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Molecular and clinical prognostic factors in BFM-treated childhood acute lymphoblastic leukemia patients: a single institution series

We analyzed the impact on outcome of minimal residual disease (MRD) and clinical factors in 97 children with acute lymphoblastic leukemia. Unfavorable factors were early-B and -T immunophenotype, hyperleukocytosis, non-response to steroids, detection of MRD at the end of induction therapy. A level of MRD ≥ 1/10³ leads to a lower probability of disease-free survival.

Sir,

Ninety-seven children with acute lymphoblastic leukemia (ALL) were treated in our institution according to the ALL-BFM protocol[1] from November 1986 to December 1997. The polymerase chain reaction (PCR) protocol used for detection of IgH and TCRδ gene rearrangements in diagnostic bone marrow and follow-up samples was similar to those previously published.[2-4] In diagnostic samples specific bands from PCR reactions were separated and recovered from a 2% low melting temperature agarose gel. A PCR second round was carried out to generate the probe using the ELJH/Bam 5'TGAGGAGACGGTGACCAGGATCCCTTGGCCCCAG3' and FRA/Pst 5'ACACCTGCAGTGTATTACTGT3'primers.[3]

Following restriction digestion with Bam HI and Pst1, the amplified fragment was labeled using the random primer method with dig-11-dUTP digoxigenin. Following hybridization, filters were washed under astringent conditions, detected by chemoluminescence and visualized using X-rays. Sensitivity of IgH and TCRδ probes ranged from 1/500 to 1/10,000, and from 1/500 to 1/1,000 respectively.

The patients' characteristics are shown in Table 1. With a median follow-up of 6 years (range 1-135 months) the probability of disease-free survival (DFS) was 0.75 (0.04 SD). There were 20 relapses, 15 in the bone marrow, 3 combined, and 2 extramedullary. DFS probability was negatively affected by the following factors: complete response (CR) not achieved by day +33 ($p< 0.0001$), early-B and T immunophenotype ($p< 0.01$), leukocytes ≥100x10⁹/L ($p= 0.02$), no response to steroids ($p= 0.0002$), and presence of minimal residual disease (MRD) at the end of induction ($p = 0.0009$). Patients included in the MRD study (n=36) were in CR but in 14 cases MRD was detected. A threshold value of 1:10³ was used to separate patients who were MRD negative (<1:10³) or MRD positive (≥1:10³). With a median follow-up of 50 months (range 22-81) DFS probability for these patients was 0.80 (0.07 SD). Six patients relapsed between 21 and 50 months after diagnosis, all of them in the MRD positive group. DFS probability was 1.0 in the MRD negative group whereas in the MRD positive group it was 0.52 (0.14 SD) (log-rank test; $p< 0.0009$; 95% CI 0.01-0.3). There was no correlation between level of MRD and clinical data (Table 2). Reports on MRD in childhood B-precursor ALL have suggested that PCR positivity and MRD level at the end of the induction predict clinical outcome.[3,5,6] However, in other studies prolonged persistence of MRD correlates with outcome better than a single PCR positive/negative determination at a given time.[7,8] Our data suggest that patients with MRD ≥ 1/10³ at the end of induction

Table 1. Patients' characteristics (n=97).

Variable	*Number of patients (%)*
Sex	
Male	58 (59.8)
Female	39 (40.2)
Age (years)	
<1	0 (0)
1-9	83 (85.6)
>10	14 (14.4)
White blood cell count (x10⁹/L)	
<10	45 (46.4)
10-<50	30 (30.8)
50-<100	13 (13.4)
>100	9 (8.2)
Hemoglobin (g/100 mL)	
<10	74 (76.2)
>10	23 (23.8)
Central nervous system involvement	1 (1)
Mediastinal involvement	12 (12.3)
Immunophenotype	
early-B ALL	15 (15.5)
c-ALL	63 (64.9)
pre B ALL	1 (1)
T-ALL	16 (16.5)
null ALL	2 (2.1)
Prednisone response (peripheral blood blasts, day 8)	
<1x10⁹/L	89 (91.8)
>1x10⁹/L	8 (8.2)
Chromosome study (n=25)	
Hyperdiploid	14 (56)
Diploid	8 (32)
Pseudodiploid	2 (8)
Hypodiploid	1 (1)

Table 2. Patients' characteristics with regard to MRD positivity.

Variable	*MRD negative n=22*	*MRD positive n=14*	*p value*
Age (years), median (range)	4 (1-12)	5 (2-12)	n.s.
Gender			
Male	14	5	n.s.
Female	8	9	
WBC count (x10⁹/L), median (range)	11.5 (1.3-85)	5 (2-132)	n.s
Estimated disease-free survival	100%	52%	0.0009

had a lower probability of DFS than patients with MRD < 1/10[3]. These data are comparable to those previously reported.[4-6,9,10] In summary, MRD after induction therapy is a relevant prognostic factor in children with B-precursor ALL. Our study indicates the need for MRD evaluation by PCR in all patients at the end of the induction period.

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Key words

Childhood ALL, minimal residual disease.

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Chronic lymphocytic leukemia and Hodgkin's disease. Clinicopathologic study of three cases with good prognosis

Lymphadenopathy during the course of chronic lymphocytic leukemia is common. However, when patients develop associated symptoms, a lymph node biopsy is warranted. We report three patients with chronic lymphocytic leukemia who subsequently developed Hodgkin's disease and who achieved complete remission after chemotherapy and radiotherapy.

Sir,

B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia, affecting older patients; 10% develop diffuse large cell lymphoma (Richter's syndrome)[1]. Hodgkin's disease (HD) sometimes occurs in patients with a longstanding history of CLL[2] and survival is usually short. We report 3 cases of HD in CLL patients. All patients achieved complete remission (CR) of HD.

Case #1. In 1990 a 62-year old man was diagnosed as having Binet stage A CLL. Chlorambucil was administered over 3 years. In 1997 axillary lymphadenopathies appeared with anemia and thrombocytopenia. Lymph node biopsy showed Reed Sternberg (RS) cells. According to the Ann Arbor classification he had stage III disease. Blood lymphocyte immunophenotyping and bone marrow biopsy are detailed in Table 1. Three courses of intensive chemotherapy (Stanford V regimen) produced CR. When last seen in 1999 he was still in CR from HD.

Case #2. In 1990 a 62-year old woman developed a Binet stage A CLL (Table 1). In 1996 lymphadenopathies and night sweats appeared. Lymph node biopsy revealed RS cells and $CD5^+$ lymphocytes. The patient was considered as having stage III disease according to the Ann Arbor classification. She received 3 courses of intensive chemotherapy (Stanford V regimen) followed by radiotherapy. She achieved a CR. In 1999 she was still in CR from HD.

Case #3. In 1991 a 60-year old man was diagnosed as having a Binet stage C CLL. He received 11 courses of mitoxantrone, etoposide, and steroids. Only a partial response was achieved (Table 1). In 1995, he developed a CLL related cytopenia. Six courses of fludarabine were delivered. In 1996 submandibular lymph nodes and splenomegaly appeared. Lymphadenopathy biopsy showed RS cells. Three courses of MOPP/ABVD were completed by radiotherapy. Thirty-six months later he was still in CR from HD.

The occurrence of HD in the course of CLL has been reported in the literature.[3-6] In Fayad's study,[4] 7 CLL patients out of 1,374 developed HD (0.6%) with a mean interval of 45 months. The median age upon diagnosis of HD was 71 years. They all received chemotherapy, but only one achieved CR.

In contrast, the prognosis of our patients remained as good as those of patients with HD without CLL. This could be because they were younger or because of the late occurrence of HD and intensive chemotherapy. Nevertheless, such an outcome is rare. Butts[5] reported two cases of HD in patients with CLL, with-