Health Outcomes of Infants with Vitamin B_{12} Deficiency Identified by Newborn Screening and Early Treated

Ulrike Mütze, MD[1], Magdalena Walter, PhD[1], Mareike Keller, MSc[1], Gwendolyn Gramer, MD[1], Sven F. Garbade, PhD[1], Florian Gleich, Dipl.-Vw[1], Dorothea Haas, MD[1], Roland Posset, MD[1], Sarah C. Grünert, MD[2], Julia B. Hennermann, MD[3], Eva Thimm, MD[4], Junmin Fang-Hoffmann, MD[1], Steffen Syrbe, MD[5], Jürgen G. Okun, PhD[1], Georg F. Hoffmann, MD[1], and Stefan Kölker, MD[1]

Objective To evaluate the clinical outcomes at age 1.5 ± 0.5 years of infants with vitamin B_{12} deficiency identified by newborn screening (NBS).

Study design Prospective multicenter observational study on health outcomes of 31 infants with vitamin B_{12} deficiency identified by NBS. Neurodevelopment was assessed by the Denver Developmental Screening Test.

Results In 285 862 newborns screened between 2016 and 2019, the estimated birth prevalence of vitamin B_{12} deficiency was 26 in 100 000 newborns, with high seasonal variations (lowest in summer: 8 in 100 000). Infants participating in the outcome study (N = 31) were supplemented with vitamin B_{12} for a median (range) of 5.9 (1.1-16.2) months. All achieved age-appropriate test results in Denver Developmental Screening Test at age 15 (11-23) months and did not present with symptoms characteristic for vitamin B_{12} deficiency. Most (81%, n = 25) mothers of affected newborns had a hitherto undiagnosed (functional) vitamin B_{12} deficiency, and, subsequently, received specific therapy.

Conclusions Neonatal vitamin B_{12} deficiency can be screened by NBS, preventing the manifestation of irreversible neurologic symptoms and the recurrence of vitamin B_{12} deficiency in future pregnancies through adequate treatment of affected newborns and their mothers. The high frequency of mothers with migrant background having a newborn with vitamin B_{12} deficiency highlights the need for improved prenatal care. *(J Pediatr 2021;235:42-8)*.

See editorial, p 19

Vitamin B_{12} (cobalamin) deficiency in the first year of life is often diagnosed with significant delay following the onset and progression of severe, often irreversible symptoms, such as muscular hypotonia, epileptic seizures, and developmental delay.[1-4] Newborn screening (NBS) for vitamin B_{12} deficiency was shown to be feasible, identifying an unexpectedly high birth prevalence between 3.3 in 100 000 (ie, 1 in 30 000) newborns[5] and 18.7 in 100 000 (ie, 1 in 5355) newborns[6,7] in Germany compared with 0.88 in 100 000 (ie, 1 in 113 600) newborns with vitamin B_{12} deficiency identified in the US.[8]

Population-based studies from Nepal, India, Mexico, and Turkey found low vitamin B_{12} levels in 30%-40% of infants.[9-12] A randomized, placebo controlled trial in 107 healthy Norwegian infants showed low vitamin B_{12} status, including vitamin B_{12} and holotranscobalamin (Holo-TC) in serum, homocysteine in plasma, and methylmalonic acid (MMA) in urine and plasma, in most infants responsive to vitamin B_{12} supplementation.[13] Selective testing of Swedish infants with neurologic symptoms demonstrated a prevalence of vitamin B_{12} deficiency of 1 in 350 infants during the first year of life.

NBS studies have mainly focused on technical feasibility and diagnostic quality. Studies on clinical outcomes of individuals after early identification by NBS with subsequent diagnosis and early therapy are indispensable[14] to evaluate the

C3	Propionylcarnitine
DDST	Denver Developmental Screening Test
Holo-TC	Holotranscobalamin
Met	Methionine
MMA	Methylmalonic acid
NBS	Newborn screening

From the [1]Division of Child Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Heidelberg; [2]Department of General Pediatrics, Adolescent Medicine and Neonatology, Medical Center, University of Freiburg, Faculty of Medicine, Freiburg; [3]Villa Metabolica, Department of Pediatric and Adolescent Medicine, Mainz University Medical Center, Mainz; [4]Department of General Pediatrics, Neonatology, and Pediatric Cardiology, University Children's Hospital, Heinrich Heine University Düsseldorf, Düsseldorf; and [5]Division of Pediatric Epileptology, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany

The long-term observational study (Long-Term Outcome of Patients with Inherited Metabolic Diseases after Diagnosis by Expanded Newborn Screening) and the newborn screening pilot study (Extension of Newborn Screening by 26 Additional Metabolic Disorders) are generously supported by the Dietmar Hopp Foundation, St. Leon-Rot, Germany (2311221, DH2011117, and 2311220 [to S.K. and G.H.]). U.M. is supported by an Olympia Morata research fellowship of the Medical Faculty Heidelberg, Germany (F.206852). The authors confirm independence from the sponsor; the content of the article has not been influenced by the sponsor. The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2021.02.009

clinical benefit of affected individuals from NBS programs. The aim of this study was to evaluate the health outcomes of infants with vitamin B_{12} deficiency identified by NBS and treated early.

Methods

Since August 1, 2016, NBS for vitamin B_{12} deficiency using second tier strategies was initiated in a prospective single-center pilot study evaluating an extension of the German NBS panel by additional 26 metabolic and genetic conditions at the NBS laboratory of Heidelberg University Hospital, Germany (NBS study).[6,7] As described previously,[6] the NBS strategy for vitamin B_{12} deficiency includes 2 different second-tier algorithms to assess both pathways possibly affected by vitamin B_{12} deficiency: L-methionine (Met) synthetase and L-methylmalonyl-CoA mutase: (1) NBS first tier: Met (cut-off low) and/or Met-to-phenylalanine ratio (<cut-off low), second tier: homocysteine (>cut-off high) and (2) NBS first tier: elevated propionylcarnitine (C3) and C3-to-acetylcarnitine ratio (C3-to-acetylcarnitine ratio >cut-off high, C3 and C3-to-acetylcarnitine both >cut-off high, C3 > alarm limit), second tier: MMA. NBS was positive if, following a positive first tier, homocysteine and/or MMA were elevated.[6] According to recent diagnostic algorithms on vitamin B_{12} status,[13,15] a positive NBS test result for vitamin B_{12} deficiency was confirmed if the serum vitamin B_{12} level was below the normal range or if biomarkers indicating functional vitamin B_{12} deficiency, such as homocysteine in plasma, MMA in urine and/or plasma were found to be elevated, and/or Holo-TC in serum was decreased, even if vitamin B_{12} levels were still in the low normal range. Inherited disorders of cobalamin metabolism presenting with similar NBS patterns[16] were excluded by further confirmatory diagnostics in individuals with highly suggestive laboratory findings (ie, C3 >10 μmol/L; homocysteine >30 μmol/L) and, for all, by response of the metabolic measures following at least 1 week of oral vitamin B_{12} supplementation.

Treatment of Vitamin B_{12}-Deficient Individuals Identified by NBS

Infants with confirmed diagnosis of vitamin B_{12} deficiency were treated and followed according to previously described recommendations[6,17]: initially, oral vitamin B_{12} supplementation (500 μg per day) for 3 days, followed by 100 μg per day for at least 2-8 weeks until normalization of vitamin B_{12} level and the functional markers (Holo-TC, MMA in urine and plasma, and homocysteine in plasma). With normalization of the vitamin B_{12} status, oral vitamin B_{12} supplementation was reduced to a maintenance dose (ie, 5 μg per day) at least until nutrition containing appropriate amounts of vitamin B_{12} were reliably introduced into the diet.

Study Population and Study Design

Families whose newborns with vitamin B_{12} deficiency were detected by NBS were asked to participate in a prospective multicenter observational study on clinical and cognitive outcomes of individuals with inherited metabolic diseases detected by NBS at Heidelberg University Hospital, Germany (Outcome study, DRKS-ID: DRKS00013329).[14,18] The study was approved by the local ethics committee of the coordinating site (Medical Faculty of Heidelberg, application no. S-104/2005) and consecutively by the participating study sites. For the present study on outcome of patients with vitamin B_{12} deficiency, inclusion criteria were date of first NBS sampling at or after August 1, 2016, positive NBS result, subsequent confirmation of vitamin B_{12} deficiency as published previously,[6,7] and written informed consent of legal guardians before enrollment. Comprehensive regular follow-up information was obtained at ages 1.5 ± 0.5 years by structured clinical examination, recording of medical history, analysis of medical records, and Denver Developmental Screening Test (DDST). As participants had to reach 12 months of age for neuropsychological testing, outcome analysis was restricted to screened individuals born between August 1, 2016 and July 31, 2019. The cut-off date for data analysis was July 31, 2020. All eligible individuals were identified and families were asked to participate either during regular follow-ups at the study site or, for patients followed up elsewhere, by mail or phone.

Statistical Analyses

All statistical analyses were performed using R, a language for statistical computing and graphics (https://www.r-project.org). Missing data that could not be retrieved and implausible data that could not be verified were treated as case-wise missing for the respective analysis. Prevalence and 95% CI were computed with exact method by Collett[19] using R package "epiR." Poisson regression was applied to compare prevalence rates between seasonal periods, followed by post-hoc comparisons with Tukey method (R package "emmeans"). Linear mixed effect models (R package "nlme") were used to analyze response anthropometrics with predictor age in years.

Results

Study Cohort

A total of 484 089 neonates born between August 1, 2016 and December 31, 2019 were screened at the Dietmar Hopp Metabolic Center, Heidelberg University Hospital, Germany; 59% (n = 285 862) also participated in the NBS pilot study. In total, suspected vitamin B_{12} deficiency was confirmed in 74 (34 female, 40 male) individuals, resulting in an estimated birth prevalence (95% CI) of 25.9 (20.3-32.5) in 100 000 newborns (ie, 1 in 3863 [3849-3877] newborns).

Birth prevalence showed significant seasonal variations ($P < .0001$; Poisson regression, **Figure 1**). Although 8.2 (3.5-16.2) of 100 000 newborns born between May and August were identified by NBS to have vitamin B_{12} deficiency, birth prevalence significantly increased to 44.6 (32-60.5) in 100 000 newborns between September and

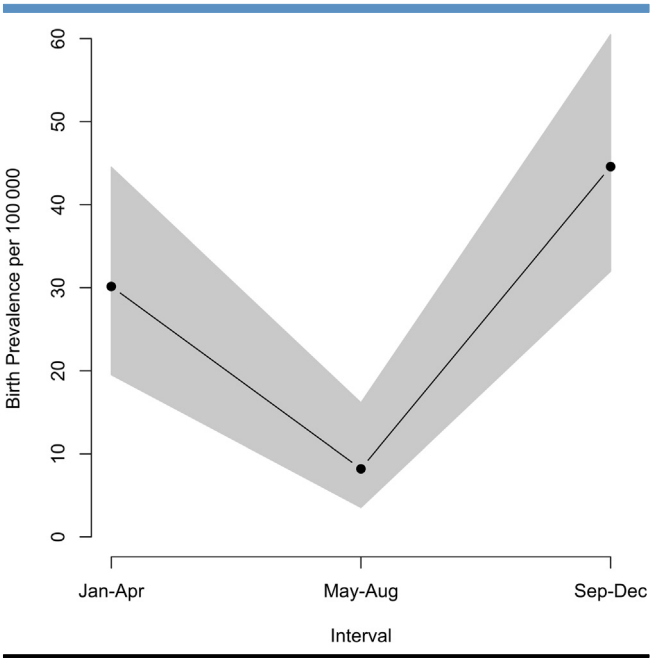

Figure 1. Birth prevalence of neonatal vitamin B_{12} deficiency. Birth prevalence and 95% CI (*color-shaded area*) of neonatal vitamin B_{12} deficiency identified by NBS analyzing the years 2017-2019 showed significant seasonal variations; 8.2 (3.5-16.2) in 100 000 newborns with vitamin B_{12} deficiency were born between May and August. In contrast, birth prevalence was 44.6 (32.0-60.5) in 100 000 newborns between September and December ($P < .0001$), and 30.2 (19.5-44.5) in 100 000 newborns between January and April ($P = .0038$), respectively.

December ($P < .0001$, Tukey contrast) and 30.2 (19.5-44.5) in 100 000 newborns between January to April ($P = .0038$, Tukey contrast), respectively. No significant difference in birth prevalence was found between the birth intervals January-April and September-December ($P = .2721$).

We are not aware of a false negative NBS test result for vitamin B_{12} deficiency during the study interval except for a twin birth in whom 1 sibling was detected by NBS, and additional tests also confirmed low vitamin B_{12} status in the other. Furthermore, 5 patients who had participated in the NBS study with unremarkable NBS results (no positive first tier) were admitted to the hospital with neurologic symptoms (infantile seizures, microcephaly, muscular hypotonia) at a median (range) age of 2.5 (0.5-8.5) months and were found to have vitamin B_{12} deficiency at this age.

All eligible patients with vitamin B_{12} deficiency detected by NBS born between August 1, 2016 and July 31, 2019 (n = 60) were asked to participate in the outcome study; 31 (18 male) infants were enrolled.

Confirmatory Diagnosis and Maternal Origin of Vitamin B_{12} Deficiency

NBS results and confirmatory diagnostics of the participants of the outcome study are summarized in **Table I**. Although single measures have been in the normal range, every child fulfilled the NBS and confirmatory diagnosis criteria mentioned in the Methods section. An hitherto unknown vitamin B_{12} deficiency was confirmed in 81% of mothers giving birth to a newborn with vitamin B_{12} deficiency. The etiology of maternal vitamin B_{12} deficiency (n = 25, missing: 6; multiple answers possible) varied comprising altered nutritional habits during pregnancy (28%) because of nausea, food aversion and hyperemesis gravidarum, treatment with antacids (8%) because of gastroesophageal reflux, malabsorption because of (autoimmune) gastritis (8%) and other gastrointestinal disorders (8%), vegetarian (8%) or vegan (8%) diet, or without known reason (12%). In 10 mothers, however, no efforts have been made to elucidate the origin of vitamin B_{12} deficiency although this had been clearly recommended.

Impact of Education, Language Skills, and Family Background

To assess additional factors influencing occurrence of maternal vitamin B_{12} deficiency, and children's development, data on parental background, education, language skills, and maternal vitamin B_{12} deficiency were analyzed. Migrant background of mothers having a neonate with vitamin B_{12} deficiency identified by NBS was more frequent (68%; n = 21) than in the general population of Southwest Germany in 2019 (28.2%),[20] which is predominantly served by the NBS laboratory at Heidelberg University Hospital, or Germany (25.4%),[21] respectively. Mothers of affected individuals were born in Germany (32%), Syria (23%), Turkey (16%), Russia (10%), and other European (13%) or Central Asian (6%) countries. Mothers' education (n = 28) was similar to the German reference population[22] with highest achievement being primary (10.7%; n = 3), lower secondary (35.7%; n = 10), upper secondary education (28.6%; n = 8), and university degree (25%; n = 7). However, in 19% (6 of 31) of affected families the German language skills of both parents did not allow them to understand the relevant information on the origin of vitamin B_{12} deficiency in mother and child and the need for treatment. Here, medical and study information were given in English and/or in the language of the family via interpreter.

Treatment and Clinical Outcome

Individuals with neonatal vitamin B_{12} deficiency participating in the outcome study were asymptomatic at the time of the first NBS report. Confirmatory diagnosis was delayed in 1 infant despite intensive tracking by the NBS laboratory. At diagnosis and start of treatment (116 days) this patient presented with mild muscular hypotonia.[6] In another infant, confirmatory tests revealed macrocytic anemia.

Vitamin B_{12} supplementation was applied according to recommendations and was discontinued in 26 children at the median age (IQR, range) of 7.0 (4.0-10; 2.8-16.8) months, following a treatment interval of 5.9 (3-9.1; 1.1-16.2) months. Last recorded vitamin B_{12} status (n = 25, missing: 6) at a median age (IQR, range) of 13 (4-15; 2-23) months, was in the normal range in all but 1 (96%) individual who still

Table I. Results of NBS and confirmatory diagnostics

	First NBS (DBS)	Second NBS (DBS)	Confirmatory diagnosis (plasma, serum, urine)	
	n Median (IQR, range)			Reference values
Age at sampling [completed d]	30 2 (2-2; 0-43)	29 12 (10-15; 2-58)	31 19 (13-27; 7-110)	NA
C3 (DBS) [μmol/L]>	30 2.63 (1.65-3.44; 0.91-10.19)	29 1.35 (0.97-2.02; 0.64-4.54)	NA	0-5.5
C3-to-C2 ratio (DBS)	30 0.11 (0.09-0.18; 0.05-0.37)	29 0.12 (0.08-0.15; 0.04-0.9)	NA	0-0.22
Met (DBS) [μmol/L]	30 10 (9-13; 6-47)	29 15 (12-18; 9-58)	NA	11-35
Met-to-Phe ratio (DBS)	30 0.23 (0.21-0.29; 0.15-0.68)	29 0.38 (0.31-0.44; 0.20-0.63)	NA	0.26-0.56
Hcy (DBS or plasma) [μmol/L]	30 15 (12.2-18.2; 3.1-28.5)	29 15 (9.3-19.4; 4.9-33.8)	30 23.5 (20-30; 3-45)	DBS 0.1-12 Plasma 2-14
MMA (DBS or plasma) [μmol/L]	29 2.24 (1.48-4.00; 0-31.01)	26 2.90 (2.05-5.61; 1.20-34.40)	21 2.82 (1.05-5.58; 0.17-162.60)	DBS 0-2.35 Plasma 0-0.26
MMA (urine) [mmol/mol creatinine]	NA	NA	27 43 (14-123; 4-500)	<10
MCA (urine) [mmol/mol creatinine]	NA	NA	N = 25 13 (6-21; 2-66)	<9
3-OH-PA (urine) [mmol/mol creatinine]	NA	NA	24 49 (33-92; 9-439)	< 170
Vitamin B_{12} (serum) [pmol/L]	NA	NA	31 118 (97-155; 38-245)	160-670
Maternal vitamin B_{12} status % (N):				
Vitamin B_{12} deficiency	NA	NA	71.0% (22)	NA
Functional vitamin B_{12} deficiency	NA	NA	9.7% (3)	NA
No vitamin B_{12} deficiency	NA	NA	19.4% (6)	NA

DBS, dried blot spots; *Hcy*, homocysteine; *MCA*, methylcitrate; *NA*, not applicable.
Metabolites used for NBS in DBS are as previously published[6]: C3-to-acetyl carnitine (C2) ratio, L-methionine (Met), Met-to-phenylalanine (Phe) ratio, Hcy, and MMA. Confirmatory diagnosis consists of vitamin B_{12} level (serum), homocysteine (plasma), and MMA (plasma), and of MMA, MCA, and 3-hydroxypropionic acid (3-OH-PA) in urine. Maternal vitamin B_{12} status was recorded.

presented with a low serum vitamin B_{12} level (155 pmol/L). At last visit, at a median age (IQR, range) of 1.2 (1.1-1.5; 0.2-1.9) years, only 5 children were still supplemented with vitamin B_{12} due to low dietary intake of vitamin B_{12}-containing natural food because of a vegetarian diet or picky eating habits.

Neurodevelopmental status and health outcomes were assessed by DDST (n = 29, missing: 2) and a standardized physical examination at a median (IQR; range) age of 15 (14-18; 12-23) months, revealing age-appropriate global and subdomain results in all tested children (n = 29; **Table II**) except for 1 child with selectively delayed development in the subdomain language. At examination and last visit, none of the 29 examined children showed neurologic symptoms characteristic of vitamin B_{12} deficiency, such as severe developmental delay, muscular hypotonia, or epileptic seizures. Iron deficiency anemia was identified in 3 infants and microcephaly (head circumference below −2 SDS) in 1 infant with familial microcephaly. These clinical manifestations, however, were not considered to be directly related to neonatal vitamin B_{12} deficiency. For 2 participants, families had to cancel the study visit and outcome evaluation but reported development of their children to be normal at least until age 18 months.

Growth data, excluding the preterm born child for analysis, were in the normal range compared with reference populations for weight and height[23] and for head circumferences.[24] Height SDS and weight SDS did not change over the observation period (birth to 1.5 years visit; height: coefficient = 0.02 SDS per year, SE = 0.14, P = .8822; weight: coefficient = 0.09 SDS per year, SE = 0.13, P = .4985), but head circumference SDS decreased (coefficient = −0.54 SDS per year, SE = 0.14, P = .0001; **Figure 2**). This effect disappeared if neonatal (first 28 days of life) anthropometrical data were excluded from the analysis (coefficient = −0.29 SDS per year, SE = 0.16, P = .0797).

Parents' Perspective and Expectations on Development

At time of DDST, parents were asked about the perceived development of their child compared with peers: 97% (27 of 29) of parents stated that their child had comparable or accelerated development (100% physical, 100% social, 97% intellectual, 89% language) and all expected their children to develop normally. However, early diagnosis of vitamin B_{12} deficiency in their child was stated to be a mild to moderate (24%) or severe (14%) burden for the family, although the parents stated it has been no burden (93%) for their child.

Discussion

Neurocognitive and clinical outcomes of infants after neonatally identified vitamin B_{12} deficiency were excellent. No

Table II. Clinical outcomes of screened and early treated individuals with neonatal vitamin B_{12} deficiency

			Individuals with neonatal vitamin B_{12} deficiency n Median (IQR, range)
Age at birth [gestational wk]			31 39 (38-40; 30-42)
Age at first NBS result [d]			31 9 (7-12; 2-68)
Age at diagnosis [d]			31 23 (18-31; 10-116)
Age at first treatment [d]			31 25 (18-31; 6-116)
Age at DDST and examination [mo]			29 15 (14-18; 12-23)
DDST (n = 29)	**n Age appropriate**	**n Accelerated**	**n Retarded**
Global	26	3	0
Personal-social	23	6	0
Fine motor and adaptive	27	2	0
Gross motor	25	4	0
Language	25	3	1
	Height SDS*	**Weight SDS***	**Head circumference SDS†**
Anthropometric data		**n Mean (SD)**	
U1 pediatric preventive check-up (d 1)	27 −0.6 (1.1)	27 −0.6 (0.8)	21 0.0 (1.2)
U2 pediatric preventive check-up (d 3-10)	28 −0.6 (1.1)	28 −1.0 (0.9)	28 −0.1 (1.2)
U3 pediatric preventive check-up (wk 4-6)	28 0.1 (0.8)	28 0.1 (0.8)	28 −0.2 (1.0)
U4 pediatric preventive check-up (mo 3-4)	24 0.3 (0.7)	24 0.3 (0.8)	24 −0.3 (1.1)
U5 pediatric preventive check-up (mo 6-7)	28 −0.1 (1.0)	28 0.0 (0.9)	28 −0.7 (1.1)
U6 pediatric preventive check-up (mo 10-12)	26 −0.4 (1.0)	26 −0.4 (0.8)	28 −0.5 (1.4)
Study visit (1.5 y ± 0.5)	20 −0.4 (1.0)	20 −0.3 (0.9)	19 −0.8 (1.1)

Process times of NBS, diagnosis, and treatment of the participants to the outcome study (n = 31). DDST results are displayed globally and in the 4 subdomains. SDS for anthropometric data recorded at the governmental recommended pediatric preventive check-ups in the first years of life (U1-U6) and at study visit (1.5 ± 0.5 years) were calculated using cohort references (height and weight[23]; head circumference[25]).

*Calculated using German reference values for height and weight.[24]

†Calculated using British reference values for head circumference[24] (no head circumference references included in the German references[24]).

child with neonatal vitamin B_{12} deficiency had neurologic symptoms at last visit, growth was normal, and vitamin B_{12} status was sufficient in 96%. Head circumference SDS, although still in the normal range, slightly decreased in the first years of life in the children with neonatally diagnosed and subsequently treated vitamin B_{12} deficiency. The relevance of this finding remains unclear and may reflect incidental variations due to small cohorts, families with various ethnic backgrounds, and measurement inaccuracies of head circumference. Furthermore, this effect disappeared if neonatal measurements were excluded.

Birth prevalence for neonatal vitamin B_{12} deficiency analyzing more than 3 years of NBS, was higher (25.9 in 100 000 [ie, 1 in 3863] newborns) than previously reported in the NBS algorithm description (18.7 in 100 000 [ie, 1 in 5355] newborns).[6,7] This was, similar to prevalence in Estonia (1 in 3000)[25] and Italy (1 in 5000)[26] but significantly higher than in the US (1 in 113 600).[8] These differences might be explained by discrepant detection rates, but might also highlight differences in eating habits, prenatal care, and adherence to micronutrient supplementation during pregnancy in different populations and study cohorts. The reasons for maternal vitamin B_{12} deficiency were various, often due to altered eating habits in pregnancy, and vegetarian or vegan diets were less frequently identified as underlying cause. The higher frequency of mothers with a migrant background (68%) having a newborn with vitamin B_{12} deficiency compared with the reference population (25%-28%)[20,21] suggests specific ethnic subgroups or eating habits leading to an increased risk of vitamin B_{12} deficiency.[27-29] Individuals with migrant background have less access to health care systems[30] and, in particular, prenatal care during pregnancy, including current recommendations on nutrition and vitamin supplementation before and during

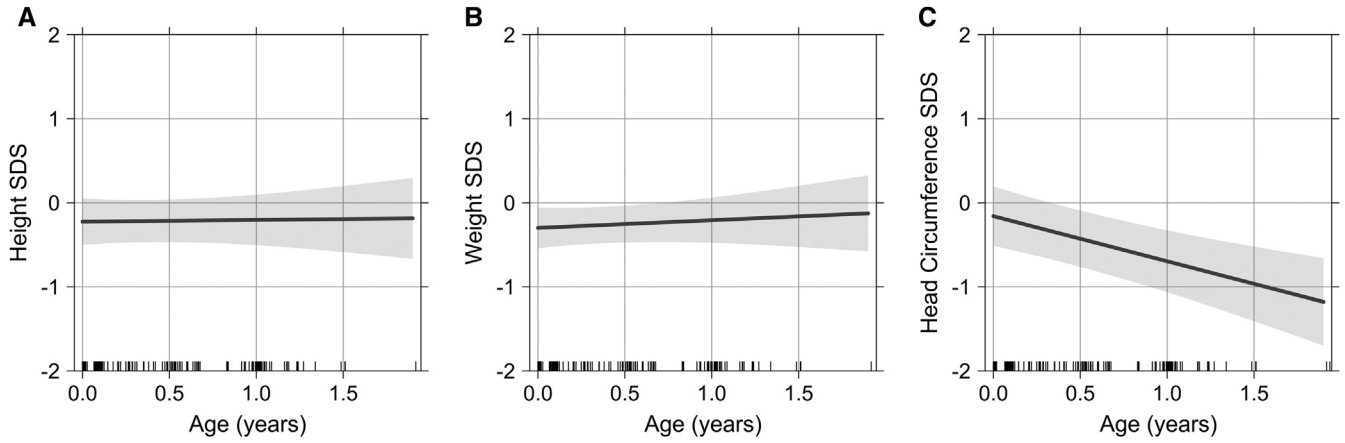

Figure 2. Variation of growth and thriving until age 1.5 years. **A,** Median height SDS; **B,** weight SDS[23]; and **C,** head circumference SDS[24] with 95% CI (*color-shaded areas*) until age 1.5 ± 0.5 years. Height SDS and weight SDS remained constant, but head circumference SDS decreased during the observational period (coefficient = −0.54 SDS per year, SE = 0.14, *P* = .0001).

pregnancy.[31] Maternal serum vitamin B_{12} levels during the first trimester of pregnancy correlated with NBS markers of infant vitamin B_{12} deficiency,[32] in 20%-30% of pregnant woman worldwide vitamin B_{12} levels fall below 150 pmol/L.[33,34] The cohort born in summer, showed the lowest birth prevalence of vitamin B_{12} deficiency in our study. This is similar to other cohorts[35-37] where seasonal variations with less energy or fat intake especially in summer were demonstrated. Caring gynecologists and midwives should be vigilant about vitamin B_{12} content in mothers' diet and vitamin supplementation in pregnancy and breastfeeding period.

Inclusion in the prospective outcome study registry for individuals with vitamin B_{12} deficiency identified by NBS (52%) was lower than for inherited metabolic diseases identified by NBS (66%).[14] This may be due to the fact that individuals with vitamin B_{12} deficiency have not been exclusively treated and followed by specialized metabolic centers. In addition, individuals with low or no clinical impairment are less bonded to medical care givers, resulting in lower recruitment to outcome studies.[14] Finally, the cohort with a high proportion of migrant background comprises a high number of refugees with frequently changing residences. Considering vitamin B_{12} deficiency as a candidate to be included in national NBS programs, there is need for highly effective NBS tracking to ensure treatment and follow-up in all patients identified.

Therapy was not randomized for ethical reasons; hence, we cannot present information about the development of children that did not receive vitamin B_{12} supplementation. The one child with delayed start of treatment had developed mild muscular hypotonia at 4 months of age, reversible after treatment.

In the majority of identified individuals, a previously unknown maternal (functional) vitamin B_{12} deficiency (81%) was associated with neonatal vitamin B_{12} deficiency. Subsequent breastfeeding without supplementation of mother and child would have probably aggravated and manifested the deficiency. Vitamin B_{12} deficiency might manifest later, as in our 5 patients with neurologic symptoms and vitamin B_{12} deficiency after a normal NBS. Clinicians have to be aware of this during diagnostic workup on developmental delay and infantile spasms, shown to be highly correlated with decreased vitamin B_{12} levels up to a prevalence of 1 in 350 infants.[2]

The impact of NBS for vitamin B_{12} deficiency is multiplied because early diagnosis in affected newborns also fosters diagnosis and adequate therapy of vitamin B_{12} deficient mothers and reduces the risk of vitamin B_{12} deficiency-related health hazards for prospective pregnancies. To avoid neonatal vitamin B_{12} deficiency, information on vitamin B_{12} content of diet and vitamin supplementation during pregnancy as well as consideration of vitamin B_{12} deficiency as a differential diagnosis of maternal anemia, should be a component of prenatal care. ■

We are indebted to all patients and their parents who participated in this study, for their motivation and trust. We thank Elena Boyd, BA, Division of Child Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany for critically proofreading the manuscript. E.B. declares no funding sources or conflicts of interest.

Submitted for publication Oct 8, 2020; last revision received Feb 3, 2021; accepted Feb 4, 2021.

Reprint requests: Ulrike Mütze, MD, Division of Child Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany. E-mail: Ulrike.Muetze@med.uni-heidelberg.de

Data Statement

Data sharing statement available at www.jpeds.com.

References

1. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. J Pediatr 1992;121(5 Pt 1):710-4.
2. Irevall T, Axelsson I, Naumburg E. B12 deficiency is common in infants and is accompanied by serious neurological symptoms. Acta Paediatr 2017;106:101-4.
3. Jain R, Singh A, Mittal M, Talukdar B. Vitamin B12 deficiency in children: a treatable cause of neurodevelopmental delay. J Child Neurol 2015;30:641-3.
4. Yilmaz S, Serdaroglu G, Tekgul H, Gokben S. Different neurologic aspects of nutritional B12 deficiency in infancy. J Child Neurol 2016;31:565-8.
5. Röschinger W, Sonnenschein S, Schuhmann E, Nennstiel-Ratzel U, Roscher AA, Olgemöller B. Neue Zielerkrankungen im Neugeborenenscreening. Monatsschrift Kinderheilkunde 2015;163:142-9.
6. Gramer G, Fang-Hoffmann J, Feyh P, Klinke G, Monostori P, Mütze U, et al. Newborn screening for vitamin B(12) deficiency in Germany: strategies, results, and public health implications. J Pediatr 2020;216:165-72.e4.
7. Gramer G, Fang-Hoffmann J, Feyh P, Klinke G, Monostori P, Okun JG, et al. High incidence of maternal vitamin B_{12} deficiency detected by newborn screening: first results from a study for the evaluation of 26 additional target disorders for the German newborn screening panel. WJP 2018;14:470-81.
8. Hinton CF, Ojodu JA, Fernhoff PM, Rasmussen SA, Scanlon KS, Hannon WH. Maternal and neonatal vitamin B_{12} deficiency detected through expanded newborn screening–United States, 2003-2007. J Pediatr 2010;157:162-3.
9. Cuevas-Nasu L, Mundo-Rosas V, Shamah-Levy T, Humaran IM-G, Ávila-Arcos MA, del Rosario Rebollar-Campos M, et al. Prevalence of folate and vitamin B_{12} deficiency in Mexican children aged 1 to 6 years in a population-based survey. Salud Publica Mex 2012;54:116-24.
10. Koc A, Kocyigit A, Soran M, Demir N, Sevinc E, Erel O, et al. High frequency of maternal vitamin B_{12} deficiency as an important cause of infantile vitamin B12 deficiency in Sanliurfa province of Turkey. Eur J Nutr 2006;45:291-7.
11. Ng'eno BN, Perrine CG, Whitehead RD, Subedi GR, Mebrahtu S, Dahal P, et al. High Prevalence of vitamin B_{12} deficiency and no folate deficiency in young Children in Nepal. Nutrients 2017;9:72.
12. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr 2007;86:1302-9.
13. Bjorke-Monsen AL, Torsvik I, Saetran H, Markestad T, Ueland PM. Common metabolic profile in infants indicating impaired cobalamin status responds to cobalamin supplementation. Pediatrics 2008;122:83-91.
14. Mütze U, Garbade SF, Gramer G, Lindner M, Freisinger P, Grünert S, et al. Long-term outcome of individuals with inherited metabolic diseases identified through newborn screening. Pediatrics 2020;146:e20200444.
15. Hannibal L, Lysne V, Bjørke-Monsen AL, Behringer S, Grünert SC, Spiekerkoetter U, et al. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. Front Mol Biosci 2016;3:27.
16. Huemer M, Kožich V, Rinaldo P, Baumgartner MR, Merinero B, Pasquini E, et al. Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. J Inherit Metab Dis 2015;38:1007-19.
17. Gramer G, Hoffmann GF. Vitamin B_{12}-Mangel im Neugeborenen- und Säuglingsalter – Ursachen, Früherkennung, Diagnostik und Vorstellung eines primär oralen Behandlungsschemas. Monatsschrift Kinderheilkunde 2020.
18. Lindner M, Gramer G, Haege G, Fang-Hoffmann J, Schwab KO, Tacke U, et al. Efficacy and outcome of expanded newborn screening for metabolic diseases—report of 10 years from South-West Germany. Orphanet J Rare Dis 2011;6:44.
19. Collett D. Modelling binary data. Boca Raton (FL): Chapman and Hall; 1999.
20. Statistisches Bundesamt. Tabellen Migrationshintergrund-Länder. Accessed May 29, 2020. https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bevoelkerung/Migration-Integration/Tabellen/migrationshintergrund-laender.html
21. Statistisches Bundesamt. Tabellen Migrationshintergrund. Accessed August 17, 2020. https://www.destatis.de/DE/ZahlenFakten/GesellschaftStaat/Bevoelkerung/MigrationIntegration/Migrationshintergrund/Tabellen/TabellenMigrationshintergrundGeschlecht.html
22. Statistisches Bundesamt. Tabellen Bildungsabschluss. Accessed August 17, 2020. https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bildung-Forschung-Kultur/Bildungsstand/Tabellen/bildungsabschluss.html
23. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V, et al. Perzentile für den Body-mass-Index für das Kindes-und Jugendalter unter Heranziehung verschiedener deutscher. Stichproben 2001;149:807-18.
24. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med 1998;17:407-29.
25. Reinson K, Künnapas K, Kriisa A, Vals MA, Muru K, Õunap K. High incidence of low vitamin B12 levels in Estonian newborns. Mol Genet Metab Reports 2018;15:1-5.
26. Scolamiero E, Villani GR, Ingenito L, Pecce R, Albano L, Caterino M, et al. Maternal vitamin B12 deficiency detected in expanded newborn screening. Clin Biochem 2014;47:312-7.
27. Jeruszka-Bielak M, Isman C, Schroder TH, Li W, Green TJ, Lamers Y. South Asian ethnicity is related to the highest risk of vitamin B12 deficiency in pregnant Canadian women. Nutrients 2017;9:317.
28. Korani M, Rea DM, King PF, Brown AE. Maternal eating behaviour differs between ethnic groups: considerations for research and practice. Matern Child Nutr 2018;14:e12630.
29. Schroder TH, Sinclair G, Mattman A, Jung B, Barr SI, Vallance HD, et al. Pregnant women of South Asian ethnicity in Canada have substantially lower vitamin B12 status compared with pregnant women of European ethnicity. Br J Nutr 2017;118:454-62.
30. Razum O, Zeeb H, Meesmann U, Schenk L, Bredehorst M, Brzoska P, et al. Migration und Gesundheit. 2008. Gesundheitsberichterstattung des Bundes, Robert Koch-Institut; 2008. http://www.gbe-bund.de/pdf/migration.pdf
31. Koletzko B, Cremer M, Flothkötter M, Graf C, Hauner H, Hellmers C, et al. Diet and lifestyle before and during pregnancy: practical recommendations of the Germany-wide healthy start-young family network. Geburtshilfe Frauenheilkd 2018;78:1262-82.
32. Dayaldasani A, Ruiz-Escalera J, Rodríguez-Espinosa M, Rueda I, Pérez-Valero V, Yahyaoui R. Serum vitamin B12 levels during the first trimester of pregnancy correlate with newborn screening markers of vitamin B12 deficiency. Int J Vitam Nutr Res 2014;84:92-7.
33. Sukumar N, Rafnsson SB, Kandala NB, Bhopal R, Yajnik CS, Saravanan P. Prevalence of vitamin B_{12} insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis. Am J Clin Nutr 2016;103:1232-51.
34. Sukumar N, Saravanan P. Investigating vitamin B_{12} deficiency. BMJ 2019;365:l1865.
35. Aparicio-Ugarriza R, Rumi C, Luzardo-Socorro R, Mielgo-Ayuso J, Palacios G, Bibiloni MM, et al. Seasonal variation and diet quality among Spanish people aged over 55 years. J Physiol Biochem 2018;74:179-88.
36. Ma Y, Olendzki BC, Li W, Hafner AR, Chiriboga D, Hebert JR, et al. Seasonal variation in food intake, physical activity, and body weight in a predominantly overweight population. Eur J Clin Nutr 2006;60:519-28.
37. Van Staveren WA, Deurenberg P, Burema J, De Groot LC, Hautvast JG. Seasonal variation in food intake, pattern of physical activity and change in body weight in a group of young adult Dutch women consuming self-selected diets. Int J Obes 1986;10:133-45.