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New chromosome abnormalities and lack of *BCL-6* gene rearrangements in Argentinean diffuse large B-cell lymphomas

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Cerretini R, Noriega MF, Narbaitz M, Slavutsky I. New chromosome abnormalities and lack of *BCL-6* gene rearrangements in Argentinean diffuse large B-cell lymphomas.

Abstract: *Objectives*: Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphomas. Cytogenetic studies have revealed a broad spectrum of clonal genetic abnormalities and complex karyotypes. The purpose of this study was to contribute to the understanding of the genomic alterations associated with this group of lymphomas. *Methods*: Cytogenetic, fluorescence *in situ* hybridization (FISH) and molecular analyses were performed in 30 cases with DLBCL: 20 *de novo* DLBCL (*dn*-DLBCL) and 10 DLBCL secondary to follicular lymphoma (S-DLBCL). *Results*: A total of 37 different structural chromosomal rearrangements were found: 27% translocations, 54% deletions, and 19% other alterations. Chromosomes 8, 6, 2, and 9 were the most commonly affected. Interestingly, translocation t(3;14)(q27;q32) and/or *BCL-6* gene rearrangements were not observed either by cytogenetic studies or by FISH analysis. Fifteen novel cytogenetic alterations were detected, among them translocations t(2;21)(p11;q22) and t(8;18)(q24;p11.3) appeared as sole structural abnormalities. Translocation t(14;18)(q32;q21) and/or *BCL-2-IGH* gene rearrangements were the genomic alterations most frequently observed: 50% of S-DLBCL and 30% of *dn*-DLBCL. Deletions del(4)(q21), del(6)(q27), del(8)(q11), and del(9)(q11) were recurrent. The most common gains involved chromosome regions at 12q13-q24, 7q10-q32, and 17q22-qter; 6q was the most frequently deleted region, followed by losses at 2q35-qter, 7q32-qter, and 9q13-qter. Four novel regions of loss were identified: 5q13-q21, 2q35-qter (both recurrent in our series), 4p11-p12, and 17q11-q12. *Conclusions*: These studies emphasize the value of combining conventional cytogenetics with FISH and molecular studies to allow a more accurate definition of the genomic aberrations involved in DLBCL.

Key words: cytogenetic; diffuse large B-cell lymphoma; fluorescence *in situ* hybridization; molecular studies; *BCL-2* gene; *BCL-6* gene

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Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma (NHL) in Western countries, representing 40% of all adult cases. This group of tumors encompasses heterogeneous clinical, morphological, immunophenotypic, cytogenetic, and molecular genetic features that are reflected in a highly variable clinical course. DLBCL may arise *de novo* (*dn*-DLBCL) or, alternatively, may develop from the transformation of a follicular lymphoma (FL). The occurrence of such a transformation is usually associated with a worse outcome and a rapidly progressive clinical course refractory to treatment and short survival. However, *dn*-DLBCL is aggressive but potentially curable with multiagent chemotherapy (1). Therefore, the knowledge of novel biological prognostic markers for predicting treatment outcome and risk assessment is needed.

Chromosomal analysis of NHL has enabled establishment of associations between clonal karyotypic abnormalities and biological and clinical features of the disease (2). However, the complexity and sub-optimal chromosome morphology often result in partially characterized karyotypes. Fluor-

escence *in situ* hybridization (FISH) analysis constitutes a useful tool to define these cytogenetically unidentifiable marker chromosomes. Several recurring translocations have been identified as single alterations, associated with tumor histology, and other cytogenetic abnormalities have been reported to be of prognostic significance in different subtypes of NHL (1, 3). However, few cytogenetic and FISH studies describing structural and numerical aberrations are available for DLBCL and the information about the prognostic impact of karyotypic abnormalities is also scarce (4–8). Approximately 50% of cases exhibit chromosomal translocations including one of the *Igs* gene sites, which lead to deregulated expression of a variety of genes. Among them, translocations t(3;14)(q27;q32) and t(14;18)(q32;q21) involving *BCL-6* and *BCL-2* genes are the most frequent (35% and 20–30%, respectively) (9–12). The remaining cases display diverse types of chromosomal rearrangements that include translocations, deletions, and other types of aberrations involving cytogenetically unidentifiable chromosome segments and marker chromosomes.

Taking into account these issues, the purpose of our study was to contribute to the understanding of the genomic alterations associated with this group of lymphomas. The combination of conventional cytogenetics with FISH and molecular studies allowed a more accurate definition of the genomic aberrations involved in DLBCL. Novel chromosome rearrangements were found and recurrent chromosomal gains and losses and specific breakpoints are described. Interestingly, translocation t(3;14)(q27;q32) and/or BCL-6 rearrangements were not observed.

Materials and methods

Patients

The study group consisted of 30 patients with DLBCL, 20 *dn*-DLBCL (10 men and 10 women, median age: 57.8 yrs, range: 26–82 yrs), and 10 DLBCL secondary to an FL (S-DLBCL) (five men and five women; median age: 56.2 yrs, range: 18–78 yrs) admitted to our institute between 1996 and 2004. Only patients with chromosomal alterations were included in this study. All patients were histologically reclassified by a trained hematopathologist according to the World Health Organization classification (1) and staged by the Ann Arbor staging system (13). Histologic examination revealed a diffuse proliferation of large lymphoid cells with vesicular nuclei with fine chromatin and two- to four membrane-bound nucleoli. Immunohistochemical analysis included the following monoclonal antibodies: CD20, CD3, CD10, BCL-2 (DAKO, Carpinteria, CA, USA). B-cell phenotype (CD20+) was confirmed in all patients. None of *dn*-DLBCL patients had a prior history of FL and their histopathological studies did not show remnant follicular structures. Patients with S-DLBCL had previous diagnosis of FL: grade 1 for cases 2 and 3, and grade 2 for the remaining ones. Patients were studied at diagnosis for *dn*-DLBCL and before therapy for S-DLBCL. Age, sex, clinical stage at diagnosis, and time of survival (months) are shown in Table 1. Eighty percent of the patients had advanced clinical stages (III and IV). Six cases presented with extranodal disease: central nervous system (two cases), testis, breast, buccal mucosa, and gastrointestinal tract involvement were seen (one case each).

Cytogenetic, FISH and Molecular Studies

Tumor samples were obtained from: lymph nodes (14 patients), bone marrow (15), and buccal mucosa (1) (Table 1). Cytogenetic analysis was performed by standard methods using 24–48 h unstimulated culture in RPMI 1640 medium (GIBCO BRL, New York) with 15% of fetal calf serum and L-glutamine. Metaphase chromosomes were banded by the GTG (G-banding-Trypsin-Giemsa) method. Karyotype data were described according to the International System for Human Cytogenetic Nomenclature (ISCN) (14).

FISH analysis was performed according to manufacturer's protocols. Abnormalities found in G-banded metaphases were subsequently confirmed using an extensive panel of total chromosomes and alpha satellite DNA probes (CAMBIO, Cambridge, UK; Vysis, Downers Grove, IL, USA). For each case, five to 10 metaphases were analyzed using the Quips Pathvision (Applied Imaging, Santa Clara, CA, USA). An LSI IGH/BCL-2 (14q32/18q21) dual fusion, LSI BCL-6 (3q27) dual fusion break apart, LSI BCR/ABL (9q34/22q11) extra signal, and LSI p53 (17p13) probes (Abbott Vysis, Downers Grove, IL, USA) were also used. A minimum of 400 interphase nuclei were scored to evaluate the locus-specific probes in patients and controls. The cut-off for positive values (mean of normal control + 3 SD), determined from samples of 10 cytogenetically normal individuals, was 0.9%, 1.3%, and 1% for fusion signals of an LSI IGH/BCL-2, LSI BCL-6 and LSI BCR/ABL probes, respectively, and 5.4% for monosomy of LSI p53 probe.

Genomic DNA was isolated from fresh tissues by conventional phenol–chloroform extraction and ethanol precipitation. PCR detection for the MBR (Major Breakpoint Region)-JH (consensus J region of the immunoglobulin heavy chain locus) and mcr (minor cluster region)-JH rearrangements

Table 1. Clinical features and cytogenetic findings in 30 cases of DLBCL

Case	Age/sex	Clinical stage at diagnosis	Sample	Combined karyotypes (GTG and FISH)	Survival (months)
Diffuse large B-cell lymphoma arising from a follicular lymphoma (FL)					
1	64/M	IIIA	LNB	46, XY, **der(6)t(1;6)(q21;p12)**.ish der(6)(wcp6+)[5]/46,XY[10]	111[d]
2	18/F	IIIA	LNB	46,XX,**t(2;21)(p11;q22)**[3]/46,XX[7]	89[d]
3	78/F	IIIB	LNB[#]	46–47,XX,−1,+2,+3,−4,del(6)(q21),+12,t(14;18) (q32;q21)[cp14]	120[d]
4	67/F	IVA	BM[#]	46,XX,t(2;8)(p12;q24),t(14;18)(q32;q21).ish t(14;18)(wcp18+;wcp18+)[8]/46,XX[8]	58[d]
5	63/M	IVA	BM	46,XY,+8[5]/46,XY[8]	48
6	65/M	IVB	LNB[#]	46,XY,del(6)(q25),t(14;18)(q32;q21)[7]/46,XY, del(6)(q25),t(9;22)(q34;q11), t(14;18)(q32;q21).ish t(9;22) (ABL+;BCR+,ABL+)[3][1]	6[d]
7	61/F	IVB	BM[#]	44–45,XX,−18,−19[cp6]/46,XX[5]	31[d]
8	33/M	IVB	LNB[#]	47–48,XY,+2, **del(4)(p11)**, **del(5)(q13q21)**, del(6)(q21),+del(7)(q32),+del(9)(q11), +**r(12)(p13q24)**,−13, t(14;18)(q32;q21),del(17)(p11), t(12;19)(p13;q13).ishr(12)(wcp12+), **psu dic (19;12)(q13;p13)** (wcp19+; wcp12+),del(17)(p11)(p53−)[cp13][2]	40
9	61/M	IVB	BM	46,XY,del(1)(q42)[5]/46,XY[7]	39
10	52/F	IVB	LNB	46,XX,del(9)(q11)[8]	26
de novo-DLBCL					
11	50/F	IA	LNB	46,XX,**ins(1)(q11p22p36.3)**,i(7)(q10).ish 1(wcp1+)[10]/46,XX[2]	55
12	36/M	IIA	BM[#]	46,XY.ish X(wcpX+)[5]/47,XXY.ish X(wcpXx2)[12]	66
13	71/F	IIA	LNB	51,XX,+del(4)(q21),+5,+7,+8,+12,+dmin[6]	44
14	44/M	IIA	BM	46,XY[4]/47,XY,+mar[4]	10[d]
15[e]	54/F	IIA	LNB	45,XX,del(2)(q31),del(11)(q21),−20.ishdel(11)(wcp11+)[7]/46,XX, del(2)(q31),del(11)(q21)x2,−20.ish del(11)(wcp11x2)[3]	75[d]
16	72/M	IIA	LNB	49,XY,+7,**t(8;9)(q22;p24)**,+9,+12,t(14;18)(q32;q21).ish t(8;9)(wcp8+, wcp9+; wcp8+, wcp9+)[6]	19[d]
17	26/F	IIIA	LNB	46,XX,**del(2)(q35)**,del(6)(q25)[cp4]/46,XX[7]	39
18	69/M	IIIB	LNB	47,XY,**t(8;18)(q24.1;p11.3)**,+12[6]	15[d]
19	68/F	IVA	LNB[#]	48,XX,+6,del(7)(q32),del(8)(p11),**der(16)(12;16)(q13;q24)**, +21.ish der(16)(wcp12+,wcp16+)[10]/46,XX[3]	151[d]
20	75/F	IVA	BM	46,XX.ish 11(D11Z1x2)[24]/47,XX,+11.ish 11(D11Z1x3)[4][3]	42
21	69/F	IVA	BM	46,XX,del(5)(p15),del(7)(q32)[cp9]/46,XX[9]	12[d]
22[e]	62/M	IVA	BM[#]	46,XY.ish Y(wcpY+)[4]/47,XYY.ish Y(wcpYx2) [7]/48,XYY,+22[3]	24[d]
23[e]	56/M	IVA	BM	44–45,X,−Y,−8[cp5]/46,XY[5]	96
24[e]	66/F	IVA	BM	46,XX,del(6)(q27)[6]/46,XX[6]	11[d]
25	82/F	IVA	BM	47,XX,+**del(5)(q13)**,**der(16)t(12;16)(q13;p13)**.ish der(16)(wcp12+,wcp16+)[6]/46,XX[4]	33[d]
26	37/M	IVA	BM	46–47,XY,del(8)(q11),+18[cp9]/46,XY[4]	27
27	40/F	IVB	LNB	45–49,XX,t(4;8)(q33;q12),del(6)(q23),−8,del(9)(q13)x2,add(11)(q23), t(14;18)(q32;q21)x2,+18,add(19) (q13).ish **t(4;11;19;8)(q33;q23;q13;q12)** (wcp4+,wcp8+;wcp4+,wcp11+;wcp11+;wcp8+), del(9)(wcp9×2), t(14;18)(wcp14×2,wcp18×2;wcp14×2,wcp18×2),18(wcp18+)[cp10]	150[d]
28[e]	50/M	IVB	BUM[#]	47–49,XY,−6,−13,+14,+16,del(17)(p11),+**del(17)(q11q21)**,+18,−22.ishdel(17)(p13)(p53−)[cp12][4]	6[d]
29	51/M	IVB	BM	46,XY,del(6)(q25)[6]/46,XY[6]	9
30[e]	82/M	IVB	BM	48,XY,+12,+i(17)(q10)[8]/46,XY[3]	3[d]

F, female; M, male; d, death patient; BM, bone marrow; LNB, lymph node biopsy; BUM, buccal mucosa; e, extranodal disease; cp, composite karyotype; ND, non-determined; Bold, new alterations; #, FISH and/or molecular BCL-2/IgH analysis; MBR-JH+, cases 3, 6, 7, 12, 22, and 28; mcr-JH+, cases 4, 8, and 19; Case 9 Bcl-2/IgH negative.
[1]FISH BCR/ABL: 25%.
[2]FISH TP53: 30%.
[3]FISH CEP11: 12%.
[4]FISH TP53: 11.7%.

of the t(14;18) translocation were performed by nested and long distance-PCR (LD-PCR) as previously described (15). Primers described by Gribben *et al.* (16) and Akasaka *et al.* (17) were used for nested and LD-PCR, respectively.

Results

G-band/FISH karyotypes are presented in Table 1. In all cases, the chromosome complement was close to 46, ranging from 44 to 51 chromosomes. Forty percent of S-DLBCL and 30% of *dn*-DLBCL showed complex karyotypes with three or more alterations detected by cytogenetic, FISH, or molecular analysis.

Cytogenetic results showed a total of 37 different structural chromosomal rearrangements corresponding to: 27% translocations, 54% deletions, and 19% other alterations (Table 2). Chromosomes 8 (16.2%), 6 (13.5%), 2 and 9 (10.8% each) were the most frequently affected. Translocation t(14;18) and deletions: del(6)(q21), del(6)(q25), del(7)(q32), del(9)(q11), and del(17)(p11) were recurring abnormalities in our series (≥2 cases). Trisomies of chromosomes 12 and 18 were the most frequent numerical alterations (Table 1). Translocation

Table 2. Structural chromosomal rearrangements identified in DLBCL patients

Type of Abnormality (No of Cases)		
Translocation	Deletion	Other rearrangements
t(2;8)(p12;q24)	del(1)(q42)	**ins(1)(q11p22p36.3)**
t(2;21)(p11;q22)	del(2)(q31)	i(7)(q10)
t(4;11;19;8)(q33;q23;q13;q12)	**del(2)(q35)**	**r(12)(p13q24)**
t(8;9)(q22;p24)	**del(4)(p11)**	i(17)(q10)
t(8;18)(q24.1;p11.3)	del(4)(q21)	**psu dic(19;12)(q13;p13)**
t(9;22)(q34;q11)	del(5)(p15)	dmin
t(14;18)(q32;q21) (6)	**del(5)(q13)**	marker chromosome
der(6)(t(1;6)(q21;p21)	**del(5)(q13q21)**	
der(16)t(12;16)(q13;p13)	del(6)(q21) (2)	
der(16)t(12;16)(q13;q24)	del(6)(q23)	
	del(6)(q25)(3)	
	del(6)(q27)	
	del(7)(q32)(3)	
	del(8)(p11)	
	del(8)(q11)	
	del(9)(q11)(2)	
	del(9)(q13)	
	del(11)(q21)	
	del(17)(p11)(2)	
	del(17)(q11q21)	

Bold: novel chromosome abnormalities.

t(3;14)(q27;q32) and/or *BCL-6* gene rearrangements were not detected either by cytogenetic analyses or by FISH studies.

To the best of our knowledge, 15 (40.5%) of these chromosomal alterations have not been reported in this histological subtype (2). Among them, 66.7% were unbalanced rearrangements and 33.3% corresponded to the balanced translocations (Table 2). Translocations t(2;21)(p11;q22) and t(8;18)(q24.1;p11.3) were observed as single structural abnormalities (cases 2 and 18) (Table 1). Chromosomes 12 and 8 (three cases each) and 1 and 4 (two cases each) were the most commonly involved in this group of new anomalies.

Translocation t(14;18)(q32;q21) and/or *BCL-2* gene rearrangement were the genomic alterations most frequently observed by combined cytogenetic, FISH, and molecular analysis in the whole series. Forty percent of S-DLBCL and 10% of *dn*-DLBCL showed this rearrangement by cytogenetic studies. In S-DLBCL, FISH analysis on interphase nuclei revealed a cryptic BCL2/IgH translocation in case 7 (15% of cells). Molecular studies in this subtype of NHL showed an MBR-JH rearrangement in cases 3, 6, and 7 and the mcr breakpoint in patients four and eight, the first one detected by an LD-PCR. Case 9 was negative for both rearrangements. In *dn*-DLBCL, four cases showed the BCL-2/IgH fusion by LD-PCR: MBR-IgH in cases 12, 22, and 28 and mcr-IgH in case 19. Combined studies gave a total of 50% cases with this alteration in S-DLBCL and 30% in *dn*-DLBCL. By cytogenetic analysis, balanced translocations were usually observed as secondary changes of t(14;18) translocation (4/6 cases): t(8;9)(q22;p24), t(4;11;19;8)(q33;q23;q13;q12), t(2;8)(p12;q24), and t(9;22)(q34;q11). Translocation t(9;22)(q34;q11) was confirmed by FISH using BCR/ABL probe (25% of cells). The other patients with t(14;18) showed unbalanced rearrangements as secondary changes, being deletions the most common abnormalities.

Structural rearrangements showed a total of 70 breakpoints located at 49 different chromosomal bands (Fig. 1). The most frequent recurring breakpoints were 18q21 and 14q32 (8.6% each), followed by 6q25 and 7q32 (4.3% each), 6q21, 9q11, 12p13, 12q13, 17p11, and 19q13 (2.9% each). Chromosomes 8 (12.2%), 1 and 6 (10.2% each), and 2 and 9 (8.2% each) presented the greatest number of different breakpoints.

Losses and gains of chromosome regions as concerns the karyotypes (Table 1) were also analyzed (Fig. 2). In our series, losses (68.4%) were noted more frequently than gains. Chromosomes 2, 5, 6, 7, 8, 9, and 16 were the most commonly involved in partial losses. Overall, the long arm of chromosome 6 was the most frequently affected (26.9%). Eight regions of common cytogenetic losses (≥2 cases) were identified: 6q27-qter was the most frequent, followed by 6q25-qter, 6q23-qter, 6q21-q23, 2q35-qter, 7q32-qter, and 9q13-qter. Moreover, we found four regions of recurring cytogenetic gains: 12q13-q24 was the most frequent, followed by 12q13-qter, 12p13-q13, 7q10-q32, and 17q22-qter (Fig. 2).

Two of our 30 cases (cases 12 and 22) showed constitutional chromosome abnormalities (CCA). Both patients presented with *dn*-DLBCL and had mosaic karyotypes with sex chromosome anomalies, i.e. 46,XY/47,XXY and 46,XY/47,XYY, respectively. In addition to the CCA, case 22 showed a trisomy 22 as an acquired chromosome abnormality. Both cases were previously published (18).

Discussion

We present the G banding and FISH analyses of 30 DLBCL patients, complemented in some cases with molecular studies. To the best of our knowledge, it would be the first series of DLBCL patients cytogenetically studied from a South American country. Although, the patient sample is rather limited, interestingly our patients did not show t(3;14)(q27;q32) and/or *BCL-6* gene rearrangements. The literature refers different frequencies for these alterations. The large series of Cigudosa *et al*. (4) and Jerkerman *et al*. (5) found chromosomal abnormalities involving 3q27 in 14% and 7.5% of cases, respectively. Studies using

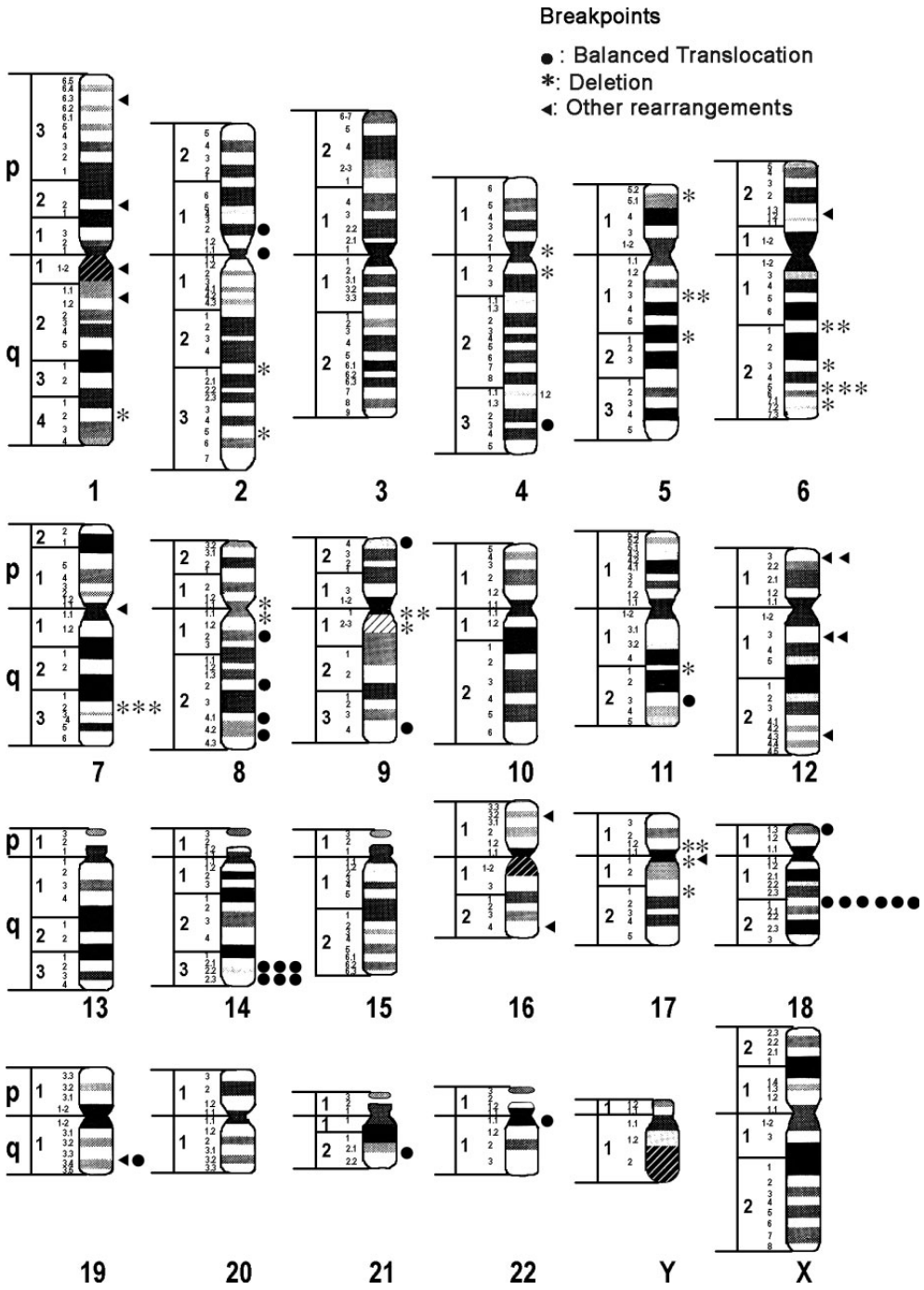

Fig. 1. Ideogram showing the distribution of the 70 chromosomal breakpoints detected in diffuse large B-cell lymphoma cases.

spectral karyotyping have detected frequencies of 9.5% (19), 25% (6) and 28% of patients (7) with these anomalies. Moreover, by Southern blot analysis, a low incidence of BCL-6 rearrangements was found in Taiwanese (14.8%) (20) and Japanese patients (14.1%) (21) with DLBCL. These findings would suggest other loci involved in lymphomagenesis in this subtype of NHL and/or geographical differences in the frequency of this alteration. Furthermore, we have compared our cytogenetic findings with those of 60 DLBCL patients with 3q27 alterations reported at Mitelman Catalog (2). No differences either in the frequency of balanced and unbalanced chromosomal rearrangements or in the pattern of affected chromosomes were found. The only exception was chromosome 1, which showed a significantly greater involvement in the published cases. It

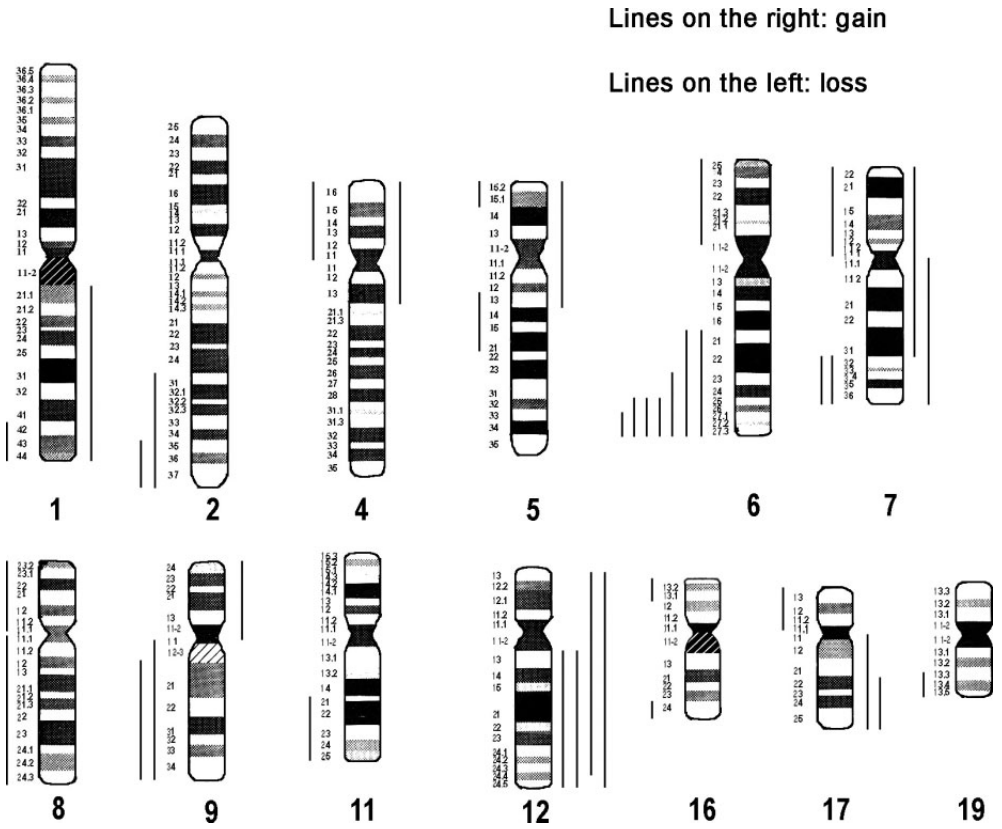

Fig. 2. Summary of chromosomal imbalances detected in 30 patients with DLBCL as concerns the karyotypes.

would suggest a similar distribution of secondary alterations in both groups of DLBCL.

Fifteen novel cytogenetic alterations were found. Chromosomes 8 and 12 were the most commonly involved, the former in balanced translocations and the latter as part of unbalanced rearrangements. Among these novel anomalies, t(2;21)(p11;q22) and t(8;18) (q24.1;p11.3) were the only ones that appeared as sole structural abnormalities. The incidence of cases with single abnormalities was similar to that of Cigudosa *et al.* (4) (6.7% vs. 7%, respectively). These findings are noteworthy taking into account that they have significance because they are considered to be primary changes involved in the genesis of the disease. Both breakpoints 8q24 and 18p11 as well as 2p11 and 21q22 have been implicated as regions containing genes involved in different hematologic neoplasms (2). In addition, t(8;18)(q24.1;p11.3) was observed in a patient with rapid progression of the disease and short survival (<2 yrs) whereas the other translocation was not associated with a poor prognosis. Six of these novel chromosomal alterations have been previously reported in other types of mature B-cell neoplasias (Table 3), suggesting a role of these structural abnormalities, though at low frequency, in the development and/or progression of these lymphoid malignancies. The remaining aberrations (40%) have not been described yet in any tumor. The analysis of these novel abnormalities permitted us to identify four novel regions of losses: 5q13-q21 and 2q35-qter (both recurrent in our series) and

Table 3. Novel structural chromosomal rearrangements in DLBCL. Comparison with published cases in other mature B cell neoplasms

Present study	Published cases	
Chromosome abnormality	Histology	References
der(6)t(1;6)(q21;p21)	Nodal marginal zone B-cell lymphoma	Ott *et al.* (22)
	B-cell lymphoma–Mature B-cell neoplasm	Shimazaki *et al.* (23)
	Multiple myeloma	Fiedler *et al.* (24)
del(2)(q35)	Multiple myeloma	Sawyer *et al.* (25)
	FL	Horsman *et al.* (26)
	CLL	Morgan *et al.* (27)
	CLL	Asou *et al.* (28)
	Hairy cell leukemia	Brito-Babapulle *et al.* (29)
del(4)(p11)	FL	Berger *et al.* (30)
del(5)(q13q21)	Multiple myeloma	Lloveras *et al.* (31)
del(5)(q13)	CLL	Buhmann *et al.* (32)
	FL	Mohamed *et al.* (33)
	FL	Whang-Peng *et al.* (34)
	Lymphoplasmacytic lymphoma	Bello *et al.* (35)
	Mature B-cell neoplasm, NOS	Hashimoto *et al.* (36)
del(17)(q11q21)	Mature B-cell neoplasm	Tsukadaira *et al.* (37)

NOS, no other specification.

4p11-p12 and 17q11-q12 observed in one case each (2). These findings could be important for further studies about sites of candidate tumor suppressor genes. In solid tumors, Comparative Genomic Hybridization (CGH) and loss of heterozygosity (LOH) studies have suggested the presence of putative tumor suppressor genes at 2q35-qter (38, 39). In hematological neoplasms, loss of 5q13 region was observed in myeloid malignancies, refractory myelodysplasias, and hairy cell leukemias. Recently, Liang *et al.* (40) have characterized a candidate tumor suppressor gene at 5q13.3, SSBP2 (single-stranded DNA-binding protein 2), involved in regulation of hematopoietic growth and differentiation. In addition, molecular studies in neurofibromatosis type 1 (NF1) have shown the inactivation of both NF1 alleles during tumorigenesis, supporting the tumor suppressor hypothesis for the *NF1* gene located at band 17q11.2 (41).

Among the remaining alterations, four deletions del(4)(q21), del(6)(q27), del(8)(q11), and del(9)(q11) were observed only once by G-banding in DLBCL (2) and appear to be recurrent in this histological subtype from our data.

Translocations affecting one of the *Ig* gene sites (14q32, 22q11, and 2p12) were noted in 20% of our cases by cytogenetic studies, in contrast to 51% of cases reported by Cigudosa *et al.* (4), possibly because of the fact that the studied populations were different in the proportion of S-DLBCL and *dn*-DLBCL patients. By M-FISH, 55–56.5% of DLBCL cases presented *IgH* gene rearrangements, corresponding to 11–25% of them having a t(14;18)(q32;q21) (6, 7). Our combined cytogenetic, FISH, and molecular analysis showed that 36.7% of our patients had t(14;18) with a higher frequency in S-DLBCL (50%) than in *dn*-DLBCL (30%). Recent data from microarray studies (42) have shown different gene expression signatures in post-transformation DLBCL compared with *dn*-DLBCL samples, being post-transformation DLBCL profiles similar to those observed in an FL. In addition, López-Guillermo *et al.* (43) found a correlation between the BCL-2 breakpoint and the clinical outcome in indolent FL, with a poor prognosis for germline patients and a higher percentage of histologic transformation in BCL-2 negative cases than those observed for patients with MBR or mcr breakpoints.

We observed one case with the rare combination of translocations t(14;18)(q32;q21) and t(2;8)(p12;q24) that involved two of the *Ig* gene sites and resulted in *BCL-2* and *MYC* rearrangements. To the best of our knowledge, this is the second DLBCL patient with both translocations to be described (44). Previous studies have indicated that *MYC* rearrangements are detectable in 5% to 15% of DLBCL patients (4, 45) associated with the translocation t(8;14)(q24;q32). Different authors (46, 47) have suggested an *MYC* deregulation as a genetic event associated with the progression of a *BCL-2* deregulated FL to DLBCL, as occurred in our patient. This translocation was always found with other secondary alterations in the karyotype. According to the literature (4), balanced translocations were the most frequent secondary aberrations to t(14;18)(q32;q21). This is in accordance with the concept that t(14;18)(q32;q21) is necessary but not sufficient by itself for cancer development and other genomic changes are required to reach the neoplastic phenotype (48). Interestingly, among these alterations, we found translocation t(9;22)(q34;q11), the cytogenetic hallmark of chronic phase of chronic myeloid leukemia (CML), that is very uncommon in DLBCL, with only two cases reported (49, 50). Our patient represents the first one in which t(9;22)(q34;q11) is associated with t(14;18)(q32;q21), a rearrangement probably related with progression of the disease, as was observed in this case that showed a very short survival (6 months).

Breakpoint analysis identified 70 breaks that were located at 49 bands and only 11 (22.4%) of them were recurrent. Each of these 11 bands has previously been reported to be involved in lymphoid tumors (2) and they cannot be considered specific of DLBCL. As reported by Cigudosa *et al.* (4), breaks at 14q32 and 18q21 corresponding to t(14;18)(q32;q21) were also the most frequent breakpoints in our series. The other recurrent breakpoints (at least 2 each) 6q21, 6q25, 7q32, and 17p11 were mainly related to deletions. These regions are regarded as common sites for secondary rearrangements in lymphomas. Breakpoints 2q35, 8q11, and 9q11 also involved in deletions were reported only once in DLBCL, and appear to be recurrent from our data [2], particularly, del(9)(q11).

Complete chromosome gains or losses may represent secondary changes that are associated with progression rather than genesis of the disease. According to the literature, trisomies of whole chromosomes 12 and 18 were the most frequent numerical alterations and always observed as secondary changes (2, 4, 6, 7). Trisomy 12 is a common numerical alteration in different histological subtypes of NHL, such as chronic lymphocytic leukemia, FL and DLBCL, suggesting that this chromosome may contain genes that are important in lymphomagenesis. As for trisomy 18, a recent article (51) has reported that 18q gains are associated with particular clinicopathologic features of the patients at presentation, progression of the diseases, and more aggressive behavior.

In reference to chromosome imbalances, most common gains involved chromosome regions 12q13-q24, 7q10-q32, and 17q22-qter, whereas 6q was the most frequently deleted region in agreement with previous studies (4, 51–53), followed by losses 2q35-qter, 7q32-qter, and 9q13-qter. Comparative genomic hybridization studies in DLBCL found that the most frequent changes were gains at X, 12, 7, 3, and 18, whereas 6q was the most frequently lost, followed by 1p, 8p, and 17p (51–53).

Finally, two DLBCL patients showed sex chromosome abnormalities as CCA, an association that was rarely observed in the literature. In fact, a number of XYY and XXY males with hematological malignancies have been reported, most of them with leukemia or myelodysplastic syndromes (54). Conversely, only sporadic cases with NHL have been described (55–59). Previous results of our laboratory in a series of patients with hematological disorders (14) did not find differences when the incidence of sex chromosome abnormalities was compared with that of the overall population, indicating that this type of CCA does not predispose to hematological malignancies.

In summary, our findings confirmed the presence of genetic instability manifested at the cytogenetic level in DLBCL, and emphasize the importance of studies combining molecular cytogenetics with a conventional G-banding technique in this subtype of NHL. We identified novel chromosomal alterations, some of them recurrent and found several regions of cytogenetic deletion pointing to sites of candidate tumor suppressor genes of potential importance in lymphomagenesis. Finally, we did not find rearrangements involving 3q27, considered the most frequent alteration in primary DLBCL.

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References

1. Harris NL. Mature B-cell neoplasms. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. Pathology & Genetics Tumours of Haematopietic and Lymphoid Tissues. World Health Organization Classification of Tumours (WHO). Lyon: IARC Press, 2001:118–187.
2. Mitelman F, Johansson B, Mertens F, eds. Mitelman Database of Catalogue of Chromosome Aberrations in Cancer. Available from URL: http://cgap.nci.nih.gov/Chromosomes/Mitelman, 2005.
3. Chaganti RS, Nanjangud G, Schmidt H, Teruya-Feldstein J. Recurring chromosomal abnormalities in non-Hodgkin's lymphoma: biologic and clinical significance. Semin Hematol 2000;**37**:396–411.
4. Cigudosa JC, Parsa NZ, Louie DC, Filippa DA, Jhanwar SC, Johansson B, Mitelman F, Chaganti RS. Cytogenetic analysis of 363 consecutively ascertained diffuse large B-cell lymphomas. Genes Chrom Cancer 1999;**25**:123–133.
5. Jerkeman M, Johansson B, Akerman M, Cavallin-Stahl E, Kristoffersson U, Mitelman F. Prognostic implications of cytogenetic aberrations in diffuse large B-cell lymphomas. Eur J Haematol 1999;**62**:184–190.
6. Dave BJ, Nelson M, Pickering DL, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Sanger WG. Cytogenetic characterization of diffuse large cell lymphoma using multi-color fluorescence in situ hybridization. Cancer Genet Cytogenet 2002;**132**:125–132.
7. Nanjangud G, Rao PH, Hegde A, Teruya-Feldstein J, Donnelly G, Qin J, Jhanwar SC, Zelenetz AD, Chaganti RSK. Spectral karyotyping identifies new rearrangements, translocations, and clinical associations in diffuse large B-cell lymphoma. Blood 2002;**99**:2554–2561.
8. Yoshioka T, Miura I, Kume M, *et al*. Cytogenetic features of de novo CD5-positive diffuse large B-cell lymphoma: chromosome aberrations affecting 8p21 and 11q13 constitute major subgroups with different overall survival. Genes Chrom Cancer 2005;**42**:149–157.
9. Ye BH, Rao PH, Chaganti RS, Dalla-Favera R. Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. Cancer Res 1993;**53**:2732–2735.
10. Bastard C, Deweindt C, Kerckaert JP, Lenormad B, Bossi A, Pezzella F, Fruchart C, Duval C, Moncondult M, Tilly H. LAZ3 rearrangements in non-Hodgkin's lymphoma: correlation with histology, immunophenotype, karyotype and clinical outcome in 217 patients. Blood 1994;**83**:2423–2427.
11. Aisenberg AC, Wilkes BM, Jacobson JO. The Bcl-2 gene is rearranged in many diffuse B-cell lymphomas. Blood 1988;**71**:969–972.
12. Jacobson JO, Wilkes BM, Kwaiatkowski DJ, Medeiros LJ, Aisenberg AC, Harris NL. Bcl-2 rearrangements in *de novo* diffuse large cell lymphoma. Association with distinctive clinical features. Cancer 1993;**72**:231–236.
13. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Zuliana M. Report of the Committee on Hodgkin's Disease Staning. Cancer Res 1971;**31**:1860–1861.
14. Mitelman F, ed. ISCN: An International System for Human Cytogenetic Nomenclature. Basel: Karger, 1995.
15. Noriega MF, De Brasi C, Narbaitz M, Slavutsky I. Incidence of BCL-2 gene rearrangements in Argentinean non-Hodgkin lymphoma patients: increased frequency of breakpoints outside of MBR and MCR. Blood Cells Mol Dis 2004;**32**:232–239.
16. Gribben LG, Freedman AS, Woo SD, Blake K, Shu RS, Freeman G, Longtine JA, Pinkus GS, Nadler LM. All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment. Blood 1991;**78**:3275–3280.
17. Akasaka T, Akasaka H, Yonetani N, Ohno H, Yamabe H, Fukuhara S, Okuma M. Refinement of the BCL-2 immunoglobulin heavy chain fusion gene in t(14;18)(q32;q21) by polymerase chain reaction amplification for long targets. Genes Chrom Cancer 1998;**21**:17–19.
18. Cerretini R, Acevedo S, Chena C, Belli C, Larripa I, Slavutsky I. Evaluation of constitutional chromosome aberrations in hematologic disorders. Cancer Genet Cytogenet 2002;**134**:133–137.
19. Fan Y-S, Rizkalla K. Comprehensive cytogenetic analysis including multicolor spectral karyotyping and interphase fluorescence in situ hybridization in lymphoma diagnosis: a

summary of 154 cases. Cancer Genet Cytogenet 2003;**143**:73–79.
20. Chen P-M, Yang M-H, Yu I-T, Lin J-T, Lin Y-C, Fan FS, Wang W-S, Yen C-C, Chiou T-J, Liu J-H. Low incidence of BCL-6 gene alterations for diffuse large B-cell lymphomas in Taiwan Chinese. Cancer 2001;**94**:2635–2644.
21. Muramatsu M, Akasaka T, Kadowaki N, Ohno H, Yamabe H, Edamura S, Doi S, Mori T, Okuma M, Fukuhara S. Rearrangement of the BCL-6 gene in B-cell lymphoid neoplasms: comparison with lymphomas associated with BCL-2 rearrangements. Brit J Haematol 1996;**93**:911–920.
22. Ott MM, Rosenwald A, Katzenberger T, Dreyling M, Krumdiek AK, Kalla J, Greiner A, Ott G, Muller-Hermelink HK. Marginal zone B-cell lymphomas (MZBL) arising at different sites represent different biological entities. Genes Chrom Cancer 2000;**28**:380–386.
23. Shimazaki C, Inaba T, Shimura K, *et al*. B-cell lymphoma associated with haemophagocytic syndrome: a clinical, immunological and cytogenetic study. Br J Haematol 1999;**104**:672–679.
24. Fiedler W, Weh HJ, Hossfeld DK. Comparison of chromosome analysis and BCL-1 rearrangement in a series of patients with multiple myeloma. Br J Haematol 1992;**81**:58–61.
25. Sawyer JR, Lukacs JL, Munshi N, Desikan KR, Singhal S, Mehta J, Siegel D, Shaughnessy J, Barlogie B. Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. Blood 1998;**92**:4269–4278.
26. Horsman DE, Connors JM, Pantzar T, Gascoyne RD. Analysis of secondary chromosomal alterations in 165 cases of follicular lymphoma with t(14;18). Genes Chrom Cancer 2001;**30**:375–382.
27. Morgan R, Chen Z, Richkind K, Roherty S, Velasco J, Sandberg AA. PHA/IL2: an efficient mitogen cocktail for cytogenetic studies of non-Hodgkin lymphoma and chronic lymphocytic leukemia. Cancer Genet Cytogenet 1999;**109**:134–137.
28. Asou N, Sanada I, Tanaka K, Hidaka M, Suzushima H, Matsuzaki H, Kawano F, Takatsuki K. Inversion of chromosome 16 and bone marrow eosinophilia in a myelomonocytic transformation of chronic myeloid leukemia. Cancer Genet Cytogenet 1992;**61**:197–200.
29. Brito-Babapulle V, Pittman S, Melo JV, Parreira L, Catovsky D. The 14q + marker in hairy cell leukaemia. A cytogenetic study of 15 cases. Leuk Res 1986;**10**:131–138.
30. Berger R, Flandrin G, Bernhein A, Valensi F, Sigaux F, Daniel M-T, Ochoa-Noguera MH. Cytogenetic studies in 32 malignant lymphomas. In: Sotto JJ, Vrousos C, Vincent F, eds. Non-Hodgkin's Lymphomas: New Techniques and Treatments. Basel: Karger, 1985:79–86.
31. Lloveras E, Granada I, Zamora L, *et al*. Cytogenetic and fluorescence in situ hybridization studies in 60 patients with multiple myeloma and plasma cell leukemia. Cancer Genet Cytogenet 2004;**148**:71–76.
32. Buhmann R, Kurzeder C, Rehklau J, Westhaus D, Bursch S, Hiddemann W, Haferlach T, Hallek M, Schoch C. CD40L stimulation enhances the ability of conventional metaphase cytogenetics to detect chromosome aberrations in B-cell chronic lymphocytic leukaemia cells. Br J Haematol 2002;**18**:968–975.
33. Mohamed AN, Palutke M, Eisenberg L, Al-Katib A. Chromosomal analyses of 52 cases of follicular lymphoma with t(14;18), including blastic/blastoid variant. Cancer Genet Cytogenet 2001;**126**:45–51.
34. Whang-Peng J, Knutsen T, Jaffe ES, Steinberg SM, Raffeld M, Zhao WP, Duffey P, Condron K, Yano T, Longo DL. Sequential analysis of 43 patients with non-Hodgkin's lymphoma: clinical correlations with cytogenetic, histologic, immunophenotyping, and molecular studies. Blood 1995;**85**:203–216.
35. Bello MJ, Rey JA, Moreno S, Martinez Castro P, Benitez J. Cytogenetic study of exudates secondary to hematologic processes. Sangre (Barc) 1988;**33**:165–170.
36. Hashimoto K, Miura I, Chyubachi A, Saito M, Miura AB. Correlations of chromosome abnormalities with histologic and immunologic characteristics in 49 patients from Akita, Japan with non-Hodgkin lymphoma. Cancer Genet Cytogenet 1995;**81**:56–65.
37. Tsukadaira A, Okubo Y, Ogasawara H, Urushibata K, Honda T, Miura I, Kubo K. Chromosomal aberrations in intravascular lymphomatosis. Am J Clin Oncol 2002;**25**:178–181.
38. Narayan G, Pulido HA, Koul S, *et al*. Genetic analysis identifies putative tumor suppressor sites at 2q35-q36.1 and 2q36.3-q37.1 involved in cervical cancer progression. Oncogene 2003;**22**:3489–3499.
39. Yamamoto N, Mizoe JE, Numasawa H, Yokoe H, Uzawa K, Shibahara T, Tsujii H, Noma H, Tanzawa H. Allelic loss of chromosome 2 in human oral squamous cell carcinoma: correlation with lymph node metastases. Oral Oncol 2003;**39**:64–68.
40. Liang H, Samanta S, Nagarajan L. SSBP2, a candidate tumor suppressor gene, induces growth arrest and differentiation of myeloid leukemia cells. Oncogene 2005;**24**:2625–2634.
41. De Luca A, Bernardini L, Ceccarini C, Sinibalde L, Novelli A, Giustini S, Daniele I, Calvieri S, Mingarelli R. Fluorescence in situ hybridization analysis of allelic losses involving the long arm of chromosome 17 in NF1-associated neurofibromas. Cancer Genet Cytogenet 2004;**150**:168–172.
42. Lossos IS, Alizadeth AA, Diehn M, Warnke R, Thorstenson Y, Oefner PJ, Brown PO, Botstein D, Levy R. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. Proc Natl Acad Sci 2002;**99**:8886–8891.
43. Lopez-Guillermo A, Cabanillas F, Mcdonell TI, *et al*. Correlation of Bcl-2 rearrangement with clinical characteristics and outcome in indolent follicular lymphoma. Blood 1999;**93**:3081–3087.
44. Knuutila S, Klefstrom J, Szymanska J, Lakkala T, Peltomaki P, Eray M, Teerenhovi L, Elonen E, Franssila KO, Kaartinen M. Two novel human B-cell lymphoma lines of lymphatic follicle origin: cytogenetic, molecular genetic and histopathological characterization. Eur J Haematol 1994;**52**:65–72.
45. Ladanyi M, Offit K, Jhanwar SC, Filippa DA, Chaganti RSK. MYC rearrangements and translocations involving band 8q24 in diffuse large-cell lymphomas. Blood 1991;**77**:1057–1063.
46. Yano T, Jaffe ES, Longo DL, Raffeld M. Myc rearrangements in histologically progressed follicular lymphomas. Blood 1992;**80**:758–767.
47. Knezevich S, Ludkovski O, Salski C, Chhanabhai M, Lam W, Klasa R, Connors JM, Dyers MJS, Gascoyne RD, Horsman DE. Concurrent translocation of BCL2 and MYC with a single immunoglobulin locus in high-grade B-cell lymphomas. Leukemia 2005;**19**:659–663.
48. Janz S, Potter M, Rabkin C. Lymphoma and leukemia associated chromosomal translocation in healthy individuals. Gene Chrom Cancer 2003;**36**:211–223.
49. Nordenson I, Lenner P, Roos G. Patient with B-cell neoplasia (immunoblastic sarcoma) and the Philadelphia chromosome. Cancer Genet Cytogenet 1983;**9**:37–43.
50. Fujii H, Yashige H, Misawa S, Tanaka S, Urata Y, Matuyama F. Ph chromosome in a patient with

non-leukemic non-Hodgkin B-cell lymphoma. Am J Hematol 1990;**35**:213–215.
51. Bea S, Colomo L, López-Guillermo A, Salaverria I, Puig X, Pinyol M, Rives S, Montserrat E, Campo E. Clinicopathologic significance and prognostic value of chromosomal imbalances in diffuse large B-cell lymphomas. J Clin Oncol 2004;**22**:3498–3506.
52. Monni O, Joensuu H, Franssila K, Knuutila S. DNA copy number changes in diffuse large B-cell lymphoma. Comparative Genomic Hibridization study. Blood 1996;**87**:5269–5278.
53. Berglund M, Enblad G, Flordal E, *et al*. Chromosomal imbalances in diffuse large B-cell lymphoma detected by comparative genomic hybridization. Mod Pathol 2002;**15**:807–816.
54. Sandberg AA. In: The Chromosomes in Human Cancer and Leukemia, 2nd edn. New York: Elsevier Science Publishing, 1990:151–172.
55. Tsung SH, Heckman MG. Klinefelter syndrome, immunological disorders, and malignant neoplasm: report of a case. Arch Pathol 1974;**98**:351–354.
56. Becher R. Klinefelter's syndrome and malignant lymphoma. Cancer Genet Cytogenet 1986;**21**:271–274.
57. Attard-Montalto SP, Schuller I, Lastowska MA, Gibbons B, Kingston JE, Eden OB. Non-Hodgkin's lymphoma and Klinefelter syndrome. Pediatr Hematol Oncol 1994;**11**:197–200.
58. Humphreys M, Lavery P, Morris C, Nevin N. Klinefelter syndrome and non-Hodgkin lymphoma. Cancer Genet Cytogenet 1997;**97**:111–113.
59. Ohyashiki K, Yoshida MA, Ohyashiki J, Block AW, Dabski K, Sandberg AA. Chromosome changes in mycosis fungoides in an XYY male. Cancer Genet Cytogenet 1985;**18**:295–302.