

Two newborns with nutritional vitamin B12 deficiency: Challenges in newborn screening for vitamin B12 deficiency

Vitamin B12 deficiency causes decreased Methionine Synthase and L-Methylmalonyl-CoA Mutase activity and results in accumulation of Homocysteine, Methylmalonic acid and Propionylcarnitine. Propionylcarnitine is included in tandem mass spectrometry-based newborn screening programs for detection of certain inborn errors of metabolism. We report two asymptomatic newborns with Vitamin B12 deficiency due to maternal deficiencies. One was detected incidentally at 3 weeks of age; the second on supplemental newborn screening based on elevated Propionylcarnitine at 2 days of age. This illustrates the potential for false negative results for Vitamin B12 deficiency screening by acylcarnitine profiling in newborn screening. Homocysteine and Methylmalonic acid may be better markers of Vitamin B12 deficiency. In conclusion, we suggest measuring Methylmalonic acid, Propionylcarnitine and Homocysteine levels in blood spots in expanded newborn screening in order to detect asymptomatic newborns with Vitamin B12 deficiency. Further studies are needed to establish the sensitivity of these three markers in screening for Vitamin B12 deficiency.

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Nutritional Vitamin B12 deficiency is usually characterized by anemia, anorexia, irritability, failure to thrive, developmental delay or regression, and irreversible neurological damage. Vitamin B12 is important for central nervous system development and studies show that even moderate Vitamin B12 deficiency can be harmful to developing infants.¹ Neurologic findings may precede any signs of megaloblastic anemia in Vitamin B12 deficient infants.³ Infants born to mothers with Vitamin B12 deficiency are at risk for developing nutritional Vitamin B12 deficiency.² Prevention and early treatment are necessary to prevent irreversible neurological damage.¹

Vitamin B12 functions as a cofactor for two enzymes. The first, Methionine Synthase, is required for the synthesis of Methionine from Homocysteine. Decreased Methionine Synthase activity results in the accumulation of total Homocysteine (tHcy) and the lack of S-Adenosyl Methionine, the universal methyl group donor for more than 100 organic reactions. The second enzyme, L-Methylmalonyl Coenzyme A Mutase, converts Methylmalonyl-CoA to Succinyl-CoA. This biochemical reaction plays an important role in the production of energy from fats and proteins. Succinyl-CoA is also required for the synthesis of hemoglobin.

Decreased activity of Methylmalonyl-CoA Mutase due to Vitamin B12 deficiency results in accumulation of Methylmalonyl-CoA, resulting in increased amounts of Methylmalonic acid (MMA) in plasma and urine, as well as Propionyl-CoA. Excess Propionyl-CoA is converted to Propionylcarnitine (C3), which provides a mechanism to remove the acyl group and liberate free CoA. MMA and tHcy in plasma and urine have been used to diagnose and monitor Vitamin B12 deficiency.⁴ Brass and Stabler reported a significant increase of Propionylcarnitine in Vitamin B12 deficient rats.⁵ Elevated levels of Propionylcarnitine have not been reported in Vitamin

B12 deficient humans. High plasma levels of Propionylcarnitine may serve as a marker of impaired Vitamin B12 function, similar to plasma MMA and tHcy.

Propionylcarnitine is now included in all tandem mass spectrometry (MS/MS)-based newborn screening programs.^{6,7} Propionic acidemia, Methylmalonic acidemia, and congenital Cobalamin defects can be detected based on the finding of increased Propionylcarnitine and/or an increased Propionylcarnitine-to-Acetylcarnitine ratio in dried blood spots by MS/MS.⁶

To date, no reports have been published of any newborns with nutritional Vitamin B12 deficiency detected by acylcarnitine profiling through newborn screening.

We report two cases of nutritional Vitamin B12 deficiency in newborns secondary to maternal Vitamin B12 deficiencies. Both infants had supplemental newborn screening through Pediatrix Medical Group, Inc.

Patients

Case 1. A full term, Caucasian, male newborn with elevated C14 (Tetradecanoyl) species detected by MS/MS on expanded newborn screening was referred to our metabolic center at three weeks of age. He was born to healthy, non-consanguineous parents after an uneventful pregnancy and delivery. His physical exam was unremarkable. The family history was noncontributory. His healthy 31-year-old mother had two previous pregnancies, which resulted in healthy children (ages 7 and 9 years).

Plasma acylcarnitine analysis at three weeks of age showed increased Tetradecadienoyl carnitine (C14:2), Tetradecenoyl carnitine (C14:1), and Tetradecanoyl carnitine (C14) (Table 1).

Table 1. Laboratory data of patients.

Newborn Screening age- 48hr	At 21 days	At 40 days	At 60 days
Patient 1			
Plasma Acylcarnitines			
C14: 2(<0.150 mmol/L)		0.210	
C14: 1(<0.260 mmol/L)	Abnormal	0.370	
C14 (<0.100 mmol/L)	Abnormal	0.120	
C3(<0.750 mmol/L)	Normal	1.24	
Plasma MMA (73-271 nmol/L)		2222	467
Plasma tHcy (5.1-13.9 nmol/L)		12.6	13.2
Urine MMA (<10 mg/g creatinine)		40	<10
Serum vitamin B12 (293-1208 pg/mL)		165	289
Hemoglobin		13.7	1728
MCV	95		
	At 10 days	At 16 days	At 28 days
Patient 2			
Plasma Acylcarnitines			
C3(<0.750 mmol/L)	Abnormal	0.950	2.24
Plasma MMA (73-271 nmol/L)			5126
Plasma tHcy (5.1-13.9 nmol/L)			30.3
Urine MMA (<10 mg/g creatinine)		66	38
Serum vitamin B12 (293-1208 pg/mL)>2000		126	
Hemoglobin		13.5	
MCV	86		

Vitamin B12 therapy started

In addition there was an elevated level of Propionylcarnitine (C3) (1.24 mmol/L; normal <0.750). Urine MMA was elevated (40mg/g creatinine; normal <10) and Methylcitrate was detected (7mg/g creatinine; normal: not detected). Subsequently, plasma MMA (2222

nmol/L; normal 73-271), 2-Methylcitrate (464 nmol/L; normal 60-228), and Cystathionine (518 nmol/L; normal 44-342) levels were found elevated. The total Homocysteine level was at the high end of normal (12.6 nmol/L; normal 5.1-13.9). The blood Vitamin B12 level was below normal (165 pg/mL; normal 293-1208). The plasma amino acid profile and carnitine levels (total and free) were normal, as was the complete blood count with differential without any findings of megaloblastic anemia on peripheral blood smear.

The patient was treated with intramuscular hydroxycobalamin. The levels of Vitamin B12 increased to 289 pg/mL in three weeks and to 1728 pg/mL in two months after treatment. The patient's plasma acylcarnitine analysis showed a normal level of C3 three weeks after the initiation of treatment. The levels of plasma MMA, 2-Methylcitrate, and urine MMA excretion normalized one month after treatment initiation. Evaluation of the mother established the diagnosis of autoimmune pernicious anemia with a Vitamin B12 level of 151 pg/mL (normal 228-1514) and an abnormal Shilling test (1.4%; normal >10%).

The patient was fed exclusively breast milk for the first month of life. Once the diagnosis of VLCAD deficiency was confirmed by plasma acylcarnitine analysis showing elevated levels of C14 and C14:1, nutritional treatment was implemented with a low-fat formula (Portagen) enriched with Medium Chain Triglycerides (MCT) and supplemented with essential fatty acids.

VLCAD enzyme activity in lymphoblasts was 23% of normal (1.1 nmol/min/mg protein; normal: 4.74). Molecular analysis of the VLCAD gene demonstrated that the patient is compound heterozygous for a previously reported mutation (1748 C>T in exon 18 causing S543L) and a unique mutation (IVS13+25G>A).

Case 2. A full term Caucasian female newborn with an elevation of C3 and increased C3:16 ratio on MS/MS expanded newborn screening was referred to our metabolic center at age 10 days. She was born to non-consanguineous parents after an uneventful pregnancy and spontaneous vaginal delivery. At birth, the infant's weight was 4.1 kg (90th-95th percentile) and her length was 53.3 cm (90th percentile). There were no neonatal complications.

The family history was significant for her mother's elective gastric bypass surgery for morbid obesity 3.5 years prior to the delivery. Three previous pregnancies resulted in healthy children (ages 2, 6, and 10 years).

At age 10 days, plasma acylcarnitines showed elevated C3 (0.950 mmol/L; normal <0.750). Urine organic acids showed elevations of MMA (66 mg/g creatinine; normal <10) and Methylcitrate (10 mg/g creatinine; normal: not detected).

Testing at age 16 days showed a persistent elevation of C3 (2.24 mmol/L; normal <0.750) as well as elevations of plasma MMA, 2-Methylcitric acid, Cystathionine, and Homocysteine. Urine organic acids showed elevations of 3-OH-Propionate, MMA, and Methylcitrate. The blood Vitamin B12 level was below normal (126 pg/mL; normal 179-891). Plasma amino acids were normal.

Intramuscular hydroxycobalamin was initiated on day of life 16 at a dose of 1 mg three times per week for two weeks. By age 28 days, the blood Vitamin B12 level had increased to >2000 pg/mL (normal 228-1514). The acylcarnitine profile normalized by age 28 days. Furthermore, the levels of MMA, 2-Methylcitrate, Cystathionine, and Homocysteine were within normal limits.

Maternal Vitamin B12 levels were measured at 183

pg/mL (normal 228-1514), thus confirming the diagnosis of maternal Vitamin B12 deficiency. Although the infant's mother had been informed of the risks of vitamin malabsorption following gastric bypass surgery, she stopped taking prenatal vitamins due to side effects including stomach upset, constipation, and severe stomach pain.

Discussion

Increased levels of plasma MMA and Homocysteine, as well as urine MMA, are usually detectable in individuals with Vitamin B12 deficiency before any hematologic or neurologic findings become evident. An elevated MMA level is viewed as a sensitive and specific marker for Vitamin B12 deficiency. Michaud *et al*⁶ reported two asymptomatic newborns with nutritional Vitamin B12 deficiency detected by newborn urinary screening at three weeks of life with elevated urine MMA. Drogari *et al*⁷ reported Methylmalonic aciduria in four breastfed newborns, ages 20-60 days, born to strict vegetarian mothers; the infants all had clinical manifestations of Vitamin B12 deficiency at the time of diagnosis. Vitamin B12 deficiency was also reported by Grange and Finlay in a breastfed infant whose mother underwent gastric bypass.¹⁰

The recent advent of tandem mass spectrometry (MS/MS) for the purpose of newborn screening in dried blood samples has facilitated early diagnosis of some metabolic disorders including amino acidopathies, organic acidopathies, and fatty acid oxidation disorders.^{6,7} Given the difficulty in diagnosing Vitamin B12 deficiency in infancy due to nonspecific clinical findings, the potentially devastating and irreversible neurologic damage in untreated infants, and a readily available treatment in the form of Vitamin B12, a case could be made for inclusion on expanded newborn screening panels. Since increased serum MMA is an early marker in Vitamin B12 deficiency, newborns with Vitamin B12 deficiency could be detected by measuring C3 levels on MS/MS. However, our experience with these two patients with Vitamin B12 deficiency suggests that not all newborns with Vitamin B12 deficiency can be detected by MS/MS expanded newborn screening because the C3 levels may not be sufficiently high during the first few days of life to be detected through expanded newborn screening.

Monsen *et al* showed that newborns with low Vitamin B12 levels had significantly elevated levels of plasma tHcy and MMA at 96-108 hours post delivery.¹¹ They also reported that there was a decrease in the infants' serum Vitamin B12 levels and an increase in plasma tHcy and MMA levels during the first 6 weeks of life. This observation may explain why our first patient did not have an elevated C3 level on the 3rd day of life, but did during the 3rd week.

Due to the low sensitivity of the newborn screening acylcarnitine profile, a negative newborn screen panel is not sufficient to rule out Vitamin B12 deficiency. Improvements in the detection of Vitamin B12 deficiency are needed to enable physicians to diagnosis infants in a timely manner. Recently, the Mayo Clinic Laboratories implemented a tandem mass spectrometry procedure for measuring Hcy and MMA.¹² The determination of MMA and Hcy in addition to C3 in dried blood samples may reduce the false negative rate and increase detection of neonatal nutritional Vitamin B12 deficiency.

In conclusion, consideration should be given to measuring MMA, C3 and Hcy levels as markers in blood spots in expanded newborn screening in order to detect asymptomatic newborns with Vitamin B12 deficiency.

Additional studies are needed to establish the optimal cutoff levels of MMA and Hcy that would minimize false positive and false negative rates and to determine if all patients with Vitamin B12 deficiency could be detected through newborn screen as early as 48-72 hours post delivery.

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