Severe Hemolytic Anemia: Atypical Presentation of Cobalamin Deficiency

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Summary: Two severe cases of hemolytic anemia are described in different pediatric age groups, both linked to severe cobalamin deficiency from distinct causes. The first case refers to an exclusively breastfed infant with vitamin deficit secondary to maternal impaired absorption. Apart from the neurological deficits present at diagnosis, he also presented with infantile epileptic spasms syndrome a few months after treatment while having normal cobalamin serum levels. The second case refers to an adolescent with long-term inadequate intake. The occurrence of severe hemolytic anemia in cobalamin deficiency is exceptionally rare.

Keys Words: megaloblastic anemia, pancytopenia, vitamin B12 deficiency

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Nutritional anemias are prevalent in pediatric age, being iron-deficient anemia the most common. A less frequent cause of anemia is cobalamin deficiency, which may result from either acquired (decreased intake or reduced absorption) or inherited abnormalities (absorption, transport, or metabolism of vitamin B12).[1] Although uncommon in developed countries, its prevalence is rising in African and Asian countries (up to 80% in Indian preschool children),[2] related to poverty and cultural vegetarian diets. Since cobalamin is an essential cofactor in methylation processes in reactions involving DNA and cell metabolism, its deficit manifests not only as ineffective hematopoiesis but also as faltering growth, developmental delay, or neurological symptoms.[1,2]

Hematological manifestations typically include macrocytic anemia, which may be transfusion-dependent. The other blood cell lineages may be affected, simulating acute leukemia or aplastic anemia.[3] Presentation as hemolytic anemia is rarely described.[4–6] Neurological manifestations are often nonspecific and include developmental delay, movement disorders, weakness, headache, epilepsy, and paresthesia.[7] These symptoms are likely related to neurotoxicity and the overstimulation of *N*-methyl-D aspartate receptors associated with elevated plasma homocysteine.[8]

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RESULTS

Case 1

A 4-month-old male infant who was exclusively breastfed and who had a downward trend of the percentile of weight and height since the previous month, presented to the emergency department with sporadic vomiting for 3 weeks, without diarrhea or other symptoms. On physical examination, he looked ill and presented with fever and pale skin and mucous membranes. Axial hypotonia and appendicular hypertonia were also noticed. Laboratory results revealed normocytic anemia without reticulocytosis, (Hb 7.9 g/dL and reticulocytes 20.160/μL), with evidence of hemolysis and schistocytes in the peripheral blood; also, he had leucopenia (4.080/μL) with neutropenia (850/μL), but a normal platelet count (Table 1). The SARS-CoV-2 polymerase chain reaction on a nasal swab was positive. On further workup, the direct antiglobulin test (DAT) was negative, and functional levels of ADAMTS13 were below 10% without inhibitors. Glucose-6-phosphate-dehydrogenase deficiency was excluded. He had undetectable viral loads for Epstein-Barr virus and Parvovirus, and a low positive viral load for cytomegalovirus (< 178 UI/mL), along with negative IgM antibodies. He was hospitalized and received 2 fresh frozen plasma units and a red blood cell (RBC) transfusion (minimal Hb 6 g/dL). He was discharged 2 weeks later with Hb 10 g/dL and normal levels of functional ADAMTS13. The genetic panel for congenital thrombotic thrombocytopenic purpura (TTP) did not identify pathogenic variants.

Two weeks later, at 5 months old and still exclusively breastfed, normocytic anemia progressively reappeared (Hb 7.2 g/dL, MCV 90.4 fL, and reticulocytes 34.300/μL), with evidence of hemolysis, but without thrombocytopenia or leucopenia. In a comprehensive new etiological analysis, a profound cobalamin deficiency (< 100 pg/mL) was detected along with an increased total serum homocysteine (147 μmol/L), normal serum methionine (9 μmol/L), and increased urinary methylmalonic acid. A primary deficiency was less probable by confirmation of normal homocysteine and methionine values on the neonatal screening test. Since he was exclusively breastfed, further investigation into maternal risk factors for cobalamin deficiency was performed.

When questioned, she referred bilateral hand numbness. Her laboratory results showed macrocytosis without anemia, and additional investigation revealed severe

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TABLE 1. Admission Laboratory Results

	Case 1	Case 2
Hemoglobin, g/dL	7.9 (9.5–13.5)	4.0 (13–16)
MCV, fL	92.5 (74–108)	111.5 (78–98)
Reticulocytes, per μL	20,160 (50,000–100,000)	30,000 (50,000–100,000)
Leukocytes, per μL	4080 (6.000–17,500)	2480 (4.500–11,000)
Neutrophils, per μL	850 (1000–8500)	780L (1800–7700)
Platelets, per μL	228,000 (150,000–400,000)	75,000 (150,000–400,000)
Peripheral blood morphology	Poikilocytosis schistocytes (8/HPF)	Schistocytes*
Direct antiglobulin test	Negative	Negative
Lactate dehydrogenase, UI/L	2031 (120–300)	2406 (135–225)
Oxaloacetate transaminase, UI/L	21 (10–34)	53 (10–34)
Glutamate transaminase, UI/L	13 (10–66)	23 (10–66)
Total bilirubin (direct), μmol/L	28.4 (7.1) (3.42–17.1)	61.6 (3.42–17.1)
Creatinine, mg/dL	0.18 (0.31–0.47)	0,67 (0.7–1.2)
Urea, mg/dL	23 (10–50)	21 (10–50)
Cobalamin,† pg/mL	< 100 (191–663)	< 100 (191–663)
Total homocysteine,† μmol/L	147 (3.2–10.7)	73.8 (3.2–10.7)

*Not confirmed on reanalysis of the blood smear.
†Different date during investigation.

cobalamin deficiency secondary to autoimmune gastritis. The infant was supplemented with intramuscular hydroxocobalamin for 3 consecutive days, started complementary feeding, and had normalization of the hemoglobin value. A mild hypotonia persisted, and 3 months later, he presented with epileptic spasms without hypsarrhythmia or regression, having normal serum cobalamin and homocysteine values at that time. Cerebral magnetic resonance imaging (MRI) revealed focal lesions with slight hypersignal on T2-weight images in the medial and posterior thalamus and *globus pallidus*. The infantile epileptic spasms syndrome (IESS) was refractory to pharmacological treatment, but responded to ketogenic diet. Since the age of 10 months, neurological examination and further acquisition of psychomotor milestones were normal. A whole exome sequencing-based genetic panel for mitochondrial and metabolic disease did not reveal anomalies. A cerebral MRI will be repeated after the age of 2.

Case 2

A 17-year-old adolescent, previously healthy, was admitted to the emergency department for a syncope. He had history of vomiting and diarrhea the previous week. On admission, he presented with pale skin and mucous membranes, and icteric sclera. Laboratory results revealed pancytopenia: macrocytic anemia (Hb 4 g/dL) without reticulocytosis, but with evidence of hemolysis, leucopenia (2480/μL) with neutropenia (780/μL) and thrombocytopenia (75,000/μL; Table 1). A peripheral blood smear was initially described with macro-ovalocytes, macrocytic platelets, and schistocytes (the last were not confirmed on reanalysis of the smear). Renal function was normal, but hemoglobinuria was detected on a urinalysis. DAT was negative, and ADAMTS13 levels were normal. He received RBC transfusions and was hospitalized for further investigation. The paroxysmal nocturnal hemoglobinuria clone was negative. Bone marrow morphology exhibited giant metamyelocytes and significant dyserythropoiesis characterized by megaloblastic features, and these findings were corroborated by histological evaluation. Having the suspicion of megaloblastic erythropoiesis, cobalamin levels were assessed, revealing a profound deficiency (< 100 pg/mL) with increased serum total homocysteine (73,8 μmol/L), decreased serum methionine (11 μmol/L), and increased urinary methylmalonic acid (32 μmol/mmol creatinine). Primary cobalamin deficiency was ruled unlikely after confirmation of the neonatal screening result. Reviewing his dietary intake, it was based on bread and cereals, with an absent uptake of fish and meat for many years. He received supplementation of intramuscular cobalamin for 3 consecutive days, and his nutritional uptake of animal protein was rectified, with a progressive blood count normalization.

Neither of the patients had other nutritional deficits. They were supplemented with folic acid for 3 months, which was started after evidence of reticulocytosis, to restore normal hemoglobin. There were no anemia relapses in the follow-up period of 16 and 22 months, respectively.

DISCUSSION

Hemolytic anemia has been reported as a rare presentation of cobalamin deficiency, so far only described in adults.[4–6,9] A presumptive mechanism for hemolysis relates to elevated levels of homocysteine, which accumulate in the absence of vitamin B12 since the conversion to methionine does not occur. The accumulated homocysteine would have a toxic effect directly in the bone marrow, and cause endothelial damage and subsequent microangiopathy; however, other pathophysiologic pathways must be involved since cases with normal homocysteine levels have also presented with hemolytic anemia.[4] Both presented cases had elevated homocysteine (147 and 21 μmol/L, respectively). Moreover, the absence of cobalamin leads to inefficient erythropoiesis as a consequence of premature dysfunction of the intramedullary precursors.[7]

Two cases of severe hemolytic anemia, caused by the same nutritional deficiency, are presented in pediatric patients of 2 different age groups and resulting from different etiologies. Both had evidence of hemolysis—elevated lactate dehydrogenase and elevated bilirubin, 1 with hemoglobinuria—though neither had reticulocytosis on presentation. Reticulocytosis is usually regarded as a characteristic feature of hemolytic anemia; hence, its absence should raise suspicion for more unusual causes of hemolysis with a medullary suppression component. As for the workup on a new onset of hemolysis, 1 should initially distinguish between immune and nonimmune etiology

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through DAT, which was negative in both, guiding for a nonimmune cause. Pediatric nonimmune hemolysis is mainly associated with intrinsic disorders of the erythrocyte, such as defects of RBC membrane, enzymatic defects or of hemoglobin synthesis, or acquired disorders, such as paroxysmal nocturnal hemoglobinuria or microangiopathic phenomena, mostly hemolytic uremic syndrome (HUS), TTP, or disseminated intravascular coagulation.[1] A peripheral blood smear can inform on the presence of schistocytes, which may be found in microangiopathic anemia.

Case 1 presented with microangiopathic anemia (normocytic nonimmune anemia with evidence of hemolysis and schistocytes on a peripheral blood smear). Despite absent thrombocytopenia, TTP was considered, and, since functional ADAMST13 was extremely low, he was treated with fresh frozen plasma transfusions with a normalization of ADAMST13 levels. Further investigation revealed negative antibodies to ADAMTS13, and the panel for congenital TTP was negative. The remaining hypothesis was that TTP was related to SARS-CoV-2 infection[10] or to a laboratory error. Anemia recurred in 2 weeks, and further investigation revealed a cobalamin deficit, which in a 5-month-old infant that is exclusively breastfed, narrows the differential diagnosis to a maternal deficiency and a primary inherited defect of metabolism. As the mother followed an omnivorous diet, she was investigated and confirmed to have autoimmune gastritis. Unrecognized pernicious anemia and vegetarian mothers commonly cause acquired cobalamin deficiency in exclusively breastfed infants.[11] This infant also presented with faltering growth and neurological symptoms, which might be related to cobalamin deficiency.

The question remains if IESS and the MRI findings identified later in life are also related to this vitamin deficit in early months of life, or if these have a different etiology, since at presentation of IESS, the cobalamin and homocysteine levels were normal. Despite most cases of IESS related to cobalamin deficiency reported in the literature have an epileptic onset temporally related with cobalamin deficit,[8] there are reported cases where the onset was 2 weeks to 10 months after vitamin supplementation (during the recovery period).[12] While the first cases respond better to treatment, the second ones are often refractory. The mechanisms of epileptogenesis are not yet clearly understood.

Case 2 presented with pancytopenia with nonimmune macrocytic hemolytic anemia but without reticulocytosis, which raised the suspicion of central etiology, either by decrease/ineffective production or by destruction/infiltration of the bone marrow. This patient had a recent history of gastroenteritis, raising concern for the differential diagnosis of microangiopathic anemia (schistocytes were initially described, though later excluded) between HUS and TTP, though both could be excluded since he had no renal dysfunction and had normal levels of ADAMTS13. Findings in bone marrow examination suggested dietary deficiency, which was confirmed through anamnesis, and later through low levels of cobalamin in serum.

Body storage of vitamin B12 is relatively high, and consequently, when a deficient intake occurs, it may take several years to deplete the storages completely and present the clinical manifestations.[2] As these cases presented in early ages, primary causes of deficient cobalamin had to be considered. These were ruled out by confirmation of normal levels of methionine and homocysteine on their neonatal screening. An adequate response to treatment without recurrences also sustained the absence of a primary defect.

Treatment guidelines for cobalamin deficiency are lacking, particularly for the pediatric population. Options reside on oral or parenteral supplementation of cyano or hydroxocobalamin, respectively.[3] Due to the severity of both cases, parenteral supplementation was preferred, with a scheme of 1–2 mg/d for 3 consecutive days, without repetition, and with an immediate correction of the vitamin B12 uptake from diet. With this scheme, neither of the patients relapsed.

CONCLUSION

Cobalamin has a fundamental role in DNA synthesis and hematopoiesis. Its deficiency may present with severe anemia, occasionally associated with other cytopenias and other organ involvement. The presentation as hemolytic anemia without reticulocytosis is rare, thereby making diagnosis a challenge. Hematological abnormalities caused by a secondary cobalamin deficiency are infrequent but preventable, and can be prevented by encouraging a balanced diet. However, even with a healthy diet, the diagnosis of a nutritional deficit should be considered since intake can be insufficient, as in the case of exclusively breasted infants, absorption can be compromised, or an underlying primary deficiency can be present.

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