

The proposed requirement for approval of coverage is a useful addition to the recommendation made in the Institute of Medicine report that journals require sex-specific analyses for publication.¹ However, if the assertion that a coverage requirement is the “best way” to ensure adequately powered tests of men and women implies that a coverage requirement will be more effective than a publication requirement, I would disagree. In relation to a publication requirement, the *JAMA* Editor’s Note accompanying my Commentary questioned whether it would occur too late in the process to be effective. Decisions regarding coverage come even later and are primarily relevant to clinical trials rather than to the full range of scientific studies providing sex-specific analyses.

The impact of either requirement will depend on its salience to researchers as they plan their studies. The publication requirement would need to be phased in since it would be problematic to apply it to studies that are already underway or have completed data collection. Over time, however, its existence should shape the design and conduct of studies. Researchers do not want to undertake studies that cannot be published. If a sex-specific requirement for publication is clearly and consistently applied, they would be highly motivated to ensure adequate enrollment of women for meaningful analysis.

It is not clear that a similar requirement by insurers would have as broad an effect. The approval process for insurance coverage is more distal to research than is publication, and publication requirements are likely to be more salient. Every researcher wants to publish the findings of his or her research, but only a subset will be concerned about insurance coverage decisions.

If it were necessary to choose a single policy, a publication requirement would appear to have greater potential for broad impact and a fundamental shift in research than a coverage requirement. However, it need not be an “either-or” choice. It may well be that both types of requirements are needed to advance women’s health. Despite existing regulations by the National Institutes of Health and Food and Drug Administration, female participants are still underrepresented in animal studies, observational research, and clinical trials.¹ The bias toward framing questions based on a male model of disease, and subsequent design and inclusion criteria, may be sufficiently ingrained in the biomedical research culture that requirements are needed at multiple levels to create lasting change.

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1. Institute of Medicine. *Women’s Health Research: Progress, Pitfalls and Promise*. Washington, DC: National Academies Press; 2010.

RESEARCH LETTER

Expanded Newborn Screening for Detection of Vitamin B₁₂ Deficiency

To the Editor: When undiagnosed, infant vitamin B₁₂ deficiency can result in anemia, failure to thrive, developmental regression, and neurological deficits and is most commonly caused by maternal vitamin B₁₂ deficiency.¹ Biochemically, vitamin B₁₂ deficiency leads to an accumulation of total homocysteine (tHcy), methylmalonic acid (MMA), and propionylcarnitine (C₃). Although vitamin B₁₂ deficiency is not a primary target of newborn screening (NBS) programs, measurement of C₃ may incidentally identify vitamin B₁₂-deficient newborns. However, first-tier screening is limited because C₃ levels may not be sufficiently high during the first days of life when most NBS samples are collected.² Levels of MMA and tHcy are more sensitive markers of vitamin B₁₂ status than serum B₁₂ levels in adults,³ have a strong inverse relationship with serum B₁₂ levels,⁴ and are usually elevated in infants who are vitamin B₁₂ deficient before hematologic or neurologic findings develop.² Through the public-private partnership between the Minnesota Department of Health, Mayo Clinic, and University of Minnesota, a lower first-tier cut-off level of C₃ was applied (>9.2 μmol/L to >5.25 μmol/L) and second-tier testing of MMA and tHcy levels in presumptive-positive NBS specimens was introduced in 2005.⁵

Methods. A population-based study of 363 649 infants born in Minnesota was conducted between January 1, 2005, and December 31, 2010. Newborn screening samples with elevated C₃ levels, a C₃ to acetylcarnitine (C₃/C₂) ratio of 0.1 or greater, or a C₃ to palmitoylcarnitine (C₃/C₁₆) ratio of 2 or greater had second-tier testing of tHcy (abnormal, >15 μmol/L) and MMA (abnormal, >5 μmol/L) on the same sample.⁵ (To convert tHcy to mg/dL, divide by 7.397.) All newborns with positive NBS results and some of their mothers underwent further laboratory testing. An additional newborn from another state whose first-tier NBS findings were normal presented at age 2 months with clinical complications of vitamin B₁₂ deficiency and required prolonged hospitalization. His original NBS sample was retrospectively analyzed to determine if the Minnesota 2-tier protocol would have led to identifying the infant as vitamin B₁₂ deficient. The University of Minnesota institutional review board approved review of medical records and waived informed consent.

Results. Of 46 newborns with abnormal C₃ levels or related ratios identified in Minnesota, 11 had vitamin B₁₂ deficiency secondary to maternal vitamin B₁₂ deficiency (TABLE 1 and TABLE 2) for a detection rate of 3.02 per 100 000 live births; 22 findings were false positives; 1 declined follow-up testing; 1 had unconfirmed vitamin B₁₂ deficiency; 3 had cobalamin C defects; and 2 had methylmalonic acidemias. Five were homozygous and 1 a carrier for transcobalamin receptor defects. Newborn screening led to the identification of vitamin

Table 1. Maternal Prenatal History, Serum B₁₂ Levels (Pretreatment, Postdelivery), and Newborn Screen Dried Blood Spot Values

Case	Prenatal History	Newborn Screening Results					
		Serum B ₁₂ pg/mL ^b	C ₃ , μmol/L ^b	C ₃ /C ₂ Ratio ^b	C ₃ /C ₁₆ Ratio ^b	Second-Tier MMA, μmol/L ^c	Second-Tier tHcy, μmol/L ^c
1 ^d	Hyperemesis gravidarum, poor weight gain	294	8.12	0.20	3.40	6.25	7.81
2 ^{d,e}	NA	116	6.57	0.21	3.22	39.00	29.20
3 ^{e,f}	Preeclampsia, preterm delivery	214	6.93	0.27	2.22	15.00	37.70
4 ^g	Treatment for syphilis, postterm delivery	207	7.60	0.21	1.80	14.40	18.10
5 ^d	B ₁₂ deficiency, anemia, treatment with hydroxyl-cobalamin, 1 mg/mo	NA	6.61	0.23	2.55	9.69	12.90
6 ^{d,e}	Hypothyroidism, iron-deficient anemia	192	5.10	0.23	1.53	5.10	27.30
7 ^{e,g}	Bariatric surgery; oral cyanocobalamin, 1 mg/d, during third trimester	75 ^h	5.26	0.22	1.67	17.00	53.00
8 ^{e,g}	Uncomplicated	197	4.22	0.30	1.95	29.00	65.00
9 ^{e,g}	Hyperemesis gravidarum	201	2.82	0.25	1.20	21.80	22.40
10 ^d	Uncomplicated	198	5.16	0.27	2.10	10.90	22.40
11 ^d	Bariatric surgery, not compliant with hydroxyl-cobalamin IM treatment	104	2.61	0.29	2.10	15.90	22.40
12 ^f	Hyperemesis gravidarum, poor weight gain, pernicious anemia diagnosed postnatally	107	4.31 ⁱ	0.13 ⁱ	1.45 ⁱ	66.80 ⁱ	81.70 ⁱ

Abbreviations: C₃, propionylcarnitine; C₂, acetylarnitine; C₁₆, palmitoylcarnitine; IM, intramuscular; MMA, methylmalonic acid; NA, not available; tHcy, total homocysteine. SI conversion factors: To convert serum B₁₂ to pmol/L, multiply by 0.7378; to convert tHcy to mg/dL, divide by 7.397.

^aMaternal vitamin B₁₂ deficiency was defined by low serum B₁₂ maternal levels or low serum B₁₂ levels in the infants on confirmatory testing and if their initial elevated urine MMA or plasma tHcy normalized while receiving vitamin B₁₂ supplementation or remained normal after discontinuing vitamin B₁₂ therapy. Another cause of low B₁₂ levels, cobalamin F deficiency, is unlikely due to positive clinical response to oral vitamin B₁₂ therapy or normalization of biochemical markers while not receiving treatment. In case 5, for which serum B₁₂ levels were not available in the infant or mother, elevated markers of MMA and tHcy indicated functional vitamin B₁₂ deficiency as they normalized after treatment.

^bSerum B₁₂ levels were abnormal if <211 pg/mL. Percentiles (1st-99th) for C₃ based on 469 279 newborn screening samples analyzed.⁵ Levels of C₃ were abnormal if >5.25 μmol/L (range, 0.6-5.1 μmol/L). C₃/C₂ ratio was abnormal if ≥0.1, and C₃/C₁₆ ratio was abnormal if ≥2.

^cBased on 200 leftover NBS blood spots for routine newborn screening and yielding normal results.⁵ Levels of MMA were abnormal if >5 μmol/L (range, 0.2-2.0 μmol/L) and tHcy abnormal if >15 μmol/L (range, 0.8-10.9 μmol/L).

^dPrenatal diet information for mother not available.

^eMother took prenatal vitamins.

^fVegetarian prenatal diet.

^gRegular prenatal diet.

^hValue used is reported serum B₁₂ during pregnancy.

ⁱRetrospective analysis from original dried blood spot specimen.

Table 2. Diet and Pretreatment Biochemical Results of Newborns Presumed Vitamin B₁₂ Deficient^a

Case	Diet	Urine MMA, μg/mg Cr ^b	Plasma C ₃ , nmol/mL ^b	Plasma tHcy, μmol/L ^b	Serum B ₁₂ , pg/mL ^b	Age at Start of Therapy, wk	Therapy
1	BM	65	0.48	14.3	126	2	Oral cyanocobalamin
2	BM 1 wk, then IF	S1 = 779; S2 = 370	3.16	S1 = 31.2; S2 = 14.3	459 ^c	2	SQ hydroxycobalamin ^d
3	BM and IF	175	2.26	34.3	198	4	Oral cyanocobalamin
4	BM and IF	120	1.06	18.0	311 ^c	2	Oral cyanocobalamin
5	BM	S1 = 39; S2 = 118	1.04	13.0	NA	21	Oral cyanocobalamin
6	BM and IF	105	2.83	20.5	337 ^c	4	Oral cyanocobalamin
7	BM	180	NA	29.3	NA	1	IM hydroxycobalamin ×1, oral cyanocobalamin
8	BM	195	NA	57.2	NA	<1	Oral cyanocobalamin, then SQ hydroxycobalamin, then multivitamin
9	BM	130	NA	27.0	182	2	Oral cyanocobalamin
10	IF	S1 = 206; S2 = 120	1.25	S1 = 14.0; S2 = 14.9	415 ^c	<2	Multivitamin
11	BM and IF	121.6	NA	21.0	665 ^c	2	Oral cyanocobalamin
12	BM	7524 ^e	NA	S1 = 40.0; S2 = 19.0 ^e	196 ^e	9	IM hydroxycobalamin, then oral cyanocobalamin

Abbreviations: BM, breast milk; C₃, propionylcarnitine; Cr, creatinine; IF, infant formula; IM, intramuscular; MMA, methylmalonic acid; NA, not available; S, sample; SQ, subcutaneous; tHcy, total homocysteine.

SI conversion factors: To convert tHcy to mg/dL, divide by 7.397; to convert serum B₁₂ to pmol/L, multiply by 0.7378.

^aMaternal vitamin B₁₂ deficiency was defined by low serum B₁₂ maternal levels or low serum B₁₂ levels in the infants on confirmatory testing and if their initial elevated urine MMA or plasma tHcy normalized while receiving vitamin B₁₂ supplementation or remained normal after discontinuing vitamin B₁₂ therapy. Another cause of low B₁₂ levels, cobalamin F deficiency, is unlikely due to positive clinical response to oral vitamin B₁₂ therapy or normalization of biochemical markers while not receiving treatment. In case 5, for which serum B₁₂ levels were not available in the infant or mother, elevated markers of MMA and tHcy indicated functional vitamin B₁₂ deficiency as they normalized after treatment.

^bMeasurements of MMA, plasma C₃, and plasma tHcy came from initial samples obtained during the first month of life unless otherwise noted. MMA level was abnormal if >14 μg/mg Cr. Plasma C₃ level was abnormal if >1.78 nmol/mL. Plasma tHcy level was abnormal if >12 μmol/L. Serum B₁₂ level was abnormal if <211 pg/mL.

^cThe elevated markers (urine MMA and plasma tHcy) indicate functional vitamin B₁₂ deficiency despite normal vitamin B₁₂ levels.

^dPatient's biochemical and clinical examination findings remained normal 7 months after discontinuing IM hydroxycobalamin therapy.

^eSamples obtained at age 2 mo after acute clinical presentation.

B₁₂ deficiency in 8 of 11 mothers whose status was previously unrecognized. All 11 newborns were asymptomatic when treatment was initiated (Table 2), and subsequently all biochemical markers normalized without clinical signs of vitamin B₁₂ deficiency. Retrospective analysis using the 2-tier protocol of the original NBS sample of the symptomatic infant screened by another state had positive results (Table 1, case 12).

Comment. A recent survey of 12 state NBS programs (including Minnesota) identified 32 cases of nutritional vitamin B₁₂ deficiency, for a rate of 0.88 per 100 000 newborns.⁶ After implementing a lower C₃ first-tier cut-off level and second-tier testing of MMA and tHcy levels in NBS specimens, the detection rate in Minnesota of vitamin B₁₂-deficient infants was considerably higher (3.02/100 000 live births). None of the 11 infants would have been identified by using the previous C₃ cut-off value, potentially explaining the reason no cases of infant vitamin B₁₂ deficiency were identified by NBS before 2005 in Minnesota.

Based on the Minnesota experience, vitamin B₁₂ deficiency occurs more frequently than is currently recognized. Given the important role vitamin B₁₂ plays in DNA synthesis and central nervous system function, and the difficulty in diagnosing vitamin B₁₂ deficiency in infancy because of nonspecific clinical findings, early identification and initiation of treatment are crucial to avoid potentially devastating and irreversible neurologic damage.

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CORRECTION

Errors in Tables and Figure: In the Original Contribution entitled "Association of Troponin T Detected With a Highly Sensitive Assay and Cardiac Structure and Mortality Risk in the General Population," published in the December 8, 2010, issue of *JAMA* (2010;304[22]:2503-2512), an error appeared in tables and a figure. The highest cTnT categories in all tables and figures should have been 0.0066- $<$ 0.014 and \geq 0.014. This article has been corrected online. A related letter to the editor appears in this issue.