Transient Abnormal Myelopoiesis in Neonates

GATA Get the Diagnosis

Melissa Bombery, MD; Jo-Anne Vergilio, MD

• Transient abnormal myelopoiesis occurs exclusively in patients with Down syndrome (constitutional trisomy 21), manifests in the neonatal period, and is characterized by circulating megakaryoblasts with varied degrees of multisystem organ involvement. In most cases, this process resolves spontaneously by 3 to 6 months of age, but for some, the disease can be fatal. Affected patients are particularly prone to develop acute megakaryoblastic leukemia in early childhood. Somatic *GATA1* mutations are believed to be pivotal in the development of transient abnormal myelopoiesis and have proven to be a marker of clonal identity in its evolution to megakaryoblastic leukemia. We describe a study case of transient abnormal myelopoiesis and review the clinical manifestations, laboratory features, natural history, molecular genetics, and postulated disease pathogenesis of this disorder.

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STUDY CASE

A 2-day-old male neonate was born via caesarean section at 36 weeks 6 days' gestation with dysmorphic features, decreased tone, and an atrioventricular septal defect. A complete blood count documented moderate leukocytosis (white blood cell count [WBC] of 58 600/μL [reference range: 9000–30 000/μL]), while review of a concurrent peripheral blood smear demonstrated leukoerythroblastic features including 10% to 15% blasts that were large with round to ovoid nuclei, fine chromatin, small distinct nucleoli, and basophilic cytoplasm with occasional cytoplasmic blebs (Figure 1, A through C). Flow cytometric analysis confirmed megakaryoblastic differentiation with variable expression of CD34, CD117, CD13, CD33, CD4, CD7, CD56, CD71, CD41, and CD61 (Figure 2, A through C). During the next 2 weeks, the WBC count normalized and blasts disappeared from circulation. Cytogenetic karyotypic and molecular genetic analyses identified isolated trisomy 21 and *GATA1* mutation, respectively, thereby confirming a diagnosis of Down syndrome (DS) with transient abnormal myelopoiesis.

DOWN SYNDROME—AN OVERVIEW

Down syndrome, or constitutional trisomy 21, is the most common human chromosomal abnormality with an incidence of approximately 1 in 700 live births.[1] Prevalence correlates with increasing maternal age. Greater than 95% of cases are secondary to chromosomal nondisjunction. Rarely, mosaicism (trisomy 21 in only a subset of cells) or translocation may occur. Typical physical traits include small stature, decreased muscle tone, upward slanted eyes, and a single central deep palmar crease. Varying degrees of developmental delay are present and multiple other medical problems can occur, particularly congenital heart defects.

Interesting associations have been documented between DS and various hematopoietic and nonhematopoietic malignancies. Throughout the course of their lifetime, patients with DS have an overall reduced risk of solid tumors.[2] In contrast, a 500-fold increased risk of acute megakaryoblastic leukemia (AMKL) is seen during early childhood (1–5 years of age).[3] In late childhood, children with DS have a 10- to 20-fold increased risk of developing acute leukemia,[4] but lymphoblastic leukemia then becomes more common.

Historically, preleukemic and leukemic phases of disease have been described during infancy and early childhood. The "preleukemic" phase manifests at, or soon after, birth with circulating megakaryoblasts that are clonal in origin. Spontaneous resolution typically occurs; hence, this phase is now referred to as *transient abnormal myelopoiesis* (previously transient myeloproliferative disorder, transient leukemia). The blasts enter a seemingly latent/quiescent phase, but are believed to acquire additional mutations through the process of clonal evolution with subsequent progression to AMKL.

TRANSIENT ABNORMAL MYELOPOIESIS

Clinical and Laboratory Manifestations

Transient abnormal myelopoiesis (TAM) is seen exclusively in DS and affects approximately 4% to 10% of neonates.[5,6] The true incidence is not known as patients may be asymptomatic and routine laboratory screening is not

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From the Department of Pathology, University of Michigan Health System, Ann Arbor.

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Reprints: Jo-Anne Vergilio, MD, Department of Pathology, University of Michigan Health System, M5242 Medical Science I, 1301 Catherine Rd, Ann Arbor, MI 48109-0602 (e-mail: jvergili@umich.edu).

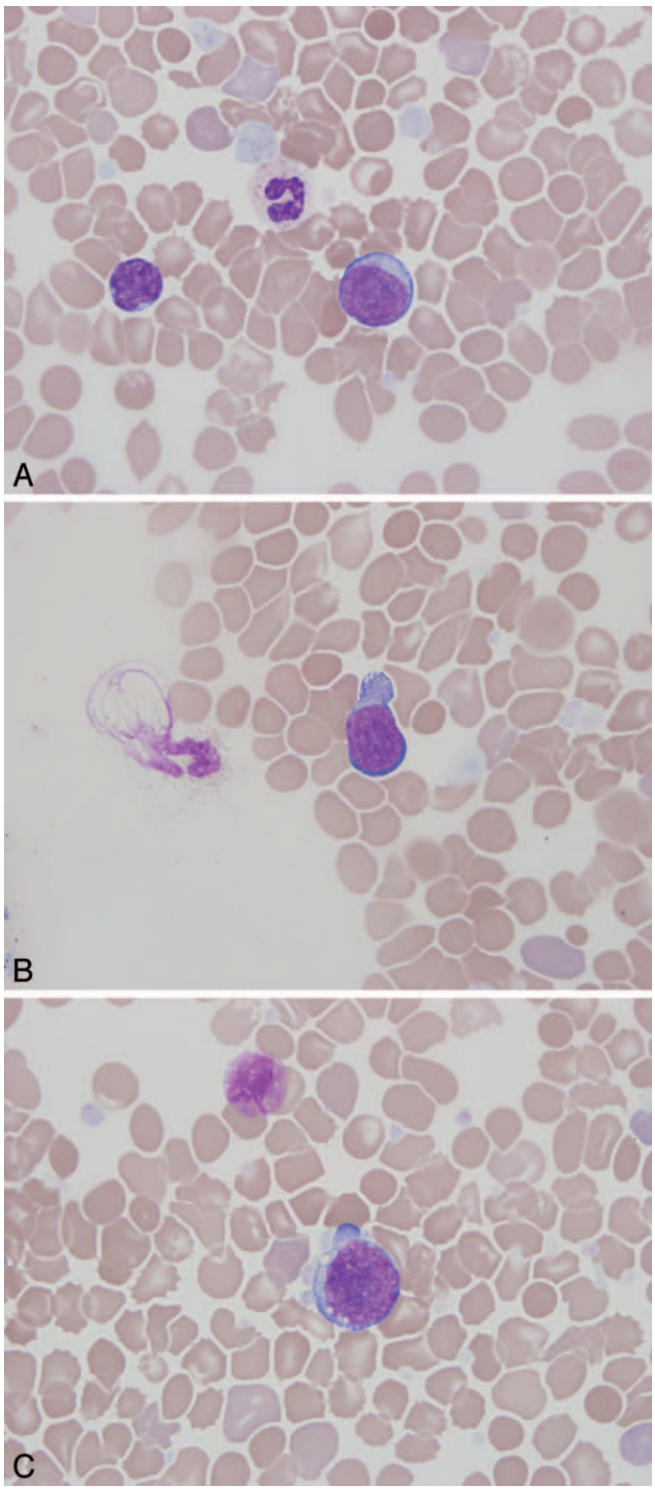


Figure 1. *Spectrum of circulating megakaryoblasts in a neonate with Down syndrome with transient abnormal myelopoiesis. The blasts may be undifferentiated in appearance with round nuclei, dispersed chromatin, small nucleoli, and scant basophilic cytoplasm (A) or may exhibit variably prominent cytoplasmic blebs (B and C) (Wright-Giemsa, original magnification ×1000 [A through C].*

generally performed. The median age of presentation is 3 to 7 days, though patients may be diagnosed at up to 2 months of life.[7] The most common clinical manifestations include hepatomegaly (60%), splenomegaly (35%–40%), jaundice (15%), pericardial effusion (15%), pleural effusion (10%–15%), ascites (10%), respiratory distress (10%), and bleeding diathesis (10%).[7] Less common features include hepatic fibrosis, hydrops fetalis, and renal failure.[2] Characteristic hematologic findings include leukocytosis (WBC > 100 000/μL in 20%–30% of cases), thrombocytopenia (40% of cases), and increased numbers of circulating blasts.[7] Approximately 10% to 25% of patients are asymptomatic; thus, the diagnosis may be established as an incidental finding during laboratory assessment for some other cause.[6,7] Occasionally, the finding of TAM may even be the first indication that a patient has trisomy 21.

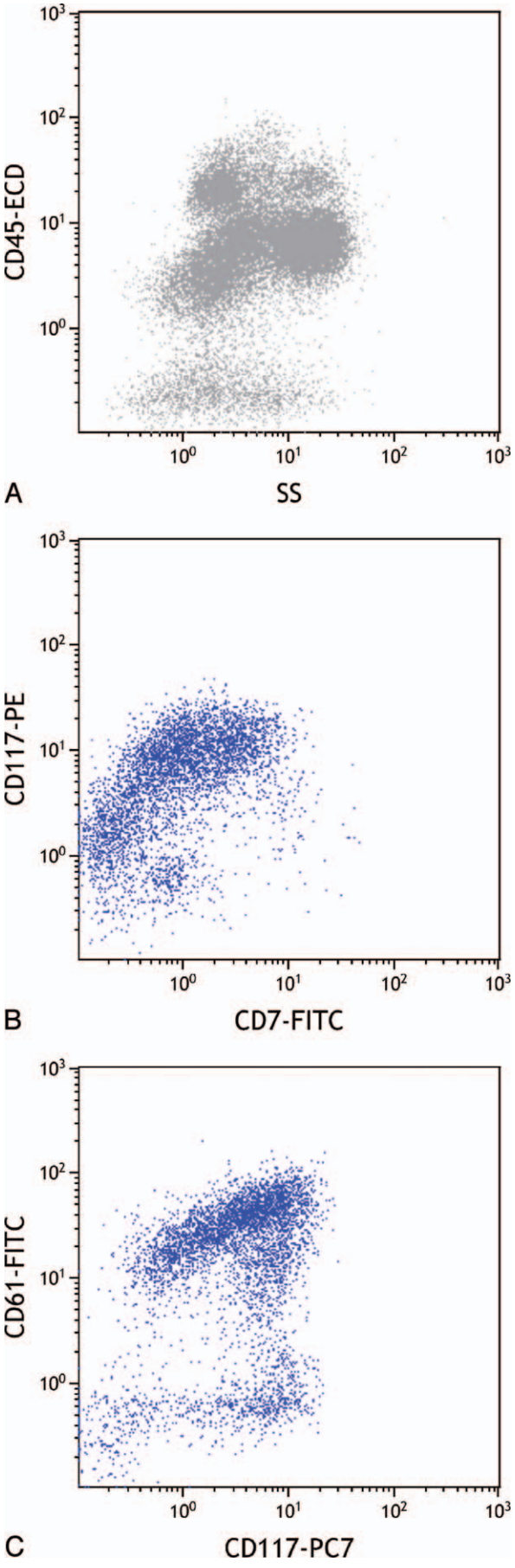
No critical threshold has been established for blast percentage in the diagnosis of TAM. Circulating blasts can often be seen in both neonates without DS, especially those who are ill or premature, and neonates with DS,[8,9] emphasizing the importance of manual peripheral smear review as well as ancillary laboratory studies in the evaluation. Morphologically, blasts typically exhibit round to ovoid nuclei, dispersed chromatin, small nucleoli, and deeply basophilic cytoplasm with cytoplasmic blebbing, a characteristic feature of megakaryoblastic differentiation (Figure 1). Phenotypically, blasts commonly express stem cell (variable CD34, CD117), myeloid (CD13, CD33), non-lineage (CD4, CD7, CD56), and megakaryoblastic/megakaryocytic (CD61, CD41, CD42) antigens.[10] Bone marrow examination is generally not indicated as marrow findings are either similar to, or less pronounced than, those in blood.

When TAM is suspected clinically, cytogenetic karyotypic analysis should be performed to establish constitutional trisomy 21, while *GATA1* mutation analysis is also recommended to document clonality of the blast population. The presence of an acquired mutation(s) in exon 2 or exon 3 of the *GATA1* gene on chromosome X establishes a diagnosis of TAM and serves as a potential marker for future disease monitoring in the development of AMKL. If a *GATA1* mutation is detected in a neonate without clinical features of DS, cytogenetic analysis should still be performed to exclude DS mosaicism.

Natural History

The natural history of TAM is quite variable. Most neonates (80% of those with documented TAM) undergo spontaneous remission within 3 to 6 months of age.[6,7,11] At the other end of the disease spectrum, neonatal (or even fetal) demise occurs in approximately 10% of patients secondary to diffuse organ infiltration by megakaryoblasts, associated parenchymal fibrosis, and subsequent multisystem failure. Independent risk factors that portend early mortality include WBC counts above 100 000/μL, preterm delivery (<37 weeks), effusions (pleural, pericardial, ascites, or hydrops), coagulopathy, bleeding diathesis, platelet count greater than 100 000/μL, low birth weight, and failure to clear peripheral blasts.[6,7,12] When these high-risk features are present, chemotherapeutic intervention is warranted.

Approximately 20% of patients with TAM develop AMKL within the first 4 years of life and this may be preceded by a myelodysplastic-like syndrome.[6,7,11] The World Health Organization 2008 classification system introduces the category "myeloid leukemia associated with DS" (herein referred to as DS-AMKL) to encompass both myelodysplastic and leukemic manifestations regardless of blast percentage, as there is neither prognostic nor therapeutic significance to finer discrimination.[13] The median age of



onset is 2 years, younger than that seen in non–DS-AMKL.[14] Patients typically manifest with low WBC count, cytopenia, organomegaly, progressive marrow fibrosis, and additional cytogenetic abnormalities (monosomy 7, trisomy 8).[14–16] Blasts in DS-AMKL are morphologically and immunophenotypically similar to those seen in TAM.[10] Patients with DS-AMKL have a favorable prognosis with 80% 3-year overall survival.[7,11] This response rate is, in part, attributed to enhanced chemosensitivity of megakaryoblasts to cytarabine. The cytidine deaminase gene functions in cytarabine catabolism, and its transcription is diminished in DS, which may lead to diminished intracellular drug metabolism and consequent increased drug efficacy.[17]

GATA1 Mutations and Disease Pathogenesis

Somatic *GATA1* mutations seem to be pivotal in the development of TAM and have proven to be a marker of clonal identity in its evolution to DS-AMKL. *GATA1* mutations were first detected in blasts from patients with DS-AMKL, but could not be demonstrated in non–DS-AMKL, in patients with DS who had other subtypes of acute leukemia, or in healthy controls, suggesting they were specific to constitutional trisomy 21.[18] Subsequently, *GATA1* mutations were detected in the blasts of patients who have DS and TAM.[19,20] And, in sequential longitudinal samples from a single patient, megakaryoblasts detected first during TAM and subsequently during DS-AMKL were both found to harbor an identical *GATA1* mutation.[16,21] Together, these findings and other corroborative reports indicate that TAM and DS-AMKL are indeed clonally related.

The *GATA1* gene is located on the X chromosome and encodes a zinc finger transcription factor that is essential for normal erythropoiesis and megakaryopoiesis. Its de novo protein product contributes to cytoplasmic maturation in megakaryocytes and organelle development in platelets, but functions as a negative regulator of megakaryocyte proliferation.[22] Various acquired mutations in exon 2, or less commonly exon 3, ultimately yield a mutant N-terminally truncated GATA1 protein (designated GATA1s) that has been detected exclusively in patients with DS.[18]

In normal human development, the liver is the primary site of fetal (in utero) hematopoiesis. With birth, the hepatic microenvironment changes such that liver hematopoiesis is down-regulated while bone marrow simultaneously assumes this primary functionality. Given the clinical and laboratory manifestations of TAM, which include leukocytosis and circulating megakaryoblasts, often with hepatomegaly, TAM has been postulated to reflect perturbation of this normal developmental process. Transient abnormal myelopoiesis may arise in utero within the fetal liver with "spontaneous resolution" reflecting the natural process of hepatic hematopoietic down-regulation.[23–25] In support of

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Figure 2. *Characteristic immunophenotypic profile of circulating megakaryoblasts in transient abnormal myelopoiesis, demonstrating positivity for CD45 (dim), CD117, CD7, and CD61. Coexpression of other markers included CD4, CD13, CD33, CD34 (weak), CD56, and CD71 (data not shown). A, CD45 versus side scatter (SS) reveals an expanded population of immature cells within the dim CD45 and low SS "blast" region. B, Blast region assessed with CD117 and CD7, demonstrating CD117 and heterogeneous CD7 positivity. C, Blast region assessed with CD61 versus CD117, confirming coexpression of CD61. Abbreviations: ECD, phycoerythrin Texas-Red; FITC, fluorescein isothiocyanate; PC7, phycoerythrin cyanin 7; PE, phycoerythrin.*

this hypothesis, trisomy 21, in the absence of *GATA1* mutation, has been shown to alter normal second-trimester hematopoiesis in fetal liver but not in fetal bone marrow. Relatively increased numbers of megakaryocyte-erythroid progenitors and relatively decreased numbers of common myeloid and granulocyte-monocyte progenitors have been detected when compared to gestation-matched normal controls.[26] Increased clonogenicity of all progenitors was also noted in this setting. These findings suggest that fetal liver in DS offers a potential substrate on which *GATA1* mutations might confer a selective growth advantage.

GATA1 mutations (both single and multiple clones) have been detected in Guthrie card blood spots of patients with DS, and concordant *GATA1* mutations were seen in identical twins, when using this methodology, supporting a prenatal origin for *GATA1* mutations.[27,28] A concordant *GATA1* mutation was also confirmed in the peripheral blood of identical twins with DS who had TAM, suggesting development of a *GATA*1 mutation in 1 twin with in utero twin-twin transmission of the clone.[29] Additionally, liver parenchyma from postmortem examination of fetuses with DS (21 and 23 weeks of gestation) also contain *GATA1* mutations; yet, these mutations were not detected in concurrent bone marrow, lending further support to the above theory of *GATA1* mutation acquisition during fetal liver hematopoiesis.[30] Lastly, *GATA* mutation in nonhematopoietic tissue is, in itself, insufficient to evoke TAM.

A multistep process of leukemogenesis has thus been postulated in which trisomy 21 represents the "initiating" event in disease pathogenesis. Trisomy 21 creates an environment, in utero, in which hematopoietic progenitor cells within fetal liver are primed for acquisition of either single or multiple somatic *GATA1* mutations that reflect a "secondary hit," thereby promoting hematopoietic dysregulation and emergence of TAM. With birth, hematopoiesis naturally transitions from fetal liver to bone marrow and the *GATA1* megakaryoblastic clone becomes quiescent. However, this clone persists over time and undergoes other yet-to-be-defined genetic and/or epigenetic events that ultimately lead to the impaired megakaryocytic differentiation and uncontrolled proliferation characteristic of DS-AMKL.

SUMMARY

Transient abnormal myelopoiesis is a preleukemic disorder that occurs only in neonates with constitutional trisomy 21. Transient abnormal myelopoiesis typically presents in the first week of life with leukocytosis, thrombocytopenia, hepatomegaly, and circulating megakaryoblasts, the latter of which contain an acquired *GATA1* mutation. Although TAM can be fatal in 10% of patients, it most often resolves spontaneously, but is believed to persist in a "quiescent" state. By 5 years of age, 20% of patients progress to AMKL following an intervening remission and/or a preceding myelodysplastic-like syndrome. Down syndrome–AMKL has a favorable prognosis with enhanced chemotherapeutic responsiveness to cytarabine.

The disease evolution of TAM and DS-AMKL is currently conceived as a sequential multistep process of leukemogenesis. Trisomy 21 represents the critical "initiating" event. *GATA1* mutation reflects a "secondary hit" to fetal liver hematopoiesis, particularly megakaryocytic-erythroid progenitors, that leads to TAM and confers some selective advantage. The subsequent events in leukemogenesis and development of DS-AMKL have yet to be defined.

Given the risk of progression to DS-AMKL, some advocate that all neonates with DS undergo routine screening for TAM with manual peripheral blood smear review and *GATA1* mutation analysis. With detection of a *GATA1* mutation, clinical assessment and routine laboratory screening is then suggested periodically throughout early childhood.

Transient abnormal myelopoiesis: GATA get the diagnosis.

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