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CLINICAL REPORT

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A cautionary tale of pyridoxine toxicity in cystathionine beta-synthase deficiency detected by two-tier newborn screening highlights the need for clear pyridoxine dosing guidelines

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Abstract

Classic homocystinuria is due to deficiency of cystathionine beta-synthase (CBS), a pyridoxine-dependent enzyme that, depending on the molecular variants, may be co-factor responsive. Elevated methionine is often used as the primary analyte to detect CBS deficiency (CBSD) on newborn screening (NBS), but is limited by increased detection of other biochemical disorders with less clear clinical significance such as methionine aminotransferase (MAT) I/III heterozygotes. Our state has implemented a two-tier NBS algorithm for CBSD that successfully reduced the number of MATI/III heterozygotes, yet effectively detected a mild, co-factor responsive form of CBSD. After initial diagnosis, newborns with CBSD often undergo a pyridoxine challenge with high-dose pyridoxine to determine responsiveness. Here we describe our NBS-identified patient with a mild form of pyridoxine responsive CBSD who developed respiratory failure and rhabdomyolysis consistent with pyridoxine toxicity during a pyridoxine challenge. This case highlights the need for weight-based dosing and duration recommendations for pyridoxine challenge in neonates.

KEYWORDS

homocystinuria, hypermethioninemia, pyridoxine responsive

1 | INTRODUCTION

The state of Michigan began screening for homocystinuria and related disorders in 2004 using an elevated methionine as the primary analyte. The methionine:phenylalanine ratio (Met/Phe) is also included in the first-tier screening algorithm as it allows for comparison of multiple amino acids that may be elevated nonspecifically due to transient dietary differences, use of total parenteral nutrition, or general liver health (Morris et al., 2017; Okun et al., 2017). In 2015, Michigan implemented a second tier assay to improve specificity of methionine and Met/Phe as primary analytes [allowing newborn screening (NBS) to keep cystathionine beta-synthase deficiency (CBSD) false positives to a minimum] given that elevated methionine is seen in a number of conditions including methionine aminotransferase (MAT) I/III heterozygotes without clear clinical significance (Huemer et al., 2015; Keller et al., 2019; Sen, Felice, Bannick, Colombo, & Conway, 2019). Currently for samples from term newborns not admitted to the neonatal intensive care unit with a methionine ≥45 μmol/L and a Met/Phe ≥1.0, the initial NBS dried blood spot is sent to a reference lab (Mayo Medical Laboratories, Rochester, MN) to perform second-tier analysis of total homocysteine. If the total homocysteine level is greater than 15 μmol/L, the sample is considered a presumptive positive and prompts a referral to a metabolic follow-up clinic. To allow for timely management of more severe cases of CBSD, NBS samples with

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methionine ≥80 μmol/L and Met/Phe ≥1.0 are considered presumptive positives and directly referred to a metabolic clinic for diagnostic testing.

Between 2004 and 2014, prior to the initiation of second-tier analysis of total homocysteine, there were 64 presumptive positive newborn screens prompting referral to a metabolic center. Confirmatory testing identified 3 confirmed cases of CBSD (none were pyridoxine responsive) and 12 cases of persistent isolated hypermethioninemia, all due to severe or partial MATI/III deficiency. There was one case of a mother with autosomal recessive MAT1A deficiency whose methionine ranged from 800 to 1,400 μmol/L whose child's methionine levels normalized with confirmatory testing (Table 1). The remaining cases were all false positives. The positive predictive value of detecting CBSD with elevated methionine over this time was 4.7% (3 true positives/64 total positive screens). Since initiation of two-tier screening, there were 7 presumptive positive referrals based on methionine elevation and elevated Met/Phe. None of these were confirmed to have CBSD. In addition, 57 samples were sent for second-tier testing. Of those 57 samples, only 3 were found to have elevated homocysteine levels. Upon confirmatory testing, two cases were found to be unaffected and the third case, as described below, was found to have pyridoxine-responsive CBSD (Table 1). The positive predictive value of detecting CBSD with second-tier screening with homocysteine was 10% (1 true positive/10 total positive screens).

Our patient was identified by NBS to have an elevated methionine (73 μmol/L, normal <45) and elevated Met/Phe ratio (1.6, normal <1.0). Second-tier testing was notable for an elevated homocysteine (21.9 nmol/ml, normal <15). Confirmatory plasma amino acids were obtained on day of life 8 that were notable for a mildly elevated methionine (54 μmol/L, normal 23–43) and elevated homocysteine (40 μmol/L, normal 5–15). Vitamin B12 (592 pg/ml) and folate (>22.3 ng/ml) levels were both normal. Molecular testing for hypermethioninemia including the *CBS*, *GNMT*, *MAT1A*, and *ACHY* genes was sent. Additional metabolites of the methionine pathway (S-adenosylmethionine and S-adenosylhomocysteine) were unable to be sent due to insufficient blood volume.

Following these confirmatory labs, the patient was diagnosed with presumptive CBSD and started on pyridoxine at a dose of 100 mg daily (31 mg/kg/day) as a trial to determine pyridoxine responsiveness without introduction of medical formula. Homocysteine measured 7 days after pyridoxine initiation was again stably elevated (44 μmol/L). With no significant reduction in homocysteine level, pyridoxine dosing was increased to 200 mg daily (54 mg/kg/day) with a plan to recheck levels in 1 week. Roughly 1 week after dose increase (~2 weeks of pyridoxine therapy), the patient suddenly developed dyspnea and cyanosis, followed by apnea, causing the caregiver to perform CPR. The patient was then transported via EMS to the Emergency Department (ED) for further evaluation.

TABLE 1 Hcy = homocysteine, Pres + = presumptive positive, T+ CBSD = true positive CBS deficiency, Hyper Met = persistent isolated hypermethioninemia, Mat MAT1A = maternal autosomal recessive MAT1A deficiency

Year	Hcy second tier	+ Second tier	Pres +	T+ CBSD	Hyper Met	Mat *MAT1A*	Changes to methionine cutoff and/or platform
2004 (October)			6	0	0	0	Met = 87 μmol/L, M/P ≥ 1.3
2005			10	0	0	0	
2006			7	0	0	0	
2007			1	0	0	0	Met = 74 μmol/L, M/P ≥ 1.3
2008			1	0	1	0	
2009			2	1	1	0	
2010			6	0	3	0	
2011			7	0	3	0	Met = 56 (switch from NeoGram to NeoBase)
2012			11	2	2	1	
2013			5	0	1	0	
2014			8	0	1	0	
2015 (January)	7	2	2	0	0	0	
2016	6	0	2	0	0	0	
2017	18	0	2	0	1	0	
2018	11	0	1	0	0	0	
2019	15	1	0	1	0	0	Met = 45 (switch from NeoBase to NeoBase2)

	Hcy second tier	+ second tier	Pres +	T+ CBSD	Hyper Met	Mat *MAT1A*
Pre-second tier			64	3	12	1
Post-second tier	57	3	7	1	1	0

Abbreviations: Hcy, homocysteine; Hyper Met, persistent isolated hypermethioninemia; Mat *MAT1A*, maternal autosomal recessive *MAT1A deficiency*; Pres +, presumptive positive; T+ CBSD, true positive CBS deficiency.

On arrival to the ED, the patient initially appeared comfortable with even and unlabored respirations, but was noted to have intermittent peripheral arterial desaturations by pulse oximetry to SpO_2 88–89% with exam. The patient continued to have intermittent apneic episodes lasting 30–40 s that progressed to more significant desaturations (SpO_2 70–79%) with subsequent bradycardia of 60–69 beats/min. Given the increased frequency of apneic episodes and bradycardia, she was intubated. The patient was then transferred to the pediatric intensive care unit for further management and close monitoring of her respiratory failure. Labs on admission were notable for elevated transaminases (AST 422, ALT 137 IU/L), elevated creatine kinase (CK) (5,973 IU/L, normal 26–180), homocysteine of 37 μmol/L, and methionine of 131 μmol/L. A broad spectrum of testing for other causes of apnea in a newborn was negative, including a full sepsis evaluation with CSF studies, viral respiratory PCR panel, brain MRI, and long-term video EEG. Pediatric Genetics was consulted for further management of homocystinuria.

Based on her clinical presentation of apnea leading to respiratory failure, elevated transaminases, and elevated CK, pyridoxine toxicity was strongly suspected and genetics recommended cessation of pyridoxine until improvement of her clinical picture and molecular confirmation of her diagnosis. Within 72 hr of pyridoxine cessation, the patient's respiratory drive had improved and she was extubated to noninvasive positive pressure ventilation for several days before being weaned to supplemental oxygen via nasal cannula. While she was critically ill, enteral feeding was held and nutrition was provided via dextrose-containing fluids and intravenous lipids.

During her admission, the results of molecular testing returned and revealed two pathogenic variants in the *CBS* gene: c.833T>C, p.Ile278Thr and c.476_477insCAGGCCC, p.Val160Argfs*4, the latter of which has not been previously described. The former is a pathogenic variant that is associated with pyridoxine-responsive CBSD. Prior to discharge and 7 days after admission, her elevations in CK, AST, and ALT continued to downtrend and pyridoxine was reinitiated at 10 mg/kg dose prior to discharge. Although she clearly did not respond clinically in a classic pyridoxine-responsive manner, genotype–phenotype correlation of CBS compound heterozygotes is difficult to predict (Morris et al., 2017), and a safe dose of pyridoxine was continued to provide potential benefit in the event that she was partially responsive to pyridoxine.

Labs performed 1 week after hospital discharge on a breast milk-based diet were notable for a normal CK, normal AST/ALT, a homocysteine of 56 μmol/L, and methionine of 268 μmol/L. Due to maternal concerns about pyridoxine toxicity, patient's pyridoxine dose was less than what we recommended (ultimately around 7–8 mg/kg/day instead of recommended 10–20 mg/kg/day). Repeat labs 1 week later revealed a homocysteine of 59 μmol/L and methionine of 589 μmol/L, but these were noted to be post-prandial samples. Preprandial labs repeated 1 week later were again notable for persistent elevations in methionine (641 μmol/L) and homocysteine (66 μmol/L). A partial methionine-restricted diet was initiated (total methionine intake of 25–30 mg/kg/day) and subsequent labs were notable for a methionine of 77 μmol/L and a homocysteine of 39 μmol/L. At 6 months of age, she has continued on pyridoxine 10 mg/kg/day and a partial methionine-restricted diet. She has attained age-appropriate milestones and has normal growth parameters. Her methionine levels are below 100 μmol/L and her homocysteine levels are below 50 μmol/L.

2 | DISCUSSION

Classic homocystinuria or CBSD is the most common inborn error of sulfur metabolism due to pathogenic variants in the *CBS* gene (Kruger, 2017). There is a wide range of presentations based on the severity of the disease and include developmental delay, ectopia lentis, Marfanoid habitus, osteoporosis, intellectual disability, thromboembolic events, and even some reports of asymptomatic adults. (Gibson, Carson, & Neill, 1964; Poloni et al., 2018). From a biochemical perspective, there is also a wide range of phenotypes including mild homocysteine elevations that are highly responsive to pyridoxine compared with pyridoxine-unresponsive forms that require extensive methionine restriction and medical management with betaine. One of the most common pathogenic variants in the *CBS* gene is c.833T>C (p.Ile278Thr) which accounts for approximately 25% of all pathogenic variants identified and is pan-ethnic (Moat et al., 2004). The p.Ile278Thr variant is associated with at least some degree of pyridoxine responsiveness (Morris et al., 2017) and results in a less severe disease phenotype (Kluijtmans et al., 1999; Shih et al., 1995). Epidemiologic studies suggest that the expected incidence of individuals with CBSD—especially when taking into account the frequency of the p.Ile278Thr allele—should be much more than the frequency of those actually identified by standard NBS methods (Gaustadnes, Ingerslev, & Rutiger, 1999). Similarly, one study suggested that NBS will only identify 25% of CBSD cases when hypermethioninemia is used as the primary analyte (Sokolová et al., 2001). The recently published guidelines for CBSD emphasize this point. The sensitivity of CBSD detection by NBS with hypermethioninemia alone is probably very low unless paired with a two-tier system (Huemer et al., 2015; Keller et al., 2019; Morris et al., 2017) as our state has implemented. To our best estimate, the incidence of CBSD in Michigan for October 2004 through January 2015 is 1:400,000 (calculated on total number of NBS performed each year with the exception of 2005 where data is not available, a stable birth rate compared with 2006 was assumed). The incidence of CBSD in Michigan between February 2015 to December 2019 is estimated to be 1:500,000 (calculated on total number of NBS performed with the exception of 2019 where data has not been reported yet and the birth rate was assumed to be stable compared with 2018). In comparison, our estimation of CBSD incidence based on clinically ascertainment prior to NBS is roughly 1 in 1,000,000 (assuming Michigan has roughly 100,000 births per year and we have on average one CBSD patient per decade who predate NBS. Our state's data supports that the two-tier screening system improves the positive predictive value of NBS while overall detecting a similar number of patients. The primary benefit of such a two-tier system, when methionine is the primary analyte for detection, is that this approach allows for lower methionine cutoffs to detect mild

forms of CBSD while resulting in a relatively low number of referrals by improving specificity with a second tier screening of total homocysteine level as has been reported elsewhere (Alodaib et al., 2012; Keller et al., 2019; Mudd, 2011; Turgeon et al., 2010). In our state, we have shown that many of the elevations of methionine found through NBS were ultimately due to confirmed or presumed mutations in *MAT1A*, the gene responsible for MAT I/III deficiency (Sen et al., 2019). However, utilizing a two-tier approach allowed NBS to successfully discriminate a true and mild case of CBSD.

Adverse effects to pyridoxine have been previously reported in other neonates with apnea after receiving high doses of pyridoxine (Mudd, Levy, & Kraus, 2001). Our patient's presentation was similar to a Japanese infant with CBSD who received 100 mg/kg for 8 days before she developed apneic respiratory failure, rhabdomyolysis, and elevated transaminases; the infant recovered fully over a few weeks after pyridoxine cessation (Shoji, Takahashi, Sato, Shoji, & Takada, 1998). There are several differences between this case and our patient. Our patient was only prescribed 200 mg daily (54 mg/kg/day) for 7 days following a 7-day period of receiving 100 mg daily (31 mg/kg/day). The neonate described in Shoji et al. also had markedly elevated methionine levels (1,340–1,630 μmol/L) whereas our patient had less significant hypermethionemia (methionine <1,000 μmol/L). Our case suggests a lower dose can cause pyridoxine toxicity in early infancy. In the guidelines for CBSD management, a pyridoxine challenge of 10 mg/kg/day is recommended for at least 7 days. However, there is a special exception for neonates detected by NBS who are presumed to be more severe and less likely to respond to pyridoxine and should receive a relatively high dose of pyridoxine (100 mg/day) for 2 weeks (Morris et al., 2017). These guidelines also report a range of doses for pyridoxine challenge of 100–500 mg/day for 1–2 weeks, which for a 3–4 kg neonate is a minimum dose of 25–33 mg/kg/day to a maximum dose of 125–167 mg/kg/day. The management guidelines proposed in GeneReviews recommends a pyridoxine challenge of a shorter duration to find the lowest possible dose to produce the maximum biochemical benefit (defined as 30% or more reduction in homocysteine). For neonates detected by NBS this would entail 100 mg/day for 2 days (25–33 mg/kg/day assuming 3–4 kg neonate), checking methionine and homocysteine levels, and two additional dose escalations over the next 4 days to 200 mg/day (50–66 mg/kg/day) and 300 mg/day (75–100 mg/kg/day) to determine pyridoxine responsiveness (Sacharow, Picker, & Levy, 2004). Commonly used biochemical textbooks vary in their recommendations for pyridoxine challenge including 100 mg daily for 2 weeks (Saudubray, Baumgartner, & Walter, 2016) to a dose escalation from 100 mg daily (25–33 mg/kg/day) to 500 mg daily (125–167 mg/kg/day) over multiple weeks (Blau, Duran, Gibson, & Dionisi-Vici, 2014).

Our case highlights the importance and risks associated with pyridoxine challenge in neonates with CBSD. The current clinical practice guidelines and literature for pyridoxine challenges does not clearly discriminate recommended doses based on age, weight, and suspicion of clinical pyridoxine responsiveness (as indicated by homocysteine level). The recommendations for dose escalation in a pyridoxine challenge may not be appropriate for all infants. It is also possible that additional factors, as yet undetermined, may predispose some neonates to toxic effects of pyridoxine at doses which are well tolerated by other neonates. Until the risks of pyridoxine toxicity are better understood, current recommendations could be strengthened by clear weight-based dosing and duration for pyridoxine therapy, especially for neonates detected by NBS when assessing for co-factor responsiveness. As molecular testing confirmation becomes more widely available, the knowledge of specific pathogenic variants may also be a crucial determinant in the pyridoxine responsiveness and the pyridoxine dose escalation chosen by providers. We propose that for neonates with CBSD detected by NBS that a pyridoxine challenge would be completed over a relatively short period of time and with a careful review with parents of the adverse side effects associated with pyridoxine toxicity including risk of apnea. Given that our patient had clinically significant apnea at 50 mg/kg/day, we would recommend starting at 10 mg/kg/day for 4 days, increasing to 20 mg/kg/day for 4 days if no appreciable change in homocysteine level, and using 40 mg/kg/day as a maximum dose for another 4 days. We recommend extreme caution in neonates for doses approaching or in excess of 200 mg daily and to use weight-based doses when possible. Pyridoxine is also used for patients with pyridoxine dependent epilepsy with a recommended maximum dose of 40 mg/kg/day for short periods of time during illnesses (van Karnebeek et al., 2015). While this disorder has a completely different mechanism, it is another helpful measure of what is generally considered safe by weight-based standards. We acknowledge that future case reports or therapeutic studies may alter our recommendations. We encourage providers to proceed with caution when selecting doses for performing neonatal pyridoxine challenges for NBS-detected patients with CBSD and follow neonates closely for pyridoxine toxicity or side effects.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ames EG: Conceptualization, patient care, data analysis, writing—original draft preparation, writing—review and editing; Scott AJ: Conceptualization; patient care, writing—original draft preparation; Conway RL: Conceptualization, data analysis, writing—review and editing, supervision; Ahmad A: Conceptualization, patient care, writing—review and editing, supervision; Pappas KB: patient care, writing—review and editing; Moloney S.M: data analysis, writing—review and editing. All authors have read and agreed to the accepted version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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