Vitamin B_{12} Deficiency in Newborns and their Mothers—Novel Approaches to Early Detection, Treatment and Prevention of a Global Health Issue*

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Summary: Vitamin B_{12} deficiency, mostly of maternal origin in newborns, is a well treatable condition but can cause severe neurologic sequelae. In women of childbearing age and pregnant women worldwide vitamin B_{12} deficiency has been reported with frequencies of 10%–50%. Children with vitamin B_{12} deficiency are asymptomatic at birth but may develop severe multisystemic symptoms, including irreversible developmental impairment in the second half-year of life. Early detection of vitamin B_{12} deficiency allows for presymptomatic treatment. This article provides an overview over the function of vitamin B_{12} and discusses causes and frequency of vitamin B_{12} deficiency in newborns, infants, and women of childbearing age. It describes novel successful approaches to newborn screening (NBS) for vitamin B_{12} deficiency and results of a pilot study which performed systematic NBS for vitamin B_{12} deficiency using so-called second-tier strategies by measuring homocysteine and methylmalonic acid in dried blood spots. Recommendations for diagnostics in mothers of children with vitamin B_{12} deficiency are described as well as results of systematic work-up in mothers and treatment and follow-up of children with vitamin B_{12} deficiency detected by NBS. Treatment options of vitamin B_{12} deficiency are presented including a newly developed standardized supplementation scheme with exclusively oral vitamin B_{12} supplementation. Recommendations for preventive approaches to vitamin B_{12} deficiency for children and mothers are stated. Many children worldwide could benefit from systematic inclusion of vitamin B_{12} deficiency into NBS panels. In addition, preventive approaches to maternal vitamin B_{12} deficiency should be implemented systematically during maternal care.
Key words: vitamin B_{12} deficiency; prevention; treatment; mother and child health; newborn screening; maternal

1 ROLE AND FUNCTION OF VITAMIN B_{12}

Vitamin B_{12} (cobalamin) is a water-soluble vitamin. The human body is unable to synthesize vitamin B_{12} and therefore relies exclusively on intake from dietary sources. Vitamin B_{12} is contained mainly in animal foods like meat, eggs, fish and milk. In the metabolic pathways vitamin B_{12} is an essential cofactor for the enzymes methionine synthase and methylmalonyl-CoA mutase. Metabolic changes due to vitamin B_{12} deficiency result from dysfunction of these enzymes, leading to increased homocysteine (tHcy), methylmalonic acid (MMA), and potentially also methylcitric acid (MCA). These markers are referred to as so called "functional markers" of vitamin B_{12} deficiency and are found elevated in nutritional vitamin B_{12} deficiency as well as in genetic disorders of cobalamin transport and metabolism. Research concerning vitamin B_{12} started as early as 1926 when Minot and Murphey described that pernicious anemia can be treated by supplementing patients' nutrition with large amounts of liver. The structure of vitamin B_{12} was first described in detail by the British scientist Dorothy Hodgkin in the year 1955[1]. For this discovery Hodgkin was awarded a Nobel Prize.

Vitamin B_{12} plays an essential role in all human cells and many organ systems, especially in tissues with a high cell turnover. It is essential for the development, myelination, and normal function of the central and peripheral nervous system. In addition, it is required for effective erythropoiesis. Vitamin B_{12} deficiency in adults can lead to reversible megaloblastic anemia with

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changes in peripheral blood and bone marrow, as well as to neurologic dysfunction including myelopathy, neuropathy, and neuro-psychiatric disturbances. Neurological and neuro-psychiatric symptoms frequently occur without or before hematological changes[2–4].

Vitamin B_{12} underlies a complex pathway of intestinal absorption and intracellular processing[5]. Uptake in the gastrointestinal system requires gastric intrinsic factor and the cubam receptor in the distal ileum[2]. The most common cause of vitamin B_{12} deficiency in adults is "pernicious anaemia" due to a lack of intrinsic factor which is caused by an autoimmune gastritis leading to the destruction of gastric parietal cells[2, 6].

2 CAUSES AND FREQUENCY OF VITAMIN B_{12} DEFICIENCY IN NEWBORNS AND WOMEN OF CHILDBEARING AGE

Vitamin B_{12} deficiency in newborns is mostly of maternal origin. Causes in the mother may be unrecognized malabsorption or nutritional deficiency, e.g. due to gastric disease or a strict vegetarian or vegan diets. Infants with vitamin B_{12} deficiency are asymptomatic at birth but may develop severe multisystemic symptoms, including irreversible developmental impairment if untreated. Symptoms in infants with vitamin B_{12} deficiency include developmental delay, muscular hypotonia, irritability, regression, feeding difficulties, and failure to thrive. Also tremors and seizures have been described. If untreated, vitamin B_{12} deficiency may result in coma or even death. Onset of clinical symptoms is usually between 4–6 months of age[2]. Diagnosis is often only established in the second half-year of life[7–9]. At that time brain imaging often shows severe brain atrophy and delayed myelinisation. Hematological changes with anemia might be present, but can also be completely absent despite severe neurological impairments.

Infants with vitamin B_{12} deficiency diagnosed symptomatically do show improvement of clinical symptoms after supplementation with vitamin B_{12}. However, the neurologic and intellectual long-term outcome after severe and especially long-term vitamin B_{12} deficiency is poor, with permanent sequelae in many children[7, 9, 10].

Vitamin B_{12} deficiency has a high incidence in women of child-bearing age and pregnant women worldwide. Reported frequencies range between 10%–50% for different populations and ethnicities[6, 11–16]. Throughout pregnancy serum cobalamin levels decline, while MMA and tHcy levels increase[12, 17]. A Canadian study found vitamin B_{12} deficiency in 10% of women in early pregnancy[13], and several consecutive studies have confirmed similar or even higher frequencies for vitamin B_{12} deficiency in pregnancy of up to 50% in different populations and ethnicities[12, 14–16, 18]. An Italian study found low vitamin B_{12} levels in about 48% of unselected healthy females admitted to hospital for delivery[11]. Especially high rates of maternal vitamin B_{12} deficiency have been reported for the Indian subcontinent and the Eastern Mediterranean[12, 15]. Vitamin B_{12} deficiency in pregnancy is associated to adverse pregnancy outcomes, such as intrauterine growth restriction[19]. Hyperhomocysteinemia—as a possible result from vitamin B_{12} deficiency—has been associated with an increased risk of pregnancy loss[19]. Children of vitamin B_{12} deficient mothers will also be vitamin B_{12} deficient at birth[15] and have a high risk to develop symptomatic vitamin B_{12} deficiency under breastfeeding by the vitamin B_{12} deficient mother. This shows that maternal vitamin B_{12} deficiency is a relevant health issue in many populations worldwide and may affect a relevant number of children born to vitamin B_{12} deficient mothers.

3 DETECTION OF VITAMIN B_{12} DEFICIENCY BY NEWBORN SCREENING (NBS)

3.1 Strategies and Prerequisites for NBS for Vitamin B_{12} Deficiency

NBS is the most successful programme of secondary prevention in medicine[20, 21]. It aims to detect treatable disorders presymptomatically, using investigations from newborns' dried blood samples (DBS) taken in the first day of life[22]. Decisions on inclusion of disorders into NBS are commonly based on the screening criteria by Wilson and Jungner[23]. According to these criteria, target disorders should be an important health problem, and there should be an accepted treatment, a recognizable latent stage and a suitable test for detection of patients. Early detection of vitamin B_{12} deficiency in newborns would allow for presymptomatic treatment, which is more effective than treatment at a symptomatic stage. As children with vitamin B_{12} deficiency are asymptomatic at birth but may develop severe multisystemic symptoms, including irreversible developmental impairment in the second half-year of life, vitamin B_{12} deficiency would be a suitable target for NBS, given that an effective screening strategy from DBS is established.

Single patients with vitamin B_{12} deficiency have been previously detected by NBS programmes screening for methylmalonic and propionic acidurias using tandem mass-spectrometry (MS). These children have been identified based on elevated concentrations of propionylcarnitine (C3), sometimes complemented by second-tier strategies[11, 24, 25]. However, retrospective evaluation of NBS samples from children diagnosed symptomatically with vitamin B_{12} deficiency showed that C3 levels alone—currently used in many panels to detect disorders of the propionate metabolism—are not

sensitive enough to detect most children with vitamin B_{12} deficiency[10, 25–27]. Therefore, new strategies for detection of vitamin B_{12} deficiency by NBS are currently evaluated in pilot studies[28–31].

3.2 Second-tier Strategies and a Novel Systematic Newborn Screening for Vitamin B_{12} Deficiency

NBS for disorders of the propionate metabolism (methylmalonic and propionic acidurias) based on elevations of C3 results in a high false-positive rate, clearly unwanted in population-based NBS programmes[32, 33]. Therefore, to increase specificity, so called second-tier strategies have been suggested for these disorders. In these strategies elevations of the respective primary markers lead to measurement of more specific metabolites for these disorders—MMA, 3-OH-propionic acid (3-OH-PA), and MCA—from the same first NBS specimen[33–35]. In NBS for classical homocystinuria and remethylation disorders, second-tier strategies with measurement of homocysteine (tHcy) from the NBS sample have been successfully evaluated[30, 36, 37].

At the Heidelberg NBS Center—one of the largest NBS centers in Germany, performing NBS for more than 140 000 newborns every year—a pilot study has been started since the year 2016 for evaluation of a possible extension of the German NBS panel, which at that time included 14 target disorders (2 endocrine and 12 metabolic disorders). This study evaluates NBS for 26 additional metabolic target disorders under the use of second-tier strategies[28]. One aim of this pilot study called "NBS 2020"* was the development and evaluation of a systematic NBS strategy for vitamin B_{12} deficiency under combination of two second-tier strategies[29]. The first strategy analyses MMA, 3-OH-PA, and MCA[35], based on abnormal first-tier results from tandem-mass spectrometry (MS) for C3 and/or C3/C2. The second method analyses tHcy[38] after an abnormal first-tier result for Met (<cut-off low) or Met/Phe (<cut-off low or > cut-off high). Patients with vitamin B_{12} deficiency were identified by elevated tHcy, elevated MMA/MCA, or a combination of both[29]. Thus, the suggested novel method effectively covers both pathways possibly affected by vitamin B_{12} deficiency: the pathway involving methionine synthase by measurement of tHcy, and that of MMA-CoA mutase by measurement of MMA and MCA. In our study this strategy identified 33 children with maternal vitamin B_{12} deficiency from a cohort of 176 702 children screened over 27 months in Germany. The most sensitive marker for vitamin B_{12} deficiency was tHcy, which was found abnormal in 30 of 33 children. However, only the combination with the second strategy measuring also MMA allowed for identification of all 33 children[29].

3.3 Severity of Vitamin B_{12} Deficiency and Recommended Confirmatory Diagnostics

With an incidence of 1 in 5300 newborns screened, vitamin B_{12} deficiency was more frequent in the Heidelberg "NBS 2020" study than every other metabolic disorder included in the German NBS panel[39]. However, this incidence also includes milder functional forms of vitamin B_{12} deficiency, which are of yet unresolved clinical significance. Of the patients identified by the pilot project in Heidelberg 25% had severe vitamin B_{12} deficiency with levels <100 pmol/L, 56% had vitamin B_{12} levels of 100–200 pmol/L, and 19% >200 pmol/L (often described as cut-off for subclinical vitamin B_{12} deficiency)[29]. With regard to the definition by the World Health Organization[5], 62% of children and 42% of mothers would be classified as having "vitamin B_{12} deficiency" (<148 pmol/L), 19% (children) and 42% (mothers) were categorized as "low vitamin B_{12}" (148–221 pmol/L), and 19% (children) and 16% (mothers) showed levels in the range of "vitamin B_{12} adequacy" (>221 pmol/L). However, it has been well described that vitamin B_{12} levels in the low normal range alone do not exclude functional deficiency, as severe functional deficiency has been documented in the presence of normal serum vitamin B_{12} levels[5]. This demonstrates that evaluation of vitamin B_{12} status should always include also the functional markers homocysteine and MMA. Recommended laboratory work-up in suspected vitamin B_{12} deficiency and for follow-up under treatment is summarized in table 1.

3.4 Incidence of Vitamin B_{12} Deficiency Detected by NBS in Different Countries

The frequency of vitamin B_{12} deficiency detected

Table 1 Laboratory work-up for diagnosis and follow-up of vitamin B_{12} deficiency in newborns, infants, and their mothers

Parameter	Material
Vitamin B_{12} status	
Vitamin B_{12}, folic acid	Serum
Holotranscobalamin (if available)	Serum
Methylmalonic acid	EDTA plasma or serum and / or urine
Homocysteine	EDTA plasma or serum
Additional parameters	
Blood count	EDTA blood
Ferritin (due to prevalent concurrent iron deficiency; initial measurement and in follow-up under treatment)	Heparin plasma or serum (depending on laboratory requirements)

by NBS differs considerably between different international reports. This may be due to true differences in incidences worldwide but is certainly also due to different cut-offs used and strategies applied, which result in different sensitivities for detection of vitamin B_{12} deficiency. For Southwest Germany the Heidelberg pilot project found vitamin B_{12} deficiency in one of 5300 newborns screened using a combination of two second-tier strategies[29]. A pilot study in the German federal state of Bavaria using a single second-tier strategy with measurement of MMA detected vitamin B_{12} deficiency in 1 of 30 000 newborns screened[31]. An Italian study using C3 as first-, and MMA as second-tier NBS test in a cohort of 35 000 children found vitamin B_{12} deficiency in 1 in 5000 newborns[11].

In some countries, vitamin B_{12} deficiency is an occasional incidental finding in NBS for methylmalonic and propionic acidemias based on elevation of C3 without a systematic approach to screen also for vitamin B_{12} deficiency. The screening programme in Estonia reported an incidence of vitamin B_{12} deficiency as high as 1 in 2500 newborns detected by elevated C3[40]. The NBS programme in the US state of Minnesota reported that no cases of vitamin B_{12} deficiency had been identified by NBS before the year 2005, followed by a detection rate of about 1 in 33 000 after lowering the C3 cut-off and implementing second-tier testing based on elevated C3 levels[25]. For 31 NBS laboratories in the USA, a questionnaire based survey reported 1 in 114 000 newborns detected with vitamin B_{12} deficiency by elevated C3 on NBS for the years 2003–2007[24]. As already described in detail, vitamin B_{12} deficiency has a high incidence of 10%–50% in pregnant women worldwide[12–16]. Therefore, it seems most likely that the very low incidences of vitamin B_{12} deficiency in some NBS programmes are rather due to undetected or unreported cases. In accordance with the results from the pilot study at the Heidelberg NBS center, demonstrating that only 4 of 33 newborns with vitamin B_{12} deficiency had elevated C3 levels in NBS, also previous reports stated normal C3 concentrations on NBS in confirmed patients with severe vitamin B_{12} deficiency[25, 26]. This demonstrates that C3 levels alone are not sensitive enough to detect these patients. The Heidelberg pilot study "NBS 2020" demonstrated that adding second-tier strategies measuring MMA and tHcy is an economical way to increase the number of children with vitamin B_{12} deficiency identified by NBS, leading to early treatment[29, 41].

In addition to different strategies for detection of vitamin B_{12} deficiency in NBS, regional differences in prenatal vitamin supplementation might contribute to the divergent reported incidences of vitamin B_{12} deficiency. In the Heidelberg pilot project "NBS 2020", 59% of mothers of children with vitamin B_{12} deficiency reported that they had not taken any vitamin supplementation before or during pregnancy at all[29]. This was a remarkable finding, as there is a general recommendation for a preconceptional folic acid supplementation for all women in Germany[42] with many of the preparations also containing vitamin B_{12}. This poor adherence to the recommendations of prenatal folic acid supplementation is in accordance with previous national surveys in Germany[43] and Switzerland[44]. This seems to particularly affect women with a migration background, possibly because of more reluctance or thresholds to use the preventive appointments of prenatal care[29, 44]. In other countries, the adherence to prenatal B-vitamin supplementation is much higher and has been reported with 93% in a Canadian study[45].

4 DIAGNOSTICS IN MOTHERS OF CHILDREN WITH VITAMIN B_{12} DEFICIENCY

Diagnostics of vitamin B_{12} deficiency in newborns and infants should always include a comprehensive work-up of the mother's vitamin B_{12} status as vitamin B_{12} deficiency in children of this age-group is mostly of maternal origin. In this context, laboratory work-up should not only include maternal vitamin B_{12} and folic acid levels but also the functional markers homocysteine in plasma and MMA (in plasma and/or urine). If available, the additional determination of holo transcobalamin may be helpful to detect a negative vitamin B_{12} balance in cases with borderline serum levels of vitamin B_{12} in the presence of functional parameters still within normal range[46]. Recommendations for laboratory work-up in children with (suspected) vitamin B_{12} deficiency and their mothers are summarized in table 1.

If laboratory results of the mother confirm vitamin B_{12} deficiency or functional vitamin B_{12} deficiency (elevation of homocysteine and/or MMA in the presence of low-normal vitamin B_{12} levels), a comprehensive work-up of possible causes of maternal vitamin B_{12} deficiency by internal medicine, consecutive treatment, and, if applicable, dietary counselling should be initiated. The Heidelberg pilot project "NBS 2020" offered and prospectively performed a thorough diagnostic work-up also for the mothers of newborns with vitamin B_{12} deficiency detected by the NBS study. This work-up for the mothers was performed in collaboration with specialists from internal medicine at the University Hospital Heidelberg and led to diagnosis of previously unrecognized gastrointestinal malabsorption, e.g. due to autoimmune gastritis, in several cases[29]. Additional diagnoses in the mothers included ulcerative colitis, gastric bypass, HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome, severe pancytopenia due to vitamin B_{12} and folic acid deficiency, and carbamazepine treatment

throughout pregnancy[29]. However, in the majority of mothers, no gastrointestinal cause for vitamin B_{12} deficiency was established. Most mothers (89%) with vitamin B_{12} deficiency in the study reported a balanced diet including meat. A vegan or vegetarian diet as explanation for maternal vitamin B_{12} deficiency was only rarely reported. However, several mothers reported feeding difficulties in pregnancy e.g. due to hyperemesis or aversion against meat. Numerous mothers had received iron supplementation during pregnancy due to anemia. Vitamin B_{12} status had not been investigated by the treating gynecologist in any of these mothers[29]. In women with anemia, coexistence of iron, vitamin B_{12}, and folic acid deficiency has been reported, with hematological changes caused by vitamin B_{12} deficiency potentially masked by those due to iron deficiency[47]. Vitamin B_{12} deficiency may cause severe symptoms in the mothers as described at the beginning of this review. In subsequent pregnancies, children would again be affected by maternal vitamin B_{12} deficiency.

5 PREVENTIVE APPROACHES TO VITAMIN B_{12} DEFICIENCY DURING MATERNAL CARE

National maternity guidelines in Germany do currently not include routine evaluation of vitamin B_{12} status during pregnancy. The German Federal Center for Nutrition recommends a balanced diet and preconceptional start of folic acid supplementation for all women. Recommendations for women following vegetarian diets include dietary counselling, and for women following vegan diets, preconceptional start of micronutrient supplementation including vitamin B_{12} as well as monitoring of micronutrient status throughout pregnancy[42]. However, a vegan diet is disadvised in pregnancy and lactation by both the German Nutrition Society (DGE) and the national network "Healthy Start – Young Family Network"[42, 48].

In the year 2016, up to 1% of the German population were reported to follow a vegan diet[48]. Although this proportion may have increased in the meantime due to current trends in nutritional choices, the proportion of women following restricted diets was very low in mothers of children with vitamin B_{12} deficiency detected by the NBS pilot study in Heidelberg[29]. This could either be explained by a high awareness of women following restricted diets for the need of vitamin supplementation in pregnancy. Alternatively, it may be that mothers with restricted diets have more reservations against study participation. As the participation rate in the pilot study "NBS 2020" is about 67%, the true incidence of maternal vitamin B_{12} deficiency in the population screened may be even higher.

As previously discussed, results from our own study and different national surveys document a poor adherence to the recommended vitamin supplementation in pregnancy[29, 43, 44]. In addition, despite risk factors like feeding difficulties or anemia, no diagnostic work-up for vitamin B_{12} deficiency had been performed by the gynecologists in mothers evaluated in our study[29]. This underlines that caregivers of pregnant women need to increase their awareness for vitamin B_{12} deficiency and that preventive approaches are necessary during maternal care. Mothers who do not suffer from gastrointestinal malabsorption would benefit from a general consequent supplementation with vitamin B_{12} containing supplements preconceptionally and during pregnancy, e.g. administered as combined vitamin preparations with folic acid. However, to detect and treat vitamin B_{12} deficiency in mothers with gastrointestinal malabsorption as early as possible, an ideal prevention strategy would include routine evaluation of vitamin B_{12} status in early pregnancy. This is necessary, as many mothers affected by atrophic gastritis have been reported to be clinically asymptomatic[49].

6 CASES OF VITAMIN B_{12} DEFICIENCY DIAGNOSED BY SELECTIVE SCREENING

During the course of the pilot project "NBS 2020", several children were diagnosed symptomatically with vitamin B_{12} deficiency by selective diagnostics in our center. These children were either born before study initiation or had undergone NBS at screening laboratories not performing NBS for vitamin B_{12} deficiency and were diagnosed based on clinical symptoms aged 2–11 months. This underlines the clinical significance of early detection of vitamin B_{12} deficiency by NBS. Retrospective analysis of the original NBS sample was possible in one child born before study initiation and showed that the strategies applied in our pilot project would have detected vitamin B_{12} deficiency in the original first NBS sample[28, 29].

In 4 children participating in the study "NBS 2020" mild vitamin B_{12} deficiency or functional vitamin B_{12} deficiency was diagnosed by selective diagnostics aged 1–8 months. Reasons for work-up in these children included abnormal NBS of a twin sibling, seizures, developmental delay after 7-month exclusive breast-feeding by a vegetarian mother, and suspicion of seizures in a child exclusively breast-fed for 6 months. These children had not fulfilled criteria for second-tier analysis in their initial NBS sample. Retrospective second-tier analysis from the initial DBS revealed normal concentrations for tHcy and MMA in 3 of these children[29]. Therefore, it seems likely that vitamin B_{12} deficiency may have developed only later in these fully breastfed children. However, it has to be noted as a limitation that also the NBS strategy using a combination of two second-tier strategies may not

always pick up mild functional vitamin B_{12} deficiency.

7 TREATMENT OF VITAMIN B_{12} DEFICIENCY AND A NOVEL STANDARDIZED ORAL SUPPLEMENTATION SCHEME FOR NEWBORNS

Although nutritional or maternal vitamin B_{12} deficiency is quite common, there is no general agreement on treatment modalities[50]. Treatment of vitamin B_{12} deficiency in newborns and infants is often performed by intramuscular application of vitamin B_{12}[50]. This regimen is however invasive and painful and therefore emotionally challenging for parents. As an alternative oral supplementation regimens have been reported for adults and children[51–53]. In the context of the pilot project "NBS 2020" at the Heidelberg NBS center, a standardized regimen of exclusively oral vitamin B_{12} supplementation has been developed and utilized successfully for children with vitamin B_{12} deficiency detected by NBS[29]. This regimen consists of oral vitamin B_{12} supplementation with 0.5 mg per day (liquid preparation) over three days, followed by 0.1 mg per day. During the first week also folic acid 0.4 mg per day is supplemented orally. After about two weeks, laboratory follow-up of vitamin B_{12} status including functional markers (table 1) has to be performed and supplementation with vitamin B_{12} 0.1 mg per day is continued until completion of laboratory results. After normalization of all markers of vitamin B_{12} metabolism (especially methylmalonic acid and homocysteine, table 1), vitamin B_{12} supplementation is continued in maintenance dosage of 5 µg/day during breastfeeding until meat-containing complementary foods or vitamin B_{12} containing formula have been reliably introduced. In the context of the Heidelberg pilot project "NBS 2020", 84% of affected newborns were treated with oral supplementation[29]. Under this regimen, rapid normaliziation of vitamin B_{12} status could be documented and all patients remained without clinical symptoms of vitamin B_{12} deficiency at short-term follow-up[29]. If parents decide to choose formula feeding for their child, vitamin B_{12} supplementation in maintanance dosage can also be stopped, due to the vitamin B_{12} content of infant formula. After stopping vitamin B_{12} supplementation, follow-up testing of vitamin B_{12} status including functional markers should always be performed.

8 VITAMIN B_{12} DEFICIENCY IN INFANTS: ROLE OF NUTRITIONAL CHOICES AND INBORN ERRORS OF COBALAMIN ABSORPTION

Vitamin B_{12} deficiency developing in infancy can be caused by delayed introduction of meat-containing complementary foods[54] in fully breast-fed children. In children with risk factors like prolonged exclusive breast-feeding or clinical symptoms like failure to thrive, diagnostic work-up including vitamin B_{12} status has to be initiated by the treating pediatrician regardless of a possibly normal NBS result concerning vitamin B_{12} deficiency.

In rare cases, vitamin B_{12} deficiency in infants may also be caused by inborn errors of gastrointestinal vitamin B_{12} absorption or systemic trafficking[50]. This should be considered especially in manifestations in later infancy or in cases without explanation of vitamin B_{12} deficiency by dietary history in mother or child. Differential diagnoses in these cases include hereditary gastric intrinsic factor deficiency (*GIF* gene), Imerslund-Gräsbeck syndrome (*AMN* or *CUBN* gene) or transcobalamin deficiency (*TCN2* gene). For this reason, a short-term follow-up of vitamin B_{12} status after initiation of oral vitamin B_{12} supplementation has to be performed by measurement of the parameters shown in table 1. In cases with suggestive clinical history or if oral supplementation does not normalize all parameters of vitamin B_{12} status, a molecular genetic work-up concerning inborn errors of gastrointestinal vitamin B_{12} absorption has to be initiated. The differentiation from genetic causes of gastrointestinal vitamin B_{12} absorption by evaluation of response to oral supplementation is an additional advantage of an exclusively oral supplementation scheme. In inborn errors of gastrointestinal vitamin B_{12} absorption like hereditary gastric intrinsic factor deficiency or Imerslund-Gräsbeck syndrome, vitamin B_{12} status will normalize under parenteral supplementation of vitamin B_{12}, but not under oral supplementation. If supplementation is started parenterally under the assumption of nutritional vitamin B_{12} deficiency, further diagnostic work-up concerning genetic causes of gastrointestinal vitamin B_{12} absorption may be delayed or even neglected – potentially leaving the underlying disorder undiagnosed.

9 VITAMIN B_{12} DEFICIENCY IN INFANTS: ROLE OF NUTRITIONAL CHOICES

It cannot be predicted whether all children with milder functional vitamin B_{12} deficiency identified by NBS pilot studies would have developed clinical symptoms without treatment. This depends on nutritional choices of the family for child nutrition and on the vitamin B_{12} status of the breastfeeding mother. Based on published case-reports, it can be postulated that also an initially milder vitamin B_{12} deficiency can result in symptomatic vitamin B_{12} deficiency in a child after prolonged exclusive breast-feeding by a vitamin B_{12} deficient mother[55]. Breast-feeding was the preferred way of child nutrition, chosen by 79% of mothers with vitamin B_{12} deficiency identified by the pilot study "NBS 2020" in Heidelberg[29]. As breast-

feeding is associated with several health benefits for child and mother[54, 56], this way of nutrition should be encouraged wherever possible. Vitamin B_{12} deficiency in the child and/or mother should not discourage from breast-feeding, given adequate supplementation and laboratory follow-up.

10 CONCLUSION AND RECOMMENDATIONS FOR PREVENTIVE APPROACHES TO VITAMIN B_{12} DEFICIENCY FOR CHILDREN AND MOTHERS

Maternal vitamin B_{12} deficiency is a relevant health issue in a large number of women in many populations worldwide. Given the negative and partly irreversible health consequences for children with vitamin B_{12} deficiency after symptomatic diagnosis, preventive approaches should be undertaken. NBS for vitamin B_{12} deficiency is feasible using second-tier strategies and leads to effective identification of moderate and severe forms of vitamin B_{12} deficiency. Presymptomatic treatment allows for normal development in affected children and additional benefits are achieved for previously undiagnosed mothers.

Many children with maternal vitamin B_{12} deficiency have already benefited from identification by NBS, especially in the context of pilot studies. Even more children could benefit in case of a systematic inclusion of vitamin B_{12} deficiency into NBS panels worldwide[41]. This should stimulate efforts to introduce second-tier testing for tHcy and MMA in all NBS programmes. In addition, preventive approaches to maternal vitamin B_{12} deficiency should be implemented already during maternal care. Systematic supplementation of vitamin B_{12} containing supplements during pregnancy would be useful for primary prevention of vitamin B_{12} deficiency in mothers unaffected by gastrointestinal malabsorption. To allow also for early identification and treatment of mothers with atrophic gastritis—who are often asymptomatic despite being severely vitamin B_{12} deficient—an ideal prevention strategy should include routine evaluation of vitamin B_{12} status in early pregnancy. These preventive approaches to vitamin B_{12} deficiency as a global health issue could help to improve mother and child health in many countries worldwide.

Conflict of Interest Statement

The authors have no conflicts of interest relevant to this article to disclose.

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