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CORRESPONDENCE

Clinical features of childhood acute myeloid leukaemia with specific gene rearrangements

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TO THE EDITOR

Specific gene rearrangements seem to distinguish distinct subsets of acute myeloid leukaemia (AML) with different features and prognosis, and some reports suggest that the epidemiological distribution of AML could vary among countries.[1,2] To date, cytogenetic examination has been used to study the frequency of these genetic alterations in a large series of children[3–5] with AML; nevertheless, in a proportion of cases, these gene rearrangements may be cryptic and undetectable by conventional cytogenetic techniques.[6,7] To evaluate the frequency of specific gene rearrangements, the corresponding clinical morphological features at diagnosis and the potential prognostic impact on patients' long-term survival, we screened by RT-PCR five different chimaeric transcripts (AML1-ETO, CBFβ-MYH11, PML-RARα, MLL-AF9 and BCR-ABL) in a series of 270 Italian children with AML, treated with AIEOP-LAM 87–92, BFM 83-93 and AIDA protocols between 1988 and 1998, and whose RNA were available and morphology had been centrally reviewed.

Of the cases, 45% were positive for one fusion gene. The frequency of AML1-ETO, CBFβ-MYH11, PML-RARα and MLL-AF9 were 14, 6.3, 20 and 4%, respectively, while BCR-ABL was observed in only two cases. The frequency of AML1-ETO, MLL-AF9 and CBFβ-MYH11 found was comparable with those of t(8;21), t(9;11) and inv(16) reported previously, suggesting that these gene rearrangements do not have geographic heterogeneity. On the contrary, PML-RARα-positive AML represented 20% of our cohort. This is a higher frequency than that reported in other studies of children from Northern Europe or the United States, but the increased frequency of promyelocytic leukaemia in Italian AML children has already been described.

Cytogenetic analysis was successfully carried out in 153 patients, while it failed or was not performed in 117 patients. Abnormalities were identified in 90/153 (59%), the frequency of t(8;21), inv(16), t(15;17) and t(9;11) was 9.8, 2, 17 and 2%, respectively; the 63 remaining cases (41%) had a normal karyotype. In the t(8;21)-positive AML cases, the most common associated alterations involved chromosome 9. Among patients with normal karyotype, RT-PCR identified 17/63 (27%) chimaeric transcripts, and six further cases with translocations undetected by cytogenetics were found among the 43 children reported to have an abnormal karyotype. Considering t(8;21), inv(16), t(15;17) and t(9;11) together, 130/153 (85%) patients, who had a successful analysis were correctly classified cytogenetically. Then, in our experience, cytogenetics showed comparable specificity (100%), but lower sensitivity (67%) than RT-PCR. A general agreement exists in considering RT-PCR more sensitive than conventional cytogenetics in identifying specific gene rearrangements and the fusion genes studied have been described in the absence of identifiable translocations. Nevertheless, the molecular technique is not capable of identifying associated abnormalities. Therefore, since the genetic studies in childhood AML allow for the identification of patient subgroups who probably can benefit from a more tailored therapy and the fusion gene identified can represent a useful target for the minimal residual disease study, ideally all cases should be studied by RT-PCR and cytogenetics: two complementary methods in the genetic characterisation of leukaemia.

Clinical data collected included FAB subtypes, gender, age, WBC count at diagnosis and extramedullary involvement, whenever available (Table 1). The low number of M7-AML was due to the scarce number of cells collected in megakaryoblastic leukaemia.

The median age at diagnosis for all patients was 7.8 (range 0–19.5). Cases with AML1-ETO and PML-RARα fusion genes were older patients (median age 8.2 and 9.5 years, respectively), while the median age of patients with CBFβ-MYH11 and MLL-AF9 was 6.7 and 3.2 years, respectively.

A strong, although not exclusive, association between M3, M4 and M5 FAB subgroups and the presence of PML-RARα, CBFβ-MYH11 and MLL-AF9 gene rearrangements, respectively, was found. The AML1-ETO-positive cases represented 43% of M2 AML; however, this gene rearrangement was frequently found in M1 patients as well. As a whole, 92% (34/37) of AML1-ETO-positive AML were classified as FAB-M1 or FAB-M2.

The median WBC count at diagnosis was significantly higher in CBFβ-MYH11-positive cases, while PML-RARα- and AML1-ETO-positive cases had the lowest values. Also, among M2 and M1 plus M2 FAB subgroups this value was significantly lower in AML1-ETO-positive AML than in negatives (16 000/μl *vs* 30 800/μl, $P=0.027$; 16 000/μl *vs* 34 200/μl, $P=0.0005$).

Extramedullary disease involved the liver (41%), spleen (37%), node (15%), CNS (8%) and other sites (9%). The FAB-M3 subtype and the presence of PML-RARα or AML1-ETO fusion genes were significantly associated with low frequency of extramedullary involvement ($P=0.0002$, $P=0.002$ and $P=0.0047$, respectively), while a correlation with the presence of extramedullary disease was found with the presence of MLL-AF9, M4 and M5 FAB subgroups and a WBC count at diagnosis higher than 20 000/μl ($P=0.024$, $P=0.017$, $P=0.018$ and $P=0.046$, respectively). CNS involvement was found in 19 patients and, in five of them, was the sole extramedullary site involved. A significant correlation was found only between CNS disease and a WBC count of $\geqslant$50 000/μl at diagnosis ($P=0.002$).

In our series, the genetic subgroups showed a characteristic clinical and morphological profile with regard to FAB subtype, age distribution, WBC count at diagnosis, extramedullary

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Table 1 Presenting clinical and biological features

	Cohort		WBC × 10^3 μl[a]		ED[b] (CNS pos)	
	n	%	Median[#] [^]	Range	%	%
Total	270	100	22.8	0.8–495	56.7	(8.0)
Sex						
Female	126	47	25.9	1.4–495	56.8	(9.6)
Male	144	53	17.4	0.8–340	56.7	(6.4)
Age (years)						
⩽1	22	8	29.3	2.9–190	60.0	(15.8)
>1⩽5	65	24	25.1	1.8–495	64.6	(6.1)
>5⩽10	85	31	24.5	0.8–425	45.1	(7.0)
>10⩽15	86	32	22.2	1–342	58.7	(9.3)
>15	12	4	11.3	1.9–41.2	41.7	(—)
FAB subgroup						
M0	12	4.5	32.0	11.7–264	70.0	(10.0)
M1	39	14.5	44.7	1–400	63.0	(7.4)
M2	58	21.5	21.6	1.4–322	50.0	(7.7)
M3	54	20	5.6[#]	0.8–290	34.0	(2.0)
M4	43	16	48.7[^]	3–425	70.7	(15.0)
M5	57	21	29.9	1.8–495	71.1	(9.6)
M6	4	1.5	NE	NE	NE	
M7	3	1	NE	NE	NE	
Gene rearrangement						
AML1-ETO	37	14	16.0[^]	1.5–329	39.3	(7.1)
CBFβ-MYH11	17	6.3	51.4[#]	11.2–220	60.0	(14.3)
PML-RARa	54	20	5.6[#]	0.8–290	38.2	(2.0)
MLL-AF9	11	4	16.7	1.8–400	91.0	(18.0)
BCR-ABL	2	0.7	NE	NE	NE	
Negative	149	55	32.2	1.0–495	65.3	(6.3)

ED = extramedullary disease; NE = not evaluable.
[a]Percentages calculated on 252 pts. for whom data were available.
[b]Percentages calculated on 236 pts. for whom data were available.
[c]Median sign test significant: [^]$P<0.05$ and [#]$P<=0.001$. Control group was total population.

disease and CNS involvement. PML-RARα-positive cases showed a strong association with the FAB-M3 subtype, a progressive increase of frequency with age, a low WBC count and only occasional extramedullary and CNS involvement. AML1-ETO-positive leukaemia demonstrated a peculiar distribution with respect to specific age groups, with the highest incidence between 5 and 10 years, and a close, but not exclusive, correlation with the FAB-M2 subtype. Furthermore, a moderate increase of the WBC count at diagnosis seems to be a typical feature of t(8;21) AML; indeed, in our patients the median WBC count was 16 000/μl, similar to values reported by other authors, while, unlike the results of other studies, we did not observe an increased frequency of CNS involvement in this group. CBFβ-MYH11 AML were associated with the FAB-M4 subtype, even if occasionally found in M2 and M5 AML, and were characterized by a high WBC count at diagnosis with frequent CNS involvement. MLL-AF9-positive AML was the group with the highest percentage of infants and the lowest median age; these cases often showed extramedullary disease and CNS involvement even though the median WBC count at diagnosis was <20000/μl.

Complete remission was achieved in 84.5% of cases. In patients with AML positive for AML1-ETO, CBFβ-MYH11, MLL-AF9 and PML-RARα, the CR rate was 91, 87.5, 91, and 84.3%, respectively. The lowest CR rate was observed in patients less than ⩽1 year (63%, $P=0.007$) and when the WBC count at diagnosis was higher than 50 000/μl (76.9%, $P=0.029$).

Univariate and multivariate survival analyses were carried out on a series of 200 patients, excluding secondary AML, Down syndrome and promyelocytic leukaemia since specific protocols, including ATRA, were implemented to treat APL over the years. Variables considered in univariate analysis were: gender, extramedullary disease, CNS involvement, presence of AML1-ETO/CBFβ-MYH11 gene rearrangements, FAB subtypes, median WBC count at diagnosis ⩽20 000/μl or >20 000/μl and age ⩽1 year or >1 year. The significant prognostic factors for both EFS and OS are listed in Table 2. Owing to the scarce number of cases and the presence of a case with Down syndrome, the MLL-AF9 gene rearrangement was not considered in the survival analysis; nevertheless, in our series 54.5% of the patients were still alive in remission. Multivariate analysis (Table 3) confirmed that the absence of the AML1-ETO and CBFβ-MYH11 gene rearrangements, a WBC count of >20 000/μl at diagnosis and an age ⩽1 year are independent unfavourable prognostic factors. A general consensus exists in considering CBFβ-MYH11-positive AML as a group characterised by better CR and better survival rates than other AML, in spite of hyperleucocytosis at diagnosis and CNS involvement, while conflicting

Table 2 Univariate survival analysis: significant variables

Variable	Pts.	Overall survival		Event-free survival	
		4 years % value (s.e.)	Log-rank P-value	4 years % value (s.e.)	Log-rank P-vaue
All patients	200	45.3 (3.6)	—	41.0 (3.6)	—
AML1ETO+CBFβ-MYH11 positive	52	58.4 (7.0)	0.024	54.7 (7.3)	0.029
AML1ETO+CBFβ-MYH11 negative	148	41.0 (4.2)		36.4 (4.0)	
WBC ⩽20000/μl	74	59.5 (5.9)	0.0038	53.5 (6.0)	0.0038
WBC >20000/μl	116	37.4 (4.6)		33.8 (4.5)	
Age ⩽1 year	19	25.3 (10.2)	0.0058	21.0 (9.3)	0.002
Age >1 year	181	47.5 (3.8)		43.2 (3.8)	

Table 3 Multivariate analysis by Cox's model

Variable	Overall survival			Event-free survival		
	Hazard ratio	95% hazard ratio confidence limits	P-value	Hazard ratio	95% hazard ratio confidence limits	P-value
AML1-ETO and CBFβ-MYH11 neg.	1760	1.056–2.933	0.030	1662	1.012–2.732	0.045
WBC >20 000/μl	1793	1.181–2.721	0.006	1725	1.148–2.592	0.009
Age ⩽1 year	1860	1.035–3.344	0.038	1941	1.101–3.422	0.022

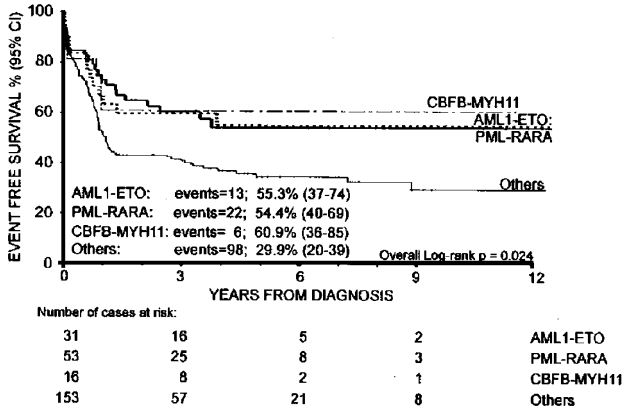

Figure 1 Event-free survival. Patients by molecular markers.

data exist with regard to AML1-ETO-positive AML. In these genetic subgroups, an improvement of the outcome in patients treated with high-dose cytarabine has been described.[8] Therefore, the poor outcome occasionally reported might be related to the absence of high-dose cytarabine in the treatment regimens. In our series, many patients received high-dose cytarabine and, thus, the favourable outcome observed in patients with AML1-ETO- and CBFβ-MYH11-positive AML could depend on the treatment received.

Finally, we performed long-term survival analysis to evaluate the events time distribution considering that the main cause of failure in AML was relapse (Figure 1). A time interval from diagnosis of 18 months or longer was significantly more frequent among AML lacking AML1-ETO and CBFβ-MYH11 transcripts than in positive cases (12.3 *vs* 2%, respectively, $P=0.02$). As a consequence, the survival probability showed a progressive decrease in the first group of patients so that their corresponding 10-year survival probability was $31.7\pm4.8\%$. On the contrary, patients with AML1-ETO- and CBFβ-MYH11-positive AML achieved a plateau at 4 years for both EFS and OS. Among infants, all events occurred within 14 months from diagnosis and were mainly due to induction death. Thus, the presence of early relapses seems to distinguish the AML1-ETO/CBFβ-MYH11-positive cases from the negative ones. These data have been not previously reported and suggest that the molecular study of minimal residual disease, performed during the first 2 years from diagnosis, could allow for the timely identification of early events in AML with rearrangement of the core binding factor genes AML1 and CBFβ, since relapses are the main cause of failure in these patients. In addition our study suggests that a long-term follow-up could be necessary to evaluate the true probability of survival in patients at high risk of late relapse.

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References

1 Forestier E, Heim S, Blennow E, Borgstrom G, Holmgren G, Heinonen K *et al.* Cytogenetic abnormalities in childhood acute myeloid leukaemia: a Nordic series comprising all children enrolled in the NOPHO-93-AML trial between 1993 and 2001. *Br J Haematol* 2003; **121**: 566–577.
2 Biondi A, Rovelli A, Cantù-Rajnoldi A, Fenu S, Basso G, Luciano A *et al.* Acute promyelocytic leukaemia in children: experience of the Italian Pediatric Hematology and Oncology Group (AIEOP). *Leukemia* 1994; **8**: 1264–1268.
3 Martinez-Climent JA, Lane NJ, Rubin CM, Morgan E, Johnstone HS, Mick R *et al.* Clinical and prognostic significance of chromosomal abnormalities in childhood acute myeloid leukemia *de novo*. *Leukemia* 1995; **9**: 95–101.
4 Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G *et al.* The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into MRC 10 trial. *Blood* 1998; **92**: 2322–2333.
5 Raimondi SC, Chang MN, Ravindranath Y, Behm FG, Gresik MV, Steuber CP *et al.* Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative Pediatric Oncology Group Study – POG8821. *Blood* 1999; **94**: 3707–3716.
6 Krauter J, Peter W, Pascherberg U, Heinze B, Bergmann L, Hoelzer D *et al.* Detection of karyotypic aberrations in acute myeloblastic leukaemia: a prospective comparison between PCR/FISH and standard cytogenetics in 140 patients with *de novo* AML. *Br J Hematol* 1998; **103**: 72–78.
7 Rowe D, Cotterill SJ, Ross FM, Bunyan DJ, Vickers SJ, Bryon J *et al.* Cytogenetically cryptic AML1-ETO and CBFβ-Myh11 gene rearrangements: incidence in 412 case of acute myeloid leukaemia. *Br J Haematol* 2000; **111**: 1051–1056.
8 Byrd JC, Dodge RK, Carrol A, Baer MR, Edward C, Stamberg J *et al.* Patients with t(8;21)(q22;q22) and acute myeloid leukaemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *J Clin Oncol* 1999; **17**: 3767–3775.