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Genetic characterization of Sézary's syndrome by conventional cytogenetics and cross-species color banding fluorescent *in situ* hybridization

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A B S T R A C T

Background and Objectives. Sézary's syndrome is a peripheral T-cell neoplasm characterized by a pruritic exfoliative or infiltrated erythroderma, lymphadenopathies, and atypical T lymphocytes in the peripheral blood. Cytogenetic studies are scarce. This study was designed to increase cytogenetic information on this disorder.

Design and Methods. Peripheral blood samples were collected from 21 patients with Sézary's syndrome (10 men, 11 women, mean age 64 years) and analyzed by conventional cytogenetics (72-hr cultures with phytohemagglutinin). For a better characterization of multiple chromosomal rearrangements, cross-species color banding (RxFISH) was used in four cases.

Results. Fifteen (71.4%) of the 21 cases showed cytogenetic aberrations, with the karyotype being complex in 14. Among the 15 patients with an abnormal karyotype, 8 presented a diploid/near-diploid karyotype and 7 a near-tetraploid karyotype. The chromosomes most frequently involved were 1, 6, 8, 9, 10, 11, and 17. The most common structural rearrangements affected 1q, 2q, 6q23-27, and 8q22. Monosomies of chromosomes 9 and 10 and trisomies of chromosome 18 were recurrently observed. A statistical trend between abnormal and complex karyotypes, the presence of monosomy 10, the number of Sézary cells, and a decreased overall survival was observed. RxFISH technology allowed the description of 27 previously undetected chromosomal abnormalities.

Interpretation and Conclusions. Abnormal karyotypes, particularly complex karyotypes, were frequently detected in patients with Sézary's syndrome. Monosomy 10 was the most frequent recurrent cytogenetic marker (73% in abnormal cases). There was a high diversity of chromosomal breakpoints. RxFISH is a useful novel technology for redefining complex karyotypes.

Key words: cutaneous T-cell lymphoma, Sézary's syndrome, cytogenetics, RxFISH.

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Sézary's syndrome is a rare peripheral T-cell lymphoproliferative disorder characterized by a pruritic exfoliative or infiltrated erythroderma, lymphadenopathies, and atypical T lymphocytes in the peripheral blood.[1-3] Neoplastic cells show convoluted nuclei and may appear as either predominantly small (Lutzner cells) or large (classical Sézary cells), or there may be a mixture of both. The proportion of atypical lymphocytes is variable and there is no consensus upon the degree required for diagnosis. However, a minimum of 1,000 Sézary cells/mm^3 is the most accepted threshold value, since Sézary cells can also be found in other diseases (e.g., discoid lupus erythematosus) and even in a small proportion of normal peripheral blood samples. Other diagnostic criteria include an elevated CD4/CD8 ratio, an increased proportion of CD4$^+$/CD7$^-$ T lymphocytes, and a clonal rearrangement of T-cell receptor (TCR) genes.[4] Because of the low incidence of Sézary's syndrome and the difficulty in obtaining metaphases, there are few cytogenetic studies on this condition. Moreover, problems on the definition of the frequent complex karyotypes have interfered with the description of recurring abnormalities.[5-14]

A recently introduced fluorescent *in situ* hybridization (FISH)-based technology, the so-called cross-species color banding (or RxFISH for Rainbow cross-species FISH), consists of a DNA probe set that allows the analysis of color-banded chromosomes.[15-19] The DNA probes are derived from bivariate fluorescence activated flow sorting of chromosomes from two gibbon species (*Hylobates concolor* and *Hylobates syndactylus*). These probes have been previously characterized as having both extensively rearranged chromosomes and a high degree

of homology to the target human DNA. Combined labeling of the probe set (3 fluorophores generating 7 colors) and its subsequent application to metaphase spreads in a single FISH generates a distinctive color banding pattern throughout the genome. The addition of colors in the karyotype has made chromosome identification simpler than during conventional banding analysis based on a gray scale.

The aim of the present study was to increase cytogenetic information on Sézary's syndrome by using conventional methods and the RxFISH method. We also assessed whether a common cytogenetic marker could be identified to define Sézary's syndrome, so that it would be possible to establish a relationship between hematologic and clinical parameters. To the best of our knowledge, this is the first study using FISH color banding technology to define complex karyotypes in Sézary's syndrome.

Design and Methods

Patients

The present series was formed of 21 patients with a previous diagnosis of Sézary's syndrome who had been recruited in a multicenter study. The diagnoses had been established between 1987 and 2002. There were 10 men and 11 women with a mean age of 64 years (range 27 to 80 years). Data from seven patients have been previously reported.[9,10] Patients were recruited from six different hospitals in Catalonia (Hospital del Mar, Hospital Universitari de Bellvitge, Hospital Clinic, Hospital de Santa Creu i Sant Pau, Hospital Universitari Vall d'Hebrón from Barcelona and Hospital Universitari Arnau de Vilanova from Lleida). The diagnoses were established according to clinical, cytological, immunophenotypic, and molecular (TCR γ chain rearrangement) criteria. The characteristics of these patients are shown in Table 1.

Conventional cytogenetics

Chromosome analyses were carried out on lymphoid cells from phytohemagglutinin-stimulated 72-hr peripheral blood cultures, following standard procedures. Briefly, cells were incubated for 72 hr with phytohemagglutinin at 37°C and harvested after exposure to the antimitotic colcemid for 30 min at 37°C. After having treated cells with hypotonic solution for 30 min at 37°C, three fixative changes were performed before the slides were prepared. Slides were aged in a slide warmer at 100°C for 1 hr to obtain G-bands and then stained with Wright's solution. Karyotypes are described according the International System for Human Cytogenetic Nomenclature.[20]

Cross-species color banding FISH (RxFISH)

RxFISH analyses were performed on those cases with a very complex karyotype for which material was available in order to characterize the multiple rearrangements better (cases #10, 11, 13 and 16). A RxFISH color chromosome analysis FISH kit (Applied Imaging, Santa Clara, CA, USA) was applied to the metaphase spreads using the procedure described by the manufacturer with modifications.[21] Results were analyzed in a Nikon Eclipse 600 fluorescent microscope using an automated filter wheel. Image acquisition was performed with an RxFISH CytoVision System (Applied Imaging). A minimum of 20 metaphases per case were studied by two independent observers.

Statistical analysis

The χ^2 test was used for the analysis of categorical variables and Student's t test or the Mann-Whitney U-test for comparison of continuous variables. Time to relapse was assessed by Cox's regression analysis. Survival curves were estimated by the Kaplan-Meier method. Curves were compared with the log-rank test. The level of statistical significance was set at $p < 0.05$.

Results

Conventional karyotypes from all patients, in four cases redefined by RxFISH, are shown in Table 2. Fifteen (71.4%) of 21 cases had cytogenetic aberrations, and in 14 of them a complex karyotype was found. Of the patients with an abnormal karyotype, 8 had a diploid or near-diploid karyotype and 7 a near-tetraploid karyotype. Among these 7 cases with a near-tetraploid clone, three patients also showed an aberrant near-diploid clone sharing chromosomal abnormalities with the near-tetraploid line, three cases showed a normal 46,XX accompanying clone, and one case presented only the near-tetraploid clone. In the remaining six cases, all cells had either a normal karyotype (n = 5) or a normal karyotype with non-clonal abnormalities (n = 1).

When the relationship between the percentages of Lutzner and Sézary cells and the modal number of the abnormal chromosome clones was analyzed, a mixture of small and large cells was observed in all cases. In those cases with a normal karyotype, the cells were predominantly Lutzner type (76% to 90% small cells), except for one case which showed a normal karyotype and in which 90% of the morphologically abnormal cells were large. Among cases with an aberrant karyotype, three cases presented a diploid or near diploid clone and small cells predominated (72% to 75% of small cells); two cases presented a near-tetraploid clone and large cells were more frequent (67% to 90% of large cells) in the peripheral blood smear. However, in

Table 1. Clinical, morphologic and molecular data of 21 Sézary's syndrome patients.

Case no.	*Age*	*Sex*	*Clinical presentation*	*% Sézary cells (numberx10⁹/L) % LC % SC*	*Skin biopsy*	*TCR rearrangement*	*Therapy*	*Status/Follow-up (months)*
1	76	F	Erythroderma	88 % (–) LC: – SC: –	MF	ND	Endoxan + Prednisone	Death related to SS/9
2	58	M	Erythroderma Adenopathies	17% (1120) LC: – SC: –	MF	ND	αIFN, Chlorambucil	Death related to SS/21
3	67	M	Erythroderma	24% (–) LC: 75% SC: 25%	MF	ND	ECP	Death related to SS/1
4	75	F	Erythroderma	46% (–) LC: – SC: –	MF	ND	CHOP	Death related to SS/26
5	76	F	Erythroderma	48% (–) LC: – SC: –	MF	ND	CHOP, AVBD, αIFN	Death related to SS/34
6	49	F	Erythroderma Adenopathies	60% (18600) LC: 10% SC: 90%	MF	ND	Chlorambucil+ Prednisone, αIFN, ECP	Death related to SS/103
7	76	F	Erythroderma	63% (7358) LC: 74% SC: 26%	MF	ND	Prednisone PUVA, MTX ECP, CHOP	Death related to SS/5
8	54	F	Erythroderma	30% (1200) LC: 90% SC: 10%	MF	Clonal	MTX, Chlorambucil+ Prednisone, PUVA, ECP	Alive/+57
9	80	M	Erythroderma Adenopathies	86% (11610) LC: 76% SC: 24%	MF	Clonal	Chlorambucil	Alive/+27
10	76	M	Erythroderma Adenopathies	60% (7860) LC: 35% SC: 65%	MF	Non clonal	Chlorambucil, Pentostatin	Death related to SS/7
11	27	M	Erythroderma Adenopathies	50% (6650) LC: 8% SC: 92%	MF	ND	Chlorambucil, Deoxycoformycin, APSCT	Death related to SS/23
12	50	M	Erythroderma	28% (4060) LC: 72% SC: 28%	MF	Clonal	Chlorambucil+ Prednisone, Deoxycoformycin, RT, CHOP	Death related to SS/26
13	35	M	Erythroderma Adenopathies	30% (4872) LC: 84% SC: 16%	MF	Clonal	PUVA,CHOP, Deoxycoformycin, αIFN, ECP, Hyper CVAD	Death related to SS/15
14	74	M	Erythroderma	25% (3475) LC: 82% SC: 18%	MF	Clonal	Chlorambucil+ Prednisone, αIFN	Death related to SS/40
15	70	F	Erythroderma	10% (-) LC: 10% SC: 90%	MF	Clonal	Chlorambucil+ Prednisone, αIFN, PUVA, ECP	Dead of pulmonary fibrosis/27
16	56	M	Erythroderma Adenopathies	20% (2060) LC:33 % SC:67%	MF	Clonal	Pentostatin+RT, Chlorambucil+ Prednisone, CHOP, 2-CDA MTX, IFNα	Alive/+29
17	70	M	Erythroderma Keratodermia	57% (1818) LC:17 % SC:83%	MF	Clonal	Chlorambucil+ Prednisone	Alive/+7
18	68	M	Erythroderma	20% (980) LC: 90% SC: 10%	MF	Non clonal	Chlorambucil+ Prednisone	Alive/+6
19	73	F	Erythroderma Adenopathies	56% (7672) LC: 85% SC:15%	MF	Oligoclonal	Chlorambucil	Alive/+17
20	78	F	Erythroderma	6% (7000) LC: 95% SC: 5%	MF	Clonal	Chlorambucil+ Prednisone	Alive/+22
21	58	F	Erythroderma	20% (6000) LC: 88% SC:12%	MF	Clonal	COP, fludarabine	Alive/+17

M: male; F: female; MF: Mycosis fungoides; ND: not done/no material available; PR: partial remission; PD: progressive disease; LC: Lutzner cells (small); SC: Sézary cells (large); TCR: T-cell receptor; α -IFN: α interferon; ECP: extracorporeal photoapheresis; CHOP: cyclophosphamide, adriamycin, vincristine and prednisone; ABVD: adriamycin, bleomycin, vinblastine, dacarbazine; PUVA: psoralen+ultraviolet A irradiation; MTX: methotrexate; APSCT: allogeneic peripheral stem cell transplantation; RT: radiotherapy; CVAD: cyclophosphamide, vinblastine, adriamycin, dexamethasone; COP: cyclophosphamide, vincristine, prednisone; 2-CDA: 2-clorodeoxyadenosine.

Table 2. Karyotypes and TCR-γ rearrangement from 21 patients with Sézary's syndrome . Chromosomal abnormalities are defined by conventional cytogenetics and by RxFISH in those cases with a complex karyotype for which material was available.

Case	*Karyotype (conventional cytogenetics and RxFISH)*	*TCR-γ rearrangement*
1	45,XX,t(1;4;11)(q31;p16;q13.1), del(2)(p21), del(6)(q23) t(6;14)(p23;q21-22),-10,-10,del(12)(p12), add(16)(p13), add(20)(q13), +mar [13]/84-88, idemx2, inc [7]/ 46,XX [10]	ND
2	44,XY,add(1)(p36),add(2)(q37),add(6)(q27),-10,-13,del(14)(q22),-15,-22, +2mar [12]/46,XY[18]	ND
3	46,XY,add(14)(q32)[10]/46,XY[10]	ND
4	47,XX,-10,+18,+19 [8]/ 46,XX [16]	ND
5	44,X,-X,del(1)(q32),-13 [20]/46,XX [16]	ND
6	89,XXXX,+1,+1,+2,+3,+3,+4,+4,+4,+5,del(5)(p11),+6,+6,+del(7)(p11), +9,+11,+11,+11,+12,+13,+13,+14, +14,+15,+15,+16,+16,+17,+17,+17, +17,+18,+20,+21,+21,+22, +22,+22,+22,+2mar [25]/46,XX [1]	ND
7	41-44,XX, del(1)(p11),der(2),der(2),-4,der(4), del(5)(q13q33),-6,-8, del(9)(p22),-10,-12,-12,-13, add(15)(q26),add(16)(q24),-17, add(17)(p13),-19, add(22)(q13),+5mar [cp8]	ND
8	46,XX [20]	Non-clonal
9	46,XY [20]/46,XY,t(2;12)(q23;q24) [1]	**Clonal**
10	48,XY, der(1)t(1;6)(q25;q25),+4, der(6)(8qter→8q22::6p23→6q25::1q25→1qter), del(6)(q23), +der(8)t(8;15)(q22;q13),-9,der(11), del(15)(q15), der(15)t(11;15)(q21;q23), der(17)t(17;18)(q21;q21),+18,der(19)t(9;19)(q11;p13), der(22), t(Y?;22)(?;q11)[16]/ 91 chromosomes with the same cytogenetic aberrations [4]	Non-clonal
11	48,XY,add(1)(p36),del(3)(p14),inv(6)(p22q12),+7,der(8)t(1;8)(p36;q24), del(9)(q13q22), +del(9)(q13q22),der(15)t(9;15)(q31;q22), der(22)t(3;22)(p21;q13) [14]/46,XY[6]	ND
12	41,X,-Y,del(2)(q34),+der(3),der(6)add(6)(p25)add(6)(q27), add(7)(p22),-8,-9,-10,-11,-13,-14, add(19)(q13.1),+20 [6]	**Clonal**
13	44,X,-X,1qh+,der(1)t(1;13)(q11;q14),der(2)add(2)(p22)add(2)(q37), der(2)(2p25→2q37::2q11→2q21), del(3)(p11),der(5)t(5;7)(p15;q22), del(6)(q21),der(6)t(3;6)(q11;q15), -10,t(10;11)(p11;p12), der(13)t(8;13)(q11;q14),add(17)(p13),der(19)t(1;19)(q21;q13)[12]/44,X,-X, 1qh+,der(1)t(1;13)(q11;q14), der(2)add(2)(p22)add(2)(q37),der(2)(2p25→2q37::2q11→2q21), del(3)(p11), der(5)(7q22→7q36::5p15→5q13::7q22→7q32),del(6)(q21),-10, 10,der(11)t(10;11)(p11;p12),+der(11), der(13)t(8;13)(q11;q14),der(17), der(19)t(1;19)(q21;q13)[20]/ 90 chromosomes with the same cytogenetic aberrations [4]	**Clonal**
14	46,XY[20]	**Clonal**
15	46,XX[20]	Non-clonal
16	89,XXYYY,+der(1),+der(1),+2,+2,+3,+3,+4,+4,+5,+5,+7,+7,+t(8;14)(q11;q11), +der(8) t(8;14)(q11;q11), +8,+9,-10,+11,+12,+12,+13,+13,+15,+15,+16, +16,+17, +18, +18,+18,+19,+19,+20,+20,+21, +21,+22,+22,+22, +mar (t(10;11)(q11;q11)) [7]	**Clonal**
17	45,XY,add(1)(p36),del(3)(p23),-10,-17,+mar [5]/ 46,XY [15]	Non-clonal
18	46,XY[20]	Non-clonal
19	46,XX [20]	**Oligoclonal**
20	82-87,XX,der(1),+2,+3,+4,+der(4),+der(4),+5,+6, del(6)(q23),+7,+der(7), +8,+9,+11,+add(11)(q24),+12, +12,+13,+14,+14,+15, +16,+der(17),+der(17),+18,+18,+19,+19,+20,+20,+21, +21,+5-10 mar [cp3]/ 46,XX[17]	**Clonal**
21	90,XX,+1,+1,+2,+2,+3,+3,+der(4),+5,+der(5),+7,+7,+8,+8,+i(8)(q10), +9,+11,+der(11),+12,+13,+13,+14,+15,+16,+add(16)(q22),+17,+17,+der(17), +18,+18,+18,+18,+19,+20,+20,+21, +21,+21,+22,+22,+5mar [2]/46,XX[13]	**Clonal**

Table 3. Common structural rearrangements and numerical chromosomal anomalies in 21 patients with Sézary's syndrome.

Data	*Chromosome bands*
Affected region	
Involvement of 1q	1q11, 1q21, 1q25, 1q31, 1q32
Involvement of 2q	2q11-q21, 2q34, 2q37
Involvement of 6q	6q15, 6q21, 6q23, 6q25, 6q27
Involvement of 8q	8q11,8q22, 8q24
Involvement of 14q	14q21-q22, 14q32
Involvement of 15q	15q22, 15q23, 15q26
Involvement of 17q	17p13
Involvement of 19q	19q11, 19q13
Involvement of 22q	22q11, 22q13
Data	*Frequency (%)*
Chromosome	
+4	3/15 (20)
-6	3/15 (20)
-9	6/15 (40)
-10	11/15 (73)
-13	4/15 (27)
-14	4/15 (27)
+18	5/15 (33)

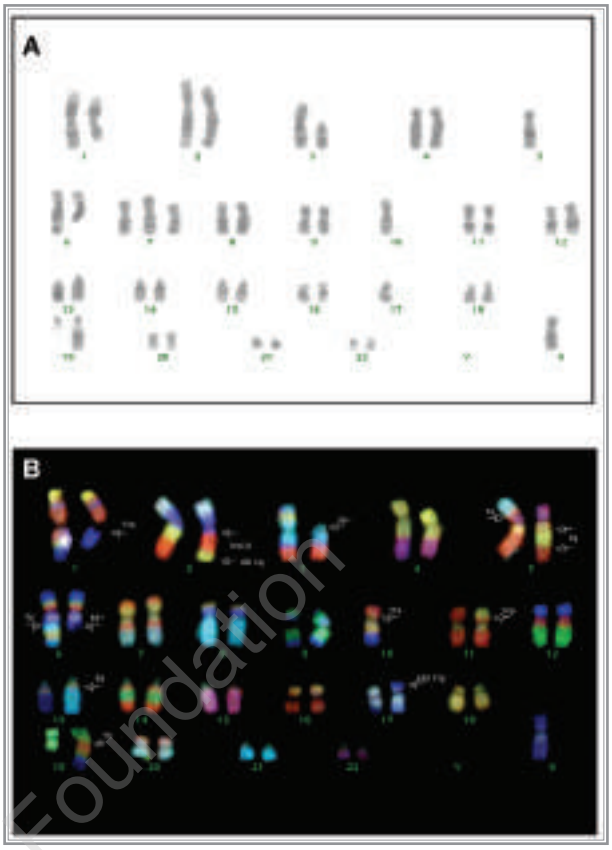

Figure 1. A. Conventional karyotype and B. RxFISH karyotype from patient #13.

two cases, the neoplastic cells were more frequently large cells (92% and 83%) but the only abnormal cytogenetic clone had a near-diploid range. Two cases showed an aberrant near-diploid clone sharing chromosomal abnormalities with the near-tetraploid line; in one of these cases, large cells predominated, whereas in the other small cells were the most important neoplastic population (Tables 1 and 2).

The frequency of chromosomal involvement was as follows: chromosome 10 in 11 cases; chromosomes 1 and 6 in 10 cases; chromosomes 8, 9, 11, and 17 in 8 cases; chromosomes 2, 19, and 22 in 7 cases; chromosomes 4, 13, 14, and 15 in 6 cases; chromosomes 3, 5, 7, and 18 in 5 cases; chromosome 20 in 3 cases; and chromosomes 21, X, and Y in 2 cases. The most common structural rearrangements affected 1p (3 cases), 1q (4 cases), 2q (3 cases), 6q (7 cases), and 8q (5 cases). Structural aberrations are detailed in Table 2. It is notable that unbalanced abnormalities, such as unbalanced translocations, additions, deletions, derivative chromosomes, and other were more common than balanced aberrations (Table 3). RxFISH technology was applied in four cases (cases 10, 11, 13, and 16) and allowed the description of 27 unpreviously detected chromosomal abnormalities (Table 2, Figure 1).

Chromosomal abnormalities did not have statistically significant implications for survival, probably because of the small sample size (21 patients). Although there were too few patients with non-complex karyotypes to measure the odds ratio for relapse accurately, a statistical trend between abnormal karyotypes ($p = 0.080$), complex karyotypes ($p = 0.210$), presence of monosomy of chromosome 10, number of Sézary cells (large) ($p = 0.375$) and a decreased overall survival was observed (Figures 2, 3 and 4).

Discussion

In the present study, we confirm the high number of chromosome abnormalities in Sézary's syndrome that has previously been reported by our group and others.[5-14,22-25] Clonal chromosome aberrations were found in 71.4% of patients. In a recent literature review,[24] 61% of the 274 cases of Sézary's syndrome showed an abnormal karyotype. The most frequent chromosomal abnormalities were losses of chromosome 10, deletions of 1p, isochromosome 17q, additions of 17p and 19p, and translocations involving 1p, 10q and 14q. On the whole, losses were more frequently observed than gains of chromosome regions or entire chromosomes. Regarding recurring structural aberrations, two cases with a der(8)t(8;17)(p11;q11) were reported by Thangavelu *et al.*[11] and recently Mao *et al.*[24] detected a der(1)t(1;10)

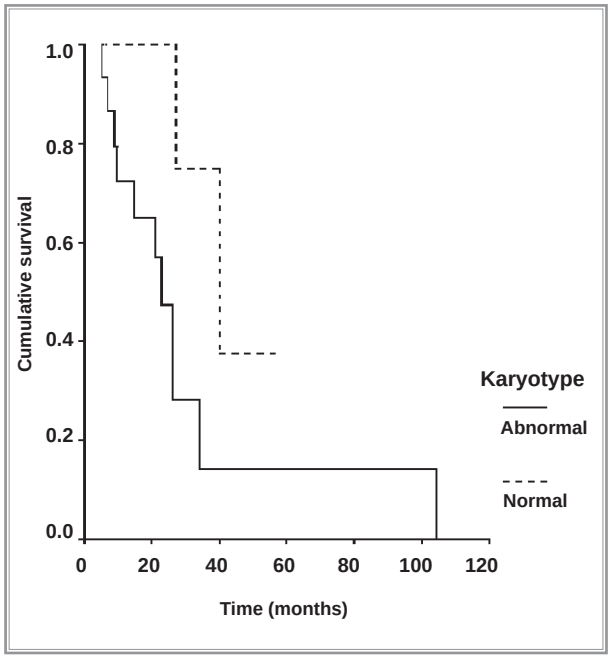

Figure 2. Relation between presence of cytogenetic abnormalities and cumulative survival (p=0.08).

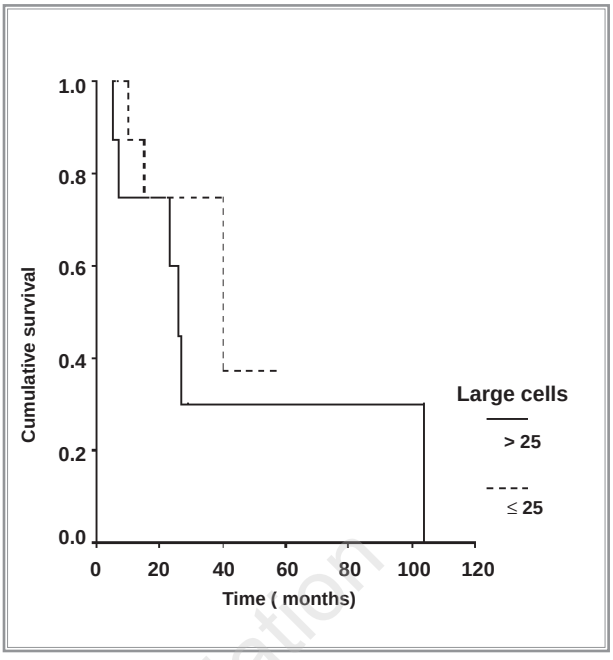

Figure 4. Relationship between the number of Sézary cells (large cells) and cumulative survival (p=0.375).

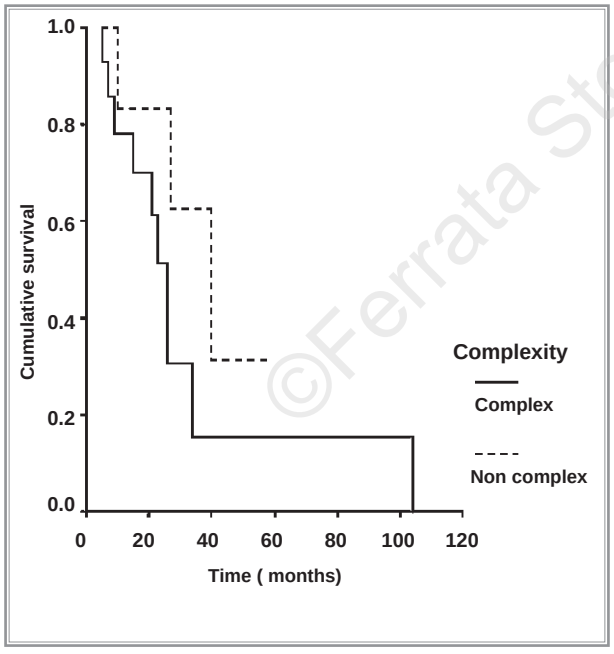

Figure 3. Relation between complexity of karyotype (a complex karyotype is defined as having three or more cytogenetic abnormalities) and cumulative survival (p=0.215).

(p2;q2) and a der(14)t(14;15)(q?;q?) in two cases each. However, a review of the previously published series reveals a high variety of complex chromosome aberrations, which were predominantly unbalanced aberrations. We did not find any cases with the above mentioned derivative chromosomes in our series. In our opinion, the finding of these aberrations in only two cases each and the lack of more reported cases with the same aberrations seem to indicate that these structural rearrangements do not represent hallmark rearrangements in Sézary's syndrome, but just reflect the high degree of chromosome instability in these patients.

Among our patients, monosomy of chromosome 10 (73% in cases showing an aberrant karyotype) was the most frequent abnormality. Studies using conventional cytogenetic methods,[5-14,24] comparative genomic hybridization,[22,23] loss of heterozygosity[26,27] and genomic microarrays[28] have extensively reported losses of one or both copies of the entire chromosome 10 or 10q regions. Scarisbrick *et al.*[26] defined an overlapping region of deletion between 10q23 and 10q24 in 23% of cases of mycosis fungoides. Losses on this region were also observed in Sézary's syndrome. Patients with allelic loss in 10q were analyzed for abnormalities on the tumor supressor gene *PTEN* (10q23.3): no mutations were found, but a homozygous deletion was detected in 2 patients. These observations may point to an important role of PTEN in the pathogenesis of mycosis fungoides and Sézary's syndrome.[23,26,27] Compararive genomic hybridization in 7 cases identified a different minimal overlapping region of loss at 10q25-q26.[22] Loss of chromosome 10 or 10q25-q26 was present in both *de novo*

Sézary's syndrome and in Sézary's syndrome evolving from mycosis fungoides. A deletion specifically defined to the mentioned region had not been previously reported in Sézary's syndrome, and the authors suggested two possible candidate genes located at 10q25-q26, namely *MXI1*[30] and *DMBT1*.[30,31] These genes had been previously described in prostate cancer and in malignant brain tumors and desmoplastic melanoma respectively. Other genes on 10q are *FAS* and *LGI1*;[32] however, no strong association between the majority of cases of Sézary's syndrome and a putative tumor supressor gene has yet been described. Concerning chromosome 1, losses in 1p have been frequently reported,[5-11] and compararive genomic hydridization has delineated a minimal region of deletion at D1S228 (1p36).[23] Previous comparative genomic hydridization studies have also shown losses of DNA copy number at 1p in various carcinomas, nodal lymphomas and leukemias.[33] With regard to 1p genes, a large number of candidates involved in the pathogenesis of cutaneous T-cell lymphomas have been indicated, including *MTS1/SA1/TFS1*, *BCL10*, *TAL-1*, *BRCD2*, *MOM1/PLASG2*, *P73* and *P18*.

In our series, we found few cases with additions on chromosome arm 1p36, which could imply a very small loss of material on the 1pter region. In addition, we found a wide variety of rearrangements affecting 1q arm (four cases), generally as complex unbalanced translocations or deletions. Losses on 1q have been previously described, but not as often as 1p deletions. Mao *et al.*[23] found 1q deletion in two cases of Sézary's syndrome, and a loss of 1q32q43 by compararive genomic hydridization in one case. In addition, a series of 1q microsatellite markers was used and allelic losses were identified at D1S397 (1q25) in 21% cases of mycosis fungoides. Chromosome 1 might be significant in Sézary's syndrome, and the genes located on both 1p and 1q arms could be hypothesized to play an important role in the pathogenesis of this disease.

The long arm of chromosome 6 was frequently affected, from q11 to q27 bands, in our patienst with Sézary's syndrome (seven cases). It is important to note that in a previous series chromosome 6 was not distinguished as an important chromosome in Sézary's syndrome, although it has been implicated in the syndrome by some authors.[6,9] Losses on 6q have been most frequently reported in mature B-cell neoplasms[34,35] and in B-cell acute lymphoblastic leukemia,[36] mainly affecting 6q21-q27 bands. However, other types of neoplasms have also presented with 6q aberrations, such as T-cell acute lymphoblastic leukemia, acute myeloblastic leukemia, T-cell prolymphocytic leukemia and myelodysplastic syndromes. In addition, a wide range of solid tumors have also shown 6q losses, such as pancreas and breast adenocarcinoma, uterine leiomyosarcoma, malignant melanoma and brain astrocytoma.[37]

In our series, in concordance with others, chromosome 8 also appeared to be affected. Detected aberrations of this chromosome were partial loss of the 8q region and a total loss of the chromosome (monosomy). In addition, among our patients, there were four cases of gains on 8q as unbalanced translocations and one case had a +i(8)(q10). Three of these cases were associated with monosomy 10, and had very complex karyotypes. It is worth pointing out that four cases with gains of 8q (instead of losses) associated with losses of 10/10q were reported by Karenko *et al.*[22] and that gains of 8q were also identified by Mao *et al.*[23] These two series both led to the suggestion that 8/8q gains were a feature of disease progression.

In our series 17p was not extensively involved, as we detected add(17)(p13) in only two cases. The 17p region has, however, been widely described to show aberrations[10,11] as the P53 tumor supressor gene is located in this region. Brito-Babapulle *et al.*[38] analyzed P53 mutations by direct DNA sequencing, allele deletions by FISH and protein accumulation by immunocytochemistry in T-prolymphocytic leukemia and Sézary's syndrome. Among 11 cases of Sézary's syndrome, P53 deletion was present in nine cases and P53 overexpression was detected in five cases. The study revealed both P53 deletion and protein accumulation without evidence of mutations in the most relevant exons described in cutaneous T-cell lymphomas. In another study of 15 Sézary's syndrome patients, a high rate of loss of heterozygosity was detected on 17p (42%).[27] Since losses of this region affect a very thin chromosome band, which can be overlooked by conventional methods or RxFISH, the application of a specific locus probe for 17p13 deletion will probably increase the frequency of detection of cases with this deletion.

Another common region of allelic loss is 9p (more precisely 9p21): P15 and P16 tumor supressor genes are mapped to this region.[27,39] P15 and P16 genes encode cyclin-dependent kinase inhibitors, which play a crucial role in the control of the cell cycle. Inactivation of these genes is frequently observed in high grade B-cell non-Hodgkin's lymphomas[40] and occasionally in patients with early cutaneous stages of mycosis fungoides.[41] In a series examined by Scarisbrick *et al.*,[27] 9p showed the highest rate of loss of heterozygosity (LOH) (46%) in patients with Sézary's syndrome, and the rate was also high in those with mycosis fungoides (16%). However, since P15 and P16 genes frequently become inactivated by hypermethylation of promoter sites and rarely by small deletions or point mutations,[42] the true prevalence of inactivation of these genes evaluated by LOH rates is underestimated.

Regarding correlations with chromosomal abnormalities and survival, we found an association between the

presence of monosomy of chromosome 10 and a decreased overall survival. No correlations were detected with other cytogenetic aberrations, probably because of the small sample size. Losses on 10q and 17p were related to more advanced stages of mycosis fungoides and Sézary's syndrome, suggesting that tumor supressor genes, such as *PTEN* (10q23), *FAS* (10q24), and *P53* (17p13) may be associated with disease progression. In contrast, losses on 1p and 9p, affecting the *P18* (1p32), *TAL1* (1p32), and *P16* (9p21) genes, might be associated with early stages of these diseases.[22,26,27]

We did find a correlation between a predominance of morphologically large Sézary cells and a worse overall survival in our series, although given the low number of cases available to run statistical studies, this association was not statistically significant. Overall, we could not find a relation between the percentage of Lutzer cells and/or Sézary cells and modal number of the abnormal chromosome clones, as some cases with a predominant large cell population showed only a near-diploid clone or *vice versa*. RxFISH seems to be an additional tool to help the study of complex karyotypes in Sézary syndrome. We have previously demonstrated the value of this new FISH technology in the study of peripheral T-cell neoplasms, in particular, T-prolymphocytic leukemia,[21] and its usefulness in other neoplasms, such as leukemias[43] and solid tumors[44] has also been shown. To our knowledge, this is the first study in which the RxFISH technique has been used to characterize chromosomal rearrangements in Sézary's syndrome. This novel technique identified between 6 to12 not previously recognized aberrations per case. Other multicolor FISH techniques have already been used in the study of complex chromosomal aberrations in Sézary's syndrome. Mao *et al.*[24] analyzed 17 of a total of 28 cases using a multiplex FISH, combined with an intensive dual-color FISH analysis using whole chromosome painting probes. They found that conventional cytogenetics and multiplex-FISH results were consistent, and new aberrations could be identified.

In conclusion, abnormal karyotypes, which were mostly complex, were frequently detected in patients with Sézary's syndrome. The most recurrent cytogenetic marker was monosomy of chromosome 10 (73% in abnormal cases). Although chromosomal rearrangements were frequent, a high diversity of chromosomal breakpoints was found. Thus, RxFISH was a useful technique to redefine complex karyotypes. Regions to which TCR genes have been mapped (14q11, 14q32, 7p15 and 7q35) were not involved. This finding suggests that the genetic basis for Sézary's syndrome is different from that involved in other T-cell malignancies.

BE was the principle author responsible for the conception and design of the study, analysis and interpretation of the data and drafting the paper. BE, MS and FS performed the conventional cytogenetics and RxFISH studies. LF, AD and SW contributed to the study with cytologic and immunophenotypic studies of the samples. RMP, FG, OS, TE and PGM revised and established the clinical criteria of the patients included in the study. FS, RMP, LF, OS and SW revised the paper citically for important intellectual content. All co-authors approved the final version of the paper. The authors are grateful to Jordi Sans-Sabrafen, MD, Teresa Vallespí, MD, Dolors Irriguible, MD, Montserrat Teixidó, MD, Beatriz Bellosillo, MD, Lurdes Zamora, Rosa M. Vilà, Rosa Navarro, Carme Melero, M. Carmen Vela, and Joan Vila for their valuable scientific and technical contributions to the study, and Marta Pulido, MD, for editing the manuscript and editorial assistance. The authors indicated no potential conflicts of interest.

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References

1. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, et al. EORTC classification for cutaneous lymphomas: a proposal from the cutaneous lymphoma study group of the European Organization for Research and Treatment of Cancer. Blood 1997;90:354-71.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW, Editors. World Health Organization classification of tumours. Tumours of hematopoietic and lymphoid tissues. IARC Press, Lyon, 2001.
3. Fink-Puches R, Zenahlik P, Bäck B, Smolle J, Kerl H, Cerroni L. Primary cutaneous lymphomas: applicability of current classification schemes (European Organization for Research and Treatment of Cancer, World Health Organization) based on clinicopathologic features observed in a large group of patients. Blood 2002;99: 800-5.
4. Russell-Jones R, Whittaker S. Sézary syndrome: diagnostic criteria and therapeutic options. Semin Cutan Med Surg 2000; 19:100-8.
5. Nowell PC, Finan JB, Vonderheid EC. Clonal characteristics of cutaneous T cell lymphomas: cytogenetic evidence from blood, lymph nodes and skin. J Invest Dermatol 1982;78:69-75.
6. Whang-Peng J, Bunn PA Jr, Knutsen T, Matthews MJ, Schechter G, Minna JD. Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma (CTCL). Cancer 1982;50:1539-53.
7. Nowell PC, Vonderheid EC, Besa E, Hoxie JA, Moreau L, Finan JB. The most common chromosome change in 86 chronic B cell or T cell tumors: a 14q32 translocation. Cancer Genet Cytogenet 1986; 19: 219-27.
8. Berger R, Baranger L, Bernheim A, Valensi F, Flandrin G, Berheimm A. Cytogenetics of T-cell malignant lymphoma. Report of 17 cases and review of the chromosomal breakpoints. Cancer Genet Cytogenet 1988;36:123-30.
9. Sole F, Tarrida N, Coll MD, Caballin MR, De Miguel A, Woessner S, et al. Cytogenetic study of a patient with the Sézary syndrome. Cancer Genet Cytogenet 1990;44: 193-6.
10. Sole F, Woessner S, Vallespi T, Perez Losada A, Florensa L, Irriguible D, et al. Cytogenetic studies in five patients with Sézary syndrome. Cancer Genet Cytogenet 1994;75:130-2.
11. Schlegelberger B, Himmler A, Bartles H, Kuse R, Sterry W, Grote W. Recurrent chromosome abnormalities in cutaneous T-cell lymphoma. Cancer Genet Cytogenet 1994;78:15-22.
12. Limon J, Nedoszytko B, Brozek I, Hellmann A, Zajaczek S, Lubinski J, et al. Chromosome aberrations, spontaneous SCE, and growth kinetics in PHA-stimulated lymphocytes on five cases with Sézary syndrome. Cancer Genet Cytogenet 1995;83:75-81.
13. Karenko L, Hyytinen E, Sarna S, Ranki A. Chromosomal abnormalities in cutaneous T-cell lymphoma and in its premalignant conditions as detected by G-banding and interphase cytogenetic methods. J Invest Dermatol 1997;108: 22-9.
14. Thangavelu M, Finn WG, Yelavarthi KK, Roenigk HH Jr, Samuelson E, Peterson L, et al. Recurring structural chromosome

abnormalities in peripheral blood lymphocytes of patients with mycosis fungoides/Sézary syndrome. Blood 1997;89: 3371-7.
15. Wienberg J, Jauch A, Stanyon R, Cremer T. Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics 1990;8:347-50.
16. Wienberg J, Stanyon R. Chromosome painting in mammals as an approach to comparative genomics. Curr Opin Genet Dev 1995;5:792-7.
17. Müller S, O'Brien PMC, Ferguson-Smith MA, Wienberg J. A novel source of highly specific chromosome painting probes for human karyotype analysis derived from primate homologues. Hum Genet 1997;101:149-53.
18. Müller S, Rocchi M, Ferguson-Smith MA, Wienberg J. Toward a multicolor chromosome bar code for the entire human karyotype by fluorescence in situ hybridization. Hum Genet 1997;100: 271-8.
19. Müller S, O'Brien PMC, Ferguson-Smith MA, Wienberg J. Cross-species colour-segmenting: a novel tool in human karyotype analysis. Cytometry 1998;33:445-52.
20. ISCN. An International System for Human Cytogenetics Nomenclature. Karger, Basel, 1995.
21. Espinet B, Sole F, Salido M, Lloveras E, Abella E, Besses C, et al. Application of cross-species color banding (RxFISH) in the study of T-prolymphocytic leukemia. Haematologica 2000;85:607-15.
22. Karenko L, Kahkonen M, Hyytinen ER, Lindlof M, Ranki A. Notable losses at specific regions of chromosomes 10q and 13q in Sézary syndrome detected by comparative genomic hybridization. J Invest Dermatol 1999;112:392-5.
23. Mao X, Lillington D, Scarisbrick JJ, Mitchell T, Czepulkowski B, Russell-Jones R, et al. Molecular cytogenetic analysis of cutaneous T-cell lymphomas: identification of common genetic alterations in Sézary syndrome and mycosis fungoides. Br J Dermatol 2002;147:464-75.
24. Mao X, Lillington DM, Czepulkowski B, Russell-Jones R, Young BD, Whittaker S. Molecular cytogenetic characterization of Sézary syndrome. Genes Chromosomes Cancer 2003;36:250-60.
25. Karenko L, Sarna S, Kahkonen M, Ranki A. Chromosomal abnormalities in relation to clinical disease in patients with cutaneous T-cell lymphoma: a 5-year follow-up study. Br J Dermatol 2003;148:55-64.
26. Scarisbrick JJ, Woolford AJ, Russell-Jones R, Whittaker SJ. Loss of heterozygosity on 10q and microsatellite instability in advanced stages of primary cutaneous T-cell lymphoma and possible association with homozygous deletion of PTEN. Blood 2000;95:2937-42.
27. Scarisbrick JJ, Woolford AJ, Russell-Jones R, Whittaker SJ. Allelotyping in mycosis fungoides and Sézary syndrome: common regions of allelic loss identified in 9p, 10q and 17p. J Invest Dermatol 2001; 117:663-70.
28. Mao X, Orchard G, Lillington DM, Russell-Jones R, Young BD, Whittaker SJ. Amplification and overexpression of JUNB is associated with primary cutaneous T-cell lymphomas. Blood 2003; 101:1513-9.
29. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV. Mutation of the MXI1 gene in prostate cancer. Nat Genet 1995;9:249-55.
30. Mollenhauer J, Wiemann S, Scheurlen W, Korn B, Hayashi Y, Wilgenbus KK, et al. DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-q26.1 is deleted in malignant brain tumors. Nat Genet 1997;17:32-9.
31. Rao UN, Bakker A, Swalsky PA, Finkelstein SD. Max interacting protein 1: loss of heterozygosity is frequent in desmoplastic melanoma. Mod Pathol 1999;12: 313-9.
32. GeneMap'99. Bethesda (MD): National Institutes of Health, National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/genemap/
33. Knuutila S, Aalto Y, Autio K. Recurrent DNA sequence copy number losses and amplification in human neoplasms evaluated by comparative genomic hybridization. http://www.helsinki.fi/~lgl_www/Loss+HLA/Loss_HLA_Cover_page.html
34. Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, et al. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. Blood 1993; 82:2157-62.
35. Stilgenbauer S, Bullinger L, Benner A, Wildenberger K, Bentz M, Dohner K, et al. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia 1999;13:1331-4.
36. Mancini M, Vegna ML, Castoldi GL, Mecucci C, Spirito F, Elia L, et al. Partial deletions of long arm of chromosome 6: biologic and clinical implications in adult acute lymphoblastic leukemia. Leukemia 2002;16:2055-61.
37. Mitelman F, Johansson B, Mertens F, eds. Mitelman Database of Chromosome Aberrations in Cancer, 2000. http://www.cgap.nci.nih.gov/Chromosomes/Mitelman.
38. Brito-Babapulle V, Hamoudi R, Matutes E, Watson S, Kaczmarek P, Maljaie H, et al. P53 allele deletion and protein accumulation occurs in the absence of p53 gene mutation in T-prolymphocytic leukaemia and Sézary syndrome. Br J Haematol 2000;110:180-7.
39. Scarisbrick JJ, Woolford AJ, Calonje E, Photiou A, Ferreira S, Orchard G, et al. Frequent abnormalities of the p15 and p16 genes in mycosis fungoides and Sézary syndrome. J Invest Dermatol 2002; 118:493-9.
40. Baur AS, Shaw P, Burri N, Delacretaz F, Bosman FT, Chaubert P. Frequent methylation silencing of p15(^{INK4b}) (MTS2) and p16(^{INK4b}) (MTS1) in B-cell and T-cell lymphomas. Blood 1999;94:1773-81.
41. Peris K, Stanta G, Fargnoli C, Bonin S, Felli A, Amantea A, et al. Reduced expression of CDKN2a/p16 in mycosis fungoides. Arch Dermatol Res 1999;291: 207-11.
42. Herman JG, Civin CI, Issa JPJ, Collector MI, Sharkis SJ, Baylin SB. Distinct patterns of inactivation of p15 (INK4B)and p16 (INK4A) characterize the major types of hematologic malignancies. Cancer Res 1997;57:837-41.
43. Teixeira MR, Micci F, Dietrich CU, Heim S. Cross-species color banding characterization of chromosomal rearrangements in leukemias with incomplete G-band karyotypes. Genes Chromosomes Cancer 1999;26:13-9.
44. Micci F, Teixeira MR, Dietrich CU, Sater G, Bjerkehagen B, Heim S. Combined RxFISH/G-banding allows refined karyotyping of solid tumors. Hum Genet 1999;104:370-5.