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Brief communication

Acute lymphoblastic leukemia following severe congenital neutropenia or *de novo* ALL?

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ABSTRACT

Acute lymphoblastic leukemia (ALL) presenting with neutropenia alone is very rare. We describe a newborn with an early life-threatening infection, severe neutropenia and bone marrow findings compatible with severe congenital neutropenia (SCN). She was treated with granulocyte colony-stimulating factor (G-CSF) with complete neutrophil recovery. Three months later she developed a pro-B ALL. We identified a rare loss of 5′-*MLL* present at the diagnosis of SCN and ALL by FISH analysis using two different *MLL* (11q23) probes. Molecular analyses for SCN causing mutations (*ELA-2*, *HAX-1* and *G6PC3*) and for somatic mutations of the *CSF3R* gene were negative. The early presence of 5′-*MLL* loss in bone marrow samples may favor the diagnosis of *de novo* ALL. Nevertheless, the genetic background for SCN is heterogeneous and a non-described mutation for SCN followed by a secondary ALL cannot be excluded. Further genetic investigation may be useful to gain insight into this rare condition in children.

1. Introduction

Acute leukemia in the first year of life is a rare condition characterized by distinct epidemiologic, clinical and biological features [1]. The annual incidence of acute leukemia in infants in the United States is reported to be approximately 30 per one million live births; of these, about 20 per million per year are ALL cases, exceeding the incidence of acute myeloid leukemia (AML) in this age-group [2]. One particular aspect of the molecular epidemiology of this condition in infants is the common association of ALL and AML with rearrangements involving the *MLL* (11q23) gene in up to 80 and 50% of cases, respectively [3], with *t*(4;11) *AF4*/*MLL* being the most frequent translocation observed in this group [4].

Severe congenital neutropenia (SCN) was first described by Kostmann in 1956 [5]. The current knowledge of the pathophysiology of SCN suggests that the condition comprises heterogeneous disorders of hematopoiesis all characterized by a maturation arrest of granulopoiesis at the level of promyelocytes with peripheral blood absolute neutrophil counts (ANC) below $0.5 \times 10/l$ [6]. Before the treatment era with granulocyte colony-stimulating factor (G-CSF), affected patients usually died of pyogenic infection during infancy or early childhood. Currently, mutations in the Neutrophil Elastase gene *ELA-2*, in the *HAX-1* and in the *G6PC3* gene were reported as the main genetic causes underlying SCN of different patterns of inheritance [6–9].

In the following article, we present the case of a newborn that developed an early life-threatening infection associated with severe neutropenia in the first week of life. She was diagnosed with severe congenital neutropenia based upon clinical and laboratorial findings, and G-CSF treatment was initiated. Three months later, the child was diagnosed with ALL. Retrospective genetic evaluations on bone marrow samples at different time-points were performed in order to search for evidence that would corroborate the diagnosis of either congenital leukemia or SCN.

2. Case report

An apparently healthy newborn developed extensive edema initiated at the site of earlobe perforation and rapidly extending throughout her left hemiface one week after birth. Concomitantly, she also presented with omphalitis. Her initial blood count (WBC) disclosed leukopenia (2000/μl) with severe neutropenia

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(ANC < 100/μl); hemoglobin levels and platelet count were normal. Subsequent blood examinations revealed the same findings with an ANC varying from 10 to 150/μl, even following the control of ear infection. Bone marrow aspirates (BMAs) demonstrated maturation arrest at the promyelocyte/myelocyte level; blast cells were present at less than 5%. Based upon clinical and cytological findings, the diagnosis of SCN was established, and treatment with G-CSF was started at a dose of 5 μg/kg subcutaneously three times weekly followed by a complete normalization of her blood counts.

Three months later, the patient developed pallor, intermittent fever and petechiae. The physical examination revealed hepatosplenomegaly. Her blood smear examination depicted anemia (8.0 g/dl), leukocytes (>50,000/μl), thrombocytopenia (20,000/μl) and 10% lymphoblasts. A new BMA revealed extensive lymphoblast infiltration (>90%) of L1-morphology. The immunophenotype of the blasts as judged from flow cytometry was in accordance with the diagnosis of a pro-B ALL ($CD19^+$, $CD45^+$ and $CD10^-$). The patient is currently under treatment according to the Brazilian protocol for pediatric acute lymphoblastic leukemia (GBTLI-99) and is in complete clinical remission. Subsequent BMAs were obtained at the end of induction (day 28), intensification (day 65) and maintenance (8 and 12 months from diagnosis).

2.1. Cytogenetic studies

Mitotic chromosome preparations were obtained from BMA withdrawn after nonstimulated culture in RPMI 1640 medium with 20% fetal calf serum. The cultures were incubated for 24 and 48 h at 37 °C and treated with colchicine (0.56 %) for the last 30 min. The cells were harvested, and slide preparation was performed according to the conventional method.

Fluorescence in situ hybridization (FISH) was performed using the commercially available probes LSI MLL break-apart rearrangement (Vysis, Downers Grove, IL, USA) and Poseidon™ Repeat Free™ *MLL* (11q23) Break (Kreatech Diagnostics, Amsterdam, The Netherlands) according to the protocol of the manufacturers. In both cases, the probe labeled in green covers a portion centromeric to the *MLL* gene breakpoint region, and the orange-labeled probe covers a portion telomeric to the BCR. The expected signal pattern for a normal cell nucleus is two green and orange signals separated by a yellow signal [green(yellow)orange]. In cells harboring *MLL* translocations, the green and orange signals appear separated without the yellow intersection.

Chromosomal aberrations were also analyzed by using the whole chromosome painting Spectrum-Orange WCP-4 and Spectrum-Green WCP-11 probes (Vysis). Before hybridization, the slides were treated with RNAse (Boehringer Ingelhein, Germany) and pepsin (Sigma) for 1 h and 15 min at 37 °C, respectively. Following hybridization, the preparations were dehydrated, dried, and counterstained with DAPI (Sigma Chemical Co., ST Louis, USA) plus the antifade reagent and analyzed in an epifluorescence microscope.

2.2. ELA-2, G6PC3 and HAX-1 gene mutation analyses

Genomic DNA was extracted from peripheral blood using standard techniques. Mutational analyses were performed by sequencing the polymerase chain reaction (PCR)-amplified exons of *ELA-2*, *HAX-1* and *G6PC3* genes and their flanking regions from both directions using a semiautomatic sequencer as described previously [6,9,10].

2.3. Molecular analyses of the CSF3R

Fragments including the mutation-sensitive region 2300–2600 were PCR-amplified and sequenced after subcloning [11]. This procedure was performed twice for the investigation of two independently prepared genomic DNAs. We analyzed 25 *Escherichia coli* clones generated from DNA from the patient at the time of diagnosis of ALL. The purified plasmids were sequenced using semiautomated sequencing.

3. Results

The retrospective analysis of bone marrow chromosome preparations at the time of diagnosis with painting probes for chromosomes 4 and 11 showed normal hybridization patterns. The FISH analysis of the *MLL* gene by the break-apart rearrangement probe (Vysis) showed the invariable deletion of the 5′ portion of the gene, presenting only red signals. Due to the rarity of this aberration, FISH analyses were also performed by using the Poseidon™ probes for the same region. The results were concordant demonstrating that this abnormality was present before leukemia diagnosis.

At the time of ALL diagnosis, chromosome preparations showed the same hybridization pattern with the loss of 5′-*MLL*. This condition was maintained in subsequent bone marrow sampling during treatment. However, chromosome preparations from sample number VI (one year after diagnosis) showed normal hybridizations with two green (yellow) orange signals (Fig. 1).

We have also screened this patient for constitutive mutations in the *ELA-2*, *G6PC3* and *HAX-1* genes as they are the known causes for SCN and for somatic mutations in the *CSF3R* gene known to be associated with leukemogenesis in SCN patients. There were no detectable mutations.

4. Discussion

Although the diagnosis of SCN relies mainly on clinical findings and morphological aspects on BMA, molecular tests have been increasingly employed to confirm this disease. Mutations in the *ELA-2* gene, in the *G6PC3* gene and in the *HAX-1* gene have been described as main genetic causes underlying SCN of different patterns of inheritance [6,9,10].

In previous studies it could be shown that somatic point mutations in the gene for the granulocyte colony-stimulating factor receptor *CSF3R* are a highly predictive marker for leukemic development in CN patients. While patients with SCN have a predisposition for AML and MDS, only three patients with SCN who developed secondary ALL have been reported [12–14]. The presence of *CSFR3* mutations in two out of these three published SCN/ALL patients suggests that there could also be different mechanisms of pathogenesis of lymphoblastic leukemia in SCN comparable to different pathways within the leukemogenesis in CN/AML patients.

Infants with ALL show some particular molecular findings, especially the common association with rearrangements involving the *MLL* (11q23) gene. The *MLL* gene plays an important role during fetal development and it is a critical regulator of *HOX* genes during hematopoiesis. This function is subverted in leukemias through cleavage, recombination and chimeric fusion with more than 50 gene partners [15,16]. However, in contrast to the diversity of *MLL* translocations, the mapping of breakpoints revealed that most translocations occur within a 8.3 kb BamH1 fragment, known as break cluster region (BCR) located between exons 8 and 14 [17].

MLL rearrangements due to translocation or other structural changes confer a poor prognosis in childhood acute leukemia and are associated with reduced response to chemotherapy regardless of presenting age [18]. The FISH analysis of the *MLL* gene showed the invariable deletion of the 5′ portion of the gene, presenting only red signals. This aberration was present at the time of initial SCN diagnosis and persisted during the first months of treatment.