Review Article

Hematopoietic Disorders in Down Syndrome

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Abstract: Patients with Down syndrome have an increased risk of developing various hematological disorders. In this article, the clinical characteristics and differential diagnosis of the hematological disorders associated with Down syndrome are reviewed, and the underlying molecular mechanisms discussed.

Key Words: Down syndrome, transient myeloproliferative disorder, acute leukemia, myelodysplastic syndrome

Introduction

Down syndrome (DS) is caused by an extra whole or partial copy of chromosome 21 and is the most common chromosomal abnormality in the live newborn (approximately 1 in 644 to 733 births in the United States) [1, 2]. The frequency of DS will probably change because of delayed maternity that is associated with increased risk of DS [3] and because of simpler and less invasive prenatal diagnosis of DS [4, 5]. Patients with DS have numerous constant and varying clinical features that include mental retardation, characteristic faces, congenital heart defects, gastrointestinal abnormalities, and hematologic abnormalities. The last is the focus of this review.

Hematologic Abnormalities in the Newborn DS

Up to 80%, 66%, and 34% of DS newborns have neutrophilia, thrombocytopenia, and polycythemia, respectively [2, 6, 7]. These findings are usually referred collectively as hematological abnormalities in newborn/neonates with DS [2, 6, 7]. We propose the catchier term, hematologic abnormalities in the newborn DS (HANDS). Thrombocytosis, anemia, and neutropenia are rarely seen in the DS newborns [2] and are usually secondary to processes independent of DS.

In general, the hematologic abnormalities in HANDS are mild, the clinical course is benign, and spontaneously resolve by 3 weeks of age [2, 6, 7]. Neutrophilia is mild (rarely exceed 30,000/µL) and is not associated with an infection. Thrombocytopenia is mild (majority with platelets counts <150,000/µL) and not associated with bleeding. Polycythemia is mild in most cases with only some exhibiting duskeness and cyanosis that correct with reduction/partial exchange transfusion. The polycythemia is usually independent of cardiac defects and hypoxia that are frequently associated with DS.

HANDS remains poorly characterized in regards to morphology, immunophenotype, and molecular mechanism. The latter likely involves the extra copy of chromosome 21 but which genes and pathways are responsible remain to be elucidated. The mechanism probably does not involve mutations to the gene GATA1 that are seen in two other hematological abnormalities of DS, transient myeloproliferative disorder (TMD) and acute megakaryoblastic leukemia (AMKL) of DS.

Transient Myeloproliferative Disorder

TMD is a disease entity unique to DS newborns and is defined as the morphologic detection of blasts in DS less than three months of age. TMD has also been referred to as transient abnormal myelopoiesis (TAM) or transient leukemia (TL). TMD is usually detected in the first week of life and spontaneously resolves by 3 months of age.
Up to 10% of all DS patients have TMD [8, 9], although more recent studies find lower percentages (3 to 6%) [2, 10]. TMD can present in the fetus [11-13] and can cause spontaneous fetal demise [9]. Hence, the prevalence of TMD in DS may be even higher because most studies do not account for cases of spontaneous fetal demise secondary to TMD.

The clinical presentation of a DS patient with TMD is variable [14]. Some clinical findings such as congenital heart disease and gastrointestinal anomalies are secondary to DS and unrelated to TMD. Other clinical findings such as hepatosplenomegaly and effusions are secondary to TMD and unrelated to DS in the absence of TMD. Although uncommon, TMD can cause severe diffuse lobular liver fibrosis with high mortality rate [15, 16] and fetal hydrops with hepatosplenomegaly [11-13]. TMD can also present with only circulating blasts without clinical symptoms [14].

TMD spontaneously resolves within 2 to 194 days with a mean of 58 days [14, 17]. TMD is associated with neonatal death secondary to liver failure, heart failure, sepsis, hemorrhage, hyperviscosity, and disseminated intravascular coagulation in 11 to 52% [14, 17, 18]. Death correlates with leukocytosis (100,000/µL), increasing organomegaly, worsening liver function, and visceral effusions. After resolution of TMD, approximately 13 to 29% develop acute megakaryocytic leukemia (AMKL) after 6 months of age with a mean age of 20 months [14, 17, 18]. AMKL occurs more frequently in TMDs that initially had additional cytogenetic abnormality beyond trisomy 21 [14]. Other TMD findings such as complete blood count, percentage of blasts, liver enzyme activities, age, and sex are not predictive of eventual AMKL [14].

TMD blasts are believed to be of fetal derivation and normally reside in organs of fetal hematopoiesis such as the liver, a model that may explain the higher percentage of TMD blasts in the peripheral blood compared to the bone marrow. In some cases of TMD, the BM biopsies can show megakaryocytic hyperplasia, dyspoietic megakaryocytes, and dyspoietic erythroid precursors [8, 9, 14]. Other cases show blastic infiltration of the bone marrow, skin, or liver. The liver biopsy can also show increased extramedullary hematopoiesis, dysplastic megakaryocytes, megakaryoblasts, and diffuse lobular fibrosis [14-16].

The blasts in TMD are almost always megakaryoblasts and are virtually indistinguishable to the blasts of acute megakaryoblastic leukemia (AMKL). Typically, the blasts are medium to large sized blasts with round to occasional binucleation, fine to slightly condensed chromatin, numerous cytoplasmic blebbing, scant to moderate basophilic cytoplasm, and occasional fine azurophilic granules consistent with platelet granules [9, 19] (Figure 1). In addition to the typical megakaryoblastic morphology, TMD blasts can resemble lymphoblast, erythroblast, myeloblast, and even monoblast, rendering correct lineage assignment difficult [20, 21]. The TMD blasts mark as megakaryoblasts by cytochemical stains. They are occasionally
Figure 2 Usual cytochemical staining patterns of megakaryoblasts in TMD and AMKL (1000x magnification).  

A. Sudan Black B. Similar negative staining is seen with myeloperoxidase and chloroacetate esterase. B. Acid phosphatase. C. Non-specific esterase using conditions resulting in diffuse cytoplasmic staining of monocytes and macrophages. Megakaryoblast (upper left) is negative while macrophage (lower right) is strongly and diffusely positive. D. Non-specific esterase using conditions resulting in multi-punctate staining of megakaryoblasts. This pattern is partially resistant to sodium fluoride.

granular to block positive for periodic acid-Schiff, and strongly positive for acid phosphatase while negative for Sudan black B, myeloperoxidase, and chloroacetate esterase (Figure 2). The nonspecific esterase staining, depending on substrate used and reaction conditions, varies from negative to multi-punctate pattern that is partially resistant to fluoride inhibition; diffuse cytoplasmic pattern inhibited by fluoride, typical of monocytic differentiation, is not seen [22]. This multi-punctate staining pattern is extremely sensitive and relatively specific for megakaryoblasts, although we have seen such pattern on some precursor B acute lymphoblastic leukemias.

Most TMD blasts resemble megakaryoblasts in electron microscopy. The blasts have cytoplasm with numerous small mitochondria, few dense granules, few alpha granules, or demarcation membranes of megakaryocytic differentiation while others show myeloperoxidase positive or ferritin-containing granules [23]. When combined with cytochemistry, electron microscopy shows that the peroxidase activity is limited to the nuclear envelope and the endoplasmic reticulum, typical of megakaryocytic lineage; this pattern is usually referred as the platelet peroxidase (PPO). PPO has been largely replaced by flow cytometry.

By flow cytometry, TMD blasts usually mark as megakaryoblasts. They express CD45, CD34, CD33, CD38, CD36, CD56, HLA-DR, CD7, and at least one of the megakaryocytic markers CD41, CD42a, or CD61 [14, 24, 25]. Less frequently, the TMD blasts express CD4, CD13, CD11b, CD265 (glycoporphin A), or
CD15. The TMD blasts do not express the more specific lymphoid markers such as CD3, CD5, CD19, and CD20. In some cases, the flow cytometry detection of the megakaryocytic antigens CD41, CD42b, or CD61 on blasts may represent reactivity to either non-megakaryoblasts with platelet satellitosis [26] or monoblasts. Platelet satellitosis can be excluded by examining the Wright stained cytospin of the flow cytometry specimen or by immunohistochemistry for CD61 on cytospin preparations [26]. Monoblasts can be excluded by diffuse non-specific esterase cytochemistry, by the absence of PPO reactivity, or by the flow cytometry detection of CD64, dim intracellular myeloperoxidase, and occasionally CD14.

The molecular mechanisms that lead to TMD are being elucidated. TMD blasts contain extra copies of chromosome 21 and occasionally additional karyotypic abnormalities [14]. Additional non-karyotypic oncogenic events must collaborate with trisomy 21 to generate TMD because TMD is seen in only a subset of neonates with trisomy 21 and TMD can occur without additional karyotypic abnormalities. Recent studies have begun to identify these collaborative steps. All TMD blasts have various somatic mutations in the X-linked gene GATA1 [27] that encodes a transcription factor critical for normal erythroid and megakaryocytic development [28]. Trisomy 21 and GATA1 mutations occur in TMDs that present in neonates who are mosaic for trisomy 21 and lack the clinical feature of DS [29, 30]. Mutations in the tyrosine kinase JAK3 are present in a subset of TMD [31, 32].

**DS-associated Acute Leukemia**

DS is associated with increased incidence of both acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML). Approximately, 1 in 100-200 DS children develops acute leukemia [33-36] with approximately equal numbers of ALL and AML. DS-associated ALL (DS-ALL) occurs throughout childhood with a median age greater than 4 years [37]. In contrast, DS-associated AML (DS-AML) occurs at a median age of 2 years [38], ranging from ages 6 months to 5 years [37]. Approximately 10-20% of DS-associated AMKL (DS-AMKL) have a preceding episode of TMD.

DS-associated acute leukemias have symptoms of cytopenias, similar to non-DS leukemias. However, DS acute leukemias have lower platelet counts than their non-DS counterparts [36, 39]. DS-AMLS have lower white blood cell count and have increased preceding history of myelodysplastic syndrome than their non-DS counterparts [36]. DS-ALLs have higher hemoglobin than their non-DS counterparts [39].

**DS-ALL**: The aspirate and biopsy findings of DS-ALL are identical to those for non-DS associated ALL. More than 90% of the DS-ALL are precursor B lymphoblastic leukemia with the remaining being precursor T lymphoblastic leukemia [34, 39-42]. Half of DS-ALLs have normal constitutive karyotype. DS-ALLs have decreased frequency of hyperdiploidy and rarely, if ever, contain the common balanced translocations, t(12;21), t(1;19), t(4;11), other 11q23 translocations, and t(9;22), that are seen in non DS-ALL cases [38, 39, 42, 43]. DS-ALLs have worse prognosis compared to non DS-ALLs [39, 42, 43]. The worse prognosis is partially secondary to the absence or decrease frequency of DS-ALLs with the favorable cytogenetics (t(12;21), hyperdiploidy, or triple trisomy of chromosomes 4, 10, and 17). DS-ALLs have similar event free survival as non-DS-ALLs that do not have the favorable cytogenetic findings [43]. However, the standard induction chemotherapy causes more toxicity (mucositis, hyperglycemia, and infection) in children with DS-ALLs. The increased toxicity seen in DS limits the use of more intense salvage therapy, resulting in decreased overall survival in DS-ALLs.

**DS-AML**: Most DS-AMLS are AMKL [35, 36, 40, 44-46] with similar aspirate smear morphology and cytochemical profile to those seen in TMD (Figures 1 and 2). By electron microscopic studies, DS-AMKLs have less differentiation with most blasts having no granules or lucent granules compared to TMD with some blasts showing alpha or dense granules of megakaryocytic differentiation [23]. On biopsy, DS-AMKLs show increased numbers of mononuclear cells in a diffuse or interstitial pattern (Figure 3), similar to other acute myeloid leukemia. The residual megakaryocytes can be dysplastic, showing hypolobated or multinucleated nuclei. DS-AMKLs can induce marrow fibrosis, resulting in a dry aspirate, or can present as a nodule mimicking metastatic round blue cell tumor. DS-AMKL may be detected on the biopsy but
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Figure 3  Bone marrow biopsy findings in AMKL. A. Extensive infiltration of the marrow by AMKL blasts (PAS stain, 400x magnification). B. Immuno-histochemistry stain for Factor VIII-related antigen is negative in the AMKL blasts despite strong reactivities seen in megakaryocytes (400x magnification).

not be represented in the bone marrow aspirate smears because of focal involvement of the specimen or associated fibrosis around the DS-AMKL blasts.

DS-AMKL is difficult to diagnose on biopsy alone because the current antibodies used for paraffin fixed tissue against glycoprotein IIIa (CD61) and factor VIII-related antigen (von Willebrand factor) may not be sufficiently sensitive enough to mark megakaryoblasts, although the more mature megakaryocytes are detected (Figure 3). If possible, immuno-histochemistry should be performed on a clot section that has not been decalcified because these antigens are acid labile and hence further compromised in a decalcified bone marrow biopsy. Touch imprints of the biopsy core could be used for cytochemical analysis or immunohistochemistry and are particularly useful in a dry aspiration. Additional biopsy cores can be submitted fresh in media, allowing the tumor cells to slowly suspend in the media +/- crushing of the core; the resulting supernatant can be analyzed by flow cytometry.

In addition to AMKL, some DS-AMLs are classified as AML-M0, M1, M2, M4, M5, or M6. M3 or acute promyelocytic leukemia is not seen in DS-AML. Even if some DS-AMLs are classified as non-AMKL, the blasts may be still megakaryoblasts given the variable morphology of megakaryoblasts and the absence of megakaryocytic markers in the flow cytometry panel of many institutions. Non-AMKL AMLs do occur in DS children but the occurrence may be coincidence and not related to DS.

By flow cytometry, most DS-AMLs are of megakaryocytic origin and have similar immunophenotype as the TMD [24, 47, 48]. Higher frequencies of CD13 and CD11b expressions can be seen in DS-associated AMKLs compared to TMD [24].

DS-AMLs and non-DS AMLs have similar cytogenetic abnormalities with few notable exceptions [36]. Both DS and non-DS associated AMLs have similar frequency of normal or constitutional trisomy 21 karyotype (approximately 25%). However, t(8;21), t(15;17), and 16q22 are rarely seen in DS-AMLs (2% combined) compared to their non-DS counterparts (24% combined). The t(1;22), commonly seen in infant non-DS associated AMKL [49, 50], is not seen in DS-AMKL or TMD.

DS-AMKL and TMD share the same morphology, immunophenotype, karyotypes, and molecular mechanisms. Both TMD and DS-AMKL have trisomy 21 and GATA1 mutations (reviewed in [51]). Despite the similarities between the blasts of TMD and DS-AMKL, their clinical courses differ, suggesting fundamental molecular differences. Validating and identifying these differences would aid in the diagnosis and better understanding of these diseases. Toward these goals, transcription profiling of small numbers of TMD and DS-AMKL has identified multiple transcripts with different expression levels [52, 53]; similar studies have identified differing transcripts in DS-AMKL versus non-DS AMKL [54, 55].

DS-AMLs have a better prognosis than non-DS-
Table 1 Distinctive features that aid in the diagnosis

<table>
<thead>
<tr>
<th>Feature</th>
<th>TMD</th>
<th>DS-AMKL</th>
<th>non-DS-AMKL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0-3m</td>
<td>6m-5y</td>
<td>Any</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>t(1;22)</td>
<td>No</td>
<td>No</td>
<td>Possible</td>
</tr>
<tr>
<td>Other cytogenetic findings</td>
<td>Maybe</td>
<td>Maybe</td>
<td>Maybe</td>
</tr>
<tr>
<td>GATA-1 mutation</td>
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<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

TMD, transient myeloproliferative disorder; DS, Down syndrome; DS-AMKL, DS associated acute megakaryoblastic leukemia; non-DS-AMKL, non DS associated acute megakaryoblastic leukemia; M, month; y, year

AMLs (68% versus 35% 4 year event free survival) provided that chemotherapy dosage is reduced to account for the higher toxicity in DS patients [36, 59]. Younger age has a favorable prognosis (86% 6 year event free survival for < 2 years of age compared to 64% for >2 years of age) [59]. Other variables such as rapid response to chemotherapy, cytogenetics, FAB classification (M7 versus others), and white blood cell count are not significant predictors of outcome [36].

Distinguishing Diagnostic Features in TMD, DS-AMKL and Non-DS-AMKL

The diagnosis of DS-ALL can be readily distinguished from DS-AML based on the immunophenotype of the blasts. However, the diagnosis of TMD versus DS-AMKL versus non-DS-AMKL is more problematic. Transcription profiling of these three entities have identified potential new diagnostic new molecular markers. However, until these markers are validated, clinical history, cytogenetics, and mutational analysis of GATA1 remain the best studies that distinguish among the megakaryoblasts of TMD, DS-AMKL, and non-DS-AMKL (Table 1). The presence of Down syndrome/trisomy 21 and GATA1 mutations support TMD or DS-AMKL and these two diagnoses are best differentiated using the age of the patient. TMD almost always occurs in DS neonates less than three months of age while TMD never occurs in DS neonates greater than 6 months of age. TMD should be considered in the differential even if DS is not suspected because the patient may be a mosaic for trisomy 21 [56-58] and may not have the characteristic physical features. Additional cytogenetic abnormality beyond trisomy 21 favors but is not diagnostic of DS-AMKL over TMD. The absence of trisomy 21 or presence of t(1;22) would indicate non-DS-AMKL.

DS-associated Myelodysplastic Syndrome

DS children can present with cytopenias, dyspoiesis, and less than 30% or 20% blasts in the peripheral blood or marrow, fulfilling the older FAB or the newer WHO criteria for myelodysplastic syndrome. The European classification of pediatric myelodysplastic & myeloproliferative diseases does not distinguish between DS-AML and DS-associated myelodysplastic syndrome (DS-MDS), grouping both as myeloid leukemia of DS [60] because DS-MDS are usually treated the same as DS-AML.

Figure 4 Focal megakaryocytic hyperplasia with megakaryocytic dysplasia (paratrabecular localization, clustering, and micromegakaryocytic forms) in a 6 month old DS patient (H&E, 20x (A) and 200 x (B) magnifications, respectively). Circled region in A contains the focal megakaryocytic hyperplasia represented in B. Patient had unexplained cytopenia without increased blasts but developed AMKL one month later.
Approximately 20% of the DS-AML is preceded by a history of MDS [36] that presents before 40 months of age [61]. Cytopenia with unexplained increased blasts in a DS child is synonymous with DS-MDS. Patients with DS-MDS are clinically well at initial diagnosis and have increased blasts in the marrow (6-29% of the marrow cellularity). A subset has a history of TMD. Most present with isolated cytopenia or bicytopenia. For those followed by observation alone, all DS-MDS progress to AML with a mean time interval of 6.3 months (ranging from 1 to 18 months).

The biopsy shows increased numbers of dysplastic megakaryocytes (small monolobated) in most cases while the aspirate smears show occasional monolobated megakaryocytes with vacuolated cytoplasm [61]. Myelofibrosis and dyserythropoiesis are frequently seen. In our experience, we have seen focal megakaryocytic hyperplasia consisting of clusters of normal and hypolobated megakaryocytes with abnormal paratrabecular localization (Figure 4).

References


[2] Henry E, Walker D, Wiedmeier SE and Centers for Disease Control and Prevention. References. or johnkimchoi1@gmail.com Fax: 215-590-0342; Email: choijo@email.chop.edu Philadelphia, PA 19104-4399. Tel: 215-590-7194; Hospital of Philadelphia, University of Pennsylvania, 802F ARC, 3615 Civic Center Blvd., Children's Hematopathology and Immunohistochemistry, Choi, M.D., Ph.D., Director of Pediatric

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Leuk Lymphoma


