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CYTOGENETIC STUDY OF 50 DE NOVO CASES OF ANLL FROM ARGENTINA

Susana Acevedo, Irma Slavutsky, Gabriela Andreoli, Irene Larripa

Departamento de Genética, División Citogenética, Instituto de Investigaciones Hematológicas "Mariano R Castex", Académia Nacional de Medicina, Buenos Aires, Argentina

ABSTRACT

Background and methods. **Consistent and specific chromosomal aberrations have been observed in an increasing number of neoplasias. In the present report, we describe the cytogenetic findings from 50 cases of *de novo* ANLL in Argentina, South America, studied at diagnosis. In addition, their relation with the FAB classification is analyzed. Children with Down's syndrome and secondary ANLL were excluded from this analysis.**

Results and conclusions. **Out of 50 banded cases studied, 11 (22%) had normal karyotype, while the remaining 39 (78%) presented abnormal metaphases with structural alterations in the majority of them. Chromosomes 7 and 22 were most frequently involved in numerical alterations in children, while chromosomes 6, 8, 14 and 16 were the ones most often involved in adults. Consistent chromosome rearrangements were observed and they were linked to specific cytomorphologic subsets. The translocations t(8;21) and t(15;17) were seen only in M2 and M3, respectively. The inversion of chromosome 16, inv(16), was a typical finding in M4, but was not restricted to this subtype. Translocation t(2;3) was observed in three cases, all M4, each with a variable chromosome pattern. These results are in accordance with cytogenetic findings in Western Europe and the USA.**

Key words: ANLL, chromosomal aberrations, cytogenetic analysis

Acute nonlymphocytic leukemia (ANLL) is a worldwide disease that has traditionally been classified according to the nature of the predominating cells as judged by cytomorphology and cytochemistry. The FAB (French, American, British) Cooperative Group includes eight morphologically distinct subgroups designated M0 through M7.[1-3]

Acquired nonrandom chromosomal abnormalities are common findings in the malignant cells of patients with *de novo* nonlymphocytic leukemia. Cytogenetic study allows identification of categories of recurrent chromosomal aberrations. Varying responses to therapy has been observed in cases with certain nonrandom aberrations,[4-7] confirming the significance of karyotype as an independent prognostic factor. Some alterations correlate with particular FAB subtypes, among them t(8;21), t(15;17), inv (16) and t(11;V) are associated with M2, M3, M4 and M5, respectively.[8-12] On average, 55% of ANLL patients with karyotypic abnormalities have only one rearrangement; the remaining 45% have two or more changes.[13] More than 25 karyotypic abnormalities, the majority structural, have now been reported as recurrent solitary changes in ANLL. Numerical aberrations, in particular +8 and –7, are also quite common.[14]

This work reports the cytogenetic findings from 50 *de novo* cases of ANLL in Argentina, South America, studied at diagnosis, as well as their relation with the FAB classification.

Correspondence: Dra. Susana Acevedo, Departamento de Genética, División Citogenética, Instituto de Investigaciones Hematológicas "Mariano R. Castex", Academia Nacional de Medicina, Pacheco de Melo 3081, 1425 Buenos Aires, Argentina.
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Patients and methods

Fifty patients with newly diagnosed ANLL, 17 of whom were children with a median age of 5 years (range 9 months-15 years) and 33 adults with a median age of 41 (range 18-76) years, were studied between 1990 and 1992.

In the present report we included only those patients who fulfilled the following requirements: unequivocal diagnosis of ANLL according to FAB morphologic and cytochemical criteria (M0-M7)[1-3] and successful cytogenetic analysis at diagnosis. Patients did not have a previous history of exposure to myelotoxic agents. Children with Down's syndrome and secondary ANLL were excluded from this analysis.

Bone marrow (BM) samples and a few cases of unstimulated peripheral blood (PB) taken at the time of diagnosis were studied. Heparinized BM or PB samples were cultured in F-10 medium supplemented with 15% fetal calf serum for 19-24 hours at 37°C. Cultures were harvested after overnight exposure to Colcemid (final concentration 1 μg/mL) and exposed to a hypotonic solution (KCl 0.075 M) for 30 min at room temperature. Afterward, cells were fixed twice in methanol:acetic acid (3:1) for 30 min. Chromosome slides were prepared by the air-drying method. Cytogenetic analysis was performed with a trypsin-Giemsa banding technique.[15] Only those cases with adequate metaphases (10-36) for cytogenetic analysis were included in this paper. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature.[16]

Results

The principal karyotypic findings are shown in Table 1. Of the 50 banded cases studied, 11 (22%) had normal karyotypes and the remaining 39 (78%) presented abnormal metaphases with structural alterations in the majority of them. The most common abnormalities were t(15,17) in 20% of the cases, inv(16) in 16% and del(7q) or –7 in 12% (6% each). Translocations t(8;21)(q22;q22) was observed in two cases with M2; inv(16) was a very common finding (especially in M4) in both pediatric and adult groups, although this marker was not restricted to this subtype. Translocation t(15;17)(q22; q11) was only seen in M3. Among miscellaneous clonal chromosomal alterations, the following markers were included: 5p– (two), 4q+ (two); moreover, t(2;3) was observed in 3 cases with the M4 subtype (one pediatric and two adult), but the breakpoints were different in each patient.

All cases studied, along with age/sex, FAB classification, chromosome category and karyotype, are shown in Table 2. The modal chromo-

Table 1. Percentage of aberrations in the different FAB subtypes.

Karyotypic abnormality	*patients*		*FAB subtypes*							
	no.	*(%)*	*M0*	*M1*	*M2*	*M3*	*M4*	*M5*	*M6*	*M7*
none	11	(22)	1	–	1	–	6	2	1	–
inv 16	8	(16)	–	–	–	–	7	–	–	1
t(8;21)	3	(6)	–	–	2	–	1	–	–	–
t(15;17)	9	(18)	–	–	–	9	–	–	–	–
t(11;V) or del(11q)	2	(4)	–	–	–	–	2	–	–	–
del(7q) or -7	6	(12)	1	–	1	–	2	1	–	1
miscellaneous clonal	11	(22)	1	1	1	–	6	1	–	1
Total	50	(100)	3	1	5	9	24	4	1	3

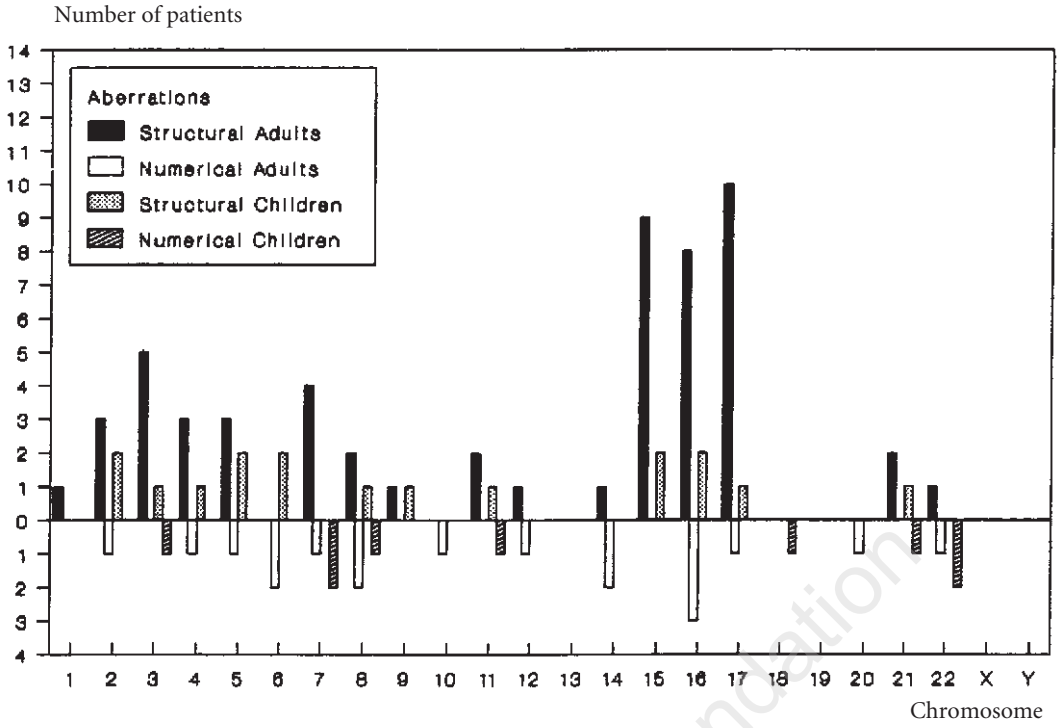

Figure 1. Numerical and structural aberrations in adults and children with ANLL.

some number was diploid in almost all patients. Thirty-five percent of children and 15% of adults had normal karyotypes. The highest proportion of abnormal karyotypes was observed in M3 and M4. We also found a high frequency of clonal abnormalities in M2 and M7, but the number of patients was too low to draw any conclusions.

Both numerical and structural abnormalities are shown in Figure 1.

Chromosomes 7 and 22 were most frequently involved in numerical alterations in children, while chromosomes 6, 8, 14 and 16 were most often involved in adults. Considering structural abnormalities, the chromosomes most frequently involved in children were 2, 5, 6, 15 and 17, while chromosomes 3, 7 15, 16 and 17 were those involved most in adults.

Discussion

Cytogenetic studies were performed in 50 untreated cases of *de novo* ANLL. The total incidence of clonal chromosomal abnormalities was 78%. A large range in the percentage of cytogenetic alterations has been described for this pathology: from 50% with the usual banding techniques[17] to more than 90% with the use of high resolution methodologies.[18-22]

When the two patient populations were separated, we observed 65% clonal abnormalities in children and 85% in adults; these values are in agreement with previous reports.[17,23]

Consistent chromosomal rearrangements were observed in these patients and they were linked to specific cytomorphologic subsets. These findings allow identification of categories of recurrent chromosomal anomalies in ANLL, and confirm the significance of karyotype as an independent prognostic factor.[12]

The translocations t(8;21) and t(15;17) were seen only in M2 and M3, respectively. The inversion of chromosome 16, inv(16), was a typical finding in M4, but was not retricted to this subtype. In the present study, we report a patient with M7 subtype who showed inv(16). Although this is a very unusual finding, it has already been described.[24] The presence of these chromosomal markers is in agreement with those reported in the literature from the USA and Western Europe.[25]

T(2;3) was observed in three cases, all M4,

Table 2. Chromosomal findings in patients with ANLL.

Patients	*Age/Sex (yrs)*	*FAB*	*Chromosome category*	*Karyotype*
children				
1	2/F	M0	NN	46,XX
2	7/M	M1	AN	46,XY/47,XY,+C
3	4/F	M2	AA	45,XX,-3,iso(11q),del(5)(p13), del(15)(q22)
4	8/M	M2	NN	46,XY
5	15/F	M3	AN	46,XX,t(15;17)(q22;q11)/46,XX
6	5/M	M4	AA	46,XX,t(2;3)(q21;q21)
7	2/M	M4	AN	45,XX,5q+,t(8;21)(q22;q22),-4/46,XX
8	3/M	M4	AN	46,XY,inv(16)(p13q22)/46,XY
9	2/M	M4	NN	46,XY
10	6/M	M4	AN	46,XY,inv(16)(p13q22)/46,XY
11	1.5/F	M4	NN	46,XX
12	5/M	M4	AN	46,XY/47,XY,+8
13	6/M	M4	NN	46,XX
14	0.75/M	M5	AA	45,XY,-7,+21,-22/46,XY, del(6)(q15)/46,XY,del(2)(p23)
15	8/M	M6	NN	46,XY
16	6/M	M7	AN	44,XY,-18,-22/46XY
17	4/F	M7	AA	45,XX,del(6)(q21),-7,del(9)(q12) -11
adults				
1	20/M	Mo	AN	46,XY,t(15;?)(q22;?)/46,XY
2	53/M	Mo	AA	46,XY,del(1)(q32)/46,XY del(7)(q32)
3	65/F	M2	AN	46,XX,t(8;21)(q22;q22)/46,XX
4	23/F	M2	AA	46,XX,t(8;21)(q22;q22)/46,XX, del(7)(q32)
5	37/F	M2	AA	46,XX,del(7)(q32)
6	47/M	M3	AN	46,XY,t(15;17)(q22;q11)/46,XY
7	76/M	M3	AN	46,XY/46,XY,inv(16)(p13q22)/ 46,XY,t(15;17)(q22;q11)
8	57/F	M3	AN	46,XX/47,XX,t(15;17)(q22;q11), +10
9	47/M	M3	AN	46,XY,t(15;17)(q22;q11)/46,XY
10	24/F	M3	AN	46,XX,t(15;17)(q22;q11)/46,XX
11	28/M	M3	AN	46,XY,t(15;17)(q22;q11)/46,XY
12	34/F	M3	AN	46,XX,t(15;17)(q22;q11)/46,XX
13	52/M	M3	AA	46,XY,t(3;5),t(15;17)(q22;q11)
14	35/F	M4	AN	45,XX,-6,inv(16)(p13q22)/46,XX
15	20/F	M4	AN	46,XY,inv(3)(q21q26)/46,XY,del(12)(p21p23),i(16p)
16	21/F	M4	AN	46,XX,4q+,-8,+16/46,XX
17	44/M	M4	AN	46,XY,4q+/46,XY
18	76/F	M4	AA	46,XX,-2,-4,-5,-6,+t (2;?;11)(q11;?;p15),-12,+14,+16,-17,+17p+,+20,+22
19	35/F	M4	AN	46,XX,inv(16)(p13q22)/46,XX
20	33/M	M4	AN	46,XY,del(7)(q22)/46,XY
21	38/F	M4	AN	46,XX,t(2;3)(q23;q25), inv(17)(q11p13)/46,XX
22	34/F	M4	AN	46,XX,-7,+8/46,XX
23	69/M	M4	NN	46,XY
24	55/F	M4	AA	46,XX,t(2;3)(q31;q21),t(9;22) (q34;q11)/46,XX,inv(16)(p13q22)
25	18/M	M4	AA	100% unspecific aneuploidy
26	19/M	M4	AN	45,XY,-16,del(11)(q23),46,XY inv(16)(p13q22),del(3)(p25)/46,XY
27	46/F	M4	AN	46,XX,inv(16)(p13q22)/46,XX
28	59/M	M4	NN	46,XY
29	34/F	M4	NN	46,XX
30	32/M	M5	NN	46,XY
31	32/F	M5	NN	46,XX
32	44/M	M5	AA	45,XY,del(5)(p13),-14
33	64/M	M7	AN	46,XY,t(4;5)(q21;q31),del(14)(q22)inv(16)(p13q22)/46,XY

each one with a different breakpoint. Although this translocation has already been reported, the breakpoints and arms involved were different with respect to our cases.[26]

The chromosomes most frequently involved in losses were 7 and 22 in children, and 6 and 7 in adults. These results are in accordance with previous series.[17, 23, 27]

In general, children displayed morphologic and cytogenetic characteristics similar to those of adults.[28, 29] Our data demonstrated that the incidence of chromosomal findings was similar in both populations for markers such as t(8;21), t(2;3) and –7/7q–, while inv(16) and t(15;17) were seen in adults more frequently.

It is important to notice that structural alterations involving chromosome 7 were not seen in our pediatric patients, who presented only monosomy 7. On the contrary, adults showed greater incidence of structural alterations involving this chromosome. Large series published in the literature reveal similar results;[17, 23] miscellaneous clonal aberrations were observed in all subtypes of ANLL.

The incidence of t(15;17) and –7/7q–, which we found in a considerable number of patients, is comparable to the incidence of these aberrations reported in different geographic regions.[25] We observed a high proportion of t(15:17) (90%) in M3, similar to what has been reported in Western Europe and the USA. The incidence of –7 (6%) and t(8;21) (40%) in our series was in accord with that found in Western Europe. The low number of cases with other subtypes did not allow us to draw any other conclusions.

Comparing the frequencies of cytogenetic alterations in series of patients with the same diagnosis, Johanson[25] found a significant geographic heterogeneity in neoplasias associated with chromosomal aberrations. Our findings show that Argentina presents similarities with Western Europe and the USA.

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