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Relevance of presenting white blood cell count and kinetics of molecular remission in the prognosis of acute myeloid leukemia with CBFβ/MYH11 rearrangement

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ABSTRACT

Background and Objectives. **The detection of CBFβ/MYH11 transcripts by RT-PCR has became a valuable and widely used technique in the accurate cytogenetic and molecular classification of acute myeloid leukemia (AML), but the clinical value of RT-PCR for monitoring minimal residual disease (MRD) during follow-up remains unclear.**

Design and Methods. **We analyzed the factors predicting relapse and the value of MRD monitoring by RT-PCR in a series of 16 patients with CBFβ/MYH11-positive AML (15 M4Eo; 1 M4). Fifteen were newly diagnosed cases (CR1) and one was studied after first relapse (CR2). Eight patients had clinical relapse 6 to 19 months after the achievement of CR.**

Results. **Presenting WBC count had a significant prognostic influence on disease-free survival (*p*=0.001). All four patients with a WBC count >100x10⁹/L relapsed, while only four additional relapses occurred among the eleven patients who had an initial WBC count below 100x10⁹/L. With regards to molecular monitoring, all relapses but one occurred in patients who showed persistent RT-PCR positivity during hematologic remission. By contrast, conversion to a repeatedly PCR-negative status was observed in the seven patients who remained in CR1 after a median follow-up of 48 months (range 31-79 months), as well as in the transplanted patient who was monitored in CR2. In these patients PCR-positivity could be detected up to 24 months after diagnosis (median time to conversion to PCR-negative: 8 months).**

Interpretation and Conclusions. **In conclusion, marked hyperleukocytosis (>100x10⁹/L) confers poor prognosis to the patient with CBFβ/MYH11-positive AML. In addition, slow kinetics of molecular remission was observed in this subset of AML, but the CBFβ/MYH11 fusion transcript is no longer detectable in long-term survivors, indicating that molecular remission is an important therapeutic goal.**
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Key words: acute myeloid leukemia, CBFβ/MYH11, MRD

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The pericentric inversion of chromosome 16 [inv(16)(p13q22)] and the translocation t(16;16)(p13;q22) are karyotypic rearrangements strongly correlated with the acute myeloid leukemia (AML) subtype M4Eo, and occasionally described in other myeloid malignancies, including AML M2, M4 without eosinophilia, M5, myelodysplastic syndromes, and blast crisis of chronic myelogenous leukemia.[1-3] In patients with AML, presence of this abnormality in leukemic blasts at diagnosis has been associated with prolonged disease-free survival and a relatively favorable outcome.[2,4-6]

Recent cloning of the 16q and 16p breakpoints allowed the identification of two genes, the core binding factor β (CBFβ) and the smooth muscle myosin heavy chain (MYH11) genes, which are fused into a CBFβ/MYH11 hybrid gene as a result of inv(16) or t(16;16).[7] Depending on distinct breakpoint locations, ten types of fusion transcripts (A-J) have been described,[8] with the so-called A form accounting for more than 90% of cases.[9]

Several studies have shown that reverse transcriptase-polymerase chain reaction (RT-PCR) allows refined diagnosis at the molecular level and sensitive monitoring of residual disease in AML patients with this abnormality. However, because only small series have been analyzed to date, the clinical value of RT-PCR monitoring in this particular subset remains unclear.[3,9-18] In the present study, we analyze factors predicting relapse, and the significance of RT-PCR monitoring of minimal residual disease (MRD) in a series of 16 patients with CBFβ/MYH11-positive AML.

Design and Methods

Patients

Sixteen patients with CBFβ/MYH11-positive AML diagnosed and treated in four Spanish hospitals between 1995 and 1999 are included in this study. Fifteen were newly diagnosed cases and one was studied after first relapse. The series included all patients with molecularly documented CBFβ/MYH11-positive AML in whom follow-up PCR tests were performed. The main clinico-biological features including FAB classification, karyotype and CBFβ/MHY11 transcript type in individual patients, together with type of treatment and clinical outcome, are reported in Table 1.

Induction treatment consisted in all cases of standard 7+3 combinations of cytosine arabinoside and anthracycline (daunorubicin or idarubicin) with or without etoposide. Post-remission therapy included high-dose cytosine arabinoside (HDARAC), in 9 patients, or standard consolidation chemotherapy, in the remaining 6 patients. Seven of the patients underwent an autologous peripheral blood stem cell transplantation (PBSCT) and one an allogeneic bone marrow transplantation. HDARAC consisted in 1-3 cycles of cytosine arabinoside, 1-3 g/m^2 x 3 days. Intensification with HDARAC was occasionally combined with idarubicin (12 mg/m^2 x 3 days) or mithoxantrone (12 mg/m^2 x 3 days). The patient (case #16) studied in second complete remission (CR2) had been initially treated with cytosine arabinoside, daunorubicin and etoposide for induction and consolidation followed by autologous PBSCT with BUCY4 as conditioning regimen. The patient relapsed nine months later and was then treated with BAVC[19] followed by unpurged autologous BMT, using the marrow harvested as back-up at the time of first complete remission (CR1).

RT-PCR studies

Bone marrow samples were collected for molecular studies at diagnosis, after completion of induction, after consolidation and during follow-up. RNA was extracted by the guanidium-thiocyanate/phenol-chloroform method of Chomczynsky and Sacchi.[20] One microgram of total RNA was reverse transcribed using 200 U MMLV reverse transcriptase (Promega, Madison, WI, USA), 0.5 µg of random primers, 20 units of RNAasin and 0.5 mM dNTP in a final volume of 25 µL made up in MMLV buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$. Following RNA denaturation at 70ºC for 5 min, the reverse transcription was carried out at 42ºC for 60 min, and MMLV reverse transcriptase was finally inactivated by heating at 95ºC for 5 min.

For the CBFβ/MYH11 amplification, the laboratories at Hospital Nuestra Señora del Pino (Gran Canaria), Hospital Universitario La Fe (Valencia) and Hospital Sant Pau (Barcelona) employed the nested PCR method described by Poirel *et al*.[12] while the laboratory at Hospital Clínico (Salamanca) followed the guidelines of the *BIOMED 1 concerted action for standardization of MRD in acute leukemias*.[21] The four laboratories showed a similar sensitivity level that allowed detection of the rearrangement in a 10^{-5} dilution of RNA from a patient at diagnosis in RNA from an AML case without CBFβ/MYH11 rearrangement. Both methods allowed identification of the different breakpoints described for such rearrangements.

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Table 1. Clinical and biological characteristics of patients at presentation and treatment outcome.

Pt.	Gender /age	WBC (x10^9/L)	FAB	Cytogenetics	RT-PCR transcript	Induction treatment	Consolidation	Time PCR + (months)	Clinical outcome (months)
Patients in CR1									
1	M/44	173	M4Eo	ND	A	DA	DA	6	Relapse, 6/Death, 8
2	M/60	26	M4Eo	inv(16)(p13;q22)	A	DAE	DAE	7	Relapse, 7/Death, 9
3	F/52	214	M4Eo	del(7)(q22), inv(16)(p13;q22), t(11;13)(q23;q12)	A	IA/CNS	MA + ABSCT	9	Relapse, 9/Death, 11
4	M/32	187	M4	inv(16)(p13;q22)	A	DA	DA + HDARAC [2] + ABSCT	12	Relapse, 12/Alive, +19
5	M/22	59	M4Eo	11p+, inv(16)(p13;q22)	A	IAE	HDARAC [2]	5	Relapse, 13/Alive, +33
6	F/33	175	M4Eo	del(16)(q22)	A	IA	HDARAC [1] + ABSCT	15	Relapse, 15/Death, 21
7	F/14	16	M4Eo	ND	A	IA	MA	19	Relapse, 19/Alive, +21
8	F/42	5	M4Eo	inv(16)(p13;q22)	A	IAE	HDARAC [2]	19	Relapse, 19/Alive, +23
9	F/41	23	M4Eo	inv(16)(p13;q22)	C	IAE	HDARAC [3]	3	CCR, +31
10	F/10	70	M4Eo	inv(16)(p13;q22)	A	DA	HDARAC [2] + ABSCT	18	CCR, +33
11	M/51	70	M4Eo	t(16;16)(p13;q22)	A	DA	IA	9	CCR, +42
12	M/67	2	M4Eo	inv(16)(p13;q22)	A	IA	HDARAC [2]	1	CCR, +48
13	M/30	19	M4Eo	ND	A	DAE	DAE + Allogeneic BMT	7	CCR, +64
14	F/34	15	M4Eo	ND	A	IA	IA + HDARAC [1] + ABSCT	8	CCR, +59
15	M/60	12	M4Eo	Normal	A	DAE	DAE + HDARAC [1] + ABSCT	24	CCR, +79
Patient in CR2									
16	M/32	97	M4Eo	ND	A	Autologous BMT		6#	CCR, +61

A= cytosine arabinoside; D= daunorubicin; I= idarubicin; E= etoposide: M= mithoxantrone; CNS= central nervous system prophylaxis; HDARAC = high dose cytosine arabinoside [Nº cycles]; BMT= bone marrow transplantation; ABSCT= autologous peripheral blood stem cell transplantation. #from autologous BMT.

Cytogenetics

Cytogenetic analyses were performed according to standard methods. Chromosomal abnormalities were described according to the International System for Human Cytogenetics Nomenclature.[22]

Statistical methods

Unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate,[23] log-rank tests and their generalizations.[24]

Results

Table 1 summarizes clinico-biological features of patients at presentation and treatment outcome. The patient's median age was 37 years (range 10-67) and their median WBC count was 41×10^9/L (range 2-214). According to the FAB classification, 15 cases were defined as M4Eo and 1 as M4 without eosinophilia. Ten of 11 patients with evaluable karyotypes had chromosome 16 abnormalities. This was the sole chromosome aberration in 8 cases [inv(16)(p13;q22), 6 patients, t(16;16)(p13;q22), one and del(16)(q22), one patient], whereas in two patients inv(16) (p13;q22) was associated with either del(7)(q22) and t(11;13), or with 11p+. The other patient had an apparently normal karyotype. Except for one patient who showed the type C fusion (case #9), all other cases had the type A CBFβ/MYH11 transcript.

As of October 1999, eight patients had clinical relapse at 6 to 19 months from the achievement of CR. Presenting WBC count had a significant prognostic influence on disease-free survival (p=0.001). As shown in Figure 1, the most discriminant cut-off value was 100×10^9/L. In fact, all four patients with WBC count >100×10^9/L relapsed, while only four additional relapses occurred among the eleven patients who had an initial WBC count below 100×10^9/L. At present, four of the eight relapsed patients remain alive and well in second continuous complete remission. This was obtained with second-line chemotherapy (FLAG-Ida, one patient), anti-CD33 (one patient) or allogeneic bone marrow transplantation (two patients).

With regards to molecular monitoring, all relapses but one (case #5) occurred in patients who showed persistent RT-PCR positivity during hematologic remission. By contrast, conversion to a repeatedly PCR-negative status was observed in the seven patients who remained in CR1 after a median follow-up of 48 months (range 31-79 months), as well as in the transplanted patient who was monitored in CR2. In this series of patients who finally converted to being PCR-negative and remained in continuous CR, PCR-positivity could be detected up to 24 months after diagnosis (median 7.5 months, range 1-24 months) (Figure 2).

Three out of six patients who underwent autologous PBSCT (ABSCT) relapsed at 3, 6 and 9 months and they had all tested persistently RT-PCR positive prior to hematologic relapse. The remaining three autografted patients and one additional patient who was transplanted from an HLA-identical sibling remain in continuous CR and are RT-PCR negative at +33, +59, +64 and +79 months. After transplantation, these patients obtained PCR negativity at 3, 5, 13 and 17 months, respectively. The patient studied at relapse (case #16) converted to being PCR negative 6 months after unpurged autologous BMT and presently remains in second complete remission at +61 months.

Discussion

This study shows that, in patients with CBFβ/MYH11-positive AML, marked hyperleukocytosis (WBC count above 100×10^9/L) is a powerful prognostic factor of relapse, and that molecular monitoring of minimal residual disease (MRD) provides additional information in order to predict a patient's outcome.

WBC count is a well-known prognostic factor in all types of acute leukemia. However, apart from acute promyelocytic leukemia, there is scarce information on the prognostic influence of this presenting feature

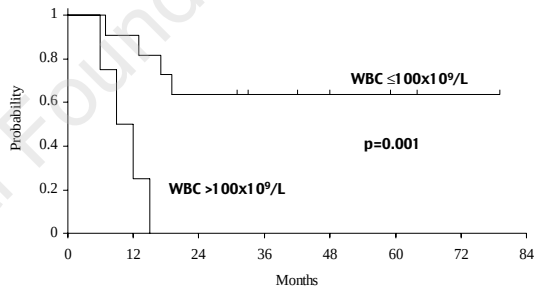

Figure 1. Disease-free survival according to presenting WBC count.

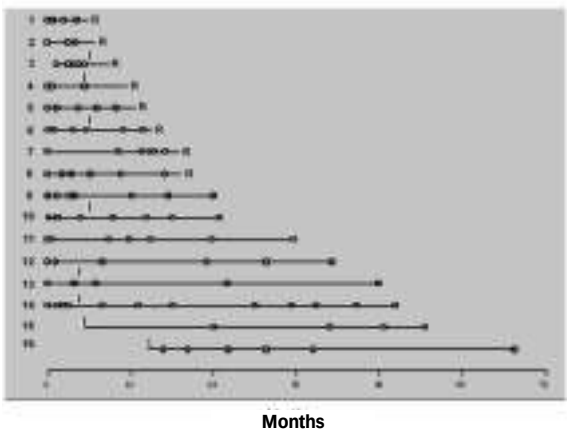

Figure 2. Detection of residual disease by RT-PCR in bone marrow samples. ● RT-PCR positive result; ❍ RT-PCR negative result; arrow: PB or BM transplantation; R: relapse.

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in the particular setting of the so-called *good prognosis* AMLs. These latter, which include t(8;21) and inv(16) AMLs, are regarded nowadays as leukemias highly responsive to chemotherapy, particularly to schemes incorporating HDARAC in the post-remission phase.[25] Interestingly, however, some recent studies have pointed to a heterogeneous clinical course of t(8;21) AML, depending on some diagnostic features such as association with extramedullary disease or CD56 expression.[26,27] To our knowledge, no studies have analyzed the prognostic impact of initial clinical characteristics in the subset of inv(16) or CBFβ/MHY11 AMLs. Our results suggest that CBFβ/MYH11-positive AML with very high WBC counts at diagnosis should be considered as a very poor prognosis leukemic form, probably requiring a different therapeutic approach from that used in patients with moderate or no hyperleukocytosis. Although this finding should be interpreted cautiously due to the heterogeneity of the post-remission therapy administered in our series, it should be noted that five of our patients relapsed after receiving intensive post-remission therapy (HDARAC and/or ABSCT).

Several investigators have reported on RT-PCR monitoring of MRD in AML patients with the CBFβ/MYH11 rearrangement.[3,9-18] In keeping with these reports, our study shows that the presence of CBFβ/MYH11 transcripts remains detectable for a long time after remission induction (up to 24 months in our series) and that conversion to PCR-negativity is usually observed therafter.[9,15] In addition, although a PCR-negative result does not preclude the possibility of relapse, especially in the early phases of the disease.[11,18] In our study, the only relapse recorded among patients who achieved molecular remission occurred 13 months after diagnosis. All patients in long-term remission (> 2 years) have undetectable CBFβ/MYH11 transcripts using the RT-PCR sensitivity level of 10^{-5}. These results support the concept that molecular remission is one of the goals to be achieved in all patients with AML with CBFβ/MYH11 rearrangement.

It is interesting to observe that the kinetics of molecular remission varies considerably in AMLs with distinct fusion proteins such as CBFβ/MYH11, PML/RARα and AML1/ETO, probably depending on the different sensitivity of the RT-PCR assays employed for each fusion. Hence, for the AML1/ETO rearrangement,[28] which is detectable in 10^{-6} dilutions, the transcript has been found in the majority of patients in long-term remission.[29] In the case of the CBFβ/MYH11 fusion, a slightly lower sensitivity ($1:10^{-5}$) is obtained,[10,12] which could explain the late negativization in most long-term survivors (within the second year after CR achievement). Finally, for the PML/RARα transcript, which is detected at lower sensitivity ($1:10^{-4}$),[30] the conversion to negative occurs earlier in most patients, i.e. after induction in 50% and at completion of consolidation in more than 90% of cases, respectively.[31,32]

In conclusion, marked hyperleukocytosis (>100 $\times 10^9$/L) confers poor prognosis to the CBFβ/MYH11-positive AML subset usually included as a whole entity in the "low risk" category. Monitoring of the fusion gene by RT-PCR indicates slow kinetics of molecular remission in this subset with delayed conversion to PCR-negativity at a median time of 7.5 months. Unlike AML1/ETO, which can be detected in long-term survivors while in remission, the CBFβ/MYH11 fusion is no longer detectable in long-term survivors, indicating that molecular remission is an important therapeutic goal. Prospective studies with longer follow-up are warranted to determine the prognostic value of MRD detection precisely. Besides, the quantification of the transcripts with recently developed real time PCR technology should provide additional insights on the predictive value of monitoring MRD in this leukemia.

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Disclosures

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Manuscript processing

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Potential implications for clinical practice

- Hyperleukocytosis is a powerful prognostic factor of relapse in patients with CBFβ/MYH11-positive AML. Molecular monitoring of minimal residual disease provides additional information in order to predict the outcome.

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