than that reported in other indolent lymphomas such as HCL, SLL and MZL.[6,27,28] However, the frequency of the alterations in BL/BCL is lower than in DLBCL[10,29] and MCL.[7]

A similar overall incidence of genomic changes was observed in patients with BL *vs* BCL (74 *vs* 79%). However, some differences between both diseases BL and BCL were detected. Thus, the incidence of changes per case was higher in BCL than in BL (4.3 ± 2 *vs* 2.7 ± 3) (Table 3). Also, three out of the 19 (16%) BL cases had high-level amplifications as compared to 7% of BCL patients. These data indicate that BCL is more predisposed to carry chromosomal abnormalities than cells from BL. Gains on chromosome arms 12q, Xq and 22q were the most frequent single genomic imbalances, suggesting a role as an early event in the clonal evolution of BL/BCL. In addition, gains of 20q, 22q, 8q and 9q, as well as losses on 13q were more frequent in BCL patients (Figure 3). The most frequent genomic imbalances observed in BL/BCL were gains on 12q without differences between BL and BCL. Gains of whole chromosome 12 have been detected by CGH in indolent lymphomas,[6,28,30] primary mediastinal B-cell lymphoma[31] and primary gastrointestinal large-cell lymphoma.[32] Interestingly, in diffuse large B-cell lymphoma (DLBCL), the abnormalities of 12q are uncommon, although they had been reported to be relatively frequent in transformed DLBCL arising from follicle center cell lymphoma.[27,32–34] Gains on 12q are found in 52% of the transformed DLBCL and in follicular lymphomas.[24,25] In the present study, the consensus region was located at 12q21–q25, telomeric to the region reported in blastoid MCL.[7] This region has been frequently gained in mediastinal lymphomas,[31] follicular lymphoma[24,35] and in primary large B-cell lymphoma of the gastrointestinal tract.[32] All these data suggested a commonly gained region at 12q for the aggressive NHL, different to the region observed in SLL or in blastoid MCL. Abnormalities on chromosome 13 were observed in 30% of patients with BL/BCL. Losses were more frequent than gains (17 *vs* 13%) and the losses affected BCL patients more frequently. Partial or full monosomy of 13 chromosome is usually found in B-cell chronic lymphocytic leukemia[36] and multiple myeloma patients.[37–40] In NHL, the frequencies of losses on 13q ranged from 4% in follicular lymphomas[23] to 49% in MCL.[7] Gains on 12q as well as losses on 13q have been reported as most frequent in cases with aggressive histopathologic features.[24] This suggests a role of these aberrations in later stages of clonal evolution.

Gains on Xq were frequent in the present series, without differences between BCL and BL patients. Gains of X chromosome have been detected usually as a secondary chromosomal abnormality in NHL.[41] The incidence of gains on Xq detected by CGH in B-cell NHL, ranges from 7% in SMZL to 26% in FL.[9,23] By contrast, in DLBCL the incidence of Xq gains reaches more than 70% of cases of primary mediastinal B-cell lymphoma.[42] Moreover, within MCL patients the changes of Xq are more frequent in that of blastoid MCL variant (40%).[7]

Five high-level DNA amplifications involving three different regions have been found in five out of the 46 patients with BCL/BL. Three cases (two BL and one BCL) displayed 1q amplification. In the present series, all the three cases with amp (1q) had a consensus region at 1q23–q31 and extranodal involvement. Previous CGH studies have reported high-level amplification of 1q in one case of DLBCL[34] and in six cases of extranodal malignant lymphoma.[29]

Several reports have correlated the presence of cytogenetic abnormalities with the outcome of NHL patients. There is evidence that so-called 'secondary' chromosome changes could play an important role in determining the clinical phenotype of lymphoid tumours.[3,4,24,43] Recent studies have reported on the impact of CGH on NHL disease-free survival (Table 4) showing that the increased number of gains or losses were significantly associated with a short survival.[7,9,22,25,29,32,44] In the present series, we failed to demonstrate an association between the number of chromosomal changes and the survival of BL/BCL. However, abnormalities on 1q (gains, high-level amplifications or translocations) and gains on 7q were associated with a poor

Table 4 Prognostic values of CGH aberrations in B-cell NHL based on data reporting reference

Diagnosis/reference	*Genomic changes*	*Relation with survival (P value)*
NHL[22]	>3 gains	<0.001
	−11q21–q23	<0.001
	−17p	<0.001
	+6p	<0.001
	−6cen–q24	0.04
	−8p	0.002
	−9p21–ter	0.02
	−13q13–21	0.01
	+3q21–qter	0.04
	+8q23–ter	0.07
PLBL-GT[32]	>2 gains	0.022
	Amplification	
MCL[7]		
	>4 gains	0.02
	+3q	0.02
	+12q	0.03
	−9p	0.02
FL[24]	−6q25q26	0.0001
SMZL[9]	Losses	0.05

P-value obtained from multivariate analysis (Cox's regression test).Abbreviations according to WHO classification of lymphoid neoplasms: NHL, non-Hodgkin's lymphoma; PLBL-GT, primary large B-cell lymphoma of gastrointestinal tract; MCL, mantle cell lymphoma; FL, follicular lymphoma; SMZL, splenic marginal zone lymphoma.

outcome. Abnormalities on 1q21 detected by cytogenetics and CGH have been associated with a shorter survival in NHL[32,43] and have been reported as an independent cytogenetic risk factor for patients with centroblastic lymphoma.[45] Regarding gains of chromosome 7, it has been reported that chromosome 7 is probably involved in the progression of NHL.[46] All these data suggest that both abnormalities on 1q and 7q could contribute to differentiate a subgroup of BL/BCL patients with adverse prognosis. CGH can detect such genomic aberrations in tumor tissues and can therefore be used in the context of clinical trials.

In summary, most patients with BCL/BL showed chromosomal imbalances. The patients with BCL had more chromosomal abnormalities than patients without BM involvement, particularly gains at 8q, 9q, 14q, 20q and 22q, as well as losses on 13q and 4q. Concerning outcome, the presence of 1q abnormalities and gains on 7q were associated with an adverse prognosis.

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