A Novel GATA-1 Mutation in a Neonate with Transient Abnormal Myelopoiesis without Down Syndrome

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INTRODUCTION

Transient abnormal myelopoiesis (TAM) is a rare disorder usually diagnosed in newborns with Down syndrome, less frequently in mosaic trisomy 21 and only sporadically in phenotypically normal infants. In addition to the association with trisomy 21, TAM is also associated with mutations in the GATA-1 gene. We report a case of TAM with a novel mutation in the GATA-1 gene. A newborn boy was found to have leukocytosis (52.9 x 10^9/l) with 50% myeloid blast forms. Fluorescence in situ hybridization (FISH) on peripheral blood showed trisomy 21 limited to the blast forms. Further cytogenetic studies revealed that he did not have constitutional trisomy 21 or mosaic trisomy 21. Sequencing studies performed on DNA isolated from a peripheral blood sample containing blast forms showed a frameshift mutation in the GATA-1 gene (c.148_149dup CC) implying premature termination. A diagnosis of transient myeloproliferative disorder spectrum was made. The patient was treated with a short course of cytarabine with resolution of leukocytosis and a blast count of less than 0.5% at one month of age. Follow-up at 2 years after diagnosis showed normal peripheral blood counts and sustained molecular remission with normal karyotype.

Key Words: myeloproliferative disorder, Down syndrome, GATA-1 gene

Case Report

From a clinical perspective, most TAM patients are asymptomatic and TAM is found as a result of a routine medical checkup or incidental blood examination performed for an unrelated illness. The majority of patients remain well and the disease resolves within the first 3 months of life in most cases without any therapy. However, rarely, clinical complications including cardiopulmonary failure, hyperviscosity, splenic necrosis and progressive hepatic fibrosis may develop. A few children experience life threatening or even fatal clinical complications. In addition, 20-30% of TAM patients develop non-transient AML 1-3 years later.1,2

Biologically, TAM is characterized by the presence of immature myeloid cells (TAM blasts) in the peripheral blood and in bone marrow. The TAM blasts often have basophilic cytoplasm and cytoplasmic blebbing suggestive of megakaryoblastic derivation. The majority of blasts are positive for CD34, CD56, CD117, CD13, CD33, CD7, CD4 dim, CD41, CD42, TPO-R, IL-3R, CD36, CD61, CD71, and negative for myeloperoxidase, CD15, CD14 and glycoporphin A. The morphologic and immunophenotypic features of TAM blasts are similar to those of the blasts in myeloid leukemia associated with Down syndrome (ML-DS).3

Here we report a TAM case that occurred in a child without Down syndrome. Trisomy 21 was present in the TAM blasts only. In addition, a novel mutation of GATA-1 gene (c.148_149dup CC) was identified in the TAM blasts.

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CASE REPORT

A baby boy was born at 40 weeks gestation to a 24 year old G1P1 mother. His APGAR scores were 9 at 1 minute and 9 at 5 minutes. There was no significant family or social history. The family origins were in South East Asia. A complete blood count (CBC) was performed at birth due to suspected chorioamnionitis. The CBC showed a hemoglobin level of 9.9 g/dl, a platelet count of 121 x 10^9/l, and a white cell count of 52.9 x 10^9/l with 50% myeloid blast forms. The blast forms had moderate amount of basophilic cytoplasm with some resembling pronormoblasts and others showing cytoplasmic undulations suggestive of megakaryocytic differentiation. The CBC and peripheral smear also revealed mild microcytic hypochromatic anemia with target cells and mild thrombocytopenia. Flow cytometry performed on the peripheral blood showed that the blast forms (55% of total cellularity) had an immature myeloid immunophenotype (CD45 dim, CD33+, CD117+). These cells expressed markers associated with erythroid (CD235/Glycoporphin) and megakaryocytic (CD41 subset) differentiation. Aberrant expression of CD7 was also seen. Most of these cells were negative for CD34 (however a small subset was positive). The blast forms were also negative for CD13, CD14, CD10, CD19, HLA-DR and other B and T cell markers. Subsequent flow cytometry performed on bone marrow showed similar findings.

Bone marrow biopsy showed a predominance of immature large myeloid forms constituting about 50% of the cellularity against a background of maturing erythroids and myeloids. Rare megakaryocytes were also noted and showed normal morphology. Immunohistochemical staining for CD117 was positive in about 20% of total cellularity.

Immunohistochemical staining for parvovirus and cytomegalovirus was negative.

Fluorescence in situ hybridization (FISH) performed on peripheral blood revealed both trisomy 21 and disomy 21 cells, with trisomy 21 being restricted to the blast forms only. To rule out Down syndrome, FISH was performed on cells derived patient’s mucosal buccal swab. All of the somatic cells revealed only two copies of chromosome 21. To confirm the patient’s constitutional karyotype, analysis was performed on skin fibroblasts and showed a normal male karyotype (46, XY). Following these findings an evaluation by Clinical Genetics was performed and confirmed the absence of clinical features associated with Down syndrome.
Upon review of all the data, especially the age of the patient (neonate) and blast cytomorphology consistent with megakaryoblastic derivation and presence of trisomy 21 in the blast forms a diagnosis of transient abnormal myelopoiesis spectrum was favored, though myeloid leukemia was considered because of the high percentage of the blast forms.

Subsequently a sequencing study on DNA isolated from diagnostic peripheral blood was performed to reveal a frameshift mutation in exon 2 of the GATA-1 gene (c.148_149dup CC), resulting in premature termination (p.Ser51Argfs*87). Although different GATA-1 mutations have been identified in transient myeloproliferative disorder and megakaryoblastic leukemia in children, this particular mutation had not been reported.

Due to the patient’s microcytic anemia with target red cells and the family origin from South East Asia, thalassemia was suspected and molecular testing was performed. The patient was found to be heterozygous for the deletion of two alpha globin genes in cis (α- / α α), consistent with alpha thalassemia trait of the South East Asian type.

The patient was treated with a short course of cytarabine (1 mg/kg, q12 hrs, x 7 doses total). Blood counts at one month of age showed resolution of his leukocytosis and a blast count of less than 0.5%. At 6 months, a bone marrow biopsy revealed normal trilineage hematopoiesis and only 1% of cells with trisomy 21 by FISH. At this point bone marrow aspirate submitted for GATA-1 gene sequencing was negative for exon 2 GATA-1 mutations. Follow-up at 2 years after diagnosis showed normal blood counts and sustained molecular remission with normal karyotype.

DISCUSSION

The pathogenesis of TAM is not well understood, but a multistep process of leukemogenesis has been proposed. Trisomy 21 is regarded as an initiating event (the first hit). Several genes on chromosome 21 (RUNX1, BACH1, ETS2 and ERG) have been under active investigation for possible involvement in TAM. It is has been proposed that trisomy 21 creates an environment within the fetal liver, where hematopoietic progenitor cells are primed for acquisition of a somatic GATA-1 mutation (the second hit) leading to dysregulated myeloid maturation and TAM. At birth, hematopoiesis transitions from the fetal liver to bone marrow.
Mutations of the GATA-1 gene (located at Xp11.23) have been reported in nearly all TAM patients. In normal circumstances, a full-length GATA-1 protein produced by translation from methionine at codon 1 (Met1) and a shorter protein (GATA-1s) is produced by alternative splicing from Met84 on exon 3. Point mutations and frameshift mutations in GATA-1 mutations have been reported in TAM, all localized to exon 2 or exon 3, resulting in the loss of the full-length GATA-1 protein with preserved production of the short GATA-1s protein. In our case, the blasts exhibit a frameshift mutation in exon 2 (c.148_149dup CC), with predicted premature termination (p.Ser51Argfs*87) of the full length GATA-1 protein translation and unaffected GATA-1s translation. This molecular change is classical for TAM.

While in most cases TAM the blasts are negative for glycophorin A, a subset of blasts in this case expressed both glycophorin A and CD41, consistent with mixed erythroid and megakaryocytic phenotype. TAM with erythroid differentiation has been reported previously. While the blasts in myeloid leukemia of DS typically show megakaryocytic differentiation, blasts in TAM tend to be more heterogeneous and express antigens related to multiple hematopoietic cell lineages, including megakaryocytes, granulocytes, erythroid cells, stem cells, and certain lymphoid markers.

Mild thrombocytopenia often accompanies TAM. Our patient presented with mild microcytic hypochromic anemia with target cells in addition to mild thrombocytopenia. The anemia and target cells can be explained by the patient’s alpha thalassemia trait. His platelet count normalized as the TAM blasts decreased.

The treatment for TAM is controversial. Some patients are observed without any treatment, while others receive leukapheresis or chemotherapy. Cytarabine is the agent of choice as it has been shown to reduce mortality, major concern being myelosuppression. In our case, a short course of cytarabine (1 mg/kg, q12 hrs, x 7 doses total) was administered, partly due to the unusual presentation and the possibility of this being a non-transient leukemia. The patient’s blast count in blood reduced rapidly after the short course and no more treatment was required. Follow up studies showed complete cytogenetic and molecular remission, confirming the diagnosis of TAM. The patient is disease free 2 years after initial diagnosis.

CONFLICT OF INTEREST
The authors have no conflict of interest to disclose.

REFERENCES