Screening for vitamin B12 deficiency and other target diseases (homocystinuria, propionic acidaemia and methylmalonic aciduria) in extended newborn screening (ENS)[1]

EXTRACT

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Institut für Qualität und Wirtschaftlichkeit im Gesundheitswesen
Im Mediapark 8
50670 Köln
Germany

Phone: +49 221 35685-0
Fax: +49 221 35685-1
E-mail: berichte@iqwig.de
Internet: www.iqwig.de

This report was prepared in collaboration with external experts.

The responsibility for the contents of the report lies solely with IQWiG.

According to §139b (3) No. 2 of Social Code Book (SGB) V, Statutory Health Insurance, external experts who are involved in the Institute's research commissions must disclose "all connections to interest groups and contract organizations, particularly in the pharmaceutical and medical devices industries, including details on the type and amount of any remuneration received". The Institute received the completed "Form for disclosure of potential conflicts of interest" from each external expert. The information provided was reviewed by a Committee of the Institute specifically established to assess conflicts of interests. The information on conflicts of interest provided by the external experts and external reviewers is presented in Chapter A8 of the full report. No conflicts of interest were detected that could endanger professional independence with regard to the work on the present commission.

External expert

- Anibh Das, Hanover Medical School (MHH), Hanover, Germany

IQWiG thanks the external expert for his collaboration in the project.

Patient and family involvement

Patients and/or family members were consulted during the preparation of the report. Anja Simon, Hanna Benz and 2 other people participated in the discussion. Its aim was to obtain information on the following topics: expectations with regard to the screening test and motivation to participate, experiences with the test, consequences of results and concerns regarding the test.

IQWiG would like to thank the participants as well as the German Interest Group Phenylketonuria and Related Congenital Metabolic Disorders (DIG PKU). The participants and the DIG PKU were not involved in the preparation of the report.

IQWiG employees

- Stefan Sauerland
- Gunnar Plinke
- Konstanze Angelescu
- Marco Knelangen
- Marie Kumpf
- Fabian Lotz
- Martina Markes

- Sandra Molnar
- Yvonne Zens

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Neonatal Screening, Vitamin B12 Deficiency, Homocystinuria, Propionic Acidemia, Methylmalonic Acidemia, Benefit Assessment, Systematic Review

Key statement

Research question

The aim of this investigation is to assess the benefit of screening for vitamin B12 deficiency and other target diseases (homocystinuria [HU], propionic acidaemia [PA] and methylmalonic aciduria [MMA]) in extended newborn screening (ENS). Newborn screening for the 4 target diseases is compared with no screening. The focus of the assessment is on patient-relevant outcomes.

Conclusion

When weighing the possible benefit and harm of screening for acquired vitamin B12 deficiency, there is a hint of a benefit of screening as part of ENS. However, due to the lack of conclusive evidence from intervention studies on the screening chain and comparative intervention studies on the start of treatment, the benefit or harm of screening for HU, PA and MMA as part of ENS is unclear. But as screening for vitamin B12 deficiency alone seems technically unfeasible, joint screening for all 4 target diseases relevant here should be considered. A comparative concomitant evaluation of all target diseases is in principle possible with the existing structures and is also necessary in view of the scarce data.

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List of abbreviations

Abbreviation	Meaning
cbl	cobalamin
CBS	cystathionine-β-synthase
CoA	coenzyme A
DIG PKU	Deutsche Interessengemeinschaft Phenylketonurie und verwandte angeborene Stoffwechselstörungen (German Interest Group for Phenylketonuria and Related Congenital Metabolic Disorders)
ESPED	Erhebungseinheit für Seltene Pädiatrische Erkrankungen in Deutschland (Data Collection Unit for Rare Paediatric Diseases in Germany)
ENS	extended newborn screening
FSG	familial screening group (cases identified on the basis of a family case)
G-BA	Gemeinsamer Bundesausschuss (Federal Joint Committee)
IQWiG	Institut für Qualität und Wirtschaftlichkeit im Gesundheitswesen (Institute for Quality and Efficiency in Health Care)
ITT	intention to treat
HU	homocystinuria
HUS	haemolytic-uraemic syndrome
LDG	late diagnosis group (cases identified by clinical symptoms)
MMA	methylmalonic aciduria
MTHFR	methylenetetrahydrofolate reductase
mut0	complete failure of the methylmalonyl-CoA mutase
courage-	partial failure of the methylmalonyl-CoA mutase
NBS	newborn screening
NSG	newborn screening group (cases identified by NBS)
PA	propionic acidaemia
PPV	positive predictive value
RCT	randomized controlled trial
SR	systematic review
VOPT	verification of only positive testers

1 Background

Vitamin B12 deficiency, homocystinuria, propionic acidaemia and methylmalonic aciduria can jeopardize the age-appropriate motor and neurocognitive development of children. Depending on the underlying disease, a newborn's life may be at risk.

Vitamin B12 deficiency in newborns can be caused by maternal vitamin B12 deficiency [1] or by the rare congenital (autosomal recessive inherited) genetic disease transcobalamin II deficiency [2]. Vitamin B12 is an important co-factor for metabolic processes, particularly in the central nervous system; a deficiency can lead to irreversible neurological damage and developmental disorders [1,2]. The most sensitive parameter for detecting vitamin B12 deficiency is an elevated methylmalonic acid level in the urine; the blood homocysteine level is usually also elevated [1,2]. Genetic analysis enables the detection of transcobalamin II deficiency [2]. A prevalence of less than 1:1,000,000 is reported for this deficiency on the website https://www.orpha.net, the portal for rare diseases and orphan drugs [3]. The data on the frequency of maternal vitamin B12 deficiency varies between 1:5300 [1] to less than 1:100,000 newborns [4] and result mainly from newborn screenings for vitamin B12 deficiency. Both deficiency conditions should be treated by parenteral administration (intravenous, intramuscular) of vitamin B12 [1]. While a maternal deficiency (hereafter: acquired vitamin B12 deficiency) is only treated for a short time [1], a genetic deficiency requires lifelong parenteral supplementation [2].

Homocystinuria (HU) is a collective term for several congenital (autosomal recessive inherited) metabolic disorders that have in common an accumulation of the amino acid homocysteine in the blood or homocystine in the urine [5]. Elevated homocysteine levels and other accumulating intermediates of the homocysteine metabolism mediate the symptoms [6]. Classic HU, also known as type 1 HU, is caused by a cystathionine β-synthase (CBS) deficiency [7]. The disease manifests itself in the eyes, skeleton, vascular system, and brain [7,8]. The two subtypes of HU based on a CBS deficiency (pyridoxine-responsive and pyridoxine-non-responsive) must be distinguished from each other: the former is characterized by less severe courses and a better long-term prognosis under treatment [9,10]. The disease is detected by genetic analysis [11]. The frequency of the disease in newborns is reported to be around 1:500,000 [12]. Lifelong treatment essentially involves the administration of vitamins and, if necessary, a low-protein diet and betaine [8]. The congenital disorders of cobalamin (cbl) metabolism (cblD1, cblE and cblG), which each cause subtypes of methylcobalamin deficiency, are also summarized under HU, also referred to as HU without methylmalonic aciduria [13]. They manifest themselves particularly in the brain; metabolic stroke with irreversible neurological damage can occur in the context of banal infections [6,13]. The underlying defect is detected by genetic analysis [13]. Data on the frequency of these metabolic disorders are not available. Lifelong treatment essentially involves the administration of vitamin B12 and possibly other vitamins [6]. Hyperhomocysteinaemia, caused by an autosomal recessive

inherited methylenetetrahydrofolate reductase (MTHFR) deficiency (also known as type 2 HU), is described as the third form of HU. MTHFR deficiency often manifests itself with severe neurological symptoms [6] up to coma and death [14]. The disease is detected by genetic analysis [6]. Data on the frequency of this deficiency is not available. An essential component of treatment is the lifelong administration of high-dose betaine [6,14].

Propionic acidaemia (PA) is a rare congenital (autosomal recessive inherited) metabolic disorder and belongs to the group of organoacidopathies [5]. Due to the limited activity of propionyl coenzyme A (CoA) carboxylase, there is an accumulation of propionic acid and other toxic metabolites, which are regularly produced in various metabolic processes and are increasingly formed during catabolic processes [15,16]. This can lead to severe metabolic imbalances which, if left untreated, can lead to irreversible brain damage and even death (metabolic stroke) [5,15,16]. The diagnosis requires an elevated level of organic acids in the urine and propionylcarnitine in the blood; the confirmatory diagnosis is made by genetic analysis [15,17]. The frequency of PA varies between more than 1:50,000 [5] and less than 1:100 000 [17]. While acute treatment consists of treating the metabolic derailment and thus ensuring survival [15], long-term treatment is based on a strict protein-conscious diet (supplemented with special amino acid mixtures without precursors of propionic acid), the regular intake of carnitine to stabilize the metabolic situation [5,15,16] as well as measures to minimize the formation of propionic acid by bacteria in the intestine [5,16]. Liver transplantation in infancy to stabilize the metabolic situation is also being discussed [15,16]. In addition, monitoring and treatment of possible (long-term) damage, for example with regard to neurological, nephrological, cardiological and pancreatic complications, is indicated [15,16].

Methylmalonic aciduria (MMA) is a collective term for several rare congenital (autosomal recessive inherited) metabolic disorders which, like PA, belong to the group of organoacidopathies [5]. The frequency of MMA is given as 1:250,000 [5]. A distinction is made between MMA without HU [18] and MMA with HU [19]. MMA without HU is divided into the following subtypes: responsive to vitamin B12 [20], vitamin B12-resistant type mut0 [21], and partially vitamin B12-resistant type mut- [22].

Vitamin B12-sensitive MMA without HU is based on different cbl defects (cblA, cblB or cblD2), each of which causes adenosylcobalamin deficiency [20]. Metabolic derailments, including coma, as well as damage to the brain and its consequences are key symptoms of the disease; long-term consequences affect not only the brain but also the kidneys in particular [20]. The disease is detected by genetic analysis [15]. While acute treatment consists of treating the metabolic derailment and thus ensuring survival, long-term treatment is based on a strict protein-conscious diet (supplemented with special amino acid mixtures without precursors of methylmalonic acid), the administration of vitamin B12 and, if necessary, carnitine to stabilize

the metabolic situation [15,20] as well as measures to minimize the formation of propionic acid by bacteria in the intestine [20]. Apart from the administration of vitamin B12, the long-term treatment of vitamin B12-resistant MMA without HU is comparable [23]. However, in the case of vitamin B12-resistant MMA without HU, there are also known cases that respond to the administration of vitamin B12 [22]. Due to the severity of the disease, liver transplantation may also be considered [15,16,23]. Patients with a complete failure of methylmalonyl-CoA mutase (mut0) have a more severe course compared to those with a partial enzyme failure (mut-), both in the acute phase and in the long-term course [23]. Diagnosis of the disease involves determination of organic acids in the urine and propionylcarnitine in the blood, followed by genetic analysis [15].

MMA with HU is based on different cbl defects (cblC, cblD, cblF or cblJ), each of which simultaneously causes both methylcobalamin and adenosylcobalamin deficiency [19]. The spectrum of symptoms includes mental and physical retardation as well as ophthalmological effects in all affected individuals [19]. The disease is detected by genetic analysis [19]. Treatment consists of the administration of high doses of vitamin B12 (parenterally), biotin and folic acid [19].

HU, PA and MMA are metabolic diseases of the so-called "intoxication type". This refers to the formation of toxic metabolites in catabolic episodes due to the release of amino acids from endogenous reserves. These cannot be completely metabolized due to the metabolic defect, so that toxic metabolites are formed and poison the body. Such catabolic episodes, in the context of postpartum catabolism or banal febrile infections, can trigger metabolic crises ("metabolic stroke"), which can lead to a "residual syndrome". In this respect, the course of the disease in patients is not only determined by the genetic defect, but also by exogenous factors [6,7,15].

Dried blood dripped onto filter paper can be used to diagnose vitamin B12 deficiency, HU, PA and MMA [24,25]. For vitamin B12 deficiency, MMA and PA, this is analysed for the metabolite propionylcarnitine, among other things. The metabolite methionine is helpful for the detection of HU [24]. In Germany, according to the Paediatric Directive of the Federal Joint Committee (G-BA) [26] for extended newborn screening (ENS) carried out in Germany, venous or heel blood is collected in the 36th to 72nd hour of life, dripped onto filter paper and examined for other target diseases. Vitamin B12 deficiency, HU, PA and MMA are not yet among the target diseases screened for as part of the ENS.

The aim of newborn screening for vitamin B12 deficiency, HU, PA or MMA is to identify and treat affected children earlier before a metabolic derailment with irreversible neurological damage in the form of a residual syndrome occurs.

2 Research question

The aim of this investigation is to assess the benefit of screening for vitamin B12 deficiency and other target diseases (homocystinuria [HU], propionic acidaemia [PA] and methylmalonic aciduria [MMA]) in extended newborn screening (ENS).[2] Newborn screening for the 4 target diseases is compared with no screening. The focus of the assessment is on patient-relevant outcomes.

3 Methods

Comparative studies on the screening chain were included in the benefit assessment. In the event that such studies were not available or not available in sufficient quantity and quality, an assessment of intervention studies that enable a comparison of an earlier versus a later start of treatment, as well as of diagnostic accuracy studies as the individual components of the screening chain (linked evidence) was planned.

Comparative intervention studies on the screening chain

The target population of the benefit assessment was newborns. The test intervention was newborn screening for at least one of the target diseases. The control intervention was no newborn screening for the target diseases.

The following patient-relevant outcomes were considered for the investigation:

- mortality (overall survival, disease-specific survival),
- morbidity (e.g. developmental and growth disorders, hospitalization),
- (serious) adverse events,
- health-related quality of life of the child.

Randomized controlled trials (RCTs) were to be included in the benefit assessment. If the evidence based on RCTs was not sufficient for the benefit assessment, non-randomized comparative intervention studies and comparative cohort studies (including retrospective studies / studies with historical controls) were also to be included.

Comparative intervention studies on the start of treatment

If no comparative intervention studies on the screening chain were identified for the benefit assessment or only ones of insufficient quality, intervention studies that enabled a comparison of an earlier versus a later start of treatment were also considered for the assessment. It had to be possible to transfer how the diagnosis was made in patients who

[2] Unless explicitly stated otherwise, the term “target diseases” is used synonymously for vitamin B12 deficiency, HU, PA and MMA.

started treatment earlier to the screening situation in newborns. For the intervention to be tested, 2 different times of treatment initiation were considered. On the one hand, (a) a presymptomatic start of treatment was considered as the intervention to be tested. On the other hand, (b) an earlier start of treatment when symptoms were already present was used as the intervention to be tested. In each case, a later start of treatment with existing symptoms was used as a control intervention. The above-mentioned patient-relevant outcomes were considered for the study. RCTs were included in the benefit assessment. If no RCTs were available for the research question, studies with a lower level of evidence (retrospective comparative studies) were included.

Diagnostic accuracy studies

Insofar as a positive conclusion on benefit resulted from bringing forward the start of treatment (see the section "Information retrieval, information assessment and synthesis"), diagnostic accuracy studies were also included in the benefit assessment as part of this report. Studies with newborns were included in the assessment. All diagnostic tests or combinations of tests applied in the studies to identify at least one of the target diseases using dried blood on filter paper were considered to be index tests.

For the detection of vitamin B12 deficiency, tests that include measurement of the vitamin B12 serum level and functional markers in the urine are accepted as reference tests. For the other target diseases, the reference tests are genetic analyses to detect the underlying enzyme defect. If the results of the index test are unremarkable, follow-up can also be accepted as an alternative.

Information retrieval, information assessment and synthesis

Parallel to the preparation of the protocol (“report plan”), a search for systematic reviews was carried out in the MEDLINE database (including the Cochrane Database of Systematic Reviews) and the HTA database, as well as on the websites of the National Institute for Health and Care Excellence (NICE) and the Agency for Healthcare Research and Quality (AHRQ).

It was checked whether at least 1 high-quality and up-to-date systematic review (SR) could be considered whose information retrieval could be used as a basis (hereinafter: basic SR).

If such a basic SR was available, a supplementary search for studies for the period not covered by the basic SR was carried out in a second step. Otherwise, studies were searched for without restricting the period.

The systematic literature search for studies was carried out in the MEDLINE, Embase and Cochrane Central Register of Controlled Trials databases.

In addition, the following information sources were considered: study registries, documents submitted by the Federal Joint Committee (G-BA), reference lists, and documents provided from hearing procedures.

The selection of relevant studies was carried out by 2 persons independently of each other. Discrepancies were resolved by discussion. Data were extracted into standardized tables. Across-outcome and outcome-specific risk-of-bias criteria were evaluated to assess the qualitative certainty of results (shortened to “certainty of results” in the following text), and the risk of bias was rated as low or high in each case. The results of the individual studies were described according to outcomes.

In addition to the comparison of the results of the individual studies, meta-analyses and sensitivity analyses were performed and effect modifiers examined, provided that the methodological requirements were met.

For each outcome, a conclusion on evidence of (greater) benefit and (greater) harm was made in 4 grades regarding the respective certainty of conclusions: either proof (highest certainty of conclusions), an indication (moderate certainty of conclusions), a hint (weakest certainty of conclusions), or none of these 3 situations was present. The latter case occurred when no data were available or the available data did not allow any of the other 3 conclusions. In this case, the conclusion “There is no hint of (greater) benefit or (greater) harm” was drawn.

Finally, an assessment of benefit and harm across outcomes was performed.

4 Results

4.1 Results of information retrieval

No SR was considered as a basic SR for the purpose of identifying primary studies.

The information retrieval revealed 3 comparative intervention studies on the screening chain relevant to the research question. No planned or ongoing studies were identified. The last search for studies on the screening chain took place on 23 January 2023.

The information retrieval revealed 13 comparative intervention studies on the start of treatment that were relevant to the research question. No planned or ongoing studies were identified. The last search for comparative treatment studies took place on 14 February 2023.

The search strategies for bibliographic databases and study registries are included in the appendix.

Since neither the intervention studies on the screening chain nor the intervention studies on the start of treatment yielded any positive conclusion on the benefit of starting treatment earlier, no diagnostic accuracy studies were searched for.

An overview of all included studies for the respective research questions, including information sources and target diseases, is shown in the table below.

Table 1: Study pool of the benefit assessment

Target diseases	Study	Full-text publication (in scientific journals)	Registry entry / Results report from study registries	Other document	Data usable
Comparative intervention studies on the screening chain					
PA and MMA	Barends 2014	yes [27]	no	no	no
	Lund 2020	yes [28]	no	no	no
PA, MMA and HU	Wilson 2012	yes [29]	no	no	no
Comparative intervention studies on the start of treatment					
Vitamin B12 deficiency	***Transcobalamin II deficiency-related neonatal vitamin B12 deficiency***				
	Monagle 1995	yes [30]	no	no	no
HU	***Pyridoxine-non-responsive CBS deficiency-based HU***				
	Yap 1998	yes [31-33]	no	no	no
	Mulvihill 2001	yes [34,35]	no	no	no
	Purcell 2017	yes [36]	no	no	no
	Walter 1998	yes [37]	no	no	no
	MTHFR deficiency-based HU				
	Strauss 2007	yes [38]	no	no	no
	Yverneau 2022	yes [39]	no	no	no
PA	Liu 2022	yes [40]	no	no	no
	Haijes 2020[a]	yes [41]	no	no	no
MMA	***MMA with HU***				
	Ahrens-Nicklas 2017	yes [42]	no	no	no
	Bourque 2021	yes [43]	no	no	no
	Gizicki 2014	yes [44]	no	no	no
	He 2020	yes [45]	no	no	no

a. Part of the patient population was affected by MMA.

CBS: cystathionine-β-synthase; HU: homocystinuria; MMA: methylmalonic aciduria; MTHFR: methylenetetrahydrofolate reductase; PA: propionic acidaemia

4.2 Comparative intervention studies on the screening chain

Barends 2014 [27], Lund 2020 [28] and Wilson 2012 [29] were identified as comparative studies on the screening chain. As explained below, the results of these studies are not presented. Therefore, only a brief description of these 3 studies follows and the risk-of-bias assessment across outcomes is omitted.

The Barends 2014 study [27] presents data collected retrospectively from medical records on ENS in the Australian state of Victoria, covering all cases over a period of 12 years (February 2002 to January 2014). These are also compared with retrospectively collected data on all patients who were clinically diagnosed in the 12 years prior to the introduction of this screening in 2002. Of the target diseases of interest in the present benefit assessment, the ENS investigated includes PA and certain subtypes of MMA. For this purpose, blood was collected in the 48th to 72nd hour of life, dripped onto filter paper and examined using tandem mass spectrometry. A total of 847,418 newborns were screened during the follow-up period. Six newborns with PA and one newborn with MMA were identified. In contrast, no cases of PA and 2 cases of MMA were clinically diagnosed in the 12 years prior to the introduction of the ENS for these diseases. The authors present data on the patient-relevant outcome of hospitalization with different operationalizations.

The Lund 2020 study [28] is an assessment of ENS in Denmark, which also includes the target diseases of interest in this benefit assessment, PA, as well as subtypes of MMA that have not been conclusively described. For this purpose, in the first years of the programme from 2002 onwards, heel blood was collected on the 4th to 9th day of life and since 2009 on the 48th to 72nd hour of life, dripped onto filter paper and examined using tandem mass spectrometry. The 967,780 newborns screened between 2002 and 2019 were compared with all newborns not screened during this period (N = 82,930) and with a historical cohort of all children born and clinically diagnosed between 1992 and 2001. For this purpose, the authors planned to collect data on the frequency of diagnoses as well as data on complications of the respective diseases such as metabolic decompensation and developmental disorders. After excluding 2 false-positive cases, a total of 7 newborns with either PA or MMA were identified among those screened - no further details are given. In the same period, 1 case of PA was clinically diagnosed among those not screened. Information on the historical cohort is completely missing in the publication.

The Wilson 2012 study [29] includes an assessment of ENS in New Zealand. Of the target diseases relevant for IQWiG's benefit assessment, the analysis includes unspecified forms of HU, PA and certain subtypes of MMA. The information provided on the screening method is limited to the use of blood dripped onto filter paper and analysed using tandem mass spectrometry. The aim was to compare all cases recorded by various measures in the first 3 years after the start of the ENS (January 2007 to December 2009) with those diagnosed

clinically or due to a family case 3 years before its introduction (January 2004 to December 2006). In addition to general information on health status, the authors present data on the patient-relevant outcome of mortality. No cases of PA or MMA were reported for any of the periods considered. In contrast, there were 2 cases of clinically diagnosed HU among the unscreened children.

Some of the results of the 3 studies on the screening chain were not reported in full. It was not clear from the data to which target diseases and their subtypes the identified cases were assigned. Therefore, no reliable conclusion on the benefit (or harm) of screening for one of the target diseases can be derived.

4.3 Comparative intervention studies on the start of treatment

The following 13 studies were identified as comparative intervention studies on the start of treatment: Monagle 1995 [30], Yap 1998 [31], Mulvihill 2001 [34], Purcell 2017 [36], Walter 1998 [37], Strauss 2007 [38], Yverneau 2022 [39], Liu 2022 [40], Haijes 2020 [41], Ahrens-Nicklas 2017 [42], Bourque 2021 [43], Gizicki 2014 [44], and He 2020 [45]. As the results of the studies are not presented (see the following explanations and Section A4.3.2. of the full report), only a brief description of these studies follows, sorted according to the investigated subtypes of the 4 target diseases. Due to a lack of analysable data, a risk-of-bias assessment across outcomes was omitted.

Transcobalamin II deficiency-related neonatal vitamin B12 deficiency

For the Monagle 1995 study [30], all cases affected by transcobalamin II deficiency in an Australian children's hospital were identified retrospectively by means of a file review and analysed with regard to their long-term course. There is no information on the period in which the cases were identified. Five cases were identified, all of which presented with symptoms at less than 1 year of age (in 4 cases less than 2 months), with 1 further case only being diagnosed and thus treated for transcobalamin II deficiency at 2 years of age due to a misdiagnosis. The data could therefore be interpreted as a comparative intervention study on the start of treatment. The authors provide information on the neurological status of each case at the last available follow-up time. The follow-up periods after the start of treatment ranged from 6 months to 19 years.

The main reason why the Monagle 1995 study was not further considered is that it shows a relevant structural and observational inequality.

Pyridoxine-non-responsive CBS deficiency-based HU

The authors of Yap 1998 [31] retrospectively analysed the clinical course of all 25 patients with CBS deficiency-based HU identified during the 25 years of newborn screening (NBS) for this disorder in Ireland (1971 and 1996). They distinguished between 21 NBS-identified and 4 NBS-

overlooked (false-negative) cases that were only diagnosed clinically (during toddler age at the earliest; see below for details). The data could therefore be interpreted in the broadest sense as a comparative intervention study on the start of treatment. In the intervention group, treatment started within 8 to 42 days after birth, in the control group after diagnosis. According to the authors, the majority of the cohort (24 of 25 cases) consisted of pyridoxine-non-responsive CBS deficiency-based HU cases. At the time of analysis, the cases treated early were between 2.5 and 22.8 years old. In view of detection by NBS and the prompt start of treatment, this roughly corresponds to the length of the follow-up periods. In the control group, only 3 out of 4 children were treated. These were diagnosed between the ages of 2.4 and 7.0 years and had a follow-up period of 11.7 to 14.1 years. Information on individual patient data, contact details and the origin of the data presented lead to the conclusion that the publications Yap 1999 [32] and Yap 2001 [33] also essentially refer to the cohort on which Yap 1998 is based. As no additional relevant findings for the benefit assessment can be drawn from this, these two publications are only assigned to Yap 1998 and are not addressed further in the following text.

The main reason why the Yap 1998 study was not further considered for this report is that it exhibits a relevant structural and observational inequality. Apart from this, it should be mentioned that the data based on this population would only have been relevant for a very small proportion of those affected in the German health care context: This is because, in contrast to the pyridoxine-nonresponsive subtype, which is predominantly represented in Ireland and evaluated by the authors in the study, the pyridoxine-responsive subtype with less severe courses dominates among continental European patients with CBS deficiency-based HU [9,10].

The Mulvihill 2001 study [34] aimed to investigate the eye health of patients with CBS deficiency-based HU who were diagnosed clinically or had poor metabolic control of the disease compared to a control group of early diagnosed and well-controlled patients. For this purpose, data was collected retrospectively from the patient files of all patients with CBS deficiency-based HU who had visited the Metabolic Centre of the University Hospital in Dublin - further information on the period investigated is not available. According to the publication, the facility is the national treatment centre in Ireland for children with this disease. Based on the authors' explanations, it would be possible to allocate the data to a group diagnosed by NBS and subsequently treated and a group diagnosed clinically and subsequently treated, so that the data could be interpreted as a comparative intervention study on the start of treatment. Twenty-one cases diagnosed by NBS and 14 cases diagnosed clinically were included. NBS had been performed in 9 of the 14 symptomatic cases, which showed a negative result. While the NBS cases were all of the pyridoxine-non-responsive CBS deficiency-based HU subtype, over 70% (10/14) of the control group consisted of the same subtype. At the time of analysis, the cases identified via NBS were between 7 and 28 years old. In view of diagnosis via NBS and the

prompt start of treatment, this roughly corresponds to the follow-up periods. The cases in the control groups were diagnosed between the ages of 1.25 and 28 years and have a follow-up period of 0.5 to 32 years. At the time of analysis, these patients were between 6 and 36 years old (median: 24 years). Information on individual patient data, contact details and the origin of the data presented lead to the conclusion that Mulvihill 2004 [35] also essentially refers to the cohort on which Mulvihill 2001 is based. As no additional relevant findings for the benefit assessment can be drawn from this, this publication is only assigned to Mulvihill 2001 and not further addressed in the following text.

The main reason why the Mulvihill 2001 study was not further considered is that both the follow-up periods after the start of treatment and the age of the patients at the time of the last follow-up are not similar enough and no age-adjusted data are available. The study exhibits a relevant structural and observational inequality. Apart from this, it should be mentioned that the data based on this population would only have been relevant for a very small proportion of patients in the German health care context (see comments on Yap 1998).

Purcell 2017 [36] retrospectively analysed all patients with pyridoxine-non-responsive CBS deficiency-based HU who had visited the Metabolic Centre of the University Hospital in Dublin - further information on the period investigated is not available. According to the publication, the facility is the national treatment centre in Ireland for children with this disorder. The subject of the analysis was physical development in terms of growth and weight, which was compared between patients diagnosed by NBS and those diagnosed clinically. For this purpose, data was collected retrospectively from the patient files. The authors report that as part of the care of these patients, it was planned to collect such data at the ages of 3, 6, 9 and 12 months as well as 2, 4, 10, 12 and 18 years. Thirty-six cases diagnosed by NBS and 12 diagnosed clinically were included. At the time of analysis, the cases diagnosed by NBS were between 0.5 and 43 years old (mean 23.70 years). In view of the diagnosis via NBS and the prompt start of treatment, this corresponds approximately to the follow-up periods. The cases in the control group were diagnosed between the ages of 1.33 and 11.79 years (mean 5.09 years) and were between 11.97 and 52.59 years old at the time of the last data collection (mean 36.64 years).

The main reason why the Purcell 2017 study was not further considered is that, in view of the differences in the follow-up periods after the start of treatment between the intervention group and the control group, it is questionable whether and how all of the cases included were considered sufficiently in the analyses. Apart from this, it should be mentioned that the data based on this population would only have been relevant for a very small proportion of those affected in the German care context (see comments on Yap 1998).

It can be assumed that the studies Yap 1998 [31-33], Mulvihill 2001 [34,35] and Purcell 2017 [36] show overlaps in the patient populations, as the studies all drew on the data set of the

national treatment centre in Ireland for children with this disease. Due to a lack of usable data from these studies, this assumption was not tested.

The retrospective study Walter 1998 [37] aimed to analyse the treatment outcomes of all cases of CBS deficiency-based HU at the Royal Manchester Children's Hospital (United Kingdom) from 1962 onwards. From the information provided by the authors for each patient, it was possible to assign them to a group diagnosed by NBS and subsequently treated and a group diagnosed clinically and subsequently treated, so that the data could be interpreted as a comparative intervention study on the start of treatment. 12 cases diagnosed by NBS and 18 diagnosed clinically were included. While the NBS cases were all assigned to the pyridoxine-non-responsive CBS deficiency-based HU subtype, only 10 of the 18 cases diagnosed clinically were of this subtype. Relevant diagnostic details are not reported. At the time of analysis, the cases diagnosed by NBS were between 2 and 25 years old. In view of the diagnosis via NBS and the prompt start of treatment, this corresponds approximately to the follow-up periods. The cases in the control groups were diagnosed between the ages of 4 and 32 and have a follow-up period of 3 to 31 years. At the time of analysis, these patients were between 12 and 39 years old. The diagnoses documented include eye health, developmental disorders and scoliosis.

The main reason why the Walter 1998 study was not further considered is that both the follow-up periods after the start of treatment and the age of the patients at the time of the last follow-up are not similar enough. The study shows a relevant structural and observational inequality. In view of the small number of cases in the groups, adjustment on the basis of the available individual patient data is not feasible. In addition, essential information on the operationalization of the outcomes for the individual patient data and the way in which they were collected was missing, so that it is not possible to use the data for the benefit assessment. The criteria for a dramatic effect were also not met (see General Methods 7.0 [46]). Apart from this, it should be mentioned that the data based on this population would only have been relevant for a very small proportion of those affected in the German health care context: Because in contrast to the comparison with the subtype of pyridoxine-non-responsive CBS deficiency-based HU that can be generated from Walter 1998, the pyridoxine-responsive subtype with less severe courses dominates among continental European patients with CBS deficiency-based HU [9,10]. Further details on the Walter 1998 study can be found in Section A4.3.2 of the full report. There it serves as an example of a more detailed justification for the non-usability of data.

MTHFR deficiency-based HU

The authors of the Strauss 2007 study [38] present data on the 5 cases of MTHFR deficiency-based HU diagnosed in a clinic in the USA between October 2002 and September 2003. One of these cases was diagnosed by NBS, 4 were diagnosed clinically between the ages of 7

months and 18 years. The data could therefore be interpreted as a comparative intervention study on the start of treatment. The authors stated that the patients were to be followed at least by laboratory tests over a period of 4 years and were between 3 and 21 years old at the time of analysis. They also provided information on physical and neurological development.

The main reason why the Strauss 2007 study was not further considered is that the ages of the study population at the time of the last follow-up are not similar enough. The study shows a relevant structural and observational inequality.

According to the authors, the Yverneau 2022 study [39] is a retrospective multicentre cohort study. The publication is based on an analysis of the European Network and Registry for Homocystinurias and Methylation Defects (E-HOD Register), which was planned prospectively. This analysis identified 62 cases of MTHFR deficiency-based HU from 32 European and North American centres. This cohort was supplemented by 10 patients recruited separately in France (from 7 centres). One of the authors' aims was to investigate prognostic factors for severe neurological manifestations. This multiparametric analysis also included the comparison between earlier and later treatment initiation. Due to a lack of complete data, 64 of the 72 cases were included in these analyses. These had a follow-up period of between 94 days and 37.4 years (median: 8.2 years), although this is not consistent with the fact that 35 people were followed up for more than 11 years. At the same time, no comparative information is available on the follow-up period after the start of treatment or the age of those affected at the time of the last follow-up.

The main reason why the Yverneau 2022 study was not further considered is that the follow-up periods after the start of treatment are not similar enough and, in particular, the analyses do not show any adjustment for age at the last time of follow-up. The study shows a relevant structural and observational inequality.

Propionic acidaemia

The aim of the Liu 2022 study [40] was to retrospectively analyse the 60 cases of PA diagnosed at Beijing First University Hospital between January 2007 and December 2020 with regard to their clinical manifestation spectrum and the underlying gene variants. Among other things, the authors report data on mortality and state that 5 cases were diagnosed by NBS and the remaining 55 cases via clinical symptoms. At the time of analysis, the cases diagnosed by NBS were between 4 months and 16 years old. They had received their diagnosis between day 7 and day 11 after birth - information on details such as the time of blood sampling is missing. In contrast, the symptomatic cases received their diagnosis between 5 days and 4 years of age, having become symptomatic between 2 hours and 3.5 years of age (median 5 months) . At the time of analysis, they were between 2.4 months and 19 years old. It is unclear how many of these cases had received their diagnosis at a comparable time to the cases diagnosed

by NBS. If this were only a small proportion, the study could be interpreted as a comparative intervention study on the start of treatment.

The main reason why the Liu 2022 study was not further considered is that both the follow-up periods after the start of treatment and the age of the patients at the time of the last follow-up are not similar enough and no age-adjusted data were available. The study shows a relevant structural and observational inequality. In purely numerical terms, the data (e.g. on mortality and epileptic seizures) were also not suitable to fulfil the criteria of a dramatic effect (see General Methods 7.0 [46]).

The Haijes 2020 study [41] is a national retrospective cohort study in which the clinical course (including acute metabolic decompensation, hospitalization) of 76 of the 83 Dutch cases of PA (N = 31) or MMA (N = 45) were analysed. For this purpose, the patient files of all 6 Dutch metabolic centres were reviewed. The diagnosis period refers to the period January 1979 to July 2019. The comparisons made by the authors between patients who were diagnosed due to a previous familial index case (familial screening group, FSG) and the course of the index cases themselves could be interpreted as a comparative intervention study on the start of treatment. According to the authors, such comparisons were possible for 5 PA sibling pairs and one sibling trio as well as 3 MMA sibling pairs. The PA-FSG cases were between 0 days and 2 years old at the time of diagnosis and were between 1.4 and 33.4 years old at the time of analysis. Patients in the control group were diagnosed between 3 days and 5 years of age and were between 2.9 and 37 years old at the time of analysis. The differences between the ages at diagnosis within the PA pairs varied between 3 days and 3 years.

The main reason why the Haijes 2020 study was not further considered is that both the follow-up periods after the start of treatment and the age of the patients at the time of the last follow-up are not similar enough and, due to the age differences within the PA pairs at the time of diagnosis, the delays until the start of treatment are also not similar enough. The study shows a relevant structural and observational inequality.

MMA with HU

In the Ahrens-Nicklas 2017 study [42], all 12 cases identified by NBS and affected by cblC-deficiency-based MMA with HU that were treated in an American children's hospital (Children’s Hospital of Philadelphia) between 1999 and 2015 were retrospectively analysed with regard to their long-term course, among other things. 10 cases were diagnosed 4 to 7 days after birth, for another case the time of diagnosis was unknown. In contrast, 1 newborn with an abnormal NBS test was referred late for analysis and was therefore not diagnosed until day 34. Assuming that a disease-specific treatment started with the diagnosis, the data could be interpreted as a comparative intervention study on the start of treatment. Among

other things, the authors provide information on aspects of eye health for each case at the last available follow-up time.

The main reason why the Ahrens-Nicklas 2017 study was not further considered is that both the follow-up periods after the start of treatment (7 months to 16 years) and the age of the patients at the time of the last follow-up are not similar enough. The study shows a relevant structural and observational inequality.

The Bourque 2021 study [43] is a retrospective cohort study that included all cases affected by cblC deficiency-based MMA with HU that had been diagnosed and treated at the Children's Hospital of Toronto, Canada, by April 2016. The authors distinguished between 13 cases that had been identified as part of the NBS that had been ongoing for 10 years at the time of the analysis (newborn screening group, NSG), and 11 cases that had only been identified via clinical symptoms (late diagnosis group, LDG), as well as 2 cases that had been identified due to a familial case (FSG group). Of these, at least the data on the NSG and LDG groups could be interpreted as a comparative intervention study on the start of treatment. For all patients, data on clinical manifestations were collected retrospectively from the patient files and the authors report data on eye health, seizures and cardiovascular health, among other things. At the time of analysis, the cases in the NSG group were between 10 months and 7.6 years old. In view of detection by NBS and the prompt start of treatment, this roughly corresponds to the length of the follow-up periods. Patients in the control group were diagnosed between the ages of 1 month and 12.8 years and were between 3.5 and 18 years old at the time of the analysis.

The main reason why the Bourque 2021 study was not further considered is that both the follow-up periods after the start of treatment and the age of the patients at the time of the last follow-up are not similar enough. The study shows a relevant structural and observational inequality.

The Gizicki 2014 study [44] retrospectively analysed all cases affected by cblC deficiency-based MMA with HU that were referred to the eye clinic of a Canadian university children's hospital (Centre Hospitalier Universitaire Sainte-Justine) between 1984 and 2012 with regard to their long-term ophthalmological course. 10 cases were diagnosed as a result of urine-based NBS. In contrast, 2 cases were diagnosed only at the age of 9 months and 2 years. As the disease-specific treatment started with the diagnosis, the data could be interpreted as a comparative intervention study on the start of treatment. The authors provide information on mortality and aspects of eye health for each case at the last available follow-up time.

The main reason why the Gizicki 2014 study was not further considered is that both the follow-up periods after the start of treatment (0 to 23 years) and the age of the patients at the time

of the last follow-up (2.5 to approx. 23.5 years) are not similar enough. The study shows a relevant structural and observational inequality.

The He 2020 study [45] aimed to retrospectively investigate which factors cause different phenotypes or courses in cases affected by cblC-deficiency-based MMA with HU who are homozygous carriers of the variant most commonly found in China. The authors analysed the 149 cases diagnosed by gene sequencing at the First Beijing University Hospital from January 1998 to December 2019. These consisted of 2 cases that had been prenatally diagnosed and treated from birth, 132 cases that had been diagnosed clinically and 15 cases that had been diagnosed by NBS. For the cases diagnosed clinically, only the time window of diagnosis (3 days to 101 months) is reported and there is no information on the length of the follow-up period or the current age at the time of the analyses. This is a major reason why data or analyses on these cases could not be used for the benefit assessment. In contrast, the information on the 15 cases identified via NBS could be interpreted in the broadest sense as a comparative intervention study on the start of treatment. This is because the authors report that 10 of these children (hereinafter “NSG group”) were treated directly after receiving the diagnosis, which was the case at the age of 15 days. The remaining 5 children (hereafter referred to as “control group”) only started treatment when symptoms appeared, which was the case at the age of 1 to 4 months. The reason for this was that their parents had refused a confirmatory diagnosis and pre-symptomatic treatment. It is reported that the children in the NSG group were 2 to 8 years old at the time of the analysis and had shown normal psychomotor and physical development up to that point. In contrast, there is no information on the follow-up of the children in the control group in the publication. All children had developmental delays at the time of symptom onset. Two children also had hydrocephalus at this time and one child had seizures. Further information on these cases is only found in the discussion. The authors state that these children had severe mental and motor retardation.

The main reason why the He 2020 study was not further considered is that there was a lack of information on the operationalization of outcomes and the way in which they were collected, making it impossible to assess the clinical relevance of the developmental disorders described. In view of the short period of time (1 to 4 months) and the plasticity of the brain as well as the possibility of achieving developmental progress after the introduction of therapeutic measures, the data were not suitable to fulfil the criteria for a dramatic effect (see General Methods 7.0 [46]). Irrespective of this, the transferability of the data based on this population to the German health care context is very questionable: In contrast to the comparison of children who are homozygous carriers of the genetic variant c.609G>A, which is prevalent in China and can be generated from He 2020, this variant is only sporadically present in the Caucasian population [47].

4.4 Diagnostic accuracy studies

Since neither the intervention studies on the screening chain nor the intervention studies on the start of treatment yielded a positive conclusion on the benefit of an earlier start of treatment, no diagnostic accuracy studies were used for the benefit assessment in this report.

4.5 Summary assessment of results

Evidence map

As no data are available for a benefit-harm assessment, no evidence map is shown.

Assessment of the scope of unpublished data

No relevant study without reported results was identified (see Section A3.1.4 of the full report). Therefore, there was no restriction of the certainty of conclusions in the present benefit assessment.

4.5.1 Weighing of benefits and harms

Comparative intervention studies on the screening chain

The 3 included comparative intervention studies on the screening chain were unable to provide any meaningful data for an assessment of the benefits or harms of ENS for vitamin B12 deficiency and other target diseases. The reason for this was that some of the results of the 3 studies on the screening chain were not fully reported. It was not clear from the data to which target diseases and their subtypes the identified cases were assigned.

Comparative intervention studies on the start of treatment

For acquired vitamin B12 deficiency, no comparative intervention study on the start of treatment could be identified that could provide the information described in Section A2.2 of the full report with regard to the criteria for study inclusion. The 13 comparative intervention studies included referred to genetic vitamin B12 deficiency and subtypes of HU, PA or MMA. Although the 13 studies sufficiently met the criteria described in Section A2.2 of the full report for study inclusion, they were unable to provide evaluable data for a benefit assessment of earlier versus later treatment initiation. Almost without exception, the reason for this was that both the duration of follow-up after the start of treatment and/or the age of the patients at the time of the last follow-up were not similar enough and the studies therefore showed both a relevant structural and observational inequality. Apart from this, based on the populations analysed, in some cases the data would only have been relevant for a very small proportion of patients in the German health care context. This is because the metabolic disorders underlying their disease do not occur in the majority of the German population and are also associated with a poorer prognosis.

As neither the intervention studies on the screening chain nor the intervention studies on the start of treatment yielded a positive conclusion on the benefit of starting treatment earlier, no diagnostic accuracy studies were used for the benefit assessment in this report. It is therefore not possible to present the number of false-positive and false-negative findings in the context of the ENS. Irrespective of this, considerations on possible benefit/harm and health-related consequences (based on selected literature, the statements of the external expert and impressions from the hearing procedure) can be presented both for the genetic target diseases and for acquired vitamin B12 deficiency.

4.5.1.1 Specific aspects of the genetic target diseases

With regard to genetic diseases, it is discussed in the literature in connection with false-positive screening results that such psychological stress can have an impact on parents' and doctors' perception of the child's state of health. See, for example, Karaceper et al. 2016 [48] who found higher health care utilization in the first year of life in Ontario among children with false-positive ENS results for medium-chain acyl-CoA dehydrogenase deficiency[3] compared to children with negative results. There is no obvious reason to assume that this situation would not also be transferable to the health care context here. Even in view of the offer of multiple examinations in the first year of life ("U examinations"), it can be assumed that parents of children who have received a false-positive screening result will, for example, consult a doctor more frequently out of uncertainty in the event of symptoms in their newborn/infant than parents of children who have not been notified of any abnormal screening results. With a screening algorithm such as that of the Heidelberg Pilot Screening Programme, such effects are also conceivable for acquired vitamin B12 deficiency.

In the case of a false-negative screening result, which was observed in some of the studies consulted, an associated delay in diagnosis and treatment can mean harm for the child if parents and medical staff do not follow up such a suspicion with further clarifying diagnostic tests in the event of clinical symptoms for one of the target diseases due to the inconspicuous screening result, or only after a delay [34]. Whether and, if so, what significance this problem has in the current German health care context is unclear. Ideally, a screening offer would increase awareness of the relevant target diseases and thus make early diagnosis more likely in symptomatic patients, rather than not considering these diseases due to an inconspicuous screening result.

Possible harm due to overdiagnosis and, as a result, overtreatment is also possible in principle for the target diseases examined here and has been discussed: For example, Haijes et al. 2020 do this with reference to people with PA who were only diagnosed at the age of 48 and 56 due to cardiological symptoms [41]. Irrespective of whether earlier treatment would have

[3] A rare congenital metabolic disorder covered by the ENS in Germany (cf. [26]).

prevented these symptoms, they classify lifelong dietary treatment in patients with mild courses of PA as overtreatment or serious risk of ENS. In order to deal with the given heterogeneity in the manifestations of the diseases, the possible use of mutation analyses to identify those with mild courses has been discussed in this context (see Barends 2014 [27]). The authors are rather critical of this. However, as almost all paediatric patients diagnosed with a treatable rare, genetic metabolic disease in Germany are linked to a specialized metabolic centre, the extent of overtreatment may be limited, as the centres have special expertise in the diseases and regular follow-up checks with biochemical monitoring and nutritional assessments take place during treatment. According to experts, treatment can be completely discontinued in individual cases.

4.5.1.2 Summary assessment of the genetic target diseases

The benefit of screening for HU, MMA and PA is unclear due to a lack of analysable data. In view of the weak genotype-phenotype correlation and the solely disease-modulating treatment available to date for the treatment of the genetic target diseases considered here, there is also no basis on which to make assumptions about a patient-relevant benefit for those affected: The chronic toxicity of the toxic metabolites that continue to accumulate will result in more or less, sooner or later, cumulative morbidity, the extent of which is, however, difficult to predict. Against this background, the position that the introduction of screening for the genetic target diseases considered here should only be considered once a causal treatment has been developed and made available should be evaluated [41]. Independently of this, it should be noted that serious symptoms, including death, can develop even before the screening test is carried out or during its analysis, particularly in people with PA or MMA. The frequency of occurrence and the corresponding effects on the assessment of a possible benefit of screening are unclear.

As described, on the one hand, there is an unclear benefit, and on the other hand, there is potential harm from potential overtreatment, delayed diagnosis and treatment delays (due to false-negative screening results), and psychological distress for parents (due to false-positive screening results).

Overall, the benefit or harm of screening for HU, PA and MMA as part of the ENS remains unclear.

4.5.1.3 Specific aspects of acquired vitamin B12 deficiency

No comparative intervention studies are available for acquired vitamin B12 deficiency, neither on the screening chain nor on the start of treatment.

Nevertheless, the following (positive) conclusions on benefit can be inferred:

There is a consensus among experts that early and timely administration of vitamin B12 (starting in the neonatal period) prevents possible irreversible damage from acquired vitamin B12 deficiency in newborns. This treatment is causal, short-term and associated with a very low risk of harm to the newborn. Vitamin B12 is a cofactor for many metabolic pathways; a deficiency leads to the accumulation of neurotoxic metabolites (e.g. methylmalonic acid, homocysteine). In newborns with vitamin B12 deficiency at birth, severe morbidity and mortality are unavoidable in the medium term without any vitamin B12 supplementation. Newborns who are partially breastfed at an early age and receive infant formula are less at risk. Screening is therefore advantageous with regard to an early and timely start of treatment, especially as newborns are generally still symptom-free (latency phase) at the time of a conspicuous screening finding.

This is supported by data from the Survey Unit for Rare Paediatric Diseases in Germany (ESPED) despite the very high uncertainty associated with the data: Fewer children with symptoms were reported in the screened group than in the non-screened group (see Chapter 5).

In this context, it should be noted that there is currently no clear consensus case definition of acquired vitamin B12 deficiency that would allow identifying those newborns who would actually benefit from treatment (see Mütze et al. 2022 [49]). It is therefore not relevant that the test accuracy of a screening strategy to detect vitamin B12 deficiency was not examined in detail in this report. This is because study data on test accuracy can only be analysed if a sufficiently clear case definition is available. On the other hand, there is no doubt that the sensitivity of a screening strategy is (presumably considerably) higher than 0%, so that in any case more affected infants could be diagnosed at an early, preferably asymptomatic stage than has been the case to date. For the practical implementation of a screening strategy, however, the questionable case definition means that a screening/treatment algorithm cannot be specified with certainty at present.

On the side of harm, the following aspects are important: the burden of the waiting time from the abnormal findings of a screening test to the final diagnosis and possible short-term overtreatment through supplementation with vitamin B12.

The psychological strain on parents will also depend on the waiting time until the final diagnosis. In particular, however, the common (re-)admission to hospital of the newborn can be very unsettling for parents. If the screening algorithm does not include a specific classification of acquired vitamin B12 deficiency, this can be an additional psychological burden. In the event of an abnormal screening result, a detailed, empathetic medical discussion and a targeted and rapid clarification and treatment should take place in order to minimize the burden on the parents.

The duration of any overtreatment of acquired vitamin B12 deficiency is limited, as the condition is only treated for a short time (see Chapter 1). An exclusively oral treatment regimen for newborns with vitamin B12 deficiency is currently being tested [1]. As stated in the commenting procedure on the preliminary report, the treatment is less stressful for the affected newborn and its relatives due to the option of oral administration. However, no consensus has yet been reached on the dosage and duration of this new treatment recommendation. But according to experts, non-invasive oral treatment is not used during the period where the diagnosis is clarified. During this period, the newborns are administered high-dose vitamin B12 parenterally (intravenously if possible, otherwise intramuscularly) for at least 3 consecutive days. This is done as a precautionary measure in order to treat the diseases in question (genetic diseases of the vitamin B12-responsive subtypes of MMA and enteral absorption disorders) from the earliest possible point in time until the results of a confirmatory diagnostic test are available, thus minimizing the risk of subsequent irreversible damage. The information from the summary of product characteristics of the products used indicates a very rare frequency of side effects (<1/10000; "anaphylactic or anaphylactoid reactions" and "acne, eczematous and urticarial drug reactions") [50].

4.5.1.4 Summary assessment of acquired vitamin B12 deficiency

Overall, these considerations provide a hint of a benefit of screening for acquired vitamin B12 deficiency. Ultimately, there is a considerable benefit in individual cases (avoiding the irreversible damage of an acquired vitamin B12 deficiency) and very little harm (psychological stress and vitamin B12 administration in the event of a false-positive result).

5 Classification of the assessment result

Available evidence on the topic

Vitamin B12 deficiency, HU, PA and MMA are rare or very rare diseases. They can jeopardize the motor and neurocognitive development of children and, depending on the underlying disease, the life of a newborn may be at risk (see Chapter 1). Against this background (rarity and severity of the diseases) and the assumption that dramatic effects were conceivable and that RCTs seemed unlikely, the methods were adapted accordingly. This means that the requirements for the level of evidence were greatly reduced and comparative cohort studies (including retrospective studies / studies with historical controls) could also be included. Using this approach, 3 comparative intervention studies on the screening chain and 13 comparative intervention studies on the start of treatment could be included. However, these studies did not provide any meaningful evidence that would have been suitable for evaluating the benefits or harms of screening for vitamin B12 deficiency and other target diseases. The reasons for this are explained in Sections 4.2 and 4.3.

Possibilities of assessing ENS for the target diseases

In principle, it would be possible to assess the benefit of ENS for vitamin B12 deficiency and the other target diseases. Non-randomized studies, especially those that compare a screened cohort with an unscreened cohort and report patient-relevant outcomes, would probably also be suitable for this purpose. In view of the problems of the 3 included intervention studies on the screening chain (see Section 4.2), however, it is clear that large cohorts are required in view of the rarity of the diseases. International registries would be suitable for overcoming this problem, provided that key criteria were fulfilled such as completeness in the recording of cases as well as completeness of follow-up, uniform data collection times with suitable (i.e. validated) instruments as well as the systematic recording of diagnostic and therapeutic procedures and confounders. As some countries in Europe have established NBS (see Section A4.2 of the full report), multinational cooperation could also enable a direct population-based comparison of the advantages and disadvantages of screening. These are infants and children whose development is still progressing, so data on or analyses of patient-relevant outcomes, for example, would be required where the children in the groups to be compared were of a similar age or adequately adjusted for age at the time of data collection. As the evaluation of neuroblastoma screening over 20 years ago [51] shows, such a project in terms of a non-randomized study comparing different regions would also be conceivable at the national level in the future. The first basic prerequisites for such a project or cooperation efforts are probably already in place, as the ongoing activities surrounding the Heidelberg Pilot Screening Programme and ESPED (see following section) suggest. In the case of the latter, the problems of completeness and reporting quality in particular would have to be overcome and the duration of data collection (previously usually 1 to 2 years) adapted for the purposes of the study. The existing infrastructure for the care of those affected also suggests that the above criteria are feasible in principle: There are approximately 15 to 20 metabolic centres in Germany where almost all patients with genetic metabolic disorders are cared for. These are registered via the Working Group for Paediatric Metabolic Disorders (APS, [52]) or Working Group for Congenital Metabolic Diseases in Internal Medicine (ASIM, [53]) and are networked with each other.

Relevance of the Heidelberg Pilot Screening Programme for the benefit assessment

During the hearing on the preliminary report, the participants referred to the Heidelberg Pilot Screening Programme and the associated publications [12,24,54-56].

This is a multi-centre, single-arm study with the aim of systematically following up children and adolescents who were identified via ENS and whose diagnosis was subsequently confirmed. Compared to the existing NBS, the newborns are additionally tested for 28 target diseases (including the diseases relevant to the present assessment: HU, MMA, PA and vitamin B12 deficiency). The aim is to examine whether an extension of the existing ENS fulfils

essential criteria for population screening, particularly with regard to technical feasibility, process quality and medical benefit [57,58].

Most of the references cited in the comments on the preliminary report had already been identified and evaluated via the information retrieval carried out for the preliminary report. All other references provided, including those provided in the course of the oral debate, were checked for their relevance to the final report: No publication attributable to the Heidelberg Pilot Screening Programme is relevant for the assessment. The available publications are not usable with regard to the research question on the screening chain or on the bringing forward of treatment, as they are not comparative studies: Only patients identified early in the course of newborn screening were followed up.

A current analysis includes a total of 548,707 newborns who completed the pilot screening programme between August 2016 and September 2022 [12] and reports the prevalence and positive predictive value (PPV) of the target diseases considered here. The PPV data refer to the initial suspected diagnosis.

A birth prevalence of 1:3586 and a PPV of 0.39 are reported for acquired vitamin B12 deficiency. A PPV of 0.39 means that 39% of the cases tested positive were actually positive. Thus, assuming a sensitivity of 1 in 3586 screened newborns, only 1.5 false-positive cases would occur.

A birth prevalence of 1:137,177 and a PPV of 0.07 is reported for PA. For HU, a birth prevalence of 1:548,707 and PPV 0.13 is reported (for isolated remethylation disorders: 1:182,902; 0.06). For MMA, a birth prevalence of 1:109,741 and a PPV of 0.57 is reported (for combined remethylation disorders: 1:548,707; 0.33).

A further analysis of the ESPED for the years 2021 and 2022 included in the comments on the preliminary report aimed to determine the incidence of symptomatic vitamin B12 deficiency with neurological symptoms in the first year of life in relation to the presence of a pilot ENS for vitamin B12 deficiency [59]. The detailed analysis in an international journal announced for these data has not yet been published.

Although a cohort (N = 31) screened via the pilot screening centres (Heidelberg, Hanover, Munich) was compared with a cohort consisting of cases diagnosed clinically (N = 29) from the screening centres of Weiden, Saxony/Thuringia, Dresden, Berlin/Brandenburg, Hamburg, Greifswald, Magdeburg and Frankfurt for the ESPED analysis, the ESPED data appear to be less reliable, as it is associated with a high degree of uncertainty due to incomplete reports: Of the total of 79 reports received, only 60 data records could be used, partly due to data entries still missing at the time of analysis. In addition, of the 29 reported symptomatic cases, it is unclear whether or not screening for vitamin B12 deficiency was carried out in 11 cases.

In addition, a large number of unreported cases can be assumed: Fewer cases were reported overall from the regions where, on the one hand, there were more births overall and, on the other hand, ENS was performed in almost all children than from the smaller regions without ENS. It would be expected that an ENS should increase the number of reported cases, as pre-symptomatic children should also be detected there. This consideration presumably also explains the authors' comment when they note that a number of unreported cases can be assumed [59].

Irrespective of this, the publication contains neither information on the operationalization of the reported symptoms (described only as neurological symptoms [including developmental disorder, muscular hypotonia, epileptic seizures, cerebral atrophy]) nor references to follow-up data.

In the few reported cases, the number of children diagnosed clinically in the group of fully screened children is much lower (4 out of 688,200) than in the group without screening (14 out of 584,800). Despite all the uncertainties mentioned, this can be used to support a possible benefit of an ENS for acquired vitamin B12 deficiency.

Possible scenarios based on the findings of this report

Since preventive approaches to vitamin B12 intake in pregnant women are very helpful, but do not provide an optimal solution to the problem (see Section A4.5 of the full report), the situation with the derivation of a hint of a benefit of the ENS for acquired vitamin B12 deficiency is as follows:

In the case of a test using the usual dry blood markers, an abnormal finding over several days usually does not allow a distinction to be made between a possible acquired vitamin B12 deficiency, which would only require short-term treatment, or a genetic target disease, which would require lifelong treatment. A definitive diagnosis can only be determined in the subsequent confirmatory diagnostic tests, which take several weeks (see also A4.4 of the final report).

According to experts, an exclusive and direct screening for acquired vitamin B12 deficiency (and thus a possible exclusively oral and therefore non-invasive supplementation) is currently not feasible in the context of an ENS, as a direct determination of holotranscobalamin using dried blood on filter paper is not technically established. Overall, this means that screening for the 4 target diseases relevant here is interlinked and cannot be carried out separately - at least not in the laboratory analysis process. Nevertheless, from the available clinical-scientific data and medical considerations, a (hint of a) benefit can currently only be recognized for screening for one of the 4 target diseases. Even if, conversely, no harm has been shown from screening for the other 3 target diseases, it must still be considered in what form a screening programme might be useful. Several scenarios are conceivable:

1) Sole vitamin B12 deficiency screening: In the event of a conspicuous finding, only the relevant information regarding the acquired vitamin B12 deficiency is communicated to the parents and clarified in a targeted manner. The Children's Directive already provides for the possibility that screening results for certain diseases should not be communicated but "destroyed immediately" [26]. However, this refers to a situation in which - unlike here - no test abnormality has yet been reported. This option therefore leads to medical, ethical and legal problems.
2) Screening for all vitamin B12-associated target diseases: As the screening parameters for acquired vitamin B12 deficiency and genetic metabolic diseases are identical and exclusive and direct screening in the context of an ENS via dried blood is not established, all target diseases are included in the ENS based on a hint of a benefit for acquired vitamin B12 deficiency.
3) No screening: Since overall, there is only a hint of a benefit in the case of acquired vitamin B12 deficiency, so that overall the benefit of screening for all 4 target diseases remains questionable, none of the 4 target diseases is included in the ENS.

Overall, screening for vitamin B12 deficiency alone (option 1) hardly seems feasible. Particularly in cases where newborns are or become symptomatic shortly after birth, it would be difficult to justify ignoring or destroying the diagnostic test result potentially indicating a vitamin B12-associated metabolic disorder. When evaluating options 2 and 3, it should be borne in mind that acquired vitamin B12 deficiency occurs much more frequently than the other target diseases. Furthermore, the possible benefits, but also the possible harm of screening for genetic vitamin B12-associated target diseases must be considered; further evaluation would be useful in any case. However, as there is currently at least no discernible negative benefit-harm ratio of screening for vitamin B12-associated metabolic disorders and a benefit appears more likely, the best option for action is the introduction of screening for all vitamin B12-associated target diseases. However, health economic and possible other aspects are not taken into account in this consideration. A scientific evaluation of the screening programme is of great importance.

6 Conclusion

When weighing the possible benefit and harm of screening for acquired vitamin B12 deficiency, there is a hint of a benefit of screening as part of ENS. However, due to the lack of conclusive evidence from intervention studies on the screening chain and comparative intervention studies on the start of treatment, the benefit or harm of screening for HU, PA and MMA as part of ENS is unclear. But as screening for vitamin B12 deficiency alone seems technically unfeasible, joint screening for all 4 target diseases relevant here should be considered. A comparative concomitant evaluation of all target diseases is in principle possible with the existing structures and is also necessary in view of the scarce data.

References for English extract

Please see full final report for full reference list.

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Appendix A Search strategies

A.1 Searches in bibliographic databases

Search for systematic reviews

1. MEDLINE

Search interface: Ovid

- Ovid MEDLINE(R) ALL 1946 to November 22, 2022

The following filters were adopted:

- Systematic Review: Wong [60] – High specificity strategy

#	Searches
1	exp Vitamin B12/ or exp Vitamin B 12 Deficiency/
2	(vitamin* and (b adj1 "12")).ti,ab.
3	((vitamin* and b12) or (cobalamin* adj6 deficien*)).ti,ab.
4	or/1-3
5	exp infant/
6	(newborn* or neonat* or pediatric* or infant*).ti,ab.
7	or/5-6
8	and/4,7
9	(homocystinuria* or ((cystathionine* or mthfr*) adj6 deficien*)).mp.
10	((methylmalonic or malonic) adj6 (acid?emia* or aciduria*)).mp.
11	((propionic adj6 (acid?emia* or aciduria*)) or propionicaciduria* or propionicacidemia* or (propionyl adj1 CoA adj6 carboxylase adj6 deficien*) or (ketotic adj6 (glycinemia* or hyperglycinemia*))).mp.
12	or/8-11
13	Cochrane database of systematic reviews.jn.
14	(search or MEDLINE or systematic review).tw.
15	meta analysis.pt.
16	or/13-15
17	16 not (exp animals/ not humans.sh.)
18	and/12,17
19	18 and (english or german or multilingual or undetermined).lg.

2. International HTA Database

Search interface: INAHTA

#	Searches
1	"Vitamin B 12"[mhe]
2	"Vitamin B 12 Deficiency"[mhe]
3	((vitamin* AND (b12 OR b 12)) OR (cobalamin* AND deficien*))[Title] OR ((vitamin* AND (b12 OR b 12)) OR (cobalamin* AND deficien*))[abs]
4	#3 OR #2 OR #1
5	"Infant"[mh]
6	(newborn* OR neonat* OR pediatric* OR infant*)[Title] OR (newborn* OR neonat* OR pediatric* OR infant*)[abs]
7	#6 OR #5
8	#7 AND #4
9	(homocystinuria* OR ((cystathionine* OR mthfr*) AND deficien*))[Title] OR (homocystinuria* OR ((cystathionine* OR mthfr*) AND deficien*))[abs]
10	((methylmalonic OR malonic) AND (acidemia* OR acidaemia* OR aciduria*))[Title] OR ((methylmalonic OR malonic) AND (acidemia* OR acidaemia* OR aciduria*))[abs]
11	((propionic AND (acidaemia* OR acidemia* OR aciduria*)) OR propionicaciduria* OR propionicacidemia* OR (propionyl AND carboxylase AND deficien*) OR (ketotic AND (glycinemia* OR hyperglycinemia*)))[Title] OR ((propionic AND (acidaemia* OR acidemia* OR aciduria*)) OR propionicaciduria* OR propionicacidemia* OR (propionyl AND carboxylase AND deficien*) OR (ketotic AND (glycinemia* OR hyperglycinemia*)))[abs]
12	#11 OR #10 OR #9 OR #8

Search for primary studies: comparative intervention studies on the screening chain

1. MEDLINE

Search interface: Ovid

- Ovid MEDLINE(R) 1946 to January 20, 2023

#	Searches
1	exp Vitamin B12/ or exp Vitamin B 12 Deficiency/ or Transcobalamins/
2	(vitamin* and (b adj1 "12")).ti,ab.
3	((vitamin* and b12) or transcobalamin*).ti,ab.
4	Homocystinuria/
5	Methylenetetrahydrofolate Reductase (NADPH2)/df or "5,10-Methylenetetrahydrofolate Reductase (FADH2)"/df or "Methylenetetrahydrofolate Dehydrogenase (NADP)"/df
6	Cystathionine beta-Synthase/df
7	homocystinuria*.ti,ab.
8	(((methylenetetrahydrofolate* adj1 reductase*) or mthfr or (cystathionine adj1 beta adj1 synthase) or (methionine adj1 synthase)) adj3 defici*).ti,ab.
9	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g).ti,ab.
10	Propionic Acidemia/
11	(*Amino Acid Metabolism, Inborn Errors/ or *Metabolism, Inborn Errors/) and Propionates/bl
12	(propionic and (acid?emia* or aciduria*)).ti,ab.
13	(Methylmalonic Acid/ or Methylmalonyl-CoA Mutase/) and (Amino Acid Metabolism, Inborn Errors/ or Metabolism, Inborn Errors/)
14	(methylmalonic and (acid?emia* or aciduria*)).ti,ab.
15	(cobalamin adj3 (defici* or disease or defect*)).ti,ab.
16	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j).ti,ab.
17	(methylmalonyl adj1 CoA adj1 mutase adj3 defici*).ti,ab.
18	or/1-17
19	exp Infant/
20	(newborn* or neonat* or pediatric* or infant*).ti,ab.
21	or/19-20
22	Neonatal Screening/
23	and/18,22
24	*Mass Screening/
25	screen*.ti,ab.
26	or/24-25
27	and/18,21,26
28	or/23,27
29	(animals/ not humans/) or comment/ or editorial/ or exp review/ or meta analysis/ or consensus/ or exp guideline/
30	hi.fs. or case report.mp.
31	or/29-30
32	28 not 31
33	32 and (english or german or multilingual or undetermined).lg.

2. Embase

Search interface: Ovid

- Embase 1974 to 2023 January 20

#	Searches
1	exp B12 deficiency/ or *cyanocobalamin/ or exp cobalamin/ or transcobalamin/
2	(vitamin* and (b adj1 "12")).ti,ab.
3	((vitamin* and b12) or transcobalamin*).ti,ab.
4	homocystinuria/
5	homocystinuria*.ti,ab.
6	(((methylenetetrahydrofolate* adj1 reductase*) or mthfr or (cystathionine adj1 beta adj1 synthase) or (methionine adj1 synthase)) adj3 defici*).ti,ab.
7	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g).ti,ab.
8	propionic acidemia/
9	(propionic and (acid?emia* or aciduria*)).ti,ab.
10	methylmalonic acidemia/ or methylmalonic aciduria/
11	(methylmalonic and (acid?emia* or aciduria*)).ti,ab.
12	(cobalamin adj3 (defici* or disease or defect*)).ti,ab.
13	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j).ti,ab.
14	(methylmalonyl adj1 CoA adj1 mutase adj3 defici*).ti,ab.
15	or/1-14
16	exp infant/
17	(newborn* or neonat* or pediatric* or infant*).ti,ab.
18	or/16-17
19	newborn screening/
20	and/15,19
21	screen*.ti,ab.
22	and/15,18,21
23	or/20,22
24	23 not medline.cr.
25	24 not (exp animal/ not exp human/)
26	25 not (Conference Abstract or Conference Review or Editorial).pt.
27	26 not ((afrikaans or albanian or arabic or armenian or azerbaijani or basque or belorussian or bosnian or bulgarian or catalan or chinese or croatian or czech or danish or dutch or english or esperanto or estonian or finnish or french or gallegan or georgian or german or greek or hebrew or hindi or hungarian or icelandic or indonesian or irish gaelic or italian or japanese or korean or latvian or lithuanian or macedonian or malay or norwegian or persian or polish or polyglot or portuguese or pushto or romanian or russian or scottish gaelic or serbian or slovak or slovene or spanish or swedish or thai or turkish or ukrainian or urdu or uzbek or vietnamese) not (english or german)).lg.

3. The Cochrane Library

Search interface: Wiley

- Cochrane Central Register of Controlled Trials: Issue 1 of 12, January 2023

#	Searches
#1	[mh "Vitamin B12"] or [mh "Vitamin B 12 Deficiency"] or [mh ^"Transcobalamins"]
#2	(vitamin* and (b NEAR/1 "12")):ti,ab
#3	((vitamin* and b12) or transcobalamin*):ti,ab
#4	[mh ^"Homocystinuria"]
#5	[mh ^"Methylenetetrahydrofolate Reductase (NADPH2)"] or [mh ^"5,10-Methylenetetrahydrofolate Reductase (FADH2)"] or [mh ^"Methylenetetrahydrofolate Dehydrogenase (NADP)"]
#6	[mh ^"Cystathionine beta-Synthase"]
#7	homocystinuria*:ti,ab
#8	(((methylenetetrahydrofolate* NEAR/1 reductase*) or mthfr or (cystathionine NEAR/1 beta NEAR/1 synthase) or (methionine NEAR/1 synthase)) NEAR/3 defici*):ti,ab
#9	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g):ti,ab
#10	[mh ^"Propionic Acidemia"]
#11	([mh ^"Amino Acid Metabolism, Inborn Errors"] or [mh ^"Metabolism, Inborn Errors"]) and [mh "Propionates"]
#12	(propionic and (acid?emia* or aciduria*)):ti,ab
#13	([mh ^"Methylmalonic Acid"] or [mh ^"Methylmalonyl-CoA Mutase"]) and ([mh ^"Amino Acid Metabolism, Inborn Errors"] or [mh ^"Metabolism, Inborn Errors"])
#14	(methylmalonic and (acid?emia* or aciduria*)):ti,ab
#15	(cobalamin NEAR/3 (defici* or disease or defect*)):ti,ab
#16	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j):ti,ab
#17	(methylmalonyl NEAR/6 defici*):ti,ab
#18	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17
#19	[mh "Infant"]
#20	(newborn* or neonat* or pediatric* or infant*):ti,ab
#21	#19 or #20
#22	[mh ^"Neonatal Screening"]
#23	#18 and #22
#24	[mh ^"Mass Screening" [mj]]
#25	screen*:ti,ab
#26	#24 or #25
#27	#18 and #21 and #26
#28	#23 or #27
#29	#28 not (*clinicaltrial*gov* or *trialsearch*who* or *clinicaltrialsregister*eu* or *anzctr*org*au* or *trialregister*nl* or *irct*ir* or *isrctn* or *controlled*trials*com* or *drks*de*):so

#	Searches
#30	#29 not ((language next (afr or ara or aze or bos or bul or car or cat or chi or cze or dan or dut or es or est or fin or fre or gre or heb or hrv or hun or ice or ira or ita or jpn or ko or kor or lit or nor or peo or per or pol or por or pt or rom or rum or rus or slo or slv or spa or srp or swe or tha or tur or ukr or urd or uzb)) not (language near/2 (en or eng or english or ger or german or mul or unknown)))
#31	#30 in Trials

Search for primary studies: comparative intervention studies at the start of treatment

1. MEDLINE

Search interface: Ovid

- Ovid MEDLINE(R) 1946 to February 13, 2023

The following filters were adopted:

- RCT: Lefebvre [61] – Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity- and precision-maximizing version (2008 revision)
- Non-RCT: Search filter with best sensitivity for controlled NRS (Ovid MEDLINE, adapted from PubMed) [62]

#	Searches
1	exp Vitamin B12/ or exp Vitamin B 12 Deficiency/ or Transcobalamins/
2	(vitamin* and (b adj1 "12")).ti,ab.
3	((vitamin* and b12) or transcobalamin*).ti,ab.
4	Homocystinuria/
5	Methylenetetrahydrofolate Reductase (NADPH2)/df or "5,10-Methylenetetrahydrofolate Reductase (FADH2)"/df or "Methylenetetrahydrofolate Dehydrogenase (NADP)"/df
6	Cystathionine beta-Synthase/df
7	homocystinuria*.ti,ab.
8	(((methylenetetrahydrofolate* adj1 reductase*) or mthfr or (cystathionine adj1 beta adj1 synthase) or (methionine adj1 synthase)) adj3 defici*).ti,ab.
9	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g).ti,ab.
10	Propionic Acidemia/
11	(*Amino Acid Metabolism, Inborn Errors/ or *Metabolism, Inborn Errors/) and Propionates/bl
12	(propionic and (acid?emia* or aciduria*)).ti,ab.
13	(Methylmalonic Acid/ or Methylmalonyl-CoA Mutase/) and (Amino Acid Metabolism, Inborn Errors/ or Metabolism, Inborn Errors/)
14	(methylmalonic and (acid?emia* or aciduria*)).ti,ab.
15	(cobalamin adj3 (defici* or disease or defect*)).ti,ab.
16	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j).ti,ab.
17	(methylmalonyl adj1 CoA adj1 mutase adj3 defici*).ti,ab.
18	or/1-17
19	exp pediatrics/
20	(infan* or newborn* or new-born or perinat* or neonat* or baby or baby* or babies or toddler* or minors or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child or child* or children* or schoolchild* or schoolchild or adolescen* or juvenil* or youth* or teen* or under*age* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*).af.

#	Searches
21	(school child or school child* or school or school*).ti,ab.
22	or/19-21
23	randomized controlled trial.pt.
24	controlled clinical trial.pt.
25	(randomized or placebo or randomly or trial or groups).ab.
26	drug therapy.fs.
27	or/23-26
28	27 not (exp animals/ not humans.sh.)
29	exp cohort studies/ or exp epidemiologic studies/ or exp clinical trial/ or exp evaluation studies as topic/ or exp statistics as topic/
30	((control and (group* or study)) or (time and factors) or program or survey* or ci or cohort or comparative stud* or evaluation studies or follow-up*).mp.
31	or/29-30
32	(animals/ not humans/) or comment/ or editorial/ or exp review/ or meta analysis/ or consensus/ or exp guideline/
33	hi.fs. or case report.mp.
34	or/32-33
35	(28 or 31) not 34
36	18 and 22 and 35
37	36 and (english or german or multilingual or undetermined).lg.

2. Embase

Search interface: Ovid

- Embase 1974 to 2023 February 13

The following filters were adopted:

- RCT: Wong [60] – Strategy minimizing difference between sensitivity and specificity

#	Searches
1	exp B12 deficiency/ or *cyanocobalamin/ or exp cobalamin/ or transcobalamin/
2	(vitamin* and (b adj1 "12")).ti,ab.
3	((vitamin* and b12) or transcobalamin*).ti,ab.
4	homocystinuria/
5	homocystinuria*.ti,ab.
6	(((methylenetetrahydrofolate* adj1 reductase*) or mthfr or (cystathionine adj1 beta adj1 synthase) or (methionine adj1 synthase)) adj3 defici*).ti,ab.
7	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g).ti,ab.
8	propionic acidemia/
9	(propionic and (acid?emia* or aciduria*)).ti,ab.
10	methylmalonic acidemia/ or methylmalonic aciduria/
11	(methylmalonic and (acid?emia* or aciduria*)).ti,ab.
12	(cobalamin adj3 (defici* or disease or defect*)).ti,ab.
13	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j).ti,ab.
14	(methylmalonyl adj1 CoA adj1 mutase adj3 defici*).ti,ab.
15	or/1-14
16	exp pediatrics/
17	(infan* or newborn* or new-born or perinat* or neonat* or baby or baby* or babies or toddler* or minors or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child or child* or children* or schoolchild* or schoolchild or adolescen* or juvenil* or youth* or teen* or under*age* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*).af.
18	(school child or school child* or school or school*).ti,ab.
19	or/16-18
20	(random* or double-blind*).tw.
21	placebo*.mp.
22	or/20-21
23	and/15,19,22
24	23 not medline.cr.
25	24 not (exp animal/ not exp human/)
26	25 not (Conference Abstract or Conference Review or Editorial).pt.
27	26 not ((afrikaans or albanian or arabic or armenian or azerbaijani or basque or belorussian or bosnian or bulgarian or catalan or chinese or croatian or czech or danish or dutch or english or esperanto or estonian or finnish or french or gallegan or georgian or german or greek or hebrew or hindi or hungarian or icelandic or indonesian or irish gaelic or italian or japanese or korean or latvian or lithuanian or macedonian or malay or norwegian or persian or polish or polyglot or portuguese or pushto or romanian or russian or scottish gaelic or serbian or slovak or slovene or spanish or swedish or thai or turkish or ukrainian or urdu or uzbek or vietnamese) not (english or german)).lg.

3. The Cochrane Library

Search interface: Wiley

- Cochrane Central Register of Controlled Trials: Issue 2 of 12, February 2023

#	Searches
#1	[mh "Vitamin B12"] or [mh "Vitamin B 12 Deficiency"] or [mh ^"Transcobalamins"]
#2	(vitamin* and (b NEAR/1 "12")):ti,ab
#3	((vitamin* and b12) or transcobalamin*):ti,ab
#4	[mh ^"Homocystinuria"]
#5	[mh ^"Methylenetetrahydrofolate Reductase (NADPH2)"] or [mh ^"5,10-Methylenetetrahydrofolate Reductase (FADH2)"] or [mh ^"Methylenetetrahydrofolate Dehydrogenase (NADP)"]
#6	[mh ^"Cystathionine beta-Synthase"]
#7	homocystinuria*:ti,ab
#8	(((methylenetetrahydrofolate* NEAR/1 reductase*) or mthfr or (cystathionine NEAR/1 beta NEAR/1 synthase) or (methionine NEAR/1 synthase)) NEAR/3 defici*):ti,ab
#9	(cbld or cbld1 or cble or cblg or cbl d or cbl d1 or cbl e or cbl g):ti,ab
#10	[mh ^"Propionic Acidemia"]
#11	([mh ^"Amino Acid Metabolism, Inborn Errors"] or [mh ^"Metabolism, Inborn Errors"]) and [mh "Propionates"]
#12	(propionic and (acid?emia* or aciduria*)):ti,ab
#13	([mh ^"Methylmalonic Acid"] or [mh ^"Methylmalonyl-CoA Mutase"]) and ([mh ^"Amino Acid Metabolism, Inborn Errors"] or [mh ^"Metabolism, Inborn Errors"])
#14	(methylmalonic and (acid?emia* or aciduria*)):ti,ab
#15	(cobalamin NEAR/3 (defici* or disease or defect*)):ti,ab
#16	(cbla or cblb or cblc or cbld or cbld2 or cblf or cblj or cbl a or cbl b or cbl c or cbl d or cbl d2 or cbl f or cbl j):ti,ab
#17	(methylmalonyl NEAR/6 defici*):ti,ab
#18	#1 or #2 or #3 #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17
#19	[mh "pediatrics"]
#20	infan* or newborn* or new-born or perinat* or neonat* or baby or baby* or babies or toddler* or minors or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child or child* or children* or schoolchild* or schoolchild or adolescen* or juvenil* or youth* or teen* or under*age* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*
#21	(school child or school child* or school or school*):ti,ab
#22	#19 or #20 or #21
#23	#18 and #22
#24	#23 not (*clinicaltrial*gov* or *trialsearch*who* or *clinicaltrialsregister*eu* or *anzctr*org*au* or *trialregister*nl* or *irct*ir* or *isrctn* or *controlled*trials*com* or *drks*de*):so
#25	#24 not ((language next (afr or ara or aze or bos or bul or car or cat or chi or cze or dan or dut or es or est or fin or fre or gre or heb or hrv or hun or ice or ira or ita or jpn or ko or kor or lit or nor or peo or per or pol or por or pt or rom or rum or rus or slo or slv or spa or srp or swe or tha or tur or ukr or urd or uzb)) not (language near/2 (en or eng or english or ger or german or mul or unknown)))

#	Searches
#26	#25 in Trials

A.2 Searches in study registries

1. ClinicalTrials.gov

Provider: U.S. National Institutes of Health

- URL: http://www.clinicaltrials.gov
- Type of search: Expert Search

Search strategy
(EXPAND[Concept] "vitamin B12" OR transcobalamin OR homocystinuria OR cystathionine beta-synthase OR methylenetetrahydrofolate reductase OR mthfr OR propionic acidemia OR propionic aciduria OR methylmalonic acidemia OR methylmalonic aciduria OR cobalamin) AND AREA[StdAge] EXPAND[Term] COVER[FullMatch] "Child"

2. EU Clinical Trials Register

Provider: European Medicines Agency

- URL: https://www.clinicaltrialsregister.eu/ctr-search/search
- Type of search: Basic Search

Search strategy
(vitamin B12) OR transcobalamin OR homocystinuria* OR (cystathionine beta-synthase) OR (methylenetetrahydrofolate reductase) OR mthfr OR (propionic acidemia*) OR (propionic aciduria*) OR (methylmalonic acidemia*) OR (methylmalonic aciduria*) OR cobalamin* // Select Age Range: Under 18

3. International Clinical Trials Registry Platform Search Portal

Provider: World Health Organization

- URL: https://trialsearch.who.int
- Type of search: Standard Search

Search strategy
vitamin B12 OR transcobalamin OR homocystinuria OR cystathionine beta-synthase OR methylenetetrahydrofolate reductase OR mthfr OR propionic acidemia OR propionic aciduria OR methylmalonic acidemia OR methylmalonic aciduria OR cobalamin // Search for clinical trials in children
(vitamin B12 OR transcobalamin OR homocystinuria OR cystathionine beta-synthase OR methylenetetrahydrofolate reductase OR mthfr OR propionic acidemia OR propionic aciduria OR methylmalonic acidemia OR methylmalonic aciduria OR cobalamin) AND (child OR children OR newborn OR infant OR infants)