

- TEL-AML1 negativity. The Pediatric Hematology Working Group in the Czech Republic. *Leukemia* 1998; 12:1064-70.
- Rubnitz JE, Shuster JJ, Land VJ, et al. Case-control study suggests a favorable impact of TEL rearrangement in patients with B-lineage acute lymphoblastic leukemia treated with antimetabolite-based therapy: a Pediatric Oncology Group study. *Blood* 1997; 89: 1143-6.
 - Seeger K, Adams HP, Buchwald D, et al. TEL/AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia. The Berlin-Frankfurt-Münster Study Group. *Blood* 1998; 9:1716-22.
 - Zuna J, Hrusák O, Kalinová M, Muzíková K, Sary J, Trka J. TEL/AML1 positivity in childhood ALL: average or better prognosis? *Czech Paediatric Haematology Working Group. Leukemia* 1999; 13:22-4.
 - Knechtli CJ, Goulden NJ, Hancock JP, et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. *Blood* 1998; 92:4072-9.
 - Ladenstein R, Peters C, Gadner H. The present role of bone marrow and stem cell transplantation in the therapy of children with acute leukemia. *Ann NY Acad Sci* 1997; 824:38-64.
 - Biondi A, Masera G. Molecular pathogenesis of childhood acute lymphoblastic leukemia. *Haematologica* 1998; 83:651-9.

Use of molecular techniques to confirm true re-emergence of an original clone and to track minimal residual disease in acute lymphoblastic leukemia

We report the case of a young girl whose acute lymphoblastic leukemia (ALL) recurred as lymphoblastic lymphoma 6.6 years after diagnosis. Analysis of minimal residual disease (MRD) allowed tracking of the leukemic clone during the phases of therapy, confirming that relapse was a true re-emergence of the original clone.

Sir,

Relapse of childhood lymphoblastic leukemia 5 or more years after diagnosis is a very rare event. When it happens, a true relapse of the original clone should be confirmed to exclude a secondary malignancy. Confirmation of the relapse can be obtained by assessing the presence of the same features in the clone at diagnosis and at recurrence.¹⁻⁵ The patient, a 7-year old girl, was admitted to our hospital for the first time in November 1992, at the age of 7 years. Main symptoms included fever and intercostal pain; the total leukocyte count was $3.3 \times 10^9/L$ with 13% abnormal cells, Hb was 4.2 g/dL and the platelet count was $82 \times 10^9/L$. Bone marrow aspiration showed a subtotal infiltration by leukemic lymphoblasts (L1, PAS+ CD10+, CD19+, HLA-DR+, TdT 95%). Karyotype was normal. The patient was treated according

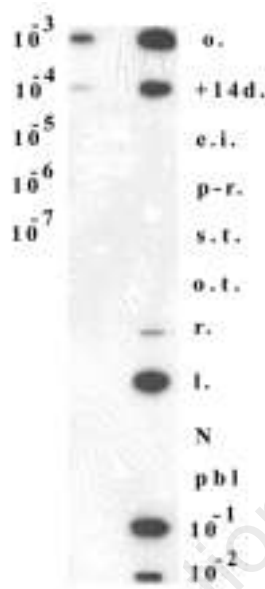


Figure 1. Dot blot of DNA of the patient, hybridized with the patient specific probe, at different time points. O, onset; + 14 d., +14 day; e.l., end of induction; p-r., pre-reinduction; s.t., stop therapy; o.f. off therapy; r., BM at time of relapse; l., lymph node at relapse; N, negative control; pbl, normal peripheral blood; 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} indicate serial dilutions of the onset DNA to test the sensitivity of the probe. The signal is detected until a dilution of 10^{-4} .

to the AIEOP 9101 protocol for low risk acute lymphoblastic leukemia (ALL).⁷ Cytological remission was achieved on day +42, at the end of the induction phase. After two years of maintenance treatment she discontinued all chemotherapy and remained in continuous remission until June 1999 (6 years and 6 months after the original diagnosis), when she presented with massive lateral cervical lymphadenopathy and a retroperitoneal abdominal mass. Biopsy of a lateral cervical lymph node was consistent with high grade lymphoblastic lymphoma. The DNA index of lymphoblasts was 1.20 and cytogenetic analysis revealed a hyperdiploid pattern in the majority of metaphases. Surface markers were consistent with the original leukemia. Bone marrow aspiration from four different sites and the spinal fluid did not show any leukemic infiltration. The patient was treated according to the AIEOP NHL non-B protocol. After the induction phase she achieved a complete clinical remission confirmed by a CT scan. She subsequently received consolidation treatment and is now in reinduction phase.

DNA at diagnosis was investigated by standard techniques for T-cell receptor gene rearrangements.⁶ The V δ 2D δ 3 rearrangement detected was sequenced and a 20mer primer spanning the junctional region was designed to monitor minimal residual disease (MRD) at day +14, end of induction, pre-reinduction, stop therapy, off therapy (+38 months), and relapse. DNA was probed with the patient specific primer. Serial dilutions of the onset DNA were analyzed to test the sensitivity of the probe, which was about 10^{-4} . The patient was positive at day +14 of therapy, but

negative at the end of the induction phase. She was still negative 38 months after diagnosis. The BM at the time of relapse showed a positive signal, revealing BM infiltration despite the morphologic negativity. The sequence obtained from the lymph node showed complete homology with the sample taken at diagnosis.

Most relapses occur within four years of diagnosis and involve an extramedullary site other than the CNS and testis in less than 2% of cases.^{7,8} Our case relapsed in 2 extramedullary sites as bulky disease. Even though BM analysis at the time of relapse had a positive PCR signal, according to standard criteria this cannot be considered as a BM relapse. The patient showed early and continuous molecular negativity at consecutive time-points. According to recent studies on MRD in ALL,⁹ this pattern should be considered as a positive prognostic factor. There is no satisfactory explanation of this late, unusual relapse. We can hypothesize that the pharmacological treatment did not eradicate the leukemic clone completely, but merely reduced the disease to a level not detectable by the probe. A second hypothesis is that some cells escaped the impact of chemotherapy in a protected site (sanctuary) and became no longer detectable as residual cells in the bone marrow.¹⁰

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Key words

Leukemia, late relapse, gene rearrangement, T-cell receptor.

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References

1. Levasseur M, Maung ZT, Jackson GH, Kernahan J, Proctor SJ, Middleton PG. Relapse of acute lymphoblastic leukaemia 14 years after presentation: use of molecular techniques to confirm true re-emergence. *Br J Haematol* 1994; 87:437-8.
2. Aydogdu I, Tayfun E, Ozcan C, Harputluoglu M, Ozen S, Hicsonmez G. Late relapse in a patient with acute lymphoblastic leukemia thirteen years after diagnosis.

Pediatr Hematol Oncol 1997; 14:491-3.

3. Frost L, Goodeve A, Wilson G, Peake I, Barker H, Vora A. Clonal stability in late-relapsing childhood lymphoblastic leukaemia. *Br J Haematol* 1997; 98:992-4.
4. Vora A, Frost L, Goodeve A, et al. Late relapsing childhood lymphoblastic leukemia. *Blood* 1998; 92:2334-7.
5. Lo Nigro L, Cazzaniga G, Di Cataldo A, et al. Clonal stability in children with acute lymphoblastic leukemia (ALL) who relapsed five or more years after diagnosis. *Leukemia* 1999; 2:190-5.
6. Pongers-Willemsse MJ, Seriu T, Stolz F, et al. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and TAL1 deletions as PCR targets: report of the BIOMED-1 CONCERTED ACTION: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; 13:110-8.
7. Conter V, Aricò M, Valsecchi MG, et al. Intensive BFM chemotherapy for childhood ALL: interim analysis of the AIEOP-ALL 91 study. *Associazione Italiana Ematologia Oncologia Pediatrica. Haematologica* 1998; 83:791-9.
8. Miniero R, Saracco P, Pastore G, et al. Relapse after first cessation of therapy in childhood acute lymphoblastic leukemia: a 10-year follow-up study. *Italian Association of Pediatric Hematology-Oncology (AIEOP). Med Pediatr Oncol* 1995; 24:71-6.
9. van Dongen JJ, Seriu T, Panzer-Grumayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet* 1998; 352:1731-8.
10. Biondi A, Masera G. Molecular pathogenesis of childhood acute lymphoblastic leukemia. *Haematologica* 1998; 83:651-9.

Cryptococcal meningitis during front-line chemotherapy for acute lymphoblastic leukemia

Fungi cause opportunistic infections in immunocompromised patients. In the case of *Cryptococcus neoformans* infection, diffusion to meninges is a typical occurrence. We report the case of a 13-year old child who owned a love-bird and who developed cryptococcal meningitis during chemotherapy for intermediate risk acute lymphocytic leukemia.

Sir,

The fungi responsible for the recent increase in mycoses are those causing opportunistic infections, including *Cryptococcus neoformans*.¹ In children undergoing bone marrow transplantation or chemotherapy for malignancies, prolonged neutropenia (absolute neutrophil count, ANC, <500/m³), mucosal breakdown, impairment of cell-mediated immunity, widespread use of broad-spectrum antibiotics, and central venous catheters are the major causes of fungal opportunistic infections, especially in case of long-term treatment with corticosteroids and/or prolonged hospitalization.^{1,2}

Cryptococcus neoformans is widely diffused