Sometimes it is better to wait: First Italian case of a newborn with transient abnormal myelopoiesis and a favorable prognosis

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Received December 9, 2015; Accepted July 21, 2016

DOI: 10.3892/ol.2016.5401

Abstract. Congenital leukemia is rare disease with an incidence of one to five cases per million births. Transient abnormal myelopoiesis (TAM), also called transient myeloproliferative disorder, is a pre-leukemia disorder that may occur in Down syndrome (DS) or non-DS infants. TAM may enter spontaneous remission; however, continual monitoring is required, as this disorder has been observed to develop into acute megakaryoblastic leukemia in 16-30% of cases. In the literature, 16 cases of TAM in non-DS infants have been reported. The case presented in the current study is, to the best of our knowledge, the first case of an Italian non-DS newborn presenting with clinical manifestations of acute leukemia at five days after birth, exhibiting a normal karyotype, trisomy 21 only in blast cells, and spontaneous remission. Chromosomal analyses on peripheral blood cells, bone marrow cells and dermal fibroblasts were conducted using a G-banding technique, and fluorescence *in situ* hybridization (FISH) was used to identify the critical regions of DS. Amplification of GATA binding protein 1 (*GATA1*) exon 2 genomic DNA was performed using polymerase chain reaction. Cytogenetic analysis of 50 peripheral blood cells and dermal fibroblasts from the patient revealed a normal karyotype: 46, XX. Conversely, cytogenetic analysis of the patient's bone marrow revealed an abnormal karyotype 47, XX+21. In order to investigate this result, FISH was performed, which identified the presence of three signals in 70% of the cells and two signals in 30% of bone marrow cells. *GATA1* sequencing revealed the substitution of a single base (c.150delG) in exon 2. Seven months after the initial analysis, FISH and cytogenetic analyses of the stimulated/unstimulated peripheral blood cells and bone marrow cells were performed, revealing that each exhibited diploid signals, as observed in a normal karyotype.

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Key words: acute megakaryoblastic leukemia, transient abnormal myelopoiesis, transient myeloproliferative disorder, Down syndrome

Introduction

Congenital leukemia is a rare disease, with a reported incidence of one to five cases per million births (1). It represents the second most common cancer in infants (while the first is neuroblastma) in newborns and typically has a poor prognosis (2). Transient abnormal myelopoiesis (TAM), also called transient myeloproliferative disorder, is a pre-leukemia disorder that occurs in Down syndrome (DS) and non-DS individuals. Morphologically, cytochemically and clinically, the presentation of TAM may be indistinguishable from acute megakaryoblastic leukemia (AMKL; commonly French-American-British type M7) (2). AMKL has a good prognosis, as it has been observed to enter spontaneous remission in ~80% of cases (3). TAM is associated with trisomy 21 in blast cells and mutations of the GATA binding protein 1 (*GATA1*) gene (4), which synthesizes a growth factor essential for the differentiation of erythroid and megakaryocytic cells. This disorder must be continually monitored, as it has been observed to develop into AMKL in 16-30% of cases during the first four years of life (5). The differentiation between TAM and AMKL is primarily based on the *GATA1* mutations, moreover the latter diverges from the former in morphological characteristics of megakaryoblasts and positive cluster of differentiation, typically CD41/42 or CD 6 on flow cytometry (6). Furthermore, AMKL blast cells exhibit an additional cytogenetic abnormalities, including extra copies of chromosome 8 (7). TAM has been identified in three contexts: Patients with DS, patients with trisomy 21 mosaicism and patients without DS. TAM occurs in ~10% of DS cases (7) and 7-16% of trisomy 21 mosaicism cases (3,8,9); however, the incidence of TAM in patients without DS is extremely rare and only 16 cases have been reported in the literature (6,10). The first case of trisomy 21-associated TAM was described erroneously as a leukemoid reaction in 1980 by Sikand *et al* (11). The present report describes the case of a newborn patient who exhibited clinical manifestations of acute leukemia five days after birth, in addition to a normal karyotype, the presence of trisomy 21 only in blast cells and spontaneous remission.

Case report

Presentation. The newborn female patient (gestational age, 37 weeks; weight, 2,570 g; length, 46.3 cm), who was a quadruplet delivered by Caesarean section, was transferred to the neonatal intensive care unit of San Carlo Hospital (Potenza, Italy) on May 2013 two days after birth, due to a pale appearance, moderate hypotonia, hepatomegaly and hypertransaminasemia. The parents were non-consanguineous, young and healthy, with no reported familial history of DS. All pregnancy infections were excluded, and vaginal and rectal swabs were negative for bacteria colonization. The initial clinical examination yielded the following results: Red blood cell count, 2,860,000/μl (4,800,000-7,200,000/μl); white blood cell count, 134,000/μl (1,500-10,000/μl); platelet count, 253,000/μl (259,000-615,000/μl); hemoglobin level, 12.10 g/dl (12,7-18,3 g/dl); hematocrit level, 36.8% (52%); prothrombin time, 36 sec (10.8-13); partial thromboplastin time, 44.9 sec (26.2-36); international normalized ratio, 1.95 (1.01); fibrinogen, 213.0 mg/dl (207-321 mg/dl); antithrombin III, 81% (60-90%); C-reactive protein level, 20 mg/dl (<0.5 mg/dl); neutrophil granulocytes, 45.5% (34%); lymphocytes, 51.3% (40%); eosinophil granulocytes, 0.1% (3.1%); and basophil granulocytes, 0.2% (0.4%). The levels of aspartate transaminase (1,011 UI/dl; normal values 15-131 UI/l), alanine transaminase (515 UI/dl; normal values 28-300 UI/l) and lactate dehydrogenase (9,957 UI/dl; 150-360 UI/l) were elevated. The patient had normal ammonia levels, and other metabolic examinations were within the normal ranges. Chest X ray was negative. Blood and urine cultures were collected and the patient commenced a course of antibiotic therapy for seven days, due to suspicion of infection (ampicillin 50 mg/kg every 12 h and gentamicin, 4 mg/kg every 24 h). Fresh plasma was administered to correct coagulation. Following two days of treatment, the platelet count began to decrease (fourth day of life, 93,000/μl; fifth day of life, 73,000/μl). Standard cytogenetic and flow cytometric analyses were performed on peripheral blood and bone marrow samples. The results indicated 65% blast cells, a feature compatible with acute leukemia (immunophenotype characteristics between M0 vs. M7) (12). Karyotype analysis of the of bone marrow and peripheral blood cells revealed trisomy 21 (46, XX+21) in all blast cells.

Six days following birth, the infant was referred to Bambino Gesù Children's Hospital (Rome, Italy). Upon physical examination, the patient exhibited no visible DS characteristics. Persistent hepatomegaly and occasional petechiae were observed. The results of the neurological examination were normal for the gestational age and the bregmatic fontanelle was normotensive and normally pulsating. The patient exhibited normal vital signs and was afebrile. An echocardiogram identified a patent foramen ovale. Ultrasonography of the brain, abdomen and kidneys was normal. Bone marrow cells immunophenotyping demonstrated that ~31% of cells were CD33 heterogeneous, CD117-positive, CD34 heterogeneous, CD71 dull, CD45 dull and DR negative. A single-base deletion (c.150delG) in exon 2 of the *GATA1* gene was identified in peripheral blood cell samples, after the admission to our center. The patient also exhibited a reduction in the platelet count, without any specific treatment, to a 40,000/μl nadir. From one month and five days, the platelet count was observed to increase again. Prior to discharge, cultures of fibroblasts and of the Epstein-Barr virus lymphoblastoid T-cell line-reactive test were negative. Considering these clinical features and the presence of a *GATA1* mutation, which could indicate transient leukemia, it was decided that chemotherapy would not be administered. The patient subsequently demonstrated a healthy clinical condition with normal values for the aforementioned clinical features. A bone marrow aspiration, conducted at the seven-month follow-up, revealed a normal karyotype and the absence of leukemic cells. Simultaneously, a bone marrow biopsy confirmed the absence of leukemic cell infiltration.

Written informed consent was obtained from the patient's family for the publication of the current case report and the accompanying images.

Flow cytometry. Flow cytometry was performed on blood or bone marrow specimens using 3- or 4-color antibody panels against a variety of lymphoid, myelomonocytic, and megakaryocytic antigens. The antibodies (and clones) used included (all antibodies from Pharmingen; BD Biosciences, San Jose, CA, USA, unless otherwise stated): anti-CD1a (HI149), CD3 (SK7), CD4 (SK3), CD5 (L17F12), CD7 (4H9), CD8 (SK1), CD10 (W8E7), CD11b (D12), CD13 (L138), CD14 (M/P9), CD15 (MMA), CD16 (NKP15), CD19 (SJ25C1), CD20 (L27), CD22 (S-HCL-1), CD33 (P67.6), CD34 (8G12), CD36 (FA6.152; Beckman Coulter, Inc., Miami, FL, USA), CD38 (HB7), CD41 (P2; Beckman Coulter), CD45 (2D1), CD56 (MY31), CD61 (RUUPL7F12), CD64 (10; Ancell, Bayport, MN), CD71 (L01.1/ M-A712), CD117 (2B8), HLA-DR (L243), surface light chains kappa (TB28-2) and lambda (1-155-2), glycophorin A (GA-R2) and appropriate isotypic control antibodies (X40, X39). Specimen processing and antibody staining were performed as previously described (13). All data were acquired using 3-color FACSort or 4-color FACSCalibur flow cytometers with Cellquest software (BD Biosciences). The data was analyzed by cluster analysis with Paint-a-Gate Software (BD Biosciences).

Cytogenetics studies. Firstly, during hospitalization, chromosomal analyses of unstimulated bone marrow of patient were conducted on 20 metaphases revealing an abnormal karyotype: 47, XX, +21 (Fig. 1B) Subsequently, the chromosomal analyses were extended to two other issues: Peripheral blood cells and dermal fibroblasts using a G-banding technique, as previously described (12,14). A total of 20 metaphases for dermal fibroblasts and 50 metaphases for peripheral blood cells, were analyzed demonstrating a normal karyotype: 46, XX (Fig. 1A), in both cases. Structural and numerical anomalies were recorded and karyotyped according to the International System for Human Cytogenetic Nomenclature (2013) or all three samples (14).

In order to investigate this result, fluorescence *in situ* hybridization (FISH) was performed using a probe specific for the 21q22.2 region. FISH was conducted as previously described (14), using a Vysis LSI 21 SpectrumOrange Probe Kit (locus 21q22.13-q22.2; Abbott Molecular, Illinois, USA) to identify the critical region of DS. Analysis of 500 nuclei from bone marrow samples identified the presence of three signals in 70% of the cells (Fig. 2A); this result represents an

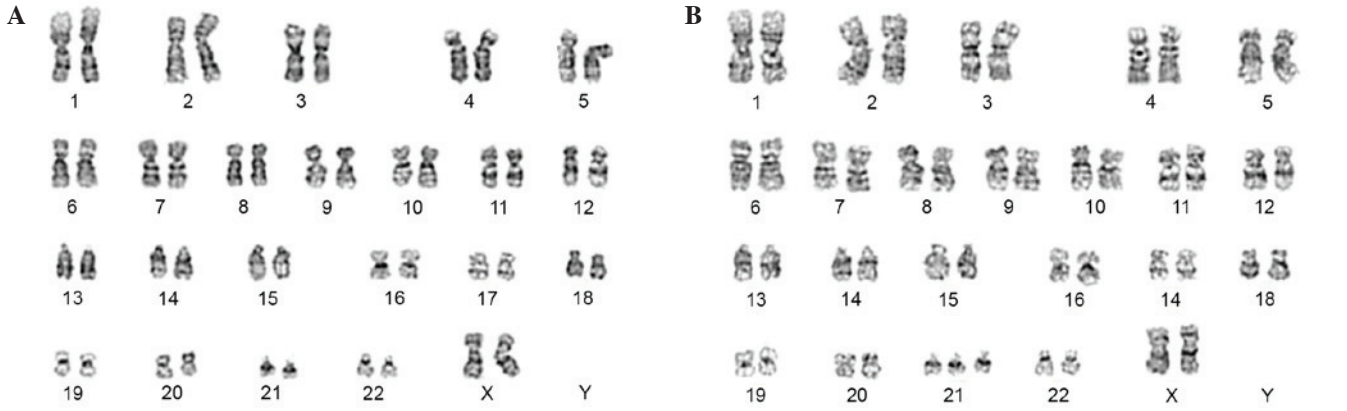

Figure 1. The G-banding karyotypes obtained from (A) peripheral blood, that reveals a normal karyotype, and from (B) bone marrow cells that presents trisomy 21.

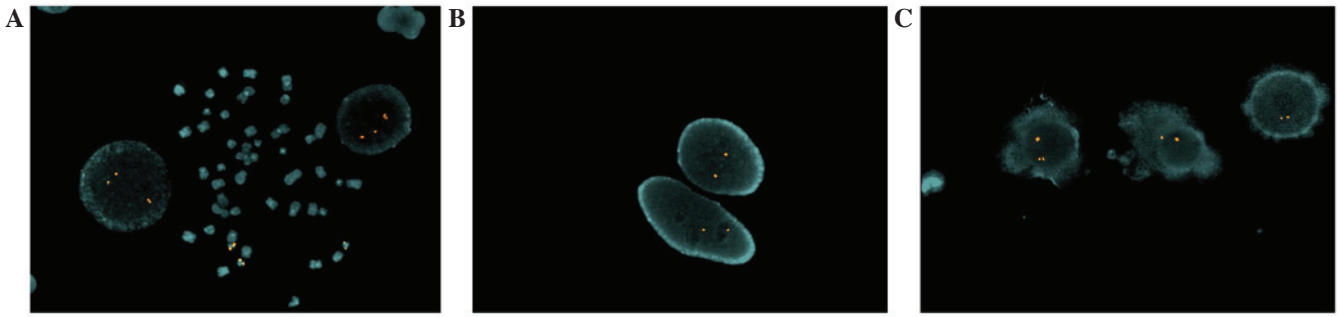

Figure 2. Fluorescence *in situ* hybridization experiments using a probe specific for the Down syndrome critical region (Vysis LSI 21 SpectrumOrange Probe; locus 21q22.13-q22.2). (A) Bone marrow cells exhibited three red signals, whereas two red signals were observed in (B) peripheral blood cells and (C) dermal fibroblasts.

abnormal condition. Instead, the remaining 30% of the cells of bone marrow exhibited two signals, as expected in normal conditions. Conversely, analysis of the peripheral blood cells and dermal fibroblasts (500 nuclei) revealed only two signals per cell (Fig. 2B and C).

All nuclei were analyzed using a Nikon Eclipse E1000 epifluorescence microscope, equipped with a CoolSNAP fx charge-coupled device camera (Photometrics, Tucson, AZ, USA). Image capturing and processing were performed using Genikon software v3.6.16 (Nikon Corporation, Tokyo, Japan).

Molecular studies. Amplification of *GATA1* exon 2 genomic DNA (genomic DNA accession no. AF196971; complementary DNA accession no. NM_002049) was performed in 20 μl containing 10 μl KAPA 2G Fast HS ReadyMix PCR Kit (Kapa Biosystems, Inc., Wilmington, MA, USA), 1 uM primers (FW-5'-TCTGTCCTCGCAGGTTAATCCR-3', RV-5'-TATTCTGACCTAGCCAAGGATCTC-3') and 2 μl of DNA (50 ng).

The cycling profile was set at 96˚C initial denaturation for 3 min, followed by 35 cycles of 10 sec at 96˚C, 10 sec at 58˚C, 2 sec at 72˚C, and a final extension at 72˚C for 5 min in a GeneAmp® PCR system 2700 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). PCR products were enzymatically purified using Illustra ExoProStar reagent containing phosphatase and exonuclease (GE Healthcare Life Sciences, Chalfont, UK). Sequencing reactions were carried out using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The reaction products were purified after sequencing by using DyeEx® 2.0 Spin Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). Direct sequencing was performed in both directions for all samples on an automated sequencer ABI Prism® 3130 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). GATA1 sequencing identified the deletion of a single base (c.150delG) in exon 2, that caused a frameshift resulted in premature sequence termination (p.Ser51Alafs*86; Fig. 3) (15).

Discussion

To the best of our knowledge, this is the first Italian case of TAM in a non-DS infant with trisomy 21 only in blast cells; this condition differs from congenital leukemia due to its spontaneous remission (2). In the present case, considering the improvement of clinical features, coagulation and inflammatory markers, in addition to the gradual increase in the number of platelets, it was assumed that acute leukemia was a transitory clinical episode and that the blasts had been progressively reduced by apoptosis.

Differentiating between TAM and congenital acute leukemia is challenging and it is important to reach a rapid

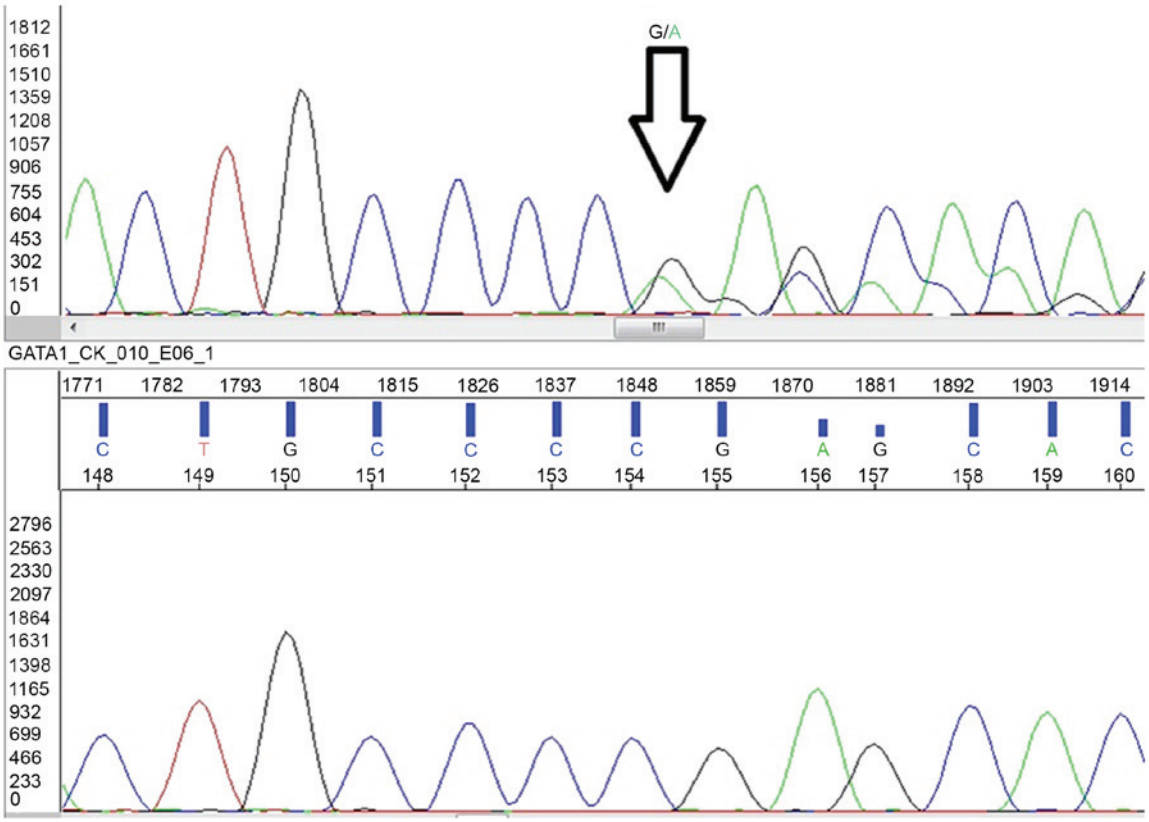

Figure 3. Substitution of a single base (c.150G>A) in exon 2 of the GATA binding protein 1 gene.

diagnosis. The initial approach is to exclude the possibility of leukemoid reactions due to other conditions, including infection, hypoxia and hemolytic disease (1). It is crucial to perform a complete blood cell count with a peripheral blood smear and a bone marrow aspiration with immunophenotyping and cytogenetic testing (1). The typical presentation of transient leukemia in infants is leukocytosis, often with a higher percentage of blasts in the peripheral blood compared with in the bone marrow (16). Hepatosplenomegaly, pericardial or pleural effusions, and liver disease are common features. The blasts are frequently of the megakaryocytic or erythroid lineages (16). In the event that a newborn with a normal karyotype and trisomy 21 only in blasts presents symptoms typical of congenital leukemia, the best approach is to 'wait and see' and to perform *GATA1* gene sequencing as soon as possible. If the patient's condition deteriorates, it may be advisable to commence low-dose chemotherapy (17). The presence of a *GATA1* mutation can confirm a diagnosis of TAM and therefore determine the non-therapeutic approach.

The *GATA1* gene is located on the short (p) arm of the X chromosome at position 11.23; the encoded protein is a transcription factor involved in the differentiation of megakaryocytic precursors of erythrocytes (6,10). The mutations are somatic, not inherited, and occur during fetal development; the increased risk of leukemia applies only to patients with an extra copy of chromosome 21 (18). *GATA1* may be involved in folate metabolism and oxidative stress in trisomy cells, modifying the repair mechanisms of DNA damage and creating pre-leukemic clones with a selective growth advantage (19). Environmental, immune and reproductive factors may contribute to the mutation process (18). The mutations on exon 2 of the *GATA1* transcription factor gene have been revealed to exhibit 100% penetrance in TAM (19). A previous study analyzed the mutation spectrum of sequence alterations at *GATA1* exon 2 in DS TAM/AMKL cases, revealing that the mutations may be insertions/deletions/duplications or base substitutions (19). The mutation detected in this case was a deletion of a single base (c.150delG), which determines an accumulation of uracil in cells under oxidative stress. Moreover, substitutions of a single base in the *GATA1* gene have been reported in 6 cases of TAM; in a retrospective series, a myelodysplastic syndrome or acute myeloid leukemia (20), often of the acute megakaryoblastic type, developed in ~30% of patients with TAM (16).

Once a diagnosis of TAM has been determined, it is necessary to follow-up with the patients to detect the possible occurrence of leukemia. In a previous study, out of 16 cases of TAM in non-DS patients, 5 developed subsequent leukemia, with 3 developing AMKL and 2 developing non-AMKL acute myeloid leukemia (10), and a total of 9 patients subsequently required chemotherapy (21).

The present case study adds another example of spontaneous remission of TAM to the literature, contributing to the discovery of an additional mutation of GATA1 gene. Furthermore, the findings of the current study may aid clinicians in decision-making when treating cases of TAM. Firstly, the best way of approaching a suspected case of TAM is waiting without starting chemotherapy. Secondly, if a case is strongly suspected to be TAM, performing *GATA1* gene sequencing may confirm the diagnostic hypothesis.

Further investigations are required to explain how blast cells disappear spontaneously, as if it obtained a selective disadvantage.

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