Transient Neonatal Myeloproliferative Disorder Without Down Syndrome and Detection of GATA1 Mutation

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Summary: Transient myeloproliferative disorder is a form of self-limited leukemia that occurs almost exclusively in neonates with Down syndrome. The authors report an unusual case of a newborn without constitutional trisomy 21 who developed undifferentiated leukemia and subsequently achieved clinical and molecular remission without chemotherapy. Cytogenetics and molecular analysis have shown trisomy 21 and GATA1 mutation restricted to leukemic cells. G-to-T transversion was detected, which is predicted to result in a premature stop codon (c.119G>T) in diagnosis samples. These findings emphasize that there must be a powerful interaction between GATA1 and trisomy 21 in leukemogenesis process.

Key Words: transient myeloproliferative disorder, trisomy 21, leukemia, GATA1 gene

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Transient myeloproliferative disorder (TMPD) is found almost exclusively in patients with Down syndrome (DS) during the newborn period.1 It has been estimated that at least 10% of DS newborns have TMPD.2 This disorder is clonal, confirmed by studies of female infants for X-linked phosphoglycerate kinase, hypoxanthine guanosine ribosyl transferase, or immunoglobulin heavy chain and T-cell antigen receptor.3,4 Spontaneous remission occurs in most patients within 3 months, although acute myeloid leukemia, usually megakaryoblastic type (AML-M7), develops 1 to 3 years later in about 25% of the infants who recover from TMPD.2

The biologic mechanism of the uniform spontaneous resolution of a disease indistinguishable from leukemia remains unclear. Recently, Wechsler et al reported the surprising finding that a gene on chromosome X, GATA1 was mutated in megakaryoblasts from each of six DS patients with AML-M7.5 GATA1 encodes a zinc finger transcription factor that has been shown to be critical for normal development of erythroid and megakaryoblastic lineages. Further reports showed that blast cells in congenital TMPD of DS carry the same type of mutations in exon 2 of GATA1 as those found in AML M7 in DS children.6 As this disorder is becoming recognized more frequently and causing difficulties in diagnosis, we would like to share our experience with the case described below.

CASE REPORT

A white girl, born at 34 weeks of gestation to a 27-year-old mother, was admitted on day 4 of life with moderate respiratory distress, feeding difficulties, and pronounced hepatosplenomegaly. Lymphadenopathy was not observed. There were no phenotypic features of DS. A complete blood count revealed a white blood cell count (WBC) of 320,000/dL with 86% blast cells, hemoglobin level (Hb) of 13.3 g/dL, and platelet count of 417,000/dL. The lactate dehydrogenase serum level was 8,260 IU/dL. Cytomorphology of bone marrow revealed 80% medium-sized blasts with prominent nucleoli and basophilic and agranular cytoplasm with frequent blebs. Immunophenotypic analysis of mononuclear cells using flow cytometry revealed CD4 89%, CD45 98%, CD19 90%, CD34 97%, CD7 65%, CD3 6%, CD13 3%, CD33 77%, and CD14 0%. Bone marrow and peripheral blood cytogenetic analyses were performed using Scheres’ method with G-banding technique. Bone marrow was cultivated using the direct method and showed trisomy 21 (47, XX, +21) in 12 of 12 metaphases analyzed. Study done on peripheral blood with phytohemagglutinin stimulation, following a modified Moorhead method,7 showed a mosaic pattern, with two cellular lineages: trisomy 21 (47, XX, +21) observed in seven metaphases and normal karyotype (46, XX) in five metaphases.

Considering these cytogenetic findings in the absence of Down’s phenotype and based on the report by Richard et al,10 we decided to provide only supportive treatment and no chemotherapy, with the agreement of her parents. She maintained a high WBC, up to 421,000/dL with 80% blasts. Over a 2-month period her clinical findings resolved and the WBC dropped to a normal standard. A second bone marrow aspirate revealed complete remission and normal karyotype (46, XX) in 34 metaphases analyzed, indicating that the trisomy 21 was nonconstitutional. A further bone marrow examination was performed at 3 months of follow-up, when the Hb level decreased to 5.2 g/dL, mean corpuscular volume (MCV) was 107 fl, the platelet count decreased to 17,000/dL, and the WBC was 10,300/dL. The feature of bone marrow aspirate was of continued

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complete remission. We found only megaloblastic changes in erythroid precursors. Although folic acid and B12 levels were not documented, therapeutic schedule with folic acid and vitamin B12 supplementation resolved the anemia and plaquetopenia. Four weeks later her hemoglobin level reached 10.3 g/dL, with MCV of 85.8 fl and platelet count 294,000/dL. The child was maintained in continuous clinical follow-up. At 5 years of age, she is developing normally and is still in continuous complete remission. Her WBC was 12.0 \times 10^9/L, Hb 12.6 g/dL, and platelet count 403,000. Cyto
genetic study performed using G banding analyzed 500 metaphases of cultured lymphocytes, confirming a normal female karyotype (46, XX).

Recently, \textit{GATA1} mutations were then screened in samples from different time points during the child’s diagnosis and follow-up. After written informed consent from the parents, genomic DNA was extracted and the exon 2 of \textit{GATA1} was PCR amplified as previously described.\textsuperscript{10} PCR products were sequenced in both directions using the DyeTerminator Kit (Amersham) as suggested by the manufacturer and analyzed in a MegaBACE 1000 automated sequencer. G-to-T transversion was detected, which is predicted to result in a premature stop codon (c.119G\textsuperscript{T}; p. Glu67X). This mutation was not detected in our patient’s peripheral blood cells at 5 years of age (Fig. 1).

**TABLE 1. Reported Cases of TMPD in Normal Newborns**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Age/Sex</th>
<th>Hb (g/DL)</th>
<th>WBC (\times 10^9/L)</th>
<th>Platelets (\times 10^9/L)</th>
<th>Hepatosple-nomegaly</th>
<th>BM Blasts (%)</th>
<th>Cytogenetics BM/PB</th>
<th>Time Karyotype Normalization Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanna, 1985\textsuperscript{13}</td>
<td>15 d/F</td>
<td>12.7</td>
<td>25.0</td>
<td>45</td>
<td>71.0</td>
<td>–</td>
<td>10</td>
<td>47XX + 21</td>
</tr>
<tr>
<td>Kalousek, 1987\textsuperscript{14}</td>
<td>7 d/M</td>
<td>15.8</td>
<td>14.2</td>
<td>20</td>
<td>50.0</td>
<td>–</td>
<td>20</td>
<td>47XY + 21/46XY</td>
</tr>
<tr>
<td>Jones, 1987\textsuperscript{15}</td>
<td>Case 1</td>
<td>2 d/F</td>
<td>9.5</td>
<td>310.0</td>
<td>73</td>
<td>130.0</td>
<td>+</td>
<td>47XX + 21</td>
</tr>
<tr>
<td></td>
<td>Case 2</td>
<td>7 d/M</td>
<td>18.0</td>
<td>82.0</td>
<td>34</td>
<td>40.0</td>
<td>–</td>
<td>46XY + t(21;21)</td>
</tr>
<tr>
<td>Ridgway, 1990\textsuperscript{16}</td>
<td>Case 1</td>
<td>12 h/M</td>
<td>9.4</td>
<td>66.0</td>
<td>53</td>
<td>295.0</td>
<td>+</td>
<td>47XY + 21</td>
</tr>
<tr>
<td></td>
<td>Case 2</td>
<td>5 d/M</td>
<td>8.7</td>
<td>92.4</td>
<td>45</td>
<td>92.0</td>
<td>+</td>
<td>53XY + 21</td>
</tr>
<tr>
<td>Faed, 1990\textsuperscript{17}</td>
<td>Case 1</td>
<td>2 d/F</td>
<td>17.7</td>
<td>17.0</td>
<td>24</td>
<td>107.0</td>
<td>+</td>
<td>47XX + 21</td>
</tr>
<tr>
<td>Brissette, 1994\textsuperscript{18}</td>
<td>Case 1</td>
<td>2 d/M</td>
<td>12.8</td>
<td>60.0</td>
<td>49</td>
<td>42.0</td>
<td>+</td>
<td>47XY + 21/46XY</td>
</tr>
<tr>
<td>Richard, 1998\textsuperscript{19}</td>
<td>Case 1</td>
<td>7 d/F</td>
<td>10.0</td>
<td>29.3</td>
<td>15</td>
<td>158.0</td>
<td>–</td>
<td>40XY + 21</td>
</tr>
<tr>
<td></td>
<td>Case 2</td>
<td>6 d/M</td>
<td>21.0</td>
<td>53.0</td>
<td>73</td>
<td>39.0</td>
<td>+</td>
<td>60XY + 21 abn 6</td>
</tr>
<tr>
<td>Slayton, 2002\textsuperscript{19}</td>
<td>Case 1</td>
<td>&lt;30 d/M</td>
<td>15.8</td>
<td>21.0</td>
<td>21</td>
<td>6.0</td>
<td>−</td>
<td>21 Trisomy 21</td>
</tr>
<tr>
<td>This report</td>
<td>Case 1</td>
<td>7 d/F</td>
<td>13.3</td>
<td>320.0</td>
<td>86</td>
<td>417.0</td>
<td>+</td>
<td>8047XX + 21/46 XX</td>
</tr>
</tbody>
</table>

BM, bone marrow; PB, peripheral blood.

**DISCUSSION**

In 1964, Engel and Hammond\textsuperscript{1} described neonates with DS and features of congenital leukemia that underwent spontaneous remission. Numerous case reports have described the condition known currently as TMPD.\textsuperscript{2,11} Confirmation of the diagnosis of TMPD is possible only when the spontaneous remission occurs, and this can take weeks or months after presentation of the symptoms. Although considered a benign disorder, one series presented an 11% mortality rate and severe clinical forms associated with hepatic fibrosis or liver dysfunction. It is estimated that 20% to 30% of DS neonates with TMPD will develop megakaryocytic leukemia (AML-M7; AMKL) within the first 4 years of life, often preceded by a myelodysplastic phase.\textsuperscript{2}

A similar condition is described in patients without phenotypic features of DS, but such patients often have constitutional trisomy 21 mosaicism.\textsuperscript{12} Table 1 summarizes the literature, with a few authors reporting TMPD in the absence of DS or constitutional mosaicism for trisomy 21, similar to our case. Unlike our patient, many of the other patients
described had moderate blastemia and an absence of systemic upset or profound marrow failure that would permit a conservative approach; our patient, in contrast, had severe hepatosplenomegaly, a high WBC, and blast cells representing almost 80% of the bone marrow nucleated cells.\textsuperscript{10,13–19} In our patient the immunophenotype results did not point to a particular subtype of cells, with a profile of undifferentiated cells (CD34/CD38+, CD34+/CD33/CD7+, and CD34/CD19+/CD10–). Slayton et al.\textsuperscript{19} using fluorescent in situ hybridization analysis (FISH) in enriched marrow subpopulations sorted by flow cytometry, determined which cell populations contained trisomy cells, at the time when the TMPD in a normal neonate with clonal trisomy 21 had resolved. The authors found trisomy 21 in erythrocyte and monocytic lineages but not in the stem cells, progenitor compartment, megakaryocytes, lymphocytes, or neutrophils. They postulated that trisomy 21 probably arises in a common myeloid progenitor, and a second mutational event led to appearance of the TMPD.

Most of these patients, after complete spontaneous hematologic and cytogenetic remission, have no evidence of recurrence or evolution to acute leukemia. There are two exceptions, described by Brissette et al in 1994\textsuperscript{18} and Richard et al in 1998.\textsuperscript{10} Both developed AML, respectively 1 year and 2 years after spontaneous resolution of TMPD; the karyotype of bone marrow in this instance presented re-emergence of trisomy 21 and in Richard’s case also evolution into a more complex karyotype.

To our knowledge, this is the first reported case of TMPD without DS with a detected GATA1 mutation. In our patient, two separate somatically acquired abnormalities were observed in leukemic cells: trisomy 21 and a premature stop codon in exon 2 of GATA1. Which of these two events occurred first? It is clear in DS with TMPD that mutations in GATA1 are the second event. Therefore, if the hypothesis presented by Slayton et al\textsuperscript{19} is correct, the non-disjunction trisomy 21 event found in our patient’s blast cells preceded the c.119G>T mutation in GATA1. The presence of both alterations in the same cell, occurring in utero, conferred a proliferative advantage, resulting in TMPD. As suggested by Slayton et al, the progenitor that acquired the double mutation would still have limited self-renewing capacity, which would explain its spontaneous remission. Therefore, even with the proliferative advantage that the combination of trisomy 21 and GATA1 mutation confers, a third genetic event that immortalizes the clone, postnatally, is probably necessary for full leukemic transformation. This scenario, initiated in utero, parallels the natural history and pathogenesis of acute leukemia in patients without DS.\textsuperscript{20}

This case is very informative because it emphasizes the point that there must be a powerful interaction between GATA1 and whatever trisomy 21 does in early hematopoietic cell differentiation.

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\textbf{REFERENCES}