

Prognostic significance of AML1-ETO fusion transcript expression in children and young adults with t(8;21) acute myeloid leukemia

We performed serial nested reverse transcription polymerase chain reaction (RT-PCR) assays to monitor minimal residual disease (MRD) in 25 patients with t(8;21) acute myeloid leukemia (AML). We found transient disappearance of the AML1-ETO transcript in most of the patients in complete remission. Durable PCR-negativity was found in a subgroup of patients and was associated with a good prognosis.

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The majority of authors consider only serial quantitative assays but not qualitative RT-PCR to predict clinical outcome in t(8;21) AML.¹⁻³ However Morschhauser et al.⁴ recently reported that all patients with conversion from PCR-positivity to PCR-negativity at a median of 6 months after diagnosis had significantly lower relapse rates than patients with persistence of the AML1-ETO transcript. We performed serial nested RT-PCR assays at short time intervals to determine whether this protocol of MRD monitoring could predict clinical outcome in the t(8;21) AML. Twenty-five patients with AML1-ETO-positive AML-M2 were studied (22 children and 3 young adults). Intensive induction chemotherapy and 1 to 3 further courses of intensive post-remission chemotherapy were used. Three patients (n. 23, 24 and 25) died during induction. Standard cytogenetic analysis of bone marrow (BM) and peripheral blood (PB) short-term cultures (24-48 hours) was performed and karyotypes were described according to the ISCN.⁵ For RNA isolation BM and PB samples were collected sequentially: at presentation, after every chemotherapy course (after recovery of blood indices) and then during complete remission (CR) at intervals of 2-4 months. RNA extraction was carried out using Tri-

zol (Invitrogen) according to the manufacturer's protocol. RNA samples were stored in 70% ethanol at -80°C until tested. The quality of RNA samples was assessed by electrophoresis in 0.8% agarose gel, and the concentration and purity of RNA were measured using a spectrophotometer.

RT-PCR was carried out according to the standard BIOMED-1 protocol.⁶ All tests were done twice to confirm the results. RNA from the Kasumi-1 cell line, carrying t(8;21)(q22;q22), served as a positive control, and RNA from the K562 cell line lacking t(8;21) and water served as negative controls. All necessary precautions were taken to eliminate cross-contamination. Amplified products from the first and second PCR steps were examined by electrophoresis on a 2% agarose gel. The sensitivity of the assay was evaluated periodically. RNA of the t(8;21)-positive cell line Kasumi-1 could be detected in K562 RNA up to a dilution of 1:10⁵, i.e. the nested PCR reached a sensitivity of 10⁻⁵. It was consistent from run to run. All results are presented in Figure 1. The standard chromosomal translocation t(8;21)(q22;q22) was found in 22 of 25 patients. In two patients (#5 and 20) an atypical translocation t(6;21;8) (p23;q22;q22) or t(7;22;21;8)(q31;q13;q22;q22) was identified. In case #18 an atypical add (21q) was found but both homologs of pair 8 looked unchanged. In patient n. 6 chromosome analysis failed. The chimeric AML1-ETO transcript was detected in all 25 patients. Three patients (n. 1, 2 and 3) were first studied by RT-PCR during long-term complete remission (CR) (at 90, 30 and 30 months). Twenty-two patients were studied for the first time before treatment.

PCR-negativity of BM and/or PB was detected at least in isolated samples in 17 of 21 patients tested at different points of their CR. We succeeded in performing serial RT-PCR assays for the AML1-ETO gene expression in 14 patients (Figure 1). From 4 to 18 assays were done per patient during a 6-37 month period. Only 5 cases demonstrated durable absence of transcript from the PB and/or BM (cases n. 3, 6, 7, 10, 12). In 4 of them CR is continuing and in one patient (case n. 6) the fusion transcript has reappeared. This patient relapsed 15 months after BM conversion to PCR-positivity. After anti-relapse treatment the patient is in 2nd CR with PCR-negativity.

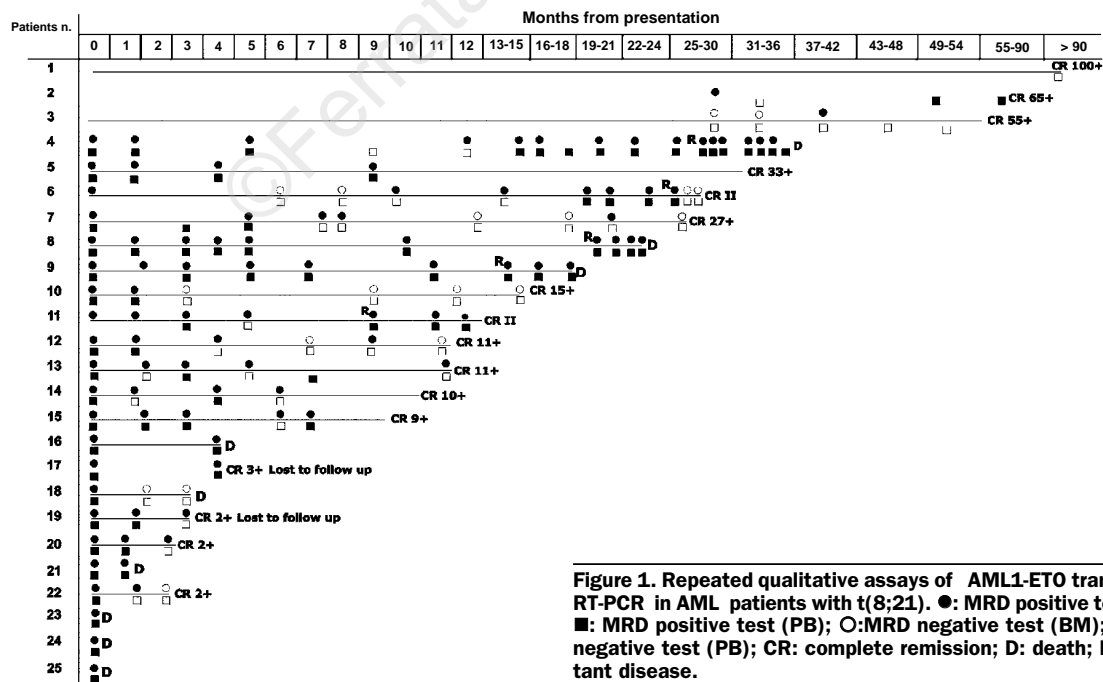


Figure 1. Repeated qualitative assays of AML1-ETO transcript by RT-PCR in AML patients with t(8;21). ●: MRD positive test (BM); ■: MRD positive test (PB); ○:MRD negative test (BM); □: MRD negative test (PB); CR: complete remission; D: death; RD: resistant disease.

Five patients relapsed at different points (from 8 to 27 months) of CR. In three of them (n. 4, 8 and 9) consistent PCR-positivity was observed during 10, 20 and 15 months before relapse. One patient (n. 11) was not studied during the 4 months before relapse. Thus, no patient relapsed after durable PCR-negativity.

We believe that durable PCR-negativity for the AML1-ETO transcript in CR is a good prognostic sign. Perhaps patients with durable PCR-negativity do not need frequent quantitative testing but conversion from durable PCR-negativity to positivity in at least two assays needs to be checked by quantitative assay.

Combination qualitative and quantitative RT-PCR might be reasonable for monitoring MRD in the t(8;21) AML. To confirm this conclusion and our suggestion for frequent monitoring a larger series of patient must be studied. Studies of PB, as an alternative to BM, for frequent testing is of current interest. This strategy also needs further investigation.

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T-immunophenotype is associated with an increased prevalence of thrombosis in children with acute lymphoblastic leukemia. A retrospective study

We retrospectively evaluated the prevalence of symptomatic thrombotic events in a group of 2,318 children with acute lymphoblastic leukemia (ALL) and found that it was 0.95%. The prevalence of thrombotic events in the patients with T-ALL was significantly greater than in the patients with non-T-ALL.

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Thrombosis is a frequent complication in children with acute lymphoblastic leukemia (ALL). The reported incidence ranges from 2.4% to 11.5%.¹⁻³ The etiopathogenesis of this complication is thought to be multifactorial: a pro-thrombotic effect of leukemic cells, genetic predisposition, central venous catheters, septic complications and use of drugs such as steroids and L-asparaginase (L-Asp) have all been implicated.¹⁻⁴ Previously we reported a greater amount of thrombin generation, especially in children with T-ALL.⁵

The aim of this study was to evaluate the prevalence of symptomatic thrombotic events in a cohort of ALL children treated according to the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) ALL '91 and '95 studies, including L-Asp combined with prednisone during induction therapy,⁵ and to examine the role of the T-ALL immunophenotype in this phenomenon.

Symptomatic thrombotic events were retrospectively evaluated by means of a questionnaire sent to each of the 43 AIEOP centers. Data were collected about the number, type and time of occurrence of the thrombotic events, the biological and immunologic features of each case, as well as the patients' clinical characteristics. Twenty-seven centers out of 43 (63%) answered and returned their questionnaires. Data were expressed as percentage, mean and median values and analyzed by Fisher's exact test and the Mann Whitney U test.

Out of a total of 2,318 ALL cases considered, 22 symptomatic thrombotic events (0.95%), confirmed by appropriate imaging methods, were reported in 22 patients (13 males and 9 females, mean age 8 years, range 3-16 years). The thrombotic events were: 11 cerebral venous thromboses, 10 deep venous thromboses (1 of the superior vena cava, 3 of the subclavian vein, 4 of the femoral vein, 2 of the popliteal vein) and one case of pulmonary thromboembolism. The main characteristics of the study population are reported in Table 1. In all cases family history for thrombosis was negative. Seventeen thrombotic events (77%) were reported during induction, 4 during re-induction and 1 during consolidation. The immunophenotype subgroups were T-ALL (n=6), common (n=13), pre-p B (n=2), and pre B (n=1). The prevalence of thrombotic events in the T-ALL patients was significantly higher than in the non-T-ALL patients [6/269 (2.23%) vs 16/2049 (0.78%); $p < 0.05$]. Five thromboses in the T-ALL patients occurred during induction and 1 during consolidation. The prevalence of thrombotic events during the induction phase in T-ALL patients was still higher than in the non-T-ALL patients [5/269 (1.86%) vs 12/2049 (0.58%); $p < 0.05$]. Moreover, T-ALL patients had a higher number of white blood cells at the onset of the disease than did non-T-ALL subjects (median values: 26,100/mm³ and 6,330/mm³, respectively; $p < 0.05$).

E. Coli L-Asp was used in 9/22 cases (Crasnitin® in 7 cases and Medac® in 2 cases), Erwinia L-Asp was used in 10/22 cases and for 3/22 cases the L-Asp source was not reported. Ten of the 22 events were reported in subjects with a central venous line (CVL). However, the influence of these two latter risk factors (L-Asp and CVL) was not evaluated because no information was obtained on their presence in the group of