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Neonatal thrombocytopenia as a presenting finding in *de novo* pyruvate kinase deficiency

Brian M Dulmovits¹, K Taylor Wild^{1,2}, John Flibotte^{1,3}, Michele P Lambert^{3,4}, Janet Kwiatkowski^{3,4}, Christopher S Thom^{1,3,*}

¹Division of Neonatology, Children’s Hospital of Philadelphia, Philadelphia, PA

²Division of Genetics, Children’s Hospital of Philadelphia, Philadelphia, PA

³Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

⁴Division of Hematology, Children’s Hospital of Philadelphia, Philadelphia, PA

Abstract

Thrombocytopenia is a common laboratory abnormality encountered in critically ill neonates. The broad differential for thrombocytopenia, and its association with potentially severe neonatal pathology, often presents a diagnostic dilemma prompting extensive evaluation. Hemolysis due to red cell enzymopathies is a rare cause of neonatal thrombocytopenia that is typically brief and self-limiting. Here, we present a case of thrombocytopenia, refractory to transfusion, associated with anemia and hyperbilirubinemia in a neonate with pyruvate kinase deficiency (PKD) arising from compound heterozygous *PKLR* mutations. The nature of the thrombocytopenia in this patient created considerable diagnostic uncertainty, which was ultimately resolved by whole exome sequencing. This case emphasizes that inherited red cell defects, such as PKD, are important to consider in cases of significant neonatal thrombocytopenia.

Keywords

thrombocytopenia; red cell enzymopathy; hemolysis; hyperbilirubinemia; whole exome sequencing

*Correspondence: Christopher S Thom, MD, PhD, Children’s Hospital of Philadelphia, Division of Neonatology, 10-052 Colket Translational Research Building, 3501 Civic Center Blvd, Philadelphia, PA 19104, USA, thomc@chop.edu.

Author Contributions

BMD and CST wrote the manuscript. BMD, KTW, and CST collected data for the manuscript. BMD, KTW, JF, ML, JK, and CST revised the manuscript. CST conceptualized the initial case study.

Statement of Ethics

Written informed consent was obtained from the patient’s family for publication of the information contained within this case report. This study was deemed exempt from oversight by the Children’s Hospital of Philadelphia Institutional Review Board.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Introduction

Thrombocytopenia is frequently encountered in the neonatal intensive care unit (NICU), affecting up to 50% of critically ill neonates [1]. Neonatal thrombocytopenia often occurs secondary to immune- or non-immune-mediated platelet destruction during sepsis, necrotizing enterocolitis, or thrombosis [2], but broad-ranging etiologies create diagnostic challenges. This is particularly true for conditions uncommonly linked with thrombocytopenia, including hemolytic red blood cell (RBC) disorders [3–5].

Pyruvate kinase deficiency (PKD), an inherited non-spherocytic hemolytic anemia resulting from *PKLR* gene mutations, possesses a variable clinical spectrum. Neonatal PKD manifestations range from asymptomatic compensated hemolytic anemia to intrauterine growth restriction (IUGR), hydrops fetalis, or fulminant hepatic failure [6, 7]. Neonates with PKD typically present with signs of hemolysis without other hematologic abnormalities, though some cases have documented brief, self-limited thrombocytopenia [8, 9]. However, ongoing hemolysis from erythrocyte enzyme and membrane defects can prolong thrombocytopenia [10]. This may be underrecognized in neonatal PKD.

We present a case of PKD associated with neonatal thrombocytopenia, anemia, hyperbilirubinemia, elevated transaminases, hepatosplenomegaly, and respiratory failure who underwent exhaustive diagnostic evaluation. Definitive diagnosis was made by whole exome sequencing (WES), demonstrating how this technology can facilitate rapid diagnosis in clinical dilemmas [11]. The patient's thrombocytopenia was somewhat prolonged, contrasting prior reports [8, 9], and persisted after transient coagulopathy resolved. Ongoing hemolysis from erythrocyte disorders like PKD is important to consider during neonatal thrombocytopenia evaluation.

Case presentation

A 3.6 kg male was born to a 30-year-old woman with no significant past medical history via spontaneous full term home vaginal delivery following uncomplicated pregnancy, labor, and delivery. Within 2 hours, the infant developed respiratory distress, abdominal distension, jaundice, hepatosplenomegaly, petechiae, and a diffuse blue macular rash, prompting NICU admission and intubation. Initial laboratory studies demonstrated leukocytosis, anemia with increased nucleated red blood cells, thrombocytopenia, coagulopathy, unconjugated hyperbilirubinemia, and elevated transaminases with normal GGT (shown in Table 1, Fig. 1a – c). He was initiated on empiric antimicrobial therapy, although blood cultures were ultimately negative. Platelet transfusions (10 mL/kg) failed to resolve persistent thrombocytopenia (shown in Fig. 1a). His bilirubin levels rose despite intensive phototherapy, requiring a double volume exchange transfusion on day of life (DOL) 4 (shown in Fig. 1c).

Prolonged thrombocytopenia with concomitant hyperbilirubinemia, anemia, and hepatosplenomegaly prompted a broad diagnostic evaluation for metabolic, hepatobiliary, infectious, oncologic, platelet, and red cell etiologies. A peripheral blood smear demonstrated anisocytosis without spherocytes or other RBC dysmorphology, along with

reticulocytosis, nucleated red blood cells, and thrombocytopenia (not shown). Both maternal and infant blood types were O, Rh-negative, and direct antiglobulin test (DAT) was negative. Tests for congenital infection, thyroid abnormalities, hemoglobin electrophoresis, and state newborn screening were unremarkable. Pyruvate kinase, hexokinase, glucose-6-phosphate dehydrogenase levels were not initially assessed. His elevated transaminases, lactate dehydrogenase (LDH) (8315; ref 600 -1200 U/L), and ferritin (638; ref 0- 478 ng/mL) levels were not to the extreme levels typical of gestational alloimmune liver disease (shown in Fig. 1d) [12]. Ultrasound imaging of the liver and spleen revealed normal echotexture of the parenchyma and absence of nodules concerning for extramedullary hematopoiesis but demonstrated hepatosplenomegaly (liver 8.4cm in greatest craniocaudal dimension; spleen 7.6cm). Further, there was no clinical or imaging evidence of thrombosis. An echocardiogram obtained for hypertension showed coronary artery dilation (left main coronary: 3.3mm, Z = 5.32; right main coronary: 2.7mm, Z = 5.62; left anterior descending: 2.8mm, Z = 8.06), which prompted concern for neonatal Kawasaki disease despite a lack of fever and laboratory evidence of ongoing inflammation as some incomplete neonatal KD cases can occur without fever or inflammation [13]. He was treated with aspirin and intravenous immunoglobulin without adverse effects. During hospitalization he required a 15 mL/kg packed RBC transfusion on DOL 21 (shown in Fig. 1b). Ultimately, no definitive diagnosis was determined, and therefore, WES was sent.

Multidisciplinary discussions initially focused on thrombocytopenia, with increased immature platelet fraction (patient range 7.0 – 10.4%; ref. 2.0-6.8) that persisted despite platelet transfusions, as a potentially key diagnostic feature. After evaluation for hemolytic processes, his thrombocytopenia remained concerning and puzzling given (i) prior literature suggesting that thrombocytopenia self-resolves in most neonates within the first 5-6 days of life [14], and (ii) the potential severity of alternative etiologies. Despite thrombocytopenia, he did not suffer bleeding complications, and his thrombocytopenia resolved in about 1 month (shown in Fig. 1a).

His clinical status stabilized, and he was discharged home on DOL 22 with aspirin and close hematologic follow up. Coronary artery ectasia and hypertension resolved at subsequent outpatient evaluation. While his platelet counts remained normal, he was readmitted on DOL 35 for symptomatic anemia requiring a 15 mL/kg packed RBC transfusion (shown in Fig. 1b). During readmission, WES confirmed a PKD diagnosis from compound heterozygous *PKLR* mutations (c.1529 G>A; p.R510Q: known variant and c.1022 G>A; p.G341D: presumed pathogenic variant). Parent genotyping demonstrated that his mother was a carrier for the R510Q mutation. His father possessed no *PKLR* mutations. These findings indicated that the G341D mutation arose *de novo*. At the time of publication, he remains transfusion-dependent.

Discussion

Persistent thrombocytopenia caused diagnostic uncertainty in this case. It is important to consider erythrocyte abnormalities and hemolytic processes in the differential for neonatal thrombocytopenia [1, 2, 10]. We speculate that this patient's thrombocytopenia may have resulted from multiple sequelae of PKD. First, hepatosplenomegaly and increased immature

platelet fraction may have reflected a consumptive thrombocytopenia from hypersplenism. Second, free hemoglobin can instigate a cascade of events that cause platelet activation, destruction, and thrombocytopenia [10]. Third, transient hepatic dysfunction may have also reduced circulating thrombopoietin (TPO) levels and platelet counts [15], as the liver represents the major source of TPO synthesis for megakaryopoiesis [16, 17]. Finally, pathologic iron loading and extramedullary hematopoiesis in the fetal liver may have affected hepatic niche formation, perturbing megakaryopoiesis during the fetal-neonatal hematopoietic transition [18]. Intriguingly, the *PKLR* mutations in our patient have not been associated with adult thrombocytopenia [19, 20]. This phenotypic discrepancy may relate to differences in neonatal megakaryopoiesis and/or platelet biology that differ from adults [21], although clarifying this association will require further study.

Neonatal thrombocytopenia may be underappreciated in PKD cases. In a multicenter study of 254 patients, 28% of patients exhibited perinatal complications including preterm birth, IUGR, transfusions, and hydrops fetalis [22]. Jaundice requiring phototherapy or exchange transfusion was the most common complication. While the incidence of thrombocytopenia was not investigated, thrombocytopenia mediated by hypersplenism has been observed in older children [6]. Indeed, we have noted consumptive thrombocytopenia in infants presenting with severe anemia. It is possible that neonatal thrombocytopenia could be attributed to other factors rather than PKD, given how frequently platelet count alterations occur in NICU patients. Case series described thrombocytopenia as a presenting laboratory finding in neonatal PKD with severe hepatic dysfunction, but the duration of thrombocytopenia was not assessed in these patients [8, 9]. Larger studies are needed to clarify the association between thrombocytopenia and PKD.

Initial management of this neonate focused on prolonged hyperbilirubinemia and thrombocytopenia, including exchange transfusion based on established guidelines [23] and platelet transfusions. Exchange transfusion may have incidentally benefitted the liver by decreasing cholestasis and resultant hepatocyte injury during ongoing hemolysis. Transient mild coagulopathy and elevated transaminases that persisted despite downtrending reticulocytosis and stable Hb may reflect mild liver injury from multifactorial etiologies. Ongoing platelet destruction and/or hypersplenism may have limited the utility of platelet transfusions. We noted that platelet transfusions were administered at higher thresholds than those currently used in some centers. This case occurred as our network adapted practices to align with current clinical studies [24, 25].

This complex case highlighted the utility of next generation sequencing (NGS) modalities, including WES or whole genome sequencing (WGS), to rapidly facilitate diagnosis [11, 26, 27]. PKD diagnosis relies on biochemical and molecular testing, but reticulocytosis, recent transfusion, and different *PKLR* mutation types may confound interpretation of standard assays [28]. Focused *PKLR* gene sequencing or NGS circumvent these pitfalls and may be preferred when multiple transfusions and/or incomplete family history complicate diagnosis.

This case reinforces the importance of maintaining a wide differential in cases of neonatal thrombocytopenia, including hemolytic processes from inherited red cell defects. Expanded

use of NGS could facilitate rapid diagnosis and timely management for atypical or complex neonates.

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Data Availability Statement

All relevant data regarding the case are included within this manuscript. Please contact the corresponding author for additional questions and information.

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Established Facts and Novel Insights

Established Facts

Thrombocytopenia is a frequently encountered laboratory finding in the neonatal intensive care unit with a broad diagnostic differential.

Pyruvate kinase deficiency possesses a variable presentation ranging from asymptomatic compensated anemia to hydrops fetalis, with previous reports describing rare brief, self-limited thrombocytopenia in the neonatal period.

Novel Insights

Pyruvate kinase deficiency (PKD) was associated with thrombocytopenia in this case, highlighting the importance of considering PKD and other hemolytic red cell defects in the differential diagnosis for neonatal thrombocytopenia.

Next generation sequencing (NGS) remains an important tool in the evaluation of critically ill neonates with significant diagnostic uncertainty.

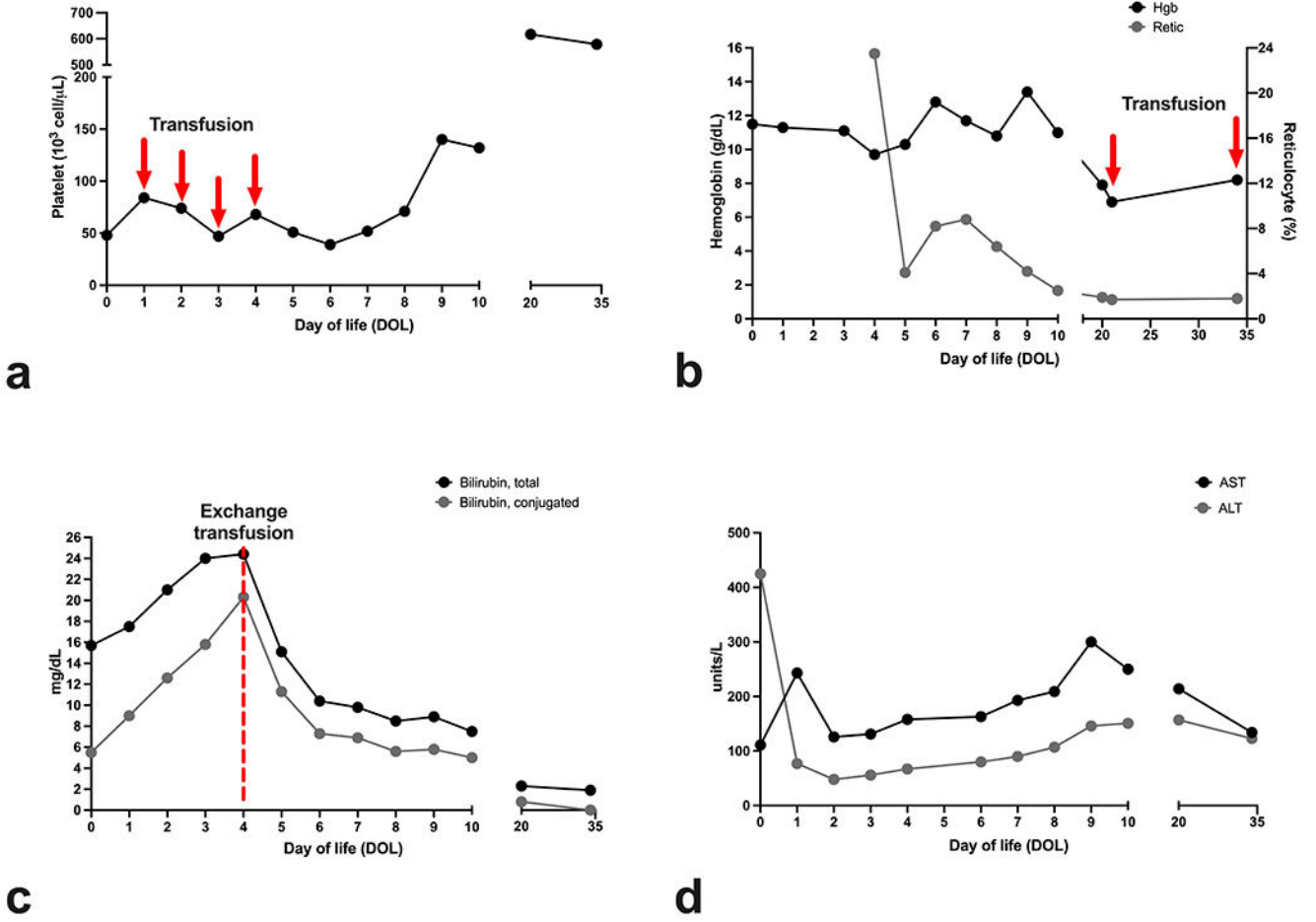


Fig. 1. Anemia, hyperbilirubinemia, and persistent thrombocytopenia associated with pyruvate kinase deficiency.
This neonate exhibited thrombocytopenia, anemia, and hyperbilirubinemia despite blood product and exchange transfusions. Trends for (a) platelets (b) hemoglobin and reticulocytes (c) bilirubin, and (d) transaminases are plotted at indicated days of life. Additional laboratory results from day of life (DOL) 0 are also shown in Table 1.

Table 1.
Selected laboratory testing on admission at 2 hours of life.

Abnormal values are bolded.

<u>Hematology</u>		<i>Reference range</i>
WBC	32.8	9 - 30 K/ μ L
Hgb	11.5	15.6 - 20.2 g/dL
Hct	38.5	46 - 60 %
Plt	48	150 - 450 K/ μ L
MCV	138	95 - 121
MCHC	29.9	29 - 37 g/dL
RDW	26	11.5 - 14.5 %
Nucleated red cells	360	17 - 20 per 100 WBC
Neutrophils	54	39 - 65 %
ANC	22	2.7 - 20 K/ μ L
Myelocytes	4	0 %
Metamyelocytes	5	0 %
Bands	4	0 - 11 %
Lymphocytes	20	23 - 29 %
Reactive lymphocytes	3	0 %
CRP	6	< 7 mg/L
<u>Coagulation studies</u>		
PT, INR	17.7, 1.5	12 - 14.6 s
aPTT	33.2	21.6 - 35.6 s
D-dimer	1.34	< 0.5 μ g/mL
Fibrinogen	267	180 - 500 mg/dL
<u>Bilirubin, liver enzymes</u>		
Bilirubin, total	15.7	0.2 - 8 mg/dL
Bilirubin, conjugated	5.5	0.3 - 0.7 mg/dL
ALT	425	< 56 U/L
AST	111	23 - 186 U/L
GGT	22	< 81 U/L