Case 28-2004: Newborn Twins with Thrombocytopenia, Coagulation Defects, and Hepatosplenomegaly

Jeffrey M. Lipton, M.D., Ph.D., Sjirk Westra, M.D., Carrie E. Haverty, M.S., Drucilla Roberts, M.D., and Nancy Lee Harris, M.D.

Newborn identical twin male infants were admitted to the neonatal intensive care unit. They were delivered at 32 weeks of gestation by a healthy 27-year-old primipara. The parents were of different ethnic backgrounds with no history of consanguinity. Prenatal screening tests revealed that the mother had group O, Rh-positive blood and was Rh-antibody–negative and immune to rubella. A test for hepatitis B surface antigen and a serologic test for syphilis were negative, as were bacterial cultures. Two days before delivery, ultrasonographic examination revealed a ring of ascites around one of the twins. An urgent delivery by cesarean section was performed because of concern about possible twin–twin transfusion.

The second infant delivered (Twin 2) was apneic and required stimulation with positive-pressure ventilation in the delivery room; his Apgar scores were 4 at one minute and 8 at five minutes. He was taken to a newborn intensive care unit, and blow-by oxygen was administered. (Although both twins had the same general syndrome, Twin 2 was sicker, and his case is presented here.) The weight was 1700 g (50th percentile for gestational age), and the length 41 cm (25th percentile). The heart rate was 152 beats per minute, and the respiratory rate was 62 breaths per minute. The blood pressure was 45/25 mm Hg. There were no dysmorphic features. Mild acrocyanosis was present, and petechiae were evident over the lower abdomen and legs. The breath sounds were coarse, with no crackles, and the heart sounds were normal. No hepatomegaly was detected; the spleen descended 3 to 4 cm below the left costal margin. There was no peripheral edema.

The results of laboratory tests showed that the levels of urea nitrogen, creatinine, fibrinogen, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were normal. Other laboratory values are shown in Tables 1 and 2. The infant’s blood type was O Rh-positive, and a Coombs’ test was negative.

An ultrasonographic study of the abdomen showed mild-to-moderate splenomegaly (Fig. 1A). There was a small amount of ascites in Morison’s pouch and around the tip of the spleen. The hepatic parenchyma appeared to be homogeneous, and there was no dilatation of the intrahepatic ducts or the common bile duct. The images of the gallblad-
der, kidneys, and portions of the pancreas showed no abnormalities.

The infant was given oxygen by nasal cannula and caffeine for episodes of apnea. An umbilical venous catheter was inserted, and an abdominal–thoracic radiograph revealed that the tip of the catheter projected over the liver. The bowel-gas pattern suggested hepatosplenomegaly (Fig. 1B). The lung volumes were low. The skeleton and soft tissues appeared normal. Ampicillin and gentamicin were administered but then discontinued after a blood culture yielded no growth at 48 hours. Breast milk and intravenous hyperalimentation were given.

Phototherapy was started on the second day of life but was discontinued because the conjugated bilirubin level rose (Table 2). At least one transfusion of platelets was given daily during the first seven days of life, and four additional transfusions of platelets were given during the second week. Initially, PLA1–negative platelets were used, with no obvious benefit. Tests of the mother for the presence of antiplatelet antibodies and the human immunodeficiency virus were negative; the results of serologic tests for toxoplasma and syphilis were negative, and those for Epstein–Barr virus and parvovirus indicated past infection.

On the fourth day of life, the infant’s urine was positive (+) for bilirubin and protein. Levels of urea nitrogen, creatinine, triglycerides, magnesium, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were normal. Other laboratory values are shown in Table 2. Pathological evaluation of the placenta revealed isolated arteritis of the umbilical cord (Fig. 2); there was no evidence of chorioamnionitis, and only small amounts of meconium were present. A cranial ultrasonographic examination revealed no abnormalities. A transfusion of packed red cells was given. The absolute neutrophil count ranged between 100 and 1200, with no obvious trend.

The conjugated bilirubin on the sixth day of life was 4.8 (Table 2). A cytomegalovirus shell-vial culture was negative. An analysis of peripheral-blood lymphocytes by flow cytometry revealed a predominance of T cells, with a normal CD4:CD8 ratio. B cells were polyclonal. On the seventh day, the axillary temperature rose to 38.8°C. Repeated chest and abdominal radiographs showed more prominent hepatosplenomegaly, without evidence of bowel obstruction or pneumatosis.

On the eighth day, examination of a stained bone marrow aspirate revealed sparse cellularity, with 6 percent myeloid precursors, 57 percent erythroid precursors, 16 percent lymphocytes, 18 percent monocytes, 1 percent eosinophils, and 2 percent promyelocytes. Myeloid maturation was normal. No megakaryocytes were present. Many large, granular lymphocytes were evident, as well as many monocytes with multilobated nuclei and pale cytoplasm. A single hemophagocytic histiocyte engulfing erythroid precursors was noted. A specimen sent for flow cytometry contained too few cells for analysis. The karyotype was 46,XY. The results of other laboratory tests are shown in Table 1.

Infused immune globulin begun on the 10th day of life did not reduce the need for blood products or prolong the half-life of platelets. On the 11th day, a screening panel of 12 tests for conditions most likely to affect newborns was negative. The hematocrit of 23.1 percent was at its lowest level to date, and a second transfusion was administered. Specimens of blood drawn for culture one week earlier were sterile. The results of laboratory tests from the 13th day of life are shown in Tables 1 and 2.

The physical and radiographic findings and lab-
Laboratory test results for Twin 1 were similar to those described above for Twin 2. A diagnostic procedure was performed.

**Differential Diagnosis**

Dr. Jeffrey M. Lipton: May we see the imaging studies?  
Dr. Sjirk Westra: Abdominal ultrasonographic images obtained on the first day of life show a liver with homogeneous echotexture, which initially was not enlarged. The spleen was homogeneously enlarged at 6.3 cm in length, which is above the upper limit of normal for a neonate (Fig. 1A). Plain radiography of the abdomen and chest performed at six days of life shows a normal chest with enlargement of the liver and spleen (Fig. 1B).

Dr. Lipton: The major components of the illness in these premature identical twin boys were fever, splenomegaly followed by hepatomegaly, thrombocytopenia, neutropenia, hypofibrinogenemia, and hyperbilirubinemia. I will first focus on the causes of thrombocytopenia in the newborn and next on the differential diagnosis of hepatosplenomegaly. I will then attempt to incorporate the constellation of findings into a single diagnosis.

**Table 2. Blood Chemical Values in Twin 2.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>At Birth</th>
<th>4th Day of Life</th>
<th>6th Day of Life</th>
<th>13th Day of Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>219</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugated bilirubin (mg/ml)†</td>
<td>0.5</td>
<td>3.9</td>
<td>4.8</td>
<td>14.3</td>
</tr>
<tr>
<td>Sodium (mmol/liter)</td>
<td>142</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium (mmol/liter)‡</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride (mmol/liter)</td>
<td>34.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>423</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>137</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/liter)</td>
<td>791</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (U/liter)</td>
<td>378</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† To convert the value for glucose to millimoles per liter, multiply by 0.05551.  
To convert the values for conjugated bilirubin to millimoles per liter, multiply by 17.1.  
‡ The normal range is 4.0 to 5.6 mmol per liter.  
§ The normal range is 19 to 22 mmol per liter.

**Figure 1. Imaging Studies in Twin 2.**  
An abdominal ultrasonographic study obtained on the first day of life (longitudinal view of left upper quadrant, Panel A) shows an enlarged homogeneous spleen (S) with a small amount of ascites (A) at the inferior pole. A frontal radiograph of the chest and abdomen (Panel B) shows enlargement of the liver (L) and spleen (S).

**Thrombocytopenia and Hepatosplenomegaly**  
Thrombocytopenia is found in up to 20 percent of newborns admitted to the neonatal intensive care unit; infection is the leading cause in the preterm infant. These twin patients had negative blood cultures and no evidence of any of the common perinatal infections. Alloimmune and autoimmune thrombocytopenia are the most common types of thrombocytopenia in a healthy newborn. The lack
of response to PLA1-negative platelets, the negative results of the platelet antibody tests in the mother, and the absence of a maternal history that would be consistent with thrombocytopenia essentially rule out a diagnosis of either type of this disorder.

There are no physical findings or laboratory results that suggest any of the rare congenital thrombocytopenic syndromes or the trisomy 13, 18, or 21 syndromes, which may present with thrombocytopenia; however, hepatosplenomegaly and coagulopathy are not features of these syndromes. (Other disorders that fall into this category are thrombocytopenia–absent radius syndrome, Fanconi’s anemia, the Hoyeraal–Hreidarsson syndrome, the Wiskott–Aldrich syndrome, the Bernard–Soulier syndrome, May–Hegglin anomaly, and congenital amegakaryocytic thrombocytopenia.) The presence of low levels of fibrinogen and elevated levels of D-dimer is consistent with disseminated intravascular coagulation and the Kasabach–Merritt syndrome, but there is no evidence of a kaposisform hemangioendothelioma, associated with the latter.

Hepatosplenomegaly and thrombocytopenia can occur in congenital leukemia, the transient myeloproliferative disorder of Down’s syndrome, osteopetrosis, metastatic neuroblastoma, and storage diseases. However, the peripheral-blood and bone marrow findings, the normal marrow karyotype, the physical examination, and the abdominal ultrasonograms rule out these disorders.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

These patients display the clinical and hematologic features of a group of disorders known as hemophagocytic lymphohistiocytosis. The signs and symptoms are either persistent or intermittent fevers with hepatosplenomegaly (often manifested initially as isolated splenomegaly), thrombocytopenia and anemia that often evolve to severe pancytopenia, coagulation abnormalities (in particular, hypofibrinogenemia), and hepatic dysfunction—all in the absence of other causes of thrombocytopenia and hepatosplenomegaly. The Histioocyte Society has developed diagnostic criteria for hemophagocytic lymphohistiocytosis. In addition to those already noted, these criteria include cytopenia in two or more cell lines; hypofibrinogenemia, hypotriglyceridemia, or both; hemophagocytosis in bone marrow, spleen, or lymph nodes; and no evidence of cancer.

Although the twin patients meet these criteria, the findings are somewhat nonspecific, particularly in sick preterm infants. Furthermore, the presence of a single hemophagocytic cell on bone marrow examination, although suggestive, cannot be accepted as diagnostic, given the difficult therapeutic alternatives facing these patients. Hemophagocytosis is evident at presentation in as few as one third of patients with verified hemophagocytic lymphohistiocytosis. Thus, when there is clinical suspicion of this disorder, convincing evidence of hemophagocytosis should be pursued by means of serial bone marrow examinations.

Clinical observations have delineated three categories of hemophagocytic lymphohistiocytosis: a familial syndrome known as familial hemophagocytic lymphohistiocytosis, hemophagocytic syndromes associated with viral infections or other types of infections, and a syndrome of macrophage activation that occurs in some patients with juvenile rheumatoid arthritis or immunodeficiency states. Aricò and colleagues proposed a diagnostic algorithm for hemophagocytic lymphohistiocytosis in patients who meet the diagnostic criteria already described in the infants under discussion.

SECONDARY HEMOPHAGOCYTIC SYNDROMES

Infectious agents such as the Epstein–Barr virus, cytomegalovirus, human parvovirus B19, bacteria, fungi, mycobacteria, and parasites should first be ruled out, as they were in these patients. The history, physical examination, and laboratory screening...
eliminate the syndrome of macrophage activation that is associated with juvenile rheumatoid arthritis. DiGeorge syndrome can be ruled out by the absence of deletion 22q11.2 on fluorescence in situ hybridization analysis, which should be performed in patients in whom this diagnosis is suspected.\textsuperscript{10,11} Hyperammonemia is characteristic of lysinuric protein intolerance, which may result in an immunodeficiency state and hemophagocytic lymphohistiocytosis.\textsuperscript{10,12} The so-called accelerated phases of the Chédiak–Higashi\textsuperscript{8,10} and Griscelli\textsuperscript{8,13} syndromes are indistinguishable from other forms of hemophagocytic lymphohistiocytosis, but both are characterized by albinism. The presence of mutations in the \textit{LYST} or \textit{RAB27A} gene, respectively, will confirm the diagnosis.\textsuperscript{13,14} The infants in the case under discussion did not have any of the physical signs or metabolic abnormalities associated with these syndromes, and they had a normal karyotype.

In male infants, a diagnosis of X-linked lymphoproliferative disease should be ruled out. In this disorder, a mutation in the \textit{SH2D1A} gene leads to an inhibitory mutation in the lymphocyte 2B4 receptor, resulting in the inability of natural killer cells to kill cells infected with the Epstein–Barr virus. This inhibition leads to the sustained proliferation of impotent cytolytic effector cells and the syndrome of hemophagocytic lymphohistiocytosis.\textsuperscript{15} The patients under discussion did not have evidence of an active Epstein–Barr viral infection.

**FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

Familial hemophagocytic lymphohistiocytosis is an inherited autosomal recessive disorder, with the underlying pathophysiology of impaired natural-killer-cell and cytotoxic T-cell activity.\textsuperscript{8,16} In 1999, two chromosomal loci linked to the disease (9q21.3–22 and 10q21–22) were identified in families with clinical and laboratory evidence of hemophagocytic lymphohistiocytosis.\textsuperscript{17,18} The two chromosomal loci defined hemophagocytic lymphohistiocytosis type 1 and hemophagocytic lymphohistiocytosis type 2, respectively. Shortly thereafter, mutations at chromosome 10q22, the locus of the perforin gene, were identified.\textsuperscript{19,20}

Perforin is a component of natural killer and cytotoxic T cells that perforates the membranes of target cells, permitting the entry of granzymes that initiate the apoptotic cell-death pathway (Fig. 3). In the absence of perforin activity, the resulting inability to kill infected target cells results in sustained natural-killer-cell and cytotoxic T-cell activity. These cells produce inflammatory cytokines (soluble interleukin-2 receptor, interleukin-6, tumor necrosis factor \(\alpha\), interleukin-10, and interleukin-12), leading to macrophage activation, dissemination, and organ infiltration and to the signs, symptoms, and laboratory abnormalities that characterize hemophagocytic lymphohistiocytosis.\textsuperscript{8,16} The presence of large granular lymphocytes, characteristic monocytes, and a single hemophagocytic histiocyte in the marrow in the patient is consistent with elevated cytotoxic T-cell and natural-killer-cell function and monocyte or macrophage activation.

Most patients with familial hemophagocytic lymphohistiocytosis are born healthy and become ill in the first two to six months of life. In addition to hepatosplenomegaly and pancytopenia, infiltration of histiocytes in other tissues, particularly the central nervous system, can occur. The initial triggering event leading to the excessive proliferation and activation of natural killer cells and cytotoxic T cells is unknown, but neonatal presentation is unusual. The finding of umbilical-cord arteritis in Twin 2 is of interest. In a recent report,\textsuperscript{21} the finding of umbilical-cord arteritis was associated with elevated levels of interleukin-6, one of the cytokines associated with hemophagocytic lymphohistiocytosis, in the umbilical-cord plasma. The finding of umbilical-cord arteritis suggests that some event resulting in the overexpression of cytokines occurred in utero in these patients.

**DIAGNOSIS OF FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

Impaired natural-killer-cell activity is the key to the diagnosis of familial hemophagocytic lymphohistiocytosis. The absence of intracytoplasmic perforin\textsuperscript{19} is a reliable marker in the 20 to 40 percent of patients with familial hemophagocytic lymphohistiocytosis type 2, which is associated with mutations of the perforin gene. The remaining familial cases may be recognized by impaired natural-killer-cell function. Hemophagocytic lymphohistiocytosis type 1, which is linked to the 9q21.3 locus, represents approximately 10 percent of all cases.\textsuperscript{16} Thus, a substantial proportion of familial cases are genetically uncategorized. Patients who have no perforin expression should undergo mutation analysis of the perforin gene. Patients with infection-associated hemophagocytic lymphohistiocytosis may be found to have transiently impaired natural-killer-cell activity. In the absence of a mutation, reevaluation of
natural-killer-cell function should be undertaken after treatment.

These twin infants probably have hemophagocytic lymphohistiocytosis. The absence of any signs of immunodeficiency or infection and their young age at presentation strongly support the diagnosis of familial hemophagocytic lymphohistiocytosis. The examination of another bone marrow specimen to confirm the presence of hemophagocytosis is essential. Evaluation by flow cytometry may reveal the absence of intracytoplasmic perforin. A positive mutation analysis of the perforin gene would confirm the diagnosis.

Dr. Nancy Lee Harris: Dr. Insoft provided the initial care for these patients. Would you comment on your thinking?

Dr. Robert M. Insoft (Neonatology): In the intensive care unit, both infants were found to have profound thrombocytopenia and leukopenia. The initial diagnoses of the neonatology team included the spectrum of congenital infections; once these had been ruled out, we realized we might be dealing with a primary familial process, since the twins presented similarly.

Dr. Harris: Dr. Friedmann supervised the initial hematologic evaluation.
Dr. Alison Friedmann (Pediatric Hematology–Oncology): The clinical diagnosis was a hemophagocytic syndrome. The hematologic team initially directed our workup toward infections with the hope that we would find something that was reversible. We strongly suspected that the condition affecting the twins was familial. In addition to obtaining studies of natural-killer-cell function and perforin expression, we performed a liver biopsy and repeated bone marrow aspiration on the more severely affected twin (Twin 2) to look for further evidence of hemophagocytosis.

Familial hemophagocytic lymphohistiocytosis.

**Pathological Discussion**

Dr. Harris: Dr. Roberts will show us the pathology of the placenta.

Dr. Drucilla Roberts: A section of the umbilical cord of Twin 2 shows inflammation through the muscle wall with focal thrombus formation (Fig. 2). Inflammatory lesions of this sort in the umbilical cord are fetal in origin and are typically a response to infection in the amniotic fluid. The differential diagnosis would also include prolonged exposure to meconium in utero.

Dr. Harris: On the repeated bone marrow–aspirate smear, we were able to identify numerous histiocytes containing phagocytized nucleated cells and erythrocytes (Fig. 4A and 4B), confirming the diagnosis of a hemophagocytic syndrome. The liver-biopsy specimen showed giant-cell hepatitis with cholestasis, extramedullary hematopoiesis, and numerous histiocytes in the portal areas and sinuses; occasional histiocytes showed hemophagocytosis (Fig. 4C). Staining for the Epstein–Barr virus and cytomegalovirus was negative. The histiocytes lacked markers of Langerhans’ cells. These findings are consistent with a hemophagocytic syndrome.

Specimens of peripheral blood obtained from the patients and their parents were sent to the laboratory of Dr. Alexandra Filipovich at the Children’s Hospital Research Foundation of the Children’s Hospital Medical Center of Cincinnati for assessment of perforin expression and natural-killer-cell function (Fig. 5 and Table 3). The patient’s cytotoxic cells contained no detectable perforin, whereas those of both parents contained variably reduced levels. Natural-killer-cell function was absent in the patient and normal in both parents. These findings are typical of patients with a perforin deficiency that is associated with familial hemophagocytic lymphohistiocytosis. Patients with infection-associated
hemophagocytic lymphohistiocytosis also have absent or decreased natural-killer-cell function, but in such cases the deficiency is accompanied by an absolute decrease in the numbers of natural killer cells, normal perforin expression, and an increase in CD8+ T cells.

22,23

Because insufficient DNA was obtained from Twin 2, only the perforin genes from Twin 1 and his parents were sequenced (Table 3 and Fig. 6). The infant had a mutation from adenine to guanine at position 665 in exon 3 as well as a transition from guanine to adenine in intron 2, which was believed to represent a polymorphism. The missense mutation was also detected in the mother, and the polymorphism in the father. Thus, the patients are heterozygous for a novel missense mutation in the perforin gene and presumably inherited a nonfunctional gene from the father as well, although a pathological mutation could not be identified. (These results were subsequently reported in

**Figure 5. Results of Flow Cytometry of Peripheral-Blood Lymphocytes.**

In the normal person (Panel A), perforin is expressed by a subgroup of CD8+ T cells, 89 percent of natural killer cells, and a subgroup of natural killer T cells. The patient's T cells and natural killer cells (Panel B) expressed no perforin. The percentage of maternal natural killer cells that expressed perforin was reduced (67 percent), and they also expressed reduced levels of perforin, with a mean channel fluorescence (MCF) of 117, as compared with 327 for normal killer cells (Panel C). The green line indicates a negative control; the purple area is the perforin expression. (Representation of data courtesy of Dr. Alexandra Filipovich, Children's Hospital Research Foundation, Children's Hospital Medical Center of Cincinnati.)
A series of 50 North American cases of familial hemophagocytic lymphohistiocytosis.24)

More than 40 distinct perforin-gene mutations have been identified.20,23-26 Slightly more than half of the families in these reports are consanguineous, and the affected patients have homozygous mutations; 35 percent of the reported patients are compound heterozygotes. Heterozygotes have been described only rarely. Most of the described mutations are unique. Recurring mutations have been found in Turkish families,18 African or African-American families,22 and Japanese families.27 There is no clear clinical difference among families with different mutations, although a late onset of the disease has been reported in a few patients with missense mutations.27 Figure 6 is a diagram of the perforin gene28 and the approximate locations of some of the reported mutations, as well as the mutation and polymorphism seen in the family of the twins under discussion.

An alternative hypothesis to that presented by Dr. Lipton (Fig. 3) has been proposed for the role of perforin deficiency in the pathogenesis of familial hemophagocytic lymphohistiocytosis. Cytotoxic T cells may modulate the immune response by causing apoptosis in activated T cells. The lack of perforin may permit activated T cells to accumulate, even in the absence of a persistent antigen.29

Dr. Harris: Dr. Friedmann, would you tell us about the initial treatment of these infants?

Dr. Friedmann: Immunosuppressive therapy with etoposide and dexamethasone was started on the 13th day of life, with the addition of cyclosporine after 6 weeks. Initially, the patients responded well, and at two months of age they were stable enough to be discharged to their home. They were referred to the Children’s Hospital in Boston to undergo bone marrow transplantation from a matched, unrelated donor.

Dr. Amy Billett (Hematology–Oncology, Children’s Hospital): Twin 2 had central nervous system disease and systemic disease that progressed despite aggressive immunosuppressive therapy, including a course of cladribine. He was never stable enough for bone marrow transplantation, and he died at the age of six months.

Dr. Leslie Lehmann (Hematology–Oncology, Children’s Hospital): Twin 1 received a bone marrow transplant at five months of age and had a relatively uneventful course for the first several weeks, with successful engraftment. Then hepatomegaly, progressive platelet consumption, and respiratory insufficiency developed, and despite aggressive medical management he died at seven months of age.

Autopsy restricted to a biopsy of the liver disclosed moderately severe chronic graft-versus-host disease and active hepatitis with a pattern that suggested a toxic or metabolic insult, with no evidence of hemophagocytic lymphohistiocytosis.

Dr. Harris: Ms. Haverty provided genetic counseling to the family after the diagnosis was established and after the twins had died.

Carrie E. Haverty: There are several important issues to consider when dealing with a prenatal diag-

### Table 3. Analysis of Natural-Killer-Cell Function, Perforin Expression, and Perforin Gene Mutation in Twin 2 and His Parents.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Perforin Expression by Flow Cytometry (%)</th>
<th>Natural Killer Lytic Units</th>
<th>Natural Killer Cells (CD16+) (3.26†)</th>
<th>Perforin Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>H222R‡ Polymorphism, intron 2</td>
</tr>
<tr>
<td>Mother</td>
<td>Decreased percentage and intensity</td>
<td>Decreased</td>
<td>Normal</td>
<td>Heterozygous for H222R</td>
</tr>
<tr>
<td>Father</td>
<td>Normal percentage, decreased intensity</td>
<td>Decreased</td>
<td>Normal</td>
<td>Polymorphism, intron 2</td>
</tr>
</tbody>
</table>

* Testing was performed in the laboratory of Dr. Alexandra Filipovich, Children’s Hospital Research Foundation, Children’s Hospital Medical Center of Cincinnati. All data are from Twin 2, unless noted.
† The normal range is 4 percent to 22 percent.
‡ A mutation from adenine to guanine at position 665 resulting in a substitution of arginine for histidine at amino acid 222 (H222R). The data are for Twin 1; there was insufficient DNA from Twin 2.
nosis of rare autosomal recessive disorders. Is the gene identified? Are there two mutations identified in the proband? Is the clinical significance of the mutations known? Is there a laboratory willing to perform prenatal diagnostic testing? How is this information communicated to the parents?

In this case, a disease-causing mutation and a polymorphism were identified. The genetic testing was done in a research laboratory that could not provide prenatal diagnostic testing. When the couple was referred to the prenatal diagnostic unit of this hospital, the mother was eight weeks into a second pregnancy, and she and her husband desired prenatal testing. We told them that there was a 25 percent risk of recurrence of the disease. The fact that only one disease-causing mutation was found does not change the risk of recurrence, but it does complicate the ability of the clinicians to offer prenatal diagnoses.

Blood samples from the parents were sent to a clinical laboratory approved by the federal guidelines of the Clinical Laboratory Improvement Amendments, which confirmed the findings. We counseled the parents that if the fetus carried the H222R mutation, we could predict that there was a 50 percent chance that the fetus was affected and a 50 percent chance it was a carrier, but we would be unable to distinguish between those two possibilities. If the mutation was not present, the fetus was neither affected nor a carrier. Determining the presence or absence of the paternal polymorphism would not be helpful, since its clinical significance is not known. Amniocentesis was performed at 15 weeks’ gestation. The H222R mutation was absent, and the parents were counseled that the fetus was predicted to be unaffected but possibly could be a carrier. This case demonstrates the complexity of the prenatal diagnosis process even in cases in which the mutations of the proband have already been identified. A healthy infant was born 15 months after the birth of the twins.

PATHOLOGICAL Diagnosis

Familial hemophagocytic lymphohistiocytosis, caused by a mutation in the perforin gene.

REFERENCES

1. Levendoglu-Tugal O, Ozkaynak ME, LaGamma E, Sherbany A, Sandoval C, Jaya-


3. Sainio S, Jarvenpaa AL, Renlund M, Ri-
ikonen S, Teramo K, Kekomaki R. Throm-
boctopenia in term infants: a population-
based study. Obstet Gynecol 2000; 95:441-
6.

boctopenia absent corpus callosum syn-
drome: third case of a distinct clinical en-


