Unusual Diffuse Liver Fibrosis Accompanying Transient Myeloproliferative Disorder in Down’s Syndrome: A Report of Four Autopsy Cases and Proposal of a Hypothesis

By Jun Miyauchi, Yushi Ito, Toshio Kawano, Yukiko Tsunematsu, and Koichi Shimizu

Transient myeloproliferative disorder (TMD), an acute leukemia-like disorder in neonates with Down’s syndrome, is characterized by spontaneous regression of abnormal blast growth. Because proliferating blasts frequently express phenotypes of megakaryocytic lineage and, as a result, this disorder resembles acute megakaryoblastic leukemia (AMKL), it would be of interest to determine whether myelofibrosis, a common complication of AMKL, is also present in TMD. Pathologic observations of four autopsy cases of TMD showed that myelofibrosis was not present in any of them, whereas intralobular diffuse liver fibrosis was present in all of them.

It is well known that children with Down’s syndrome have an increased risk of leukemia, and abnormal chromosome 21 has been suggested as playing a role in leukemogenesis. Interestingly, however, hematologic abnormalities indistinguishable from acute leukemia in neonates with Down’s syndrome spontaneously regress in most cases. This disorder is called transient myeloproliferative disorder (TMD), transient abnormal myelopoiesis, congenital or neonatal leukemoid reaction, and so on. It has been reported that leukemia rarely develops following a remission of TMD, and the nature of abnormal proliferation of the blast cells in TMD (in other words whether it is true leukemia or non-neoplastic unstable hematopoiesis) has long been controversial. Although no definite conclusion has been drawn, recent molecular analyses of restriction fragment length polymorphism performed by two groups of Japanese investigators have disclosed a genetically monoclonal origin of the blast cells, strong evidence for a neoplastic nature.

Proliferating blast cells in TMD frequently express phenotypic markers of megakaryocytic lineage, although the phenotypes of these cells may be heterogeneous and are not necessarily restricted to this lineage. On the other hand, a close association between Down’s syndrome and acute megakaryoblastic leukemia (AMKL) has been shown by many groups of investigators; leukemias in patients aged 3 years or less are almost always of this subtype, although acute lymphoblastic leukemia predominates in older children. Furthermore, both AMKL and TMD have been reported in patients with mosaic 21 trisomy. Thus, TMD and AMKL are closely related disorders, and chromosome 21 is thought to play an important role in megakaryocytopenia, particularly in younger children.

Myelofibrosis is a well-known complication of AMKL both in children and adults and is considered one of its most characteristic features. Because TMD is a similar, if not identical disorder, in that it involves abnormal proliferation of megakaryocytes, it would be of interest to know if myelofibrosis is a common complication in this disorder as well. We describe here the histopathologic findings of four autopsy cases and laboratory data of four nonautopsy cases of TMD. In contrast to our expectation, myelofibrosis was absent, whereas hepatic fibrosis or dysfunction was a common complication of TMD. The pathogenesis of this liver fibrosis is discussed.

MATERIALS AND METHODS

The diagnosis of TMD was made according to the following criteria: (1) the patient has Down’s syndrome, as suggested by characteristic clinical findings, including dysmorphic facial appearance, or is confirmed by chromosome analysis to have constitutional 21 trisomy; (2) the patient has an abnormal peripheral blood picture indistinguishable from acute leukemia, which is normalized or improved during the natural course without antileukemia therapy; and (3) the patient is a neonate. Patients who died before the hematologic remission was achieved (cases 1 and 2) were included in this study because the possibility of acute leukemia was ruled out by observation of autopsy specimens.

Autopsy cases filed with the Division of Pathology of the Clinical Laboratory, National Children’s Hospital from 1965 to 1990 were surveyed and 21 cases of Down’s syndrome were found, four of which had TMD. Pathologic slides from postmortem specimens and clinical charts of these four cases were reviewed. Azan staining, Watanabe’s silver impregnation, and Prussian blue iron staining were additionally performed by two groups of investigators. Rabbit antisera against myeloperoxidase and hemoglobin were used. Two-step indirect immunoperoxidase method was applied to 2-μm-thick paraffin-embedded sections of the formalin-fixed materials. Rabbit antisera against myeloperoxidase and hemoglobin were used.

From the Division of Pathology, Clinical Laboratory, and Divisions of Neonatology and Hematology, Department of Pediatrics, National Children’s Hospital, Setagaya, Tokyo, Japan.


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Address reprint requests to Jun Miyauchi, MD, Clinical Laboratory, National Children’s Hospital, 3-35-31 Tsukishima, Setagaya-ku, Tokyo 154, Japan.

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RESULTS

Report of Four Autopsy Cases

Case 1. A 2,600 g girl, born at 35 weeks of gestation, was observed to have hepatosplenomegaly. Her peripheral blood examination showed that her white blood cell (WBC) count was 128,000/μL with 85% blasts. Blood chemistry data showed liver dysfunction (Table 1). The patient died of gastrointestinal bleeding and massive intraperitoneal hemorrhage caused by gastric perforation at the age of 2 days.

Case 2. A 2,830 g boy, born at 38 weeks of gestation, was observed to have hepatosplenomegaly and tachypnea. His peripheral blood examination showed a high WBC count (69,000/μL) with 75% blasts and a low platelet count (1,000/μL). Blood chemistry examination found elevated total bilirubin (12.1 mg/dL); other tests were within the normal range (Table 1). Hepatosplenomegaly and tachypnea progressed and the patient died of respiratory failure resulting from marked hepatosplenomegaly at the age of 10 days.

Case 3. A 2,360 g boy, born at 39 weeks of gestation, was admitted to the hospital because of abdominal distention at 7 days of age. On admission, jaundice, hepatosplenomegaly, and respiratory disturbance were observed. Laboratory data showed an enormously high WBC count (289,000/μL) with 91% blasts in circulation, and liver dysfunction (Table 1 and Fig 1). Despite normalization of his WBC count and the disappearance of blasts from the circulation within 3 weeks, jaundice and ascites increased and hemorrhagic

Table 1. Summary of Laboratory Data

<table>
<thead>
<tr>
<th>Case no.</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Admission (age)</td>
<td>0–2 d</td>
<td>1–10 d</td>
<td>7–41 d</td>
<td>13–66 d</td>
<td>2–115 d</td>
<td>14–45 d</td>
<td>1–87 d</td>
<td>0–41 d</td>
</tr>
<tr>
<td>Term at birth (wk)</td>
<td>35 wk</td>
<td>36 wk</td>
<td>39 wk</td>
<td>34 wk</td>
<td>36 wk</td>
<td>38 wk</td>
<td>36 wk</td>
<td>40 wk</td>
</tr>
<tr>
<td>Weight at birth (g)</td>
<td>2,600</td>
<td>2,380</td>
<td>2,380</td>
<td>2,200</td>
<td>2,000</td>
<td>2,300</td>
<td>3,230</td>
<td>2,620</td>
</tr>
</tbody>
</table>

Blood chemistry:

- TB: 11.5–12.1/μL
- DB: 3.9–9.0 mg/dL
- GOT: 129–133 U/L
- GPT: 42–66 U/L
- LDH: 2,365–143 U/L
- ALP: 202–185 U/L

Peripheral blood

- RBC (10^6/μL): 453–507
- Hb (g/dL): 18.2–20.0
- PLT (10^3/μL): 38–60
- WBC (per μL): 128,000–289,000
- Blast (%): 65–75
- Erythroblast: 0

Bone marrow

- Blast (%): ND
- HB antigen: ND
- Chromosome: ND
- Outcome: Autopsy

Normal blood chemistry values for infants in our hospital are shown below with abbreviations. Two columns in a single patient represent data from two different time points, early (left) and late (right) during hospital treatment.

Abbreviations: TB, total bilirubin; Hb, hemoglobin; DB, direct bilirubin; <50% of TB; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.
diathesis became evident. The patient died of massive gastrointestinal bleeding at the age of 41 days.

Case 4. A 2,200 g girl, born at 34 weeks of gestation as one of identical twins, was admitted to our hospital at the age of 13 days for evaluation of her blood, because the other twin had been found to have a leukemia-like blood picture. Her blood also had a high WBC count with blasts. At the age of 23 days, the peripheral blood contained 22% blasts and bone marrow contained 33.2% blasts (Table 1 and Fig 2). At the age of 45 days, her WBC count and the number of blasts in circulation started increasing, and liver dysfunction progressed until her death. Although hematologic abnormalities had regressed and blasts had disappeared from the circulation by about 1 week before her death, hepatosplenomegaly and dyspnea became severe and she died at the age of 66 days.*

*The partner of the identical twin (case 5), a baby girl weighing 2,000 g at birth, had anal atresia and was admitted to our hospital for surgery at the age of 3 days. Her blood examination found a high WBC count with 12% blasts, whereas bone marrow aspiration showed only 1% blasts (Table 1). Although her abnormal blood picture showed improvement within 9 weeks, hepatomegaly and splenomegaly appeared, liver dysfunction developed, and she died of dyspnea at the age of 115 days.

**Pathologic Findings at Autopsy**

Diffuse intralobular liver fibrosis was observed in all four cases at autopsy. In case 1, the liver cell cords were disarranged and collagen fibers, which were increased along the vascular beds, surrounded the hepatocytes (Fig 3). The fibrosis was diffusely and nonzonal distributed throughout the hepatic lobules. In case 4, the hepatic architecture was destroyed, and somewhat denser but very similar diffuse fibrosis was seen (Fig 4). Although liver cell cords were highly disarranged and trapped in the fibrous tissue, the distribution of fibrosis along the vascular beds suggested a pattern of sinusoidal fibrosis. In case 3, intralobular collagenous fibrosis was dense and extensive, and disassembled hepatocytes with a pseudoductular structure were dispersed in the fibrous tissue. Here again, the distribution of fibrosis was diffuse and nonzonal (data not shown). In case 2, in which the laboratory data for liver function were normal except for elevated total bilirubin, fibrosis was the mildest among the four cases. The fibrosis was of the fine reticulin type, occupying the sinusoidal spaces and scattered diffusely (data not shown). In none of the above cases was pseudolobular nodular transformation of hepatocytes, suggesting cirrhosis, observed.

In sinusoidal spaces as well as in portal tracts in the liver, extensive extramedullary hematopoiesis with maturing cells of all three lineages was observed in all cases (Figs 3 and 4) except case 3, consistent with the diagnosis of TMD. The
presence of hematopoietic cells of each cell lineage was confirmed by immunohistochemical staining for each of the lineage-specific markers. The representative photomicrographs are shown in Fig 5. The ratio of erythroid to myeloid cells varied among the cases, but megakaryocytic cells were always prominent. Mononuclear small blastoid cells with positive staining for GPIIb/IIIa complex, a marker of megakaryocytic lineage, were detected along with mature megakaryocytes, which often had atypical nuclei. In case 3, fibrosis was severe and only a very small number of hematopoietic cells were scattered in the fibrous tissue.

Intrahepatic bile stasis was present in all cases and was particularly remarkable in cases 1, 3, and 4. The stagnant bile was present mainly in the bile canaliculi or cytoplasms of hepatocytes, whereas bile ducts in the portal tracts and extrahepatic bile ducts were normal, suggesting that the
outflow of bile was blocked in the hepatic lobules or at the junction between intralobular and interlobular bile ducts. Multinucleated giant hepatocytes suggestive of neonatal hepatitis were absent. Mild to moderate hemosiderosis was observed mainly in hepatocytes in cases 1, 3, and 4, but was undetectable in case 2.

Bone marrow had normal cellularity with maturing hematopoietic cells of all three lineages. The ratio of erythroid to myeloid cells was decreased and showed a moderate leftward shift of myeloid series in case 3, but was normal in the other cases. Monotonous blast cell growth, suggesting acute leukemia, was not present. The number of megakaryocytes was within the normal range, although small numbers of atypically shaped mature megakaryocytes similar to those found in the liver were also present in the marrow in some cases. In none of the patients was myelofibrosis evident.

Extramedullary hematopoiesis was present in other peripheral hematopoietic organs, including spleen, but was much milder than in the liver. Splenomegaly, which was possibly caused by both extramedullary hematopoiesis and portal hypertension attributable to hepatic fibrosis, was present in all the patients. Fibrosis was not present in the spleen or lymph nodes.

Cardiovascular anomalies, ie, atrial septal defect and patent ductus arteriosus, were found in cases 1 and 4, but not in the other cases.

**Laboratory Data of Other Nonautopsy Cases**

The laboratory data of the other four patients (cases 5 through 8) are included in Table 1. Blasts in peripheral blood disappeared or were reduced in number without any antileukemia therapy. However, hepatic dysfunction was found in all of these cases, as indicated by elevated bilirubin or transaminase, although it varied from only slight to severe. Two of the patients died and, unfortunately, consent for autopsy was not obtained. The other two children were discharged after their general condition improved. No viral titers, including that for hepatitis B (Table 1), were elevated.

**DISCUSSION**

TMD has the following features that are unusual for true leukemia: (1) the growth of blast cells regresses spontaneously within several weeks or months after birth without antileukemia therapy and permanent recovery is achieved, although relapse occurs on rare occasions; (2) the ratio of blasts in bone marrow is low compared with that in blood; (3) extramedullary hematopoiesis, consisting of maturing cells of three lineages but not monotonous blast cell infiltration, is seen in the peripheral hematopoietic tissues; (4) the blasts have 21 trisomy with no other clonal chromosomal abnormalities; and (5) mature hematopoietic colonies, which are usually completely inhibited in acute leukemia, are seen alongside the abnormal blast colonies in vitro. These aspects of TMD have caused controversy as to whether or not it is leukemia. However, regardless of the true nature of TMD, there is a striking common characteristic between TMD and leukemia in infants with Down’s syndrome, namely, that proliferating blast cells possess the phenotypes of megakaryocytic lineage.

Myelofibrosis is a well known complication of AMKL. Although the mechanism of myelofibrosis in AMKL is not clear, it has been suggested that cytokines, secreted by neoplastic megakaryocytes in a qualitatively and/or quantitatively abnormal manner, cause fibrosis of the bone marrow, because megakaryocytes are known to produce the cytokines that stimulate growth and collagen synthesis of fibroblasts. The possible candidates for these cytokines include transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), and platelet factor 4 (PF4). Among these, TGFβ has been shown to be a very potent stimulator of collagen synthesis and to play the most important role in the pathogenesis of myelofibrosis. However, despite the similarity of TMD to AMKL, myelofibrosis was absent in our patients and those of
In our present study. These results suggest a close association between TMD and hepatic lesions. It is known that jaundice and hepatic abnormalities are more common in children with Down's syndrome. However, relatively few detailed studies on histopathologic changes of the liver in patients with Down's syndrome have been published. Seeff et al. studied 48 patients with Down's syndrome and found that 42 had liver fibrosis, including 14 cases of cirrhosis. However, most of their cases were adults and older children, in whom the situation is different from that of neonates because institutionalized older patients with Down's syndrome are known to be at higher risk for viral hepatitis than others. Rosner and Lee surveyed 276 cases of Down's syndrome accompanying leukemia or TMD in the literature, in which 4 of 22 patients with TMD had jaundice and/or cirrhosis. However, the etiology and histology of the hepatic lesions are unfortunately not clear from the literature.

Our cases may fall clinically into the category of so-called neonatal hepatitis syndrome (or "giant cell hepatitis"), a hepatocyte disorder of unknown etiology. Although hepatic diffuse fibrosis and excessive hematopoiesis may be seen in this disorder, the lack of multinucleated giant hepatocytes in our patients does not fit with this diagnosis. Viral hepatitis caused by intrauterine infection is also an unlikely cause of hepatic fibrosis in our patients because none of the virus titers studied were elevated, and the uniform non-zonal distribution of fibrosis along the vascular beds was unlike the portal fibrosis seen in viral hepatitis. Thus, the liver fibrosis in TMD is histologically unusual and does not seem to be attributable to any of the known causes of hepatic fibrosis, including bile duct atresia, intrahepatic familial cholestatic syndromes, α1-antitrypsin deficiency, metabolic disorders, or hemosiderosis. We therefore consider that the hepatic fibrosis accompanying TMD results from certain highly unusual conditions.

The extent of hepatic fibrosis was not necessarily proportional to the age of each patient in this study; one patient only 2 days old (case 1) had extensive fibrosis, indicating that the fibrosis occurred during the fetal period. Besides TMD, hepatic fibrosis has been reported by other investigators in cases of primary myelofibrosis and osteopetrosis, both of which had sinusoidal fibrosis and extramedullary hematopoiesis in the liver, and in a patient with AMKL with hepatic involvement. A possibility has been suggested that the fibrosis is caused by cytokines elaborated by megakaryocytes in the liver. This mechanism seems to explain why liver fibrosis, but not myelofibrosis, was present in TMD, although abnormal megakaryoblastic proliferation similar to AMKL occurs in this disorder. On the basis of the above observations, we propose the following hypothesis as a mechanism of the liver fibrosis: the abnormal blasts in TMD proliferate in the liver during the fetal period, and the cytokines, including TGFB, PDGF, and PF4, which are stimulators of growth and collagen synthesis in fibroblasts, produced by these cells with megakaryocytic properties may cause fibrosis of the liver in a similar fashion to the myelofibrosis in AMKL, in which leukemia cells grow in the bone marrow.

Some other unique aspects of TMD also seem relevant to fetal hematopoiesis. An outstanding feature of TMD is its spontaneous remission. If the abnormal blasts in TMD are of fetal liver origin, a transition in the site of hematopoiesis from the liver to the bone marrow after birth could influence the proliferation of these cells and might cause their regression. The facts that the ratio of blasts in marrow is low compared with that in peripheral blood and that normal hematopoietic colony-forming cells in the bone marrow, unlike the case of acute leukemia, are not completely suppressed in TMD support our hypothesis that the major organ of abnormal cell origin is the liver and that the bone marrow is only involved by these cells.

In conclusion, we found that unusual hepatic fibrosis, histologically distinct from that caused by already known diseases, was closely associated with TMD and we proposed a hypothesis regarding the pathogenesis of this fibrosis. Although this hypothesis is based on only the results of histopathologic observations, it will be of value to test the hypothesis from various points of view because it seems to explain some specific cellular biologic aspects of TMD.

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LIVER FIBROSIS AND TMD IN DOWN’S SYNDROME


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