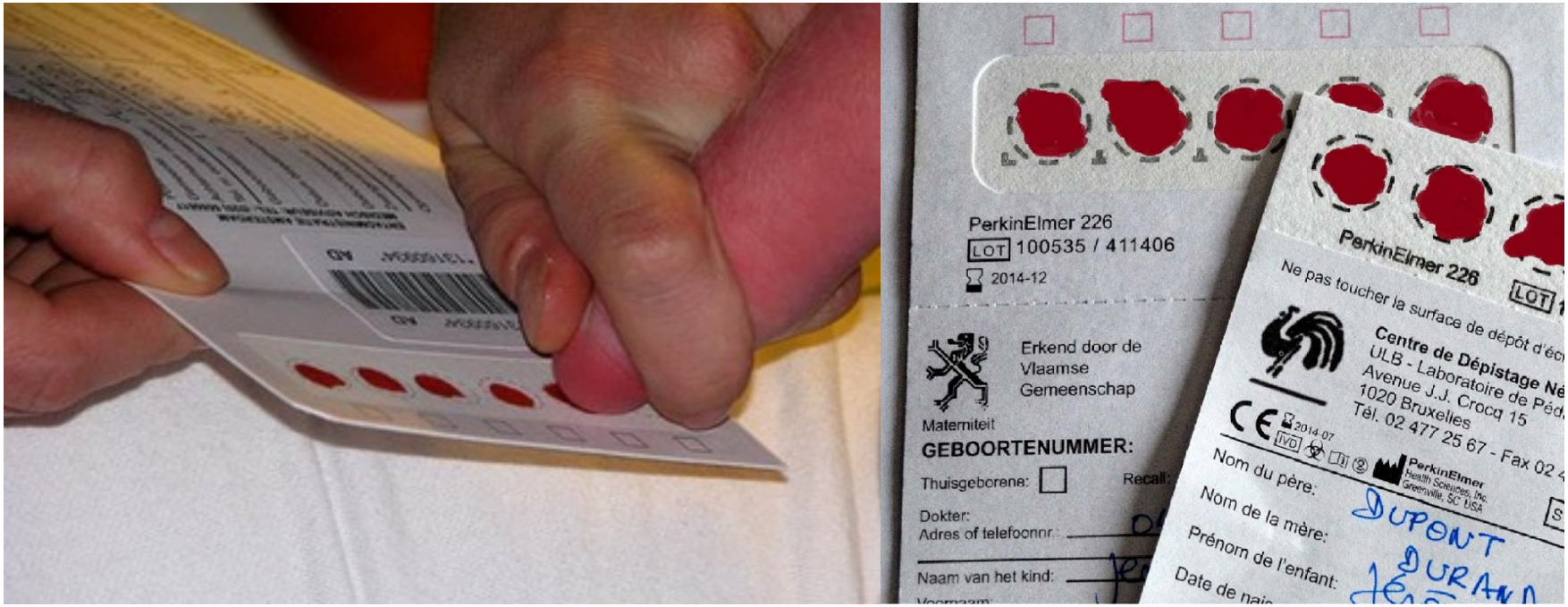
KCE REPORT 267S

Federaal Kenniscentrum voor de Gezondheidszorg
Centre Fédéral d'Expertise des Soins de Santé
Belgian Health Care Knowledge Center

# MULTI CRITERIA DECISION ANALYSIS TO SELECT PRIORITY DISEASES FOR NEWBORN BLOOD SCREENING

## SUPPLEMENT



KCE REPORT 267S
HEALTH SERVICES RESEARCH

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# MULTI CRITERIA DECISION ANALYSIS TO SELECT PRIORITY DISEASES FOR NEWBORN BLOOD SCREENING

## SUPPLEMENT

CHRIS DE LAET, GERMAINE HANQUET, ERIK HENDRICKX

# COLOPHON


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# 1. FURTHER DETAILS ON NEWBORN BLOOD SCREENING IN BELGIUM

## 1.1. Steering committee in FWB

**Le comité de pilotage est composé :**

1° d'un représentant de l'administration;

2° du coordinateur de chaque centre de dépistage;

3° d'un médecin spécialiste représentant les médecins responsables des centres spécialisés en maladies métaboliques héréditaires rares;

4° d'un médecin représentant la Société Belge de Pédiatrie;

5° d'un médecin pédiatre spécialisé en endocrinologie;

6° de deux médecins spécialistes en gynécologie-obstétrique des services de maternité représentant les associations d'hôpitaux;

7° de deux sages-femmes représentant respectivement, l'Union professionnelle des Sages-Femmes Belges (UPSFB) et l'Association

Francophone des Sages-Femmes Catholiques (AFSFC);

8° de deux représentants de l'Office de la Naissance et de l'Enfance;

9° d'un membre du Conseil supérieur de la promotion de la santé;

10° d'un expert en communication du service communautaire de promotion de la santé chargé de la communication;

11° d'un représentant du Ministre.

Les membres du comité de pilotage sont nommés par le Gouvernement pour une période de cinq ans renouvelable. Le Gouvernement nomme également, pour chaque membre effectif, un membre suppléant. Le membre suppléant ne siège qu'en l'absence du membre effectif. Un représentant de l'Institut scientifique de Santé publique ayant la qualification d'épidémiologiste est invité à chaque réunion du comité de pilotage, en qualité d'expert. Le comité de pilotage peut inviter d'autres experts de son choix. Tous les experts invités ont une voie consultative.

Le comité de pilotage accompagne le pilotage du programme par l'administration de la Communauté française. Sa composition est fixée par l'arrêté du 9 mai 2014 nommant les membres suivant :

1° en qualité de représentants de l'administration :
Tatiana PEREIRA, effectif, Jean-Michel ANTONUTTI, suppléant.
2° En qualité de coordinateur de chaque centre de dépistage :
a) François BOEMER , effectif; Roland SCHOOS, suppléant ;
b) Hilde LAEREMANS, effective; Philippe GOYENS, suppléant ;
c) Marie-Françoise VINCENT, effective; Sandrine MARIE, suppléante.
3° En qualité de représentant des médecins responsables des centres spécialisés en maladies métaboliques héréditaires rares :
Marie-Cécile NASSOGNE, effective; Corinne DE LAET, suppléante.
4° En qualité de représentant de la Société Belge de Pédiatrie :
Joëlle GOEDSEELS, effective; Catherine PIELTAIN, suppléante.
5° En qualité de médecin pédiatre spécialisé en endocrinologie :
Véronique BEAULOYE, effective; Claudine HEINRICHS, suppléante.
6° En qualité de médecins spécialistes en gynécologie-obstétrique des services de maternité représentant les associations d'hôpitaux :
a) Laura TECCO, effective; Véronique MASSON, suppléante;
b) Renaud LOUIS, effectif; Patricia STEENHAUT, suppléante.
7° En qualité de sages-femmes représentant respectivement l'Union professionnelle des Sages-Femmes Belges (UPSFB) et l'Association francophone des Sages-Femmes Belges (AFSFC) :
a) Claire MATAGNE, effective; Mélanie LAVENNE, suppléante;
b) Patricia BAEYENS, effective; Séverine DUBOIS, suppléante.
8° En qualité de représentant de l'Office de la Naissance et de l'Enfance :
a) Marie-Christine MAUROY, effective; Ingrid MORALES, suppléante;
b) Jacques LOMBET, effectif; Marianne WINKLER, suppléante.
9° En qualité de membre du Conseil Supérieur de la promotion de la santé:
Raffaele BRACCI, effectif; Michel CANDEUR, suppléant.
10 ° En qualité d'expert en communication du service communautaire de promotion de la santé chargé de la communication :
Bernadette TAEYMANS, effective; Patrick TREFOIS, suppléant.
11° En qualité de représentant de la Ministre chargée de la Santé

### 1.2. Steering committee in VG

Guidance is provided by a Steering Committee for population-wide health screening programmes which has the support of the Flemish working group for population-wide screening on congenital disorders in neonates by means of a blood sample with the responsibility to provide technical support, propose new diseases for screening, guide the programme, monitor quality indicators, and contribute to the sensitization of health professionals and institutions to neonatal screening[1]

# 2. ORIGINAL CRITERIA AND WEIGHING FROM THE INESSS REPORT (CITATION FROM THE ORIGINAL FRENCH LANGUAGE VERSION)

Citation from Côté et al :[2]

1. Incidence et prévalence au Québec
2. Gravité de la maladie
3. Disponibilité du résultat du test de dépistage en temps opportun
4. Efficacité d'un traitement précoce
5. Résultats faux positifs obtenus lors du test de dépistage (impact négatif)
6. Probabilité de résultats faux négatifs au test de dépistage (impact négatif)
7. Impact sur le système de santé

Le poids relatif de chaque critère est issu d'une pondération spécifique de chacun, dont le résultat global est soumis au consensus des participants.

Concernant les critères comme jugés pertinents quant à l'intervention évaluée, les données (probantes et contextuelles) ont été synthétisées dans des fiches synthèse par critère. Les participants ont ensuite mesuré la performance de la maladie (pondération) relativement à chaque critère retenu.

# 3. DISEASE INFORMATION ON BIOTINIDASE DEFICIENCY (LMCD)

## 3.1. Specific data sources for biotinidase deficiency

Orphanet database.[3]

Biotinidase deficiency family support group: http://biotinidasedeficiency.20m.com/

'Fiche synthèse' biotinidase deficiency from the INESSS report: https://www.inesss.qc.ca/fileadmin/doc/INESSS/Rapports/Genetique/Fiche_Synthese_BIOT.pdf

## 3.2. Background information on the disease (not to be scored)

Biotinidase deficiency, caused by mutations in the biotinidase gene (BTD gene), follows an autosomal recessive mode of inheritance. Approximately 100 mutations in the BTD gene that lead to biotinidase deficiency have been discovered. These mutations either prevent the enzyme production or reduce the enzyme activity. This disease is categorized as an organic aciduria.

Biotinidase recycles biotin, a vitamin from the B complex (B7), by extracting it from ingested food or endogenous sources (e.g. endogenous proteins). Biotin is an essential cofactor for several enzymes, known as carboxylases, which are required to process proteins, fats, or carbohydrates. Biotin is attached to these carboxylase enzymes through an amino acid (the building material of proteins) called lysine, forming a complex called biocytin. Biotinidase removes biotin from biocytin and makes it available to be reused by other enzymes.

ICD codes for LMCD are E53.8 for ICD-10 and 277.6 for ICD-9. MeSH term: 'Biotinidase Deficiency'. ORPHA79241: Biotinidase deficiency. [3]

Screening is currently performed only in the 'Vlaamse Gemeenschap' (VG).[4] For this screening the enzyme activity is measured using colorimetric or fluorimetric detection. Thresholds for each laboratory are determined on less than 10% of the average enzyme activity.[5] The therapy for detected patients is dietary, consisting in oral biotine supplementation.

## 3.3. Frequency of the disease (incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

The VG reported a birth prevalence of 2 / 100 000 in 2012-14 through the current screening period (Table 1).[5] This includes 1.5 / 100 000 cases of profound biotinidase deficiency (3 cases on 202 713 screened**).**(personal communication François Eyskens)

In Western Europe, birth prevalence of combined profound and partial biotinidase deficiency ranges between 0.9 and 2.9 / 100 000 live births,[4] and up to 4.5 in Northern America.[2, 6, 7]

**Table 1 – Birth prevalence rate of detected biotinidase deficiency in newborn screening programmes**

| Country, date | Source | Total screened | Rate cases by MS/MS per 100,000 per year |
|---|---|---|---|
| Belgium (VG), 2012-14 | Vlaams Agentschap Zorg en Gezondheid | 202 713 | 2.0 (4 cases) |
| Ontario, 2006-11 | Annual report to the Newborn and Child Screening Subcommittee[2, 8] | NA | 1.0 (6 cases) |
| US, profound deficiency, 2007-08 | Cowan et al.[2, 6] | NA | 1.3 |
| US, partial deficiency, 2007-08 | | | 3.2 |
| Worldwide, profound deficiency | INESSS report[2] | NA | 0.9 |
| Worldwide, partial deficiency | | | 0.8 |

## 3.4. Severity of the disease in untreated cases

If this condition is not recognized and treated, its signs and symptoms typically appear within the first few months of life, although it can also become apparent later in childhood in the milder forms. However, severe symptoms can even develop as early as 4 weeks postpartum: intractable epilepsy, brain damage (hearing impairment and cognitive dysfunction that do not improve under therapy with biotine supplementation.

Profound biotinidase deficiency, the more severe form of the condition, can cause seizures, hypotonia, breathing problems, hearing and vision loss, neurological problems including mental retardation, problems with movement and balance (ataxia), skin rashes, hair loss (alopecia), candidiasis. In some cases these complications might even lead to death. Affected children also have delayed development. Lifelong treatment can prevent these complications from occurring or improve them if they have already developed.

Partial biotinidase deficiency is a milder form of this condition. Without treatment, affected children may experience hypotonia, skin rashes, and hair loss, but these problems may appear only during illness, infection, or other times of stress.[7]

A European report (Weber et al.) describes follow-up results from 37 children with a profound biotinidase deficiency.[9] The majority of children with developmental retardation are those with a very low enzymatic activity as are those with an optical atrophy. Non-detected cases have a much higher probability for hearing and visual problems.

## 3.5. Timely availability of the test results

The disease is screened for by measuring the biotinidase activity on the neonatal blood sample. If positive the diagnosis should always be confirmed in an enzyme diagnostic test in serum.[4] However, if untreated or with late treatment biotinidase deficiency leads to irreversible symptoms such as hearing or vision loss and neurologic damage.[4]

The majority of infants develop symptoms between 3 and 6 months after birth.[2] Therefore the timely availability of the test result appears to be good with neonatal blood screening. In a Danish report from 2012, all three cases found though neonatal screening were asymptomatic at the moment of diagnosis.[2, 10]

## 3.6. Efficacy of early vs late treatment

The treatment is dietary (biotine supplementation) and should start as soon as possible and continued life-long.[2]. Since in patients with biotinidase deficiency the biotine recycling is blocked, a high concentration of biotine in the diet is needed to avoid the symptoms. A normal diet is, in general, deficient for this vitamin.[4] When diagnosed before symptoms occur, those patients remain symptom free and have an excellent prognosis.[4]

In Austria, it was reported that children with sub-optimal treatment did have a lower IQ than those who were treated adequately.[2]

## 3.7. Probability and impact of false positive results

Data on false positive (FP) results were derived from the INESSS report and based on international literature.[2]

- In Denmark where only profound deficiency is screened for the number of false positives is 5 / 100 000.[10]
- In a New England study the number of FP (all forms of deficiency) was reported to be 21.4 /100 000 at the first test.[11]
- In Greece a similar number of 26.7 / 100 000 was obtained.[12]

Some authors report that reasons for FP could be premature delivery or a wrong manipulation of the sample.[2]

Performance of the screening in 100 000 tests for biotinidase deficiency (assuming uncertain estimated values) are shown Table 2. False negative: are not reported in the literature (see below).

**Table 2 – Performance of the biotinidase deficiency screening test assuming average values**

| | Disease positive | Disease negative | Total | Estimated Predictive values |
|---|---|---|---|---|
| **Test positive** | 2,6 | 20,0 | 22,6 | PPV: 12% |
| **Test negative** | 0,0 | 99977,4 | 99977,4 | NPF: Cannot be calculated |
| **Total** | 2,6 | 99997,4 | 100000 | |

False positive results after screening likely result in anxiety of parents when they are contacted for referral to a paediatrician and confirmatory testing, as well as additional costs and burden on the health care system associated with confirmatory tests. No study describing this impact was found, but false positives at first screening test would involve approximately 25 newborns in Belgium per year and approximately 3 true positive cases.

## 3.8. Probability and impact of false negative results

In the literature review of the INESSS report no false negative results were reported.[2]

## 3.9. Impact on the health care system

In a cost-effectiveness analysis from 2006, Carroll et al. estimated the Incremental Cost Effectiveness Ration (ICER) of screening for biotinidase deficiency as cost saving.13 This means the screening dominates the non-screening strategy. The authors conclude, that, screening for biotinidase deficiency would be cost saving in their base-case scenario.13 However, this analysis is very dependent upon the underlying assumptions about thresholds of the test and the existing uncertainties. Therefore, this analysis should be treated with care.

The above analysis includes the capacity and financial cost for both TP and FP.

## 3.10. Key points for biotinidase deficiency

- **The frequency of biotinidase deficiency varies according to definition and country, but data from Flanders correspond to a frequency of 1.5 / 100 000 of profound biotinidase deficiency (3 cases in 202 713 screened newborns). In other Western countries similar rates are reported but with variations due to uncertainties (ranging from 0.9 to 2.9 / 100 000 life births).**
- **Profound biotinidase deficiency, the most severe form of the condition, can cause seizures, weak muscle tone (hypotonia), breathing problems, hearing and vision loss, problems with movement and balance (ataxia), skin rashes, hair loss (alopecia), and a fungal infection called candidiasis. These conditions can occacionally lead to death. Affected children also have delayed development.**

- **Partial biotinidase deficiency is a milder form of this condition. Without treatment, affected children may experience hypotonia, skin rashes, and hair loss, but these problems may appear only during illness, infection, or other times of stress.**
- **The majority of infants develop symptoms between 3 and 6 months after birth. However, symtoms can develop as early as 4 weeks postpartum. Therefore the timely availability of the test result appears to be good with neonatal blood screening. Without loss to follow-up the test results should be available on time.**
- **The therapy for detected patients is dietary, lifelong but rather cheap (biotin supplementation). When diagnosed on time, those patients remain symptom free and have an excellent prognosis. This therapy can also improve symptoms if they have already developed**
- **Estimates for Belgium correspond to 3 true positive cases yearly and 25 false positive cases after first screening.**
- **The Positive Predictive Value of a first test positive result is estimated to be about 12%.**
- **For false negative cases no estimate is possible because no case was reported.**
- **Impact on the health care system of screening for this disease: a cost-effectivenss estimate from the US estimated this screening to be cost-saving.**

# 4. DISEASE INFORMATION ON CONGENITAL ADRENAL HYPERPLASIA (CAH)

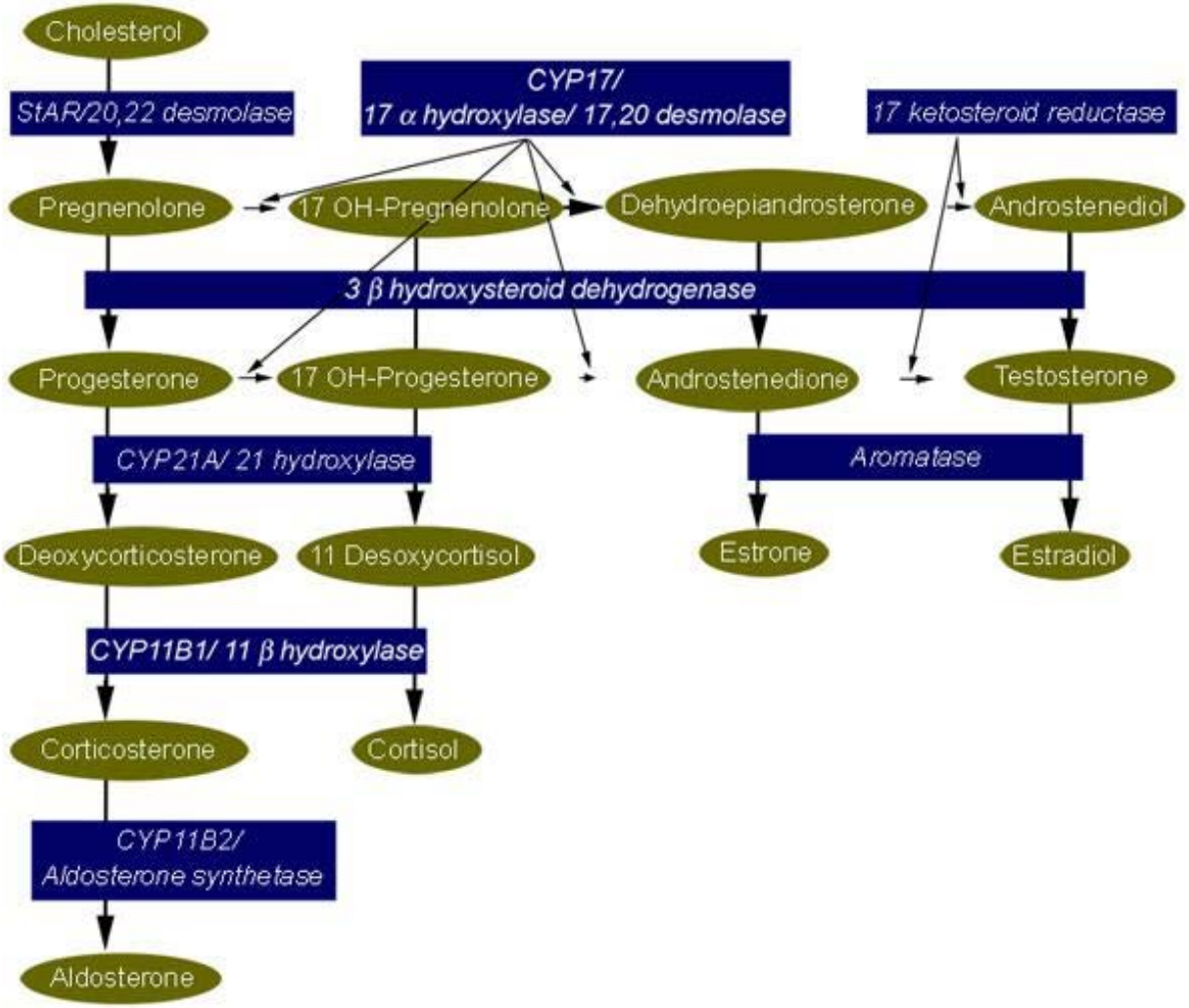
## 4.1. Background information on the disease (not to be scored)

Congenital adrenal hyperplasia (CAH) is a family of several autosomal recessive disorders resulting from mutations of genes for enzymes mediating the biochemical steps of production of cortisol from cholesterol by the adrenal glands (steroidogenesis).[14] In 92 to 95% of cases CAH is due to a defect in the steroid-21-hydroxylase enzyme resulting in a “salt-wasting” form (about 70% of affected infants) or a “simple virilising disease” (about 30% of affected infants), depending on the nature of the genetic defect.[4, 15, 16] As several genes or different mutations in each of these genes can be responsible, both severe and mild (classical and nonclassical) forms of the disease can occur within one family depending on the combination of mutations in affected individuals.[15] The second most important origin of CAH is a defect in the 11-Beta-hydroxylase enzyme (5 to 8% of cases).[15] Deficiencies of other enzymes causing CAH are very rare.[15, 17] The enzyme pathway is illustrated in Figure 1. ICD codes for CAH are E25.0 for ICD-10 and 255.2 for ICD-9. MeSH term: ‘Adrenal Hyperplasia, Congenital’. ORPHA418: Congenital adrenal hyperplasia.[3]

Most of these conditions involve excessive or deficient production of steroid hormones or their intermediate metabolites and are characterised by a hyperplasia of the outer part of the suprarenal glands. Severe cases suffer from episodic salt-wasting crises (acute adrenal insufficiency) that are life threatening because of low blood sugar levels, electrolyte disturbances and pronounced dehydration. In addition, development of primary or secondary sex characteristics can be altered in some of the affected infants, children, or adults.[15]

Currently screening is only performed in the ‘Vlaamse Gemeenschap’ (VG).[4, 5]

**Figure 1-– Steroidogenic pathway for cortisol, aldosterone, and sex steroid synthesis[18]**



## 4.2. Frequency (incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

The Vlaams Agentschap Zorg en Gezondheid reports a birth prevalence of 5.7 (up till 2010) to 6.9 / 100 000 (2012-2014) detected by neonatal screening, corresponding to an average of four to five children born with CAH in Flanders each year (Table 3).[4, 5]

In the screening centre of PCMA a total of 536 324 newborns was screened during 1988-2008 by determining the level of 17-OH-progesterone: twenty boys and thirteen girls infants were diagnosed with CAH resulting in a prevalence of 6.3 / 100 000. These results, including the proportion of infants with the salt-wasting form (69.7%), are comparable to those reported internationally.[a]

Orphanet reports worldwide a prevalence of 7.1 / 100 000 (range: one to nine) of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency.[19] For the UK approximately 5.6 / 100 000 newborns are reported to have CAH while in white populations in Italy, France, Scotland and New Zealand the estimated overall incidence was seven per 100 000.[16, 20]

For the USA an overall prevalence of 6.3 / 100 0000 population of classic adrenal hyperplasia was documented.[21] However, the disorder is more likely in selected populations (Hispanics, Yugoslavs, Ashkenazi Jews, Yupik Inuit of Alaska) where prevalence can be as high as 250 / 100 000.[14, 18]

**Table 3 – Birth prevalence rate of detected CAH in newborn screening programmes**

| Country, date | Source | Total screened | Rate cases by level of 17-OH-progesterone per 100 000 |
|---|---|---|---|
| Belgium, Flanders, till 2010 | Draaiboek 2012 – Vlaams bevolkingsonderzoek naar aangeboren aandoeningen bij pasgeborenen via een bloedstaal.[4] | 703 000 | 5.7 (40 cases) |
| Belgium, Flanders, 2012-14 | Draaiboek 26/11/2015 – Vlaams bevolkingsonderzoek naar aangeboren aandoeningen bij pasgeborenen via een bloedstaal.[5] | 202 713 | 6.9 (14 cases) |
| Belgium, PCMA, 1988-2008 | F. Eyskens[1] | 536 324 | 6.3 (33 cases) |
| Worldwide | Orphanet[19] | NA | 7.1, range between 1 and 9 |
| UK, 2007-09 | Khalid, 2012[16] | NA | 5.6 (144 cases) |
| Italy, France, | Pang et al[20] | 1 093 310 | 7.0 (77 cases) |

[a] http://www.vlaamsezorgkas.be/uploadedFiles/NLsite/Preventie/Kinderen_en_jongeren/Hielprik/AMA%20draaiboek%20PPT%203%20lanceerdag%20Fran%C3%A7ois%20Eyskens%20def.ppt.

| | | | |
|---|---|---|---|
| Scotland, New Zealand, 1980-88 | | | |
| US, whole country, 2001-10 | Therell et al[21] | 24 567 249 | 6.3 (67 cases) |

## 4.3. Severity of the disease in untreated cases

The deficiency in cortisol and potentially aldosterone induces a continuous adrenal stimulation leading to adrenal enlargement and overproduction of intermediary metabolites and adrenal androgens. In girls this leads to virilisation of the external genitalia.[4, 5, 15, 17, 18].

The symptoms of CAH vary depending upon the form of CAH and the sex of the patient. Symptoms can include:

Due to inadequate mineralocorticoids:

Episodic acute adrenal insufficiency (salt-wasting crises) is rare but severe, affects both sexes equally and represents a medical emergency. It typically develops at ten to fourteen days of age but may also occur in later life triggered by precipitating infections and/or periods of stress. Hypoglycemic seizures, electrolyte and acid-base disturbances and symptoms of dehydration are common manifestations seen in children.

If untreated, shock and cardiac arrhythmias can develop and rapidly lead to death. Boys are at higher risk because the diagnosis is generally not suspected at birth in presence of normal genitalia. Prognosis varies depending on the etiologies, but is generally correlated with the rapidity of diagnosis and medical assistance. Death is rare when the patients receive appropriate medical assistance.

Due to excess androgens:

Prenatally or at birth, girls with severe foms present with ambiguous genitalia and the extent of virilization can vary from minimal clitoromegaly to a nearly male appearance, such that it can be initially difficult to identify external genitalia as "male" or "female". A normal uterus and various degrees of abnormal vaginal development are seen. Mild forms are identified later in childhood because of precocious pubic hair, clitoromegaly, or both, often accompanied by accelerated growth and skeletal maturation. Still milder deficiencies may present in adolescence or adulthood with acne, hirsutism, oligomenorrhea, absence of breast development, sub- or infertility and/or obesity.

In boys the external genitalia are normal which explains why the clinical diagnosis is often missed in the neonatal period. If the defect is severe and results in salt wasting, these male neonates present at age one to four weeks. Patients with less severe deficiencies of 21-hydroxylase present later in childhood because of early development of pubic and axillary hair, phallic enlargement, accelerated growth velocity, accelerated skeletal maturation (leading to short stature in adulthood), advanced bone age and precocious puberty during childhood.

However, asymptomatic females and males are reported as well but in these cases the distinction with paucisymptomatic late onset is generally not clear. Confused psychosexual identity is not common with CAH.

## 4.4. Timely availability of the test results

The 17-OH-progesterone is a precursor in the cortisol synthesis. The level of this hormone is strongly increased and the detection in the Guthrie card is a reliable screening test. Symptoms can occur during the first weeks after birth and timely availability of test results is therefore important. The average age at which newborn screening results were reported was eleven days in two studies (Texas, New Zealand) and eight days in another (Sweden).[22]

The nonclassical form of CAH is not reliably detected by NBS but this is of little clinical significance because adrenal insufficiency does not occur in this type of 21-hydroxylase deficiency.[19]

## 4.5. Efficacy of early vs late treatment

The treatment consists of preventing life threatening episodes of acute adrenal insufficiency (salt-wasting crises) before the first crisis occurs and minimization of excessive growth, skeletal maturation and virilisation. This is achieved through life long administration of cortisol and mineralocorticoids with adaptation of dosage during periods of stress (e.g. surgery, infection, etc.). The treatment of the external genitalia in girls is surgical.[15, 18, 23]

Only timely treatment can avoid potentially fatal salt-wasting crises.

Gender identity in females with virilising adrenal hyperplasia is usually female if female gender assignment is made early in life, if adequate medical and surgical support are provided, and if the family (and eventually the patient herself) is given adequate education to understand the disease. However, problems with psychological adjustment are common and usually stem from the genital abnormality that accompanies some forms of CAH.

With adequate medical and surgical therapy, the prognosis is good. Short stature and reduced fertility rates are common, but fertility is possible with good metabolic control.[15]

## 4.6. Probability and impact of false positive results

Cut off 17-OH-progesterone levels for recall are set low to detect reliably all affected infants which generates high frequencies of false positive results because levels of 17-OH-progesteronne are high in the first two to three days of life, even in unaffected infants, especially if they are sick or premature. Estimated performance data are shown in Table 4.

Specimen collection prior to 24 hours of age and illness can affect this screening, as physiological stress can cause a normal elevation of the 17-OH-progesterone level.

The majority of reported false-positive results have been caused by low birth weight and premature birth, in which the 17-OH-progesterone levels are invariably higher.[24] Information collected on gestational age at birth allows to adjust the cut-off and to lower the false positive rate.[5]

The screening alone in 29 programs from thirteen countries resulted in a false-positive rate (usually found in low birth weight and premature infants) that was acceptably low (0.01–0.5%) except for three programs (0.7–2.5%).[25] Therefore the test was reported to have a low positive predictive value of about 1%.[26]

Recent practices of early discharge from the nursery and increased numbers of deliveries at birthing centres have resulted in many screening samples collected at one to two days after birth. This may result in an increased number of false-positive tests.[24]

In Belgium, recall rates for positive results varied between 0.4% and 0.8% over the observed periods (1993-2008) and one false-negative (girl, simple virilizing form) was identified.[b]

**Table 4 – Performance of the CAH screening test[b]**

| | Disease positive | Disease negative | Total | Estimated Predictive values |
|---|---|---|---|---|
| Test positive | 6.2 | 5 500.0 | 5 506.2 | PPV: 0.1% |
| Test negative | 0.18 | 94 493. | 94 493.8 | NPV: 99.1% |
| Total | 6.4 | 99 997 | 100 000 | |

*Assuming average values for FP: 550 / 100 000 neonatal screening tests (estimated from sample recall rate during 1993-2008).*

False positive results after screening likely result in anxiety of parents when they are contacted for referral to a paediatrician and confirmatory testing, as well as additional costs and burden on the health care system associated with confirmatory tests. No study describing this impact was found, but false positives would involve approximately 688 newborns in Belgium per year and between seven to nine true positive cases.

## 4.7. Probability and impact of false negative results

The screening alone in 29 programs from thirteen countries resulted in a false-negative rate of CAH screening that was negligible.[25]

For Belgium, only one false negative case was reported on a total of 536 324 newborns screened.[b]

[b] http://www.vlaamsezorgkas.be/uploadedFiles/NLsite/Preventie/Kinderen_en_jongeren/Hielprik/AMA%20draaiboek%20PPT%203%20lanceerdag%20Fran%C3%A7ois%20Eyskens%20def.ppt

Blood transfusions and treatment with hydrocortisone or dexamethasone may result in false negative screening results.[5]

Life threatening episodes of acute adrenal insufficiency can occur in severe cases when the disease is not recognised but death is rare when the patients receive appropriate emergency medical assistance in time.

Patients with less severe deficiencies of 21-hydroxylase present later in childhood because of precocious puberty and abnormal growth or for women in adulthood due to fertility problems.

## 4.8. Impact on the health care system

In Flanders screening is performed by measuring 17-OH-progesterone concentration using an ELISA test at an estimated cost of € 3.00 - 3.50 per analysis. This test only detects 21- and 11-beta-hydroxylase deficiency.[c]

Carroll et al. estimated that screening for CAH had a net cost per QALY gained with an Incremental Cost Effectiveness Ratio (ICER) of US$ 20 357 (in 2004 US dollars) but this cost was less than the $50 000 per QALY used conventionally as a benchmark for cost-effectiveness.[13] However, this analysis is very dependent upon the underlying assumptions about the occurrence and outcome of crises of acute adrenal insufficiency. Therefore, this analysis should be treated with care.

Health economic data are not readily available in Belgium but in view of the anticipated number of positive screening test results and cases, the effect on financial cost and capacity of the health system can be assumed to be low.

Impact of the treatment on health related quality of life is difficult to estimate but patients are subjected to medication and regular clinical follow up (e.g. growth problems and sub- or infertility) which adds to the burden.

## 4.9. Key points for congenital adrenal hyperplasia

- **The frequency of CAH is estimated at 5.7 to 6.9 / 100 000 neonates tested in Belgium and varies between 1.0 to 9.2 / 100 000 live births in international studies. In some selected populations, the disorder is more likely. The two most important variants constitute 92 to 95% (steroid-21-hydroxylase deficiency) and 5 to 8% (11-Beta-hydroxylase deficiency) of all cases. Other variants are exceptional. Classic CAH can present as salt-wasting disease (70% of all cases) or as simple virilising disease (30% of all cases) while in 11-Beta-hydroxylase deficiency the occurrence of salt-wasting crises is rare. In Belgium this would correspond to on average of seven to nine newborns with CAH every year.**
- **The disease can be severe when untreated due to the rare occurrence of salt-wasting crises which typically develop at ten to fourteen days of age, although they also may occur in later life. Yet death is rare when patients receive rapid and appropriate medical assistance. In simple virilising disease, the main manifestations at birth are abnormalities in varying degrees of the external genitalia in girls. Later in life, both boys and girls may experience precocious puberty, abnormal growth and sub- or infertility. However, very mild late onset and asymptomatic cases also occur but frequency estimations for the general population lack.**
- **Test results are generally available before the development of any salt-wasting crisis in studies who report on this aspect.**
- **With adequate medical and surgical therapy, the prognosis is good. Even with treatment short stature and reduced fertility rates are common, but fertility is possible with good metabolic control.**
- **The frequency of false positive results is known to be high. The majority are caused by low birth weight, premature birth, specimen collection prior to 24 hours of age and ilness. If gestational age at birth is known, cut of levels of the test can be adapted to lower the number of false positive tests. For Belgium seven true positive**

[c] Personal communication: F. Eyskens

**cases and 688 false positive cases would be anticipated at first screening.**

- **For Belgium, only one false negative case was reported on a total of 536 324 newborns screened.**
- **The impact on the health care system is expected to be rather low. The cost of a 17-OH-progesterone immunoassay is reportedly affordable and only about sevenhundred false positive newborns would have to be retested yearly. The drugs needed for the treatment of seven to eight true cases per year are inexpensive. However, the necessity for life long treatment and follow up (e.g. growth problems and sub- or infertility) adds to the burden.**

# 5. DISEASE INFORMATION ON GALACTOSEMIAS (MAINLY GALT DEFICIENCY)

## 5.1. Specific data sources for galactosaemia

Orphanet database.[3]

Galactosaemia Foundation: http://www.galactosaemia.org/

EGS: European Galactosaemia Society.[27]

'Fiche synthèse' GALT from the INESSS report: https://www.inesss.qc.ca/fileadmin/doc/INESSS/Rapports/Genetique/Fiche_Synthese_GALT.pdf

Personal opinion from consulted experts.

See also references.

## 5.2. Background information (not to be scored)

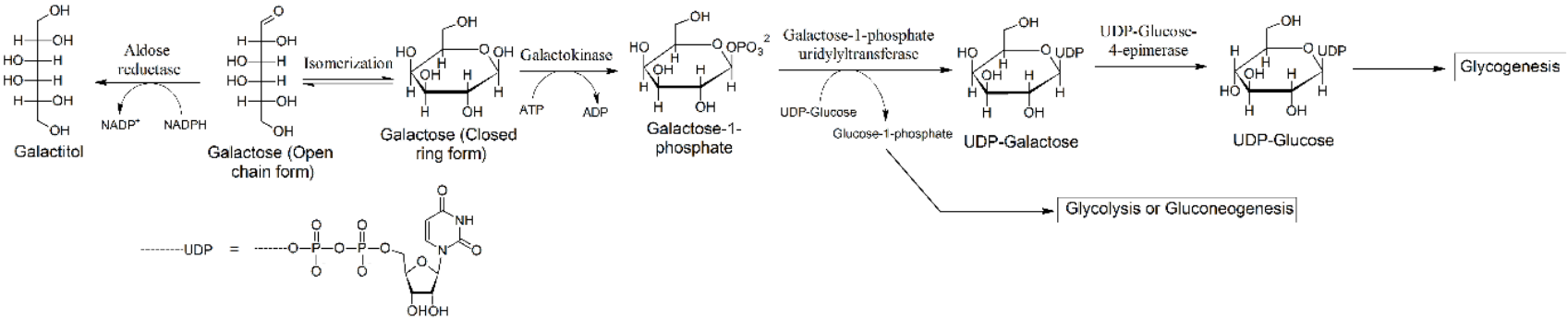
Galactosaemia follows an autosomal recessive mode of inheritance that confers a deficiency in one of the enzymes responsible for adequate galactose degradation.

Galactosaemia is a family of genetic disorders that result from a compromised ability to metabolize the sugar galactose. It is a family of disorders linked to mutation in the genes coding 3 enzymes (Leloir pathway,28 see Figure 2, that are essential for the galactose metabolism; ICD codes for Galactosaemias are E74.21 for ICD-10 and 271.1 for ICD-9. MeSH term: 'galactosemias'. ORPHA352: Galactosemia

Lactose is the main carbohydrate in breast milk and most non-soy infant formulas and is broken down into glucose and galactose in the intestine. Individuals with galactosaemia are not able to utilize galactose because an enzyme is defective or deficient. This leads to an accumulation of galactose in the blood and urine, which can cause serious health problems (see further). In the FWB screening is currently performed.

The classic galactose-1-phosphate uridyl transferase (GALT) deficiency (galactosaemia type 1) is the more severe form while also partial GALT deficiency can occur. The two other enzymes of the Leloir pathway (galactokinase/GALK/galactosaemia type 2 or UDP galactose epimerase/GALE/galactosaemia type 3) can also be deficient but those deficiencies are rarer and cause in general milder symptoms.29

**Figure 2 – Galactose pathway**



Screening is currently performed only in 'Fédération Wallonie-Bruxelles' (FWB).

The only treatment for classic galactosaemia is eliminating lactose and galactose from the diet. Even with an early diagnosis and a restricted diet, however, some individuals with galactosaemia experience long-term complications such as speech difficulties, learning disabilities, neurological impairment (e.g. tremors, etc.), and ovarian failure.

## 5.3. Frequency of the disease (incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

In the FWB screening is currently performed. Over the two years 2012-2013 screening occurred in 120 295 newborns. In 7 of them a true classic GALT was ultimately diagnosed and in 12 a partial GALT deficiency (not all centres report full data over the two years). No cases of GALK or GALE were detected, but not all centres performed these tests (or did not report them).[29, 30]

This leads to a birth prevalence for classic GALT deficiency of approximately 5 / 100 000 newborns. The estimated birth prevalence of partial GALT deficiency is at around 10 / 100 000 newborns.

Previously, screening also occurred in VG and at that moment the birth prevalence of classic GALT deficiency was 2 / 100 000, comparable to the birth prevalence in the Netherlands.(Personal Communication François Eyskens).

However, the three centres did not perform exactly the same tests neither for the kit nor for the thresholds, so results might be difficult to compare.

A total of 265 infants were detected in the first screening over this same period and needed confirmatory diagnostics.

An earlier report from the French community published in 2008 published the numbers since the beginning of the screening program for galactosaemia. In 1 697 982 newborns tested the birth prevalence of true positives for severe GALT was 1.9 / 100 000.[31]

### 5.3.1. Frequency in other countries

In the Orphanet registry: the birth prevalence of classic galactosaemia in western countries (GALT) is estimated to be 1.7 to 2.5 / 100 000).[32], and between 0.1 to 0.7 / 100 000 for GALK. The birth prevalence of GALE deficiency is unclear because this is a very exceptional condition.[33]

According to the European Galactosaemia Society galactosaemia occurs in 1.7 to 3.3 / 100 000) newborns. It occurs in people of all ethnic groups, but it is most common in people of Irish descent.[27]

A recent overview article by Pyhtila et al.[34] for the US reports an overall birth prevalence (based on up to 50 years of screening in the US and over 2500 babies with classic GALT detected) of 2 / 100 000.

In the UK: 2.2 / 100 000).[2]

In the US (Michigan): 1.7 / 100 000).[2]

## 5.4. Severity of the disease in untreated cases

There are different forms for this disease: for GALT deficiency there is the classic serious form, but also a milder form of the condition in which there is some GALT activity. This deficiency is also called galactosaemia type 1. Above, there are also 2 other enzyme deficiencies potentially involved; In general 3 variants are described depending upon the enzyme involved in the mutation.

The classic serious form of galactosaemia is caused by a deficit in galactose-1-phosphate uridyl transferase (GALT). Pregnancy and birth of a child with galactosaemia usually give no immediate indication that there is a problem.[27] Without timely treatment the disease manifests itself a few days after birth and after ingestion of milk through hypotonia, alimentary problems, vomiting, weight loss, liver and renal problems, bleedings and icterus. This can lead to death. In those severe cases the disease symptoms start very early and a diet is needed in the first days of life. [35] The diagnosis is not always straightforward without timely screening since other diseases may present similar symptoms. The final diagnosis is made by measuring the enzyme level in the baby's blood. [27]

Without intervention, classic galactosaemia is a potentially fatal disorder in infancy. With early diagnosis and dietary restriction of galactose, the acute sequelae of classic galactosaemia can be prevented or reverse. However, despite early and lifelong dietary treatment, many galactosaemic patients go

on to experience serious long-term complications including cognitive disability, speech problems, neurological and/or movement disorders and, in girls and women, ovarian dysfunction. Further, there remains uncertainty surrounding what constitutes a 'best practice' for treating this disorder.

Because these are rare diseases the occurrence of these different outcomes is difficult to quantify but in an international survey including 11 countries, many disparities in approaches to diagnosis, management and follow-up care were noted.[35]

Also impact on Health Related Quality of Life is difficult to estimate and has only been studied in small samples. Although the institution of a milk-free diet leads to a rapid clinical improvement of the acute problems, the long-term outcome is disappointing with impaired cognitive performance, speech impairment, reduce bone mineral density and female hypogonadotropic hypogonadism.[36] It is also plausible that social participation including education, social relations, living situation and HRQoL may therefore be impaired. A small study, using a chronic disease specific questionnaire found significant reductions of HRQoL in the 'positive mood'; 'social wellbeing' and 'social functioning' dimensions.[36]

A rarer but milder form is the deficit of galactokinase (GALK). This is also known as galactosaemia type 2. This can cause juvenile or adult cataract often without other signs. It can also lead to blindness.

A very rare form with mixed severity is the deficit of galactose epimerase (GALE) leading to mixed clinical signs including the signs of classic galactosaemia. This is also known as galactosaemia type 3 but is very rare.

## 5.5. Timely availability of the test results

The screening of galactosaemia is done by dosage of total galactose (galactose + galactose-1-phosphate). In case this total galactose is increased the GALT enzyme activity has to be measured (MS/MS) to confirm or exclude classic galactosaemia type 1.[2, 33] If both tests are positive further referring to a paediatric specialised centre is required.[33]

In one German screening study 2 out of 14 cases detected through NBS (14 %) did present symptoms in the first week after birth (after 4 to 8 days).[37]

## 5.6. Efficacy of early vs late treatment

A lactose-galactose restriction starting during the first 10 days after birth appears to be efficacious to prevent many complications, included neonatal death, liver and renal problems and intellectual deficiency. Treatment should be life-long and calcium and vitamin D intake should also be restricted.[2]

Efficacy of screening and treatment to reduce mortality was studied in the US: mortality is 10 times lower after the implementation of screening programs and early treatment.[38] Efficacy of treatment to prevent long-term complications appears to be limited even with early treatment,[39, 40] but cognitive functions appear not to decrease with age.[41] Patients, however, can function even with these sequels. Because of some remaining endogen production of the enzyme, the possibility of some relaxing of the diet is observed over time.[2]

## 5.7. Probability and impact of false positive results

In Belgium, this test is only performed routinely in the FWB: The screening is through the dosage of total galactose (galactose + galactose-1-phosphate).[33] When total galactose is elevated a GALT enzyme activity measurement using the MS/MS technique should be performed to confirm or reject the diagnosis of classic galactosaemia.

In the first instance test there were 14 to 16 false positive results (FP) per 100 000.[11, 42] For the confirmation test no validation data on the performance of the test are available.

Taking those uncertain average values into account and the fact that no false negative values have been reported (however it is reported those dependent upon lactose ingestion before the test) the performance of the test can be interpreted as shown in Table 5.

**Table 5 – Performance of the GAL screening test assuming average values**

| | Disease positive | Disease negative | Total | Estimated Predictive values |
|---|---|---|---|---|
| Test positive | 2,2 | 15,0 | 17,2 | PPV: 13% |
| Test negative | 0,0 | 99982,8 | 99982,8 | NPV: Cannot be calculated |
| Total | 2,2 | 99997,8 | 100000 | |

*Assuming average values for the assumptions: birth prevalence 2.2 / 100 000, FP: 15 / 100 000, FN: non reported but depending on lactose ingestions before test.*

False positive results after screening likely result in anxiety of parents when they are contacted for referral to a paediatrician and confirmatory testing, as well as additional costs and burden on the health care system associated with confirmatory tests. No study describing this impact was found, but false positives would involve approximately 19 newborns in Belgium per year and approximately 3 true positive cases.

## 5.8. Probability and impact of false negative results

No false negative results (FN) have been documented in studies.[2] However, it is essential that the newborn has had an intake of milk to allow total galactose to increase. The ingestion of colostrum during the first days of life potentially increases the risk for false negative results and clinical problems could appear later.[2]

## 5.9. Impact on the health care system

In a cost-effectiveness analysis from 2006, Carroll et al. estimated the Incremental Cost Effectiveness Ration (ICER) at $94 000.[13] This means the screening is not cost-saving, but the level of cost-effectiveness is matter of debate. The authors conclude, that, screening for galactosaemia would not be cost saving under any assumption even if tests could be performed for free.[13] However, this analysis is very dependent upon the underlying assumptions about thresholds of the test and the existing uncertainties. Therefore, this analysis should be treated with care.

The above analysis includes the capacity and financial cost for both TP and FP. Again: information is invited from Belgian experts to arrive at a reliable estimate for Belgium on how this would impact, does impact, the health care system including the possible risk for FN.

## 5.10. Key points for galactosaemia

- **The frequency of galactosaemia varies according to definition and country, but data from FWB correspond to 5 / 100 000 (7 cases in 120 295 screened newborns). In other Western countries similar rates are reported but with variations due to uncertainties (ranging from 1.7 to 3.3 / 100 000 live births).**
- **Point estimate for partial GALT frequency is ~10 / 100 000 births in Belgium.**
- **In Belgium this would correspond on average to 3 new classic GALT defiency cases each year, and approximately 12.5 partial GALT deficiency cases.**
- **The classic serious form of GALT deficiency causes early problems that, without screening, are difficult to diagnose because they are non-specific. These can quickly lead to death and a diet is needed from the first days of life.**
- **In principle the test results can and should be available on time, but symptoms can appear before test results are available in a small proportion of cases (14%, 2 out of 14 cases) in a study in over a million neonates in Germany.**
- **Early treatment has an important impact on many complications, including early mortality. However, efficacy of treatment to prevent long-term complications appears to be limited. On the longer term difficulties in speech development, neurological symptoms (tremor, ataxia, learning problems) and ovarian insuffiiency can still occur even with adequate therapy.**
- **Estimate for the screening in Belgium would be 3 true positive cases yearly and 19 false positive cases at first screening.**
- **The Positive Predictive Value of a first test positive result is estimated to be about 13%.**

- **For false negative casess no estimate is possible because none reported case were reported, but there is uncertainty about the timing of the test since milk needs to be ingested prior to the test.**
- **Impact on the health care system of screening for this disease: a cost-effectiveness estimate from the US (using total galactose as a screening tool) estimated the Incremental Cost Effectiveness Ration (ICER) for this screening at $94 000 per detected true case.**

# 6. DISEASE INFORMATION ON HOMOCYSTINURIA (HCY)

## 6.1. Background information (not to be scored)

Homocystinuria (HCY) follows an autosomal recessive mode of inheritance.

Classical homocystinuria (HCY) is an aminoacidopathy due to a deficiency of the enzyme cystathionine beta synthase (CBS), see metabolic pathway in Figure 3. At least 150 different mutations in the CBS gene have been identified since this deficiency was established.[43, 44] HCY belongs to the wider family of hypermethionemias, which may be caused by at least six genetic conditions. HCY due to CBS deficiency was the first genetic condition shown to cause hypermethioninemia.[43] ICD codes for HCY are E72.11 for ICD-10 and 270.4 for ICD-9. MeSH term: Homocystinuria. ORPHA394: Classic homocystinuria.[3]

Pyridoxine is a co-factor for CBS, and is playing a role in treatment and prognosis of HCY disease. There are two types of HCY, responsive or non-responsive to pyridoxine, each representing around half of the cases. Severity, outcome and case management differ in each group.[45]

**Figure 3 – Homocystinuria pathway, from Stabler et al 2013[46]**

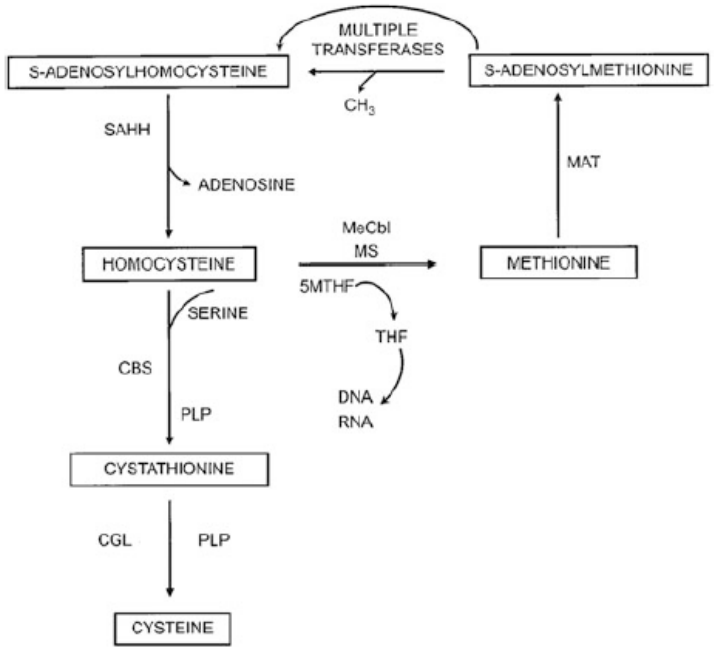



**Fig. 1** Pathways of methionine and homocysteine metabolism are shown. Homocysteine is methylated to methionine by methionine synthase (*MS*) with methyl-cobalamin (*Me-Cbl*). 5-methyltetrahydrofolate (*5-MTHF*) is demethylated to tetrahydrofolate (*THF*) in this reaction. Methionine is activated to S-adenosylmethionine by methionine adenosyltransferase (*MAT*). Multiple transferases utilize S-adenosylmethionine producing S-adenosylhomocysteine in the reactions, which can be hydrolyzed to homocysteine and adenosine by S-adenosylhomocysteine hydrolase (*SAHH*). Homocysteine can be condensed with serine by the pyridoxal phosphate (PLP)-dependent enzyme cystathionine beta-synthase (*CBS*) to form cystathionine. Cystathionine is cleaved by another PLP-dependent enzyme, cystathionine gamma-lyase (*CGL*) forming cysteine

In Belgium, the screening of new-borns for HCY started in 1974. After the responsibility for screening activities became regional in the eighties, HCY remained in the disease list on the French speaking side (Fédération Wallonie Bruxelles or FWB); it was excluded in the Flemish part of the country after the screening programme was reorganised in the nineties.

Subjects are detected by elevation of plasma levels of methionine by MS/MS. In FWB, thresholds for plasma methionine differ in each centre according to the laboratory method (range 40-60 µM/l in 2013) and represent the 99.5% percentile value of the population methionine distribution.[33] When values are above threshold, the test is repeated at the screening centre on the same sample. If the second test is above threshold, the newborn is referred to paediatricians and confirmatory tests consist in dosage of plasma methionine levels by chromatography of amino acids and dosage of plasma homocysteine.[29]

There is no specific cure for homocystinuria. Treatment options include oral pyridoxine for those responsive and low-methionine diet with amino acid, mineral and vitamin supplements. Betaine (Cystadane) is offered as adjuvant treatment to non-responsive patients. If started sufficiently early in life, these interventions may prevent the development of complications.[45]

## 6.2. Frequency of the disease (incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

In Belgium, HCY data are only available from the FWB where new-borns are screened for this disease. HCY amounted to 0.83 true cases per 100 000 in 2012-13, based on one case detected over the two-year period (Table 6).[29, 30] If the FWB rate is applied to the whole of Belgium (birth cohort 125 000 in 2013), one HCY case per year would be expected.

**Table 6 – Birth prevalence of detected HCY cases in systematic NBS by MS/MS in similar populations (EU countries)**

| Country, date | Source | Total screened | Rate cases by MS/MS per 100,000 |
|---|---|---|---|
| Belgium (FWB), 2012-13 | Annual reports from FWB neonatal screening, 2012 and 2013[29, 30] | 120 295 | 0.83 (1 case) |
| Western populations | Meta-analysis of 13 studies, Moorthie 2014[47] | NA | 0.53 (95%CI 0.31-1.89) |
| Portugal, 2004-08 | Vilarinho 2010[48] | 316 243 | 0.32 (1 case) |
| The Netherlands, 2009 | Loeber unpublished, based on Moorthie[47] | 186 128 | 1.07 (2 cases) |
| Spain, 2001-11 | AECNE unpublished[47] | 808 149 | 0.99 (8 cases) |

A systematic review and meta-analysis with search date up to May 2013 estimated a weighted pooled birth prevalence for Western populations at 0.64 and 0.53 per 100 000 of cases detected by MS/MS new-born screening and clinical diagnosis, respectively (Table 6).[47] Among selected studies in Western EU countries using NBS, the rates of cases detected by MS/MS ranged from 0.32 (Portugal) to 1.07 (Netherlands) per 100 000, but the number of detected cases was very small. No EU study presented rates of clinical cases. However, not all screening programmes identify the milder pyridoxine-responsive patients (see below).[47] Screening based on detection of CBS mutations led to higher prevalence, as high as 5 per 100 000 in Denmark.[49]

## 6.3. Severity of disease in untreated cases

The most frequent complications in untreated cases include dislocation of optic lenses (86%), mental retardation (56%), early thromboembolic episodes (16%) and bone abnormalities (24%).[45] Pyridoxine non-responsive subjects are mostly detectable in neonatal screening and, when untreated, present more severe complications compared to those pyridoxine responsive.[45] Pyridoxine responsive subjects are less detectable in the first days of life, and generally diagnosed later at childhood or adulthood based on clinical features, including the sole presence of thromboembolic episodes.[46] In a retrospective study of 629 patients in the eighties, the majority of those detected by NBS (78%) were nonresponsive to pyridoxine.[45] Non responsive subjects presented more frequent dislocation of optic lenses earlier (50% at 6 years) compared to responsive subjects (50% at 10 years).

Premature vascular events are the main life-threatening complications, and death in childhood may occur.[45] In the Mudd study, expected mortality at age 20 years was below 5% in pyridoxine responsive and at 20% in those non responsive.

## 6.4. Timely availability of the test results

HCY test results, based on MS/MS screening and confirmatory tests, are available at a timely moment to prevent preventable sequelae, as the screening is conducted in neonates and disease evolution takes years. Clinical diagnosis is rarely possible before the age of two to three years. In a New Carolina study, two HCY cases were identified based on clinical symptoms between 1985 and 1997, were older than five years at the time of diagnosis and both had been identified by NBS before any symptoms occurred.[50]

## 6.5. Efficacy of early vs late treatment

If started sufficiently early in life, oral pyridoxine (for those responsive), betaine and low-methionine diet may prevent the development of complications,[45] If not detected before, the clinical diagnosis is usually made after irreversible damage has occurred.

High level of evidence on the benefit of early treatment is lacking.[43] Cochrane reviews assessing the effectiveness of NBS diagnosis on HCY with CBS deficiency on clinical benefit compared to later clinical diagnosis

did not find any controlled clinical trials including screened population versus a non-screened population.[51] However a number of observational studies show a clinical benefit of early compared to late treatment.[43, 52-54] In Ireland, 15 patients who were detected in the neonatal period had no evidence of lens subluxation and had perfect bilateral vision compared to 14 late-diagnosed patients (median diagnosis at 4 years) who all had steadily progressive lens subluxation and only 28.6% had 20/40 vision or better.[52] The difference between the two groups was highly significant. A multicenter study of 170 treated HCY patients in Australia and Europe identified 17 vascular events (12 patients) compared to an expected number of 112 vascular events without treatment in the same group.[53] A study among 23 Irish HCY cases suggested that treatment with good biochemical control prevented mental retardation.[54]

## 6.6. Probability and impact of false positive results

Mild hypermethionemia may occur in new-borns with other harmless deficiencies, such as deficiencies of MAT (methionine adenosyltransferase) I/III. Elevated plasma homocysteine levels allow to distinguish true cases of HCY from other hypermethionemia.[43]

In FWB in 2012-13, 30 new-borns had an elevated methionine level at first MS/MS test, 25 were re-tested and five were not re-tested. After confirmation tests, 24 were false positive and only one was a true positive (25 positive per true case). The rate of false positive of a first MS/MS test was 21 per 100 000 tested children, based on 120 000 screened neonates. The positive predictive value (PPV) of the first MS/MS test was thus 4%, as HCY incidence is very low. For a first MS/MS screening test, two US studies found rates at 25.9 (62/239 415, threshold >106 µM/L) and 44.5 (210/472 255, threshold unknown) false positive per 100 000, representing respectively 63 and 209 positive cases per true case.[11, 50] If the FWB false positive rate would be applied to the whole Belgian birth cohort (125 000 in 2013), 26 new-borns with false positive results would be expected per year.

No data are available from Belgium on false positive after the repeated MS/MS test. In the US, subjects with repeated test represented a rate of false positive results per 100 000 screened new-borns around 1.7 for a ratio of 5-8 false positive per true case.[11, 50] False positive results after confirmatory testing are not reported.

The rate of false positive by MS/MS is influenced by the threshold for methionine levels and may be reduced by the use of a MS/MS second-tier testing.[55, 56] In Mayo clinic, a lower threshold of methionine (60µM/L) resulted in 290 per 100 000 samples above threshold, and second-tier testing using blood homocysteine determination by MS/MS identified one true positive on 516 samples above threshold. The positive predictive value of second-tier testing was thus 100% but such test is not yet implemented in Belgium.[57]

No study describing this impact was found. The impact of false positive results after a first screening test should be limited because samples are re-tested. A second MS/MS test would be performed in 26 newborns per year only in Belgium, and confirmatory testing would involve two to three newborns, based on US rates. Parents are contacted for referral to a paediatrician and confirmatory testing only when the second test is positive.

## 6.7. Probability and impact of false negative results

In FWB, no late HCY diagnosis with false negative result at screening has been identified by the neonatal screening programme over 2012-13.[29, 30] However, experts from reference centers are aware of false negative cases that were diagnosed at adulthood. No rate of false negative results is described in studies with longer follow-up.[50, 58] However, the comparison of HCY rates detected by NBS screening and by clinical diagnosis later in life (in a systematic review and a retrospective study) suggest that NBS screening does not identify milder pyridoxine-responsive patients, as these do not show markedly elevated levels of methionine in the neonate period.[45, 47]

In other words, pyridoxine-responsive subjects may be missed by neonatal screening when the threshold is not sufficiently low.[45] In this case, HCY disease may manifest later in life with clinical symptoms such as thromboembolic episodes.[46] High levels of methionine will also not be detected if the newborn has not ingested milk before the sample is taken. Timing of sampling will thus influence the risk of false negative results.

False negative results may result in undiagnosed cases with irreversible damages. However, missed cases are more likely to be pyridoxine-responsive and thus present milder forms of the disease.

## 6.8. Impact on the health care system

The cost of adding HCY testing would be minimal at the screening level as this is performed by the current MS/MS technique. Data on false positive after the MS/MS retest (after a first positive test) were not found for Belgium but based on US rates would amount at two false positive cases per year. These two infants would require confirmatory testing and imply unnecessary parent anxiety.

The impact of adding HCY to the NBS screening on the management of detected case would be limited as around one case per year would be detected and most management is dietary, including pyridoxine intake. Treatment with Betaine in non-responsive cases would cost 1000-2000€ per year for a 10 kg-weight child, based on retail prices.[59]

A British HTA study concluded that extension of NBS screening to include MCAD deficiency and HCY would be cost-effective at low threshold values (93% probability at a threshold value of 1000£). This favorable cost-effectiveness ratio is due to a low incremental cost of adding HCY to the existing MS/MS screening, which would be offset by savings from reduced disabilities in HCY detected cases.[60]

## 6.9. Key Points for homocystinuria

- **The frequency of HCY is low, as one case per birth cohort would be expected in Belgium. The birth prevalence was 0.83 per 100 000 in Wallonia-Brussels (2012-13) with one detected case only over the 2-year period. In other industrialised countries, it varied between 0.32 to 1.07 per 100 000 births based on small numbers of cases.**
- **The disease is severe in untreated cases, leading to irreversible damages. If untreated, half affected children will show mental retardation, 86% will develop vision problems (dislocation of the optic lenses) and many will have vascular events (thromboembolic episodes). Between 5% and 20% of cases will die before reaching 20 years of age, depending on the disease type.**
- **A positive NBS result would be available in time to prevent complications as disease progression is slow: clinical signs do rarely appear before the age of two years. NBS will thus identify cases before any symptoms or complication occur.**
- **Early treatment (pyridoxine in those responsive, betaine and diet) reduces substantially disease complications, as shown in a number of observational studies: it preserved vision, prevented mental retardation and dramatically reduced vascular events. Early treatment presents a clear clinical benefit compared to late treatment in all these studies, as shown by a significantly higher reduction of these complications.**
- **A number of false positive results are found after the first test (26 false positive cases expected per year in Belgium) but all positive results are retested on the same sample. After the retest, only two false positive cases would be expected in Belgium per year, would require confirmatory testing and imply unnecessary parent anxiety for a limited amount of time.**
- **No false negative case has been identified in Belgium. A few missed case are likely to be diagnosed at adult age only, but would involve midler (pyridoxine-responsive) cases.**
- **The impact on the health care system is expected to be limited. The cost of adding HCY detection to the current NBS would be marginal as it is performed by MS/MS. Around three cases per year would be referred to pediatricians and require confirmatory testing. Only one case would be confirmed to be a true HCY case and require case management. Cost of treatment is mainly dietary and not expensive. This screening has been considered as cost-effective in the UK.**

# 7. DISEASE INFORMATION ON TYROSINEMIA TYPE I (TYR I)

## 7.1. Background information (not to be scored)

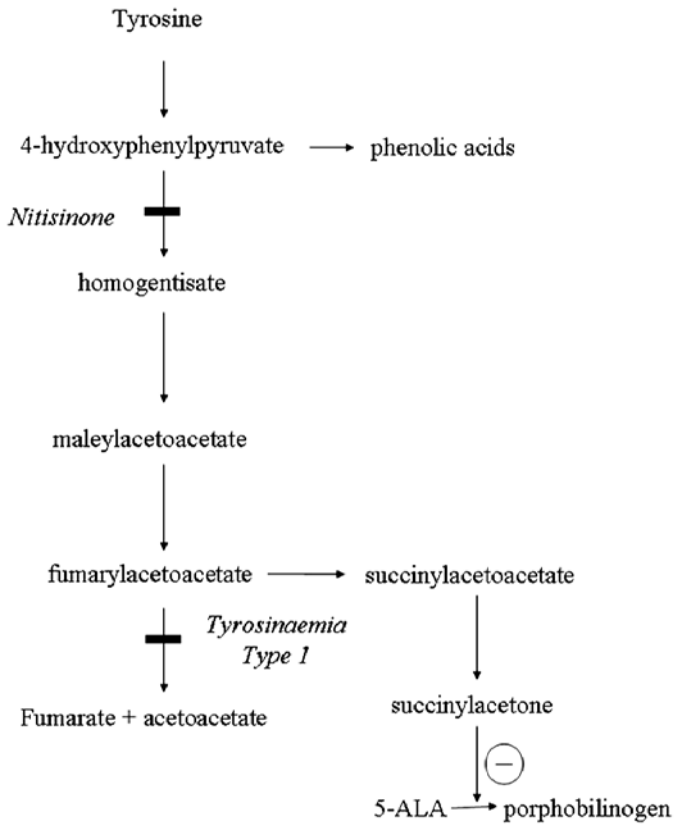
Tyrosinemia type I (TYR I), also known as hepatorenal tyrosinemia, is an aminoacidopathy due to a deficiency of the enzyme fumarylacetoacetase. It follows an autosomal recessive mode of inheritance and represents the most severe form of tyrosinemia. The enzyme pathway of TYR I is illustrated in **Figure 4**. The ICD10 code is E70.21 and MeSH term is Tyrosinemias. ORPHA882: Tyrosinemia type 1.[3]

In Belgium, the screening of newborns for TYR I started in 1974 at national level. After the responsibility for screening activities became regional in the eighties, TYR I remained in the disease list on the French speaking side (Fédération Wallonie Bruxelles or FWB), and was excluded in the Flemish part of the country after the screening programme was reorganised in the nineties.

Subjects are detected initially by elevation of tyrosine plasma levels by MS/MS. In FWB, thresholds for positive tyrosinemia differ in each centre according to the laboratory method (range 185-330 µM/L), representing the 99.5% percentile value of the population tyrosinemia distribution.[33] However, elevated tyrosine levels are not specific to TYR I;[61-63] tyrosinemia is mildly elevated in cases of TYR I in the first days of life because tyrosine accumulation may take longer to occur, while its elevation is much more frequent with other types of tyrosinemia (e.g. tyrosinemia type II and III).[64, 65] To address that, FWB screening centres lowered tyrosine thresholds and introduced testing for succinylacetone (SUAC, see **Figure 4**) by MS/MS to reduce false negative and false positive results, thus limiting the need for confirmatory testing.[33] In two centres, SUAC is tested in a two-tier strategy: any dried blood sample with tyrosinemia above threshold at the first test is then tested for tyrosinemia and SUAC. In one centre, all blood samples are directly tested for (simultaneous) tyrosinemia and SUAC. If SUAC is high, the newborn is referred to paediatricians and confirmatory tests consist in dosage of plasma tyrosine levels by chromatography of amino acids and dosage of urinary organic acids, in particular urinary SUAC.[29] If tyrosinemia is high and SUAC below threshold, the newborn is referred for suspect TYR II or III diagnosis.

An effective treatment, Nitisinone (or NTBC), is available since the nineties and should be started as soon as the diagnosis is confirmed, together with dietary treatment.[63, 66]

**Figure 4 – Enzyme pathway of Tyrosinemia Type I**



## 7.2. Frequency of the disease (incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

In Belgium, TYR I data are only available from the FWB where newborns are screened for this disease. TYR I birth prevalence amounted to 1.7 cases per 100 000 in 2012-13, based on two cases detected over the two-year period ().[29, 30] If the FWB rate is applied to the whole of Belgium (birth cohort 125 000 in 2013), two case of TYR I would be expected per year. The rates of cases detected by NBS MS/MS technique in published studies from Western EU countries show similar values, ranged from 1.26 (Portugal) to 1.47 (Italy) per 100 000 (Table 7), but are based on small numbers of cases.[48, 65]

In most EU studies, a majority of cases were diagnosed among newborns of migrant populations, in particular from Moroccan and Turkish origin. Indeed, 7/10 cases treated in Belgium in 2004 and 3/3 in Italy were from these origins, and 8/9 cases were found among migrants in Norway.[65, 67, 68]

**Table 7 – Birth prevalence rate of detected tyrosinemia type I in newborn screening programmes**

| Country, date | Source | Total screened | Rate cases by MS/MS per 100,000 per year |
|---|---|---|---|
| Belgium (FWB), 2012-13 | Annual reports from FWB neonatal screening, 2012 and 2013[29, 30] | 120 295 | 1.7 (2 cases) |
| Portugal, 2004-08 | Vilarinho 2010[48] | 316 243 | 1.3 (4 cases) |
| Italy, 2008-10 (using SUAC) | La Marca 2011[65] | 136 075 | 1.5 (2 cases) |

**: adjusted for undiagnosed cases*

## 7.3. Severity of the disease in untreated cases

TYR I is a devastating disorder of childhood that causes liver failure, painful neurologic crises, rickets, and hepatocellular carcinoma.[69] Untreated TYR I usually presents either in young infants (in the first six months of age) with severe liver involvement and/or renal tubular dysfunction, or later in the first year of life with hepatocarcinoma, cirrhosis and renal problems associated with growth failure and rickets.[63, 70] Untreated cases may also have repeated neurologic crises lasting one to seven days that can include changes in mental status, abdominal pain, peripheral neuropathy, and/or respiratory failure requiring mechanical ventilation.[70]

Among 42 and 28 TYR I untreated patients diagnosed on clinical grounds in France and Spain, liver failure was found in 91% and 68% of cases and renal problems in 86% and 36% of cases at the time of diagnosis, respectively.[71, 72] In Belgium, among the six Belgian cases presenting acute symptoms at the time of diagnosis (before 6 months of age) before being treated by NTBC in 2004, five presented hepatic failure or cytolysis and one presented rickets. Three had an IQ <85 but it is unclear whether this is due to the disease or to NTBC treatment.[67] Among 28 untreated cases in Quebec, 71% required a liver transplantation.[73]

If left untreated, most patients will die in the first years of life (before the age of 12 years), typically from liver failure and recurrent bleeding, neurologic porphyria-like crisis, or hepatocellular carcinoma.[69, 74]

## 7.4. Timely availability of the test results

TYR I test results, based on MS/MS screening for tyrosine and SUAC followed by confirmatory testing, are mostly available at a timely moment to prevent complications, as the average onset of clinical symptoms is around

16 months of age.[63] However in the most acute form, severe liver failure may appear during the neonatal period if NTBC treatment has not been initiated.[70, 74] In an international review of 168 cases (including 28 diagnosed via NBS), the most acute case (0.6%) suffered from renal dysfunction at the age of 12 days.[63] In FWB in 2012-13, two TYR I cases were detected at day 11 and 13 of life by NBS.[29, 30]

## 7.5. Efficacy of early vs late treatment

NTBC is rapidly effective when initiated during the first month of life, with improvement of liver function within a week.[70] It should be accompanied by dietary treatment consisting in low tyrosine and low phenylalanine diet. If the patient deteriorates, then liver transplantation is considered.

No clinical trial measured NTBC effectiveness but seven retrospective studies were retrieved.[63, 68, 70-73, 75] No hepatocellular carcinoma or severe liver disease developed in patients treated in the first weeks of life and none of these cases required liver transplant, with follow-up ranging 3-9 years.[71-73] In Quebec, none of the 24 early treated patients (before one month of age) required liver transplant compared to 71% among the 28 untreated patients.[73] All patients with rickets on first evaluation were cured after treatment.[72, 73] Survival under early treatment was 100% in three recent studies of 20-46 treated cases each.[63, 71-73]

Three studies comparing early vs. late NTBC treatment show that cases treated later achieved much less favorable outcomes.[63, 72, 73] In particular an international study showed that cases with treatment starting at age 1-6 months and 7-12 months have a 2.5 and 6-fold higher risk, respectively, of developing liver tumors requiring liver transplant, compared to patients with treatment starting in the neonatal period.[63] The risk was 5-fold higher of developing renal dysfunction for patients who are treated beyond 6 months of age compared to those treated before one month. In Quebec, 8% of patients (2/26) treated with NTBC after 30 days of age died, both after liver transplant, and 27% (7/26) required liver transplant, compared to no death nor liver transplant in the early treated group.[73] Early treated patients spent 0.4 days per year in hospital, compared to 3.2 days in late treated patients.[76]

Survival of TYR I cases after liver transplant has been estimated at 83% at 2-year in a study conducted in the nineties and at 40% in an 2014 international study with longer follow-up.[63, 74]

Four studies, including a Belgian study on 10 patients, noted that TYR I patients treated with NTBC are at a higher risk of impaired cognitive function (with lower IQ) and schooling difficulties, despite protein-restricted diet, compared to the normal population.[67, 68, 72, 75] However, the potential role of NTBC in impaired cognitive function among TYR I patients, possibly related to a change in plasma aminoacid (tyrosin and phenylalanine) concentrations, is still debated.[67] NTBC side-effects were mild to moderate, involving asymptomatic transient neutropenia or thrombopenia, photophobia and rash. No adverse event required interruption of NTBC treatment.[63, 70, 72]

## 7.6. Probability and impact of false positive results

TYR I is one of the metabolic disorders accounting for most false positive screening tests if only based on tyrosinemia levels.[11] As said above, tyrosine levels lack specificity as it cannot distinguish TYR I from other disorders of tyrosine catabolism and transient hypertyrosinemia.[77] In FWB in 2012-13, the rate of false positive after a first MS/MS elevated tyrosinemia ranged between 200 and 400 per 100 000 tested newborns (average 262) but was reduced to 0 per 100 000 in the centre testing simultaneously for tyrosinemia and SUAC in a first instance. If the FWB false positive rate of a first tyrosinemia test would be applied to the whole Belgian birth cohort (125 000 in 2013), 327 newborns with false positive results would be expected per year.

No Belgian data were found on false positive at SUAC test, whether as two-tier testing or as first test. In an Italian study using SUAC testing on blood spot (as single test), no false positive was described,[65] while a German study found 20 false positive cases per 100 000 based on tyrosinemia and SUAC.[58] False positive results after confirmatory testing are not described.

False positive results after screening likely result in anxiety of parents when they are contacted for referral to a paediatrician and confirmatory testing, as well as additional costs and burden on the health care system associated with confirmatory tests. No study describing this impact was found.

## 7.7. Probability and impact of false negative results

Where tyrosine level is the sole marker for TYR I, false negative results are frequent as tyrosine may take longer than a few days to accumulate in TYR I cases, and this period may exceed the duration of hospitalisation for normal deliveries.[65] Timing of sampling will thus influence the risk of false negative

results if based only on tyrosine levels.[78] For instance five TYR I cases described in Italy, the US and Australia had tyrosine levels under threshold.[50, 65, 79] In the US, one TYR I case had tyrosine level under threshold and was diagnosed at 8-month of age with failure to thrive, hypotonia and hepatomegaly.[50] TYR I was then removed from the list of NBS diseases as MS/MS for tyrosinemia on newborn samples was not felt as an adequate test for TYR I.

When SUAC blood levels are also measured, such as in FWB, no false negative results have been described, i.e. no late TYR I diagnosis with negative result at NBS was made in the retrieved studies or systematic reviews.[29, 30, 58, 63, 78, 80] However, the occurrence of false negative cases has not been properly evaluated with follow up of all screened newborns,[65, 78] and Belgian NBS experts consider that false negative cases may occur. If occurring, false negative results may result in undiagnosed cases with irreversible damages.

## 7.8. Impact on the health care system

The cost of adding TYR I testing to the NBS screening, including SUAC second tier-test, would be low as this is performed by MS/MS technique.[65, 78]

Adding TYR I to the NBS screening would detect two cases per year if we apply prevalence in the FWB to the whole of Belgium. Detection of TYR I cases may also allow the identification of the disease in siblings.[67]

Early NTBC treatment prevents death, liver transplant and neurological crises, resulting in reduced hospital days and improved quality of life.[76] The annual cost of NTBC treatment delivered by hospital pharmacies at the recommended dosage (around 1 mg per kg per day) would amount to 10 461€ (28.66€ per 5mg capsule) in a 5 kg infant and above 400 000€ in a 20 kg children for the cost of the drug only, and must be continued indefinitely (personal communication C De Laet).[66] Individualized management of NTBC treatment also entices substantial costs, involving special diet and monitoring tests (liver function, α-fetoprotein, coagulation, quantitative plasma aminoacids and SUAC levels in blood and urine). Learning difficulties are described in NTBC treated patients, and in Belgium, two patients among the ten NTBC-treated cases (20%) required special education, although no causal link could be established so far with NTBC treatment.[67]

On the other side, treatment of complications in cases not treated or treated too late also results in high costs. Liver transplantation is required in 70% of untreated cases and 27% of late treated cases,[76] its cost has been estimated in Belgium at 100 000€ in 2011,[81] its associated mortality is around 10% and it involves lifelong immunosuppressive therapy and diminished quality of life.[76]

In Quebec, early NTBC treatment reduced hospital costs from 12 980$ per person-year in untreated patients to 673$ per person-year in early treated patients, but NTBC only cost 51 493$ per person-year.[76] A British HTA study concluded that the extension of NBS screening to include TYR I would cost £13 168 (around 18 000€) per life-year gained. Although representing a favorable cost-effectiveness ratio, TYR I was one of the least cost-effective metabolic diseases detected in NBS in this evaluation, possibly due to the high cost of treatment to prevent complications.[60] However, this evaluation was conducted before the use of SUAC second tier-testing. Update of the literature review was performed by a UK systematic review and no further separate study on TYR I cost-effectiveness was found.[80]

## 7.9. Key Points for Tyrosinemia Type I

- **The birth prevalence is around 1.5 per 100 000, based on small numbers of cases. This means that two cases per birth cohort are expected in Belgium. The disease is more frequent in newborns from Turkish and North African origin.**
- **If left untreated, TYR I is a very severe disease, resulting in failure of the liver and neurological crises in young infants, or liver cancer and renal problems later in the first year of life. Around 70% cases will require liver transplant, and around 40% show renal dysfunction, depending on age. All untreated cases would die before reaching the age of 12 years.**
- **NBS results would be timely for effective management in the large majority of cases, as confirmation of cases was performed before 14 days in recent Belgian experience and nearly all untreated cases become symptomatic later (average 16 months). However the most acute cases may present symptoms before 14 days (<1% of all TYR I cases).**

- **TYR I should be treated with NTBC directly after diagnosis. Early treatment reduced substantially complications in all studies, prevented liver transplant, neurological crises and death, resulting in improved quality of life: treatment starting in the first month of age achieved 100% survival and prevented liver transplant in all studies. Late treatment, i.e. treatment after 6 months of age, has a 5 to 6-fold higher risk of developing liver tumors requiring liver transplant and renal dysfunction, compared to early treated patients.**
- **The TYR I screening as organised in FWB, i.e. including SUAC testing, should not result in false positive cases. In that case, no impact is expected on the family and the health care system. However, if screening is based on tyrosinemia level only, the probability of false positive cases is high (327 per year) and would imply a high number of recall of newborns, referrals to pediatricians and confirmatory tests for the health care system and a high amount of (transient) anxiety among parents of these newborns.**
- **The TYR I screening using SUAC as organised in the FWB, is not expected to result in false negative cases, or this would be marginal (and unknown to date). However, if the screening would only be based on tyrosinemia level, the probability of false negative results would be high, many cases would be missed and this would lead to irreversible complications in TYR I patients.**
- **The impact on the health care system of detecting TYR I would not entice a high cost and burden for the screening system: the cost of adding TYR I detection to MS/MS would be low if SUAC testing is included. Detecting TYR I cases has also the secondary benefit of detecting disease in siblings of cases. Although early treatment is very effective, it is also burdensome. The cost of the drug is high, its monitoring is demanding and NTBC may impair learning difficulties in treated patients, depending on the plasma tyrosine level. However only two new cases per year (birth cohort) would be detected and require treatment. If left untreated, most cases will require liver transplantation, with associated risk of mortality (10%), reduced quality of life and high hospital costs (around 100 000€). The screening of this disease has been considered as reasonably cost-effective in the UK, though less cost-effective compared to other metabolic inborn diseases.**

# 8. DISEASE INFORMATION ON VERY LONG CHAIN ACYL COA DEHYDROGENASE DEFICIENCY (VLCAD DEFICIENCY)

Note: previously termed long chain acyl-coenzyme A dehydrogenase deficiency (LCAD deficiency).

## 8.1. Background information on the disease (not to be scored)

Very long-chain acyl-CoA dehydrogenase (VLCAD) is an enzyme of the internal mitochondrial membrane. It catalyses the initial step of mitochondrial β-oxidation of long-chain fatty acids with a chain length of fourteen to twenty carbons. A deficiency of this enzyme is an autosomal recessive hereditary disease and prevents the conversion to energy of very long-chain fatty acids originating from foods and fat tissues. It is the second most commonly diagnosed disorder of fatty acid oxidation (FAO) and is caused by mutations in the Acyl-CoA Dehydrogenase, Very Long-Chain gene[82, 83]. The enzyme pathway is illustrated in **Figure 5**. ICD codes for VLCAD deficiency are E71.3 for ICD-10 and 277.8 for ICD-9. Mesh term: 'Acyl-CoA Dehydrogenase, Long-Chain'. ORPHA26793: Very long chain acyl-CoA dehydrogenase deficiency.[3]

Three clinical groups or phenotypes of VLCAD deficiency have been reported and are described under 8.3.[83]

A clear relationship has been shown between the severity of disease and the nature of the mutation, which results in no residual enzyme activity in patients with the severe childhood phenotype while some residual enzyme activity is retained in patients with the milder childhood and adult phenotypes.[84]

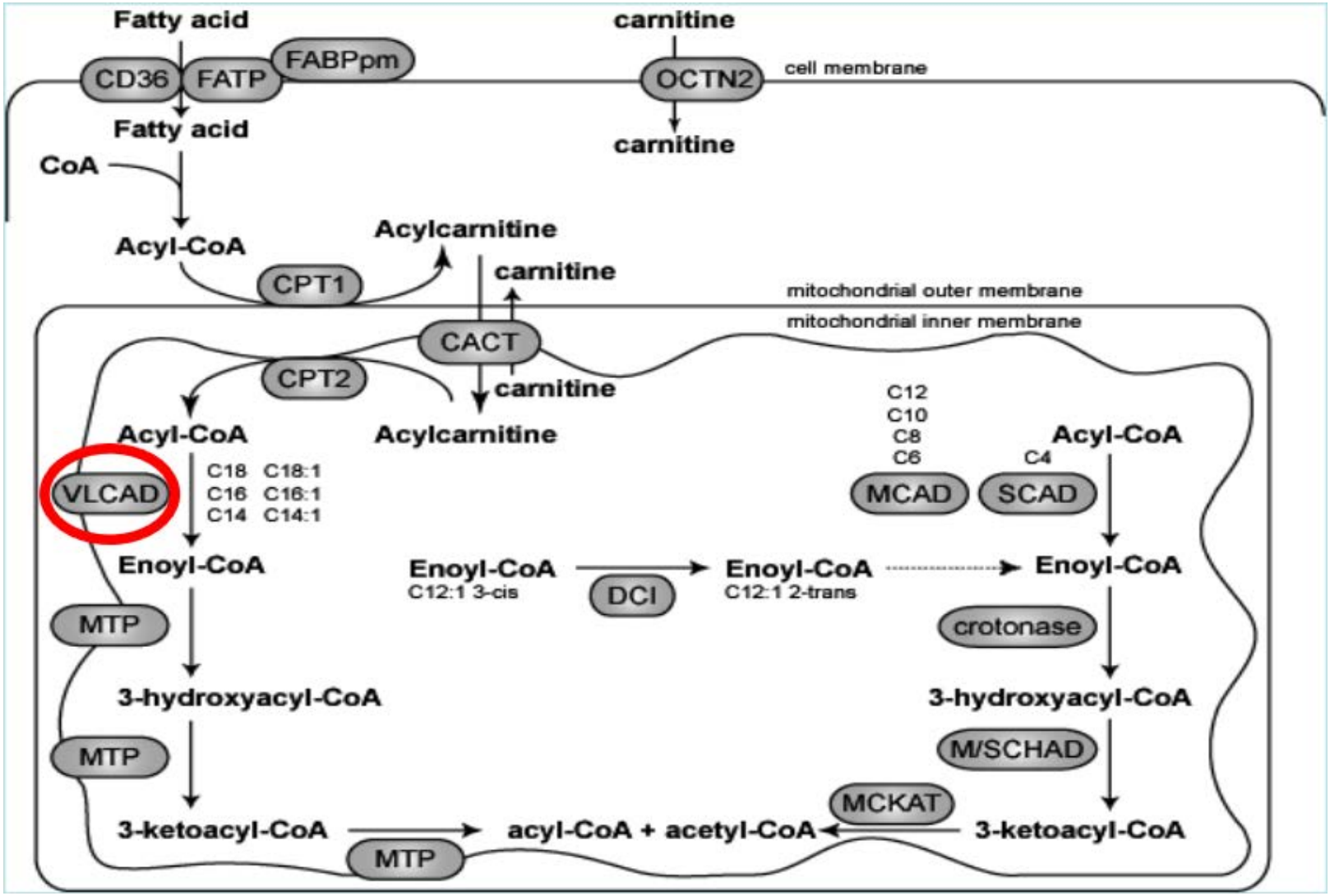
Following birth, milk (of which ~60% of calories are fat) becomes the major nutrient, and therefore, fat becomes the major energy source, especially in the heart, kidney and skeletal muscle.

The heart constantly uses fatty acids for energy. During longer periods of fasting, the liver uses acetyl CoA to generate ketone bodies. The brain adapts to fasting by switching to a ketone economy, reducing the need for glucose as the energy source. With exercise, especially prolonged exercise, slow skeletal muscles use longer-chain FAO to generate energy.

As one of the first enzymes in the FAO chain, the enzyme VLCAD controls a critical point of the respiratory chain, and also provides a pathway to the production of ketones. It would be expected that reduction at this step of FAO would impair the ability to transition successfully from foetal to neonatal life, to maintain cardiac output, to adapt to long fasting, and to generate energy for exercise and normal cerebral functioning, all of which have been observed in VLCAD deficiency. The most severe defects result in early-infantile cardiomyopathy, hepatomegaly, hypotonia, lethargy and intermittent hypoglycaemia. Very long-chain fatty acids or partially metabolized fatty acids may also build up in tissues and damage the heart, liver, and muscles. This abnormal build up causes the other signs and symptoms of VLCAD deficiency. [83]

In Belgium, screening is currently performed only in 'Fédération Wallonie-Bruxelles' (FWB). Nevertheless, also the Flemish screening centres report systematically results for VLCAD deficiency as the disease is unintentionally diagnosed during screening for MAD deficiency (personal communication).

**Figure 5 – The mitochondrial fatty acid oxidation pathway[85]**



## 8.2. Frequency (Incidence and prevalence)

Disclaimer about frequency: For rare disease frequency estimates are unreliable, therefore gross uncertainty margins are encountered even in one country for different years. Those are best estimates. The number of cases are too low to apply statistics upon them.

The FWB annual report estimates the occurrence of the condition at birth at 1.7 / 100 000 neonates.[29]

The screening centres of Ulg, UCL and ULB report no positive test result on the 94 888 neonates tested (2004-2013 included), two on the 196 595 neonates tested (2000-2013 included) and two on 361 238 neonates tested (2000-2013 included) respectively, adding up to a total of four infants (0.6 / 100 000 neonates tested) picked up in the screening over the period 2000-2013 and needing confirmatory diagnosis while the Flemish screening centre PCMA reports two confirmed cases (1.1 / 100 000) on 181 246 screened newborns for the period 2008-2012 (Table 8).[86-88 89] According to Orphanet, overall prevalence is estimated to be between one and nine per 100 000. In EU countries, it was one and two per 100 000 in Portugal and Germany, respectively (Table 8),[48] and 1.3 and 3.2 in Canada (Ontario) and Australia (Victoria).

In the US, birth prevalence was estimated at 1.6 / 100 000 in 2001–2010 (Table 8) but authors remark that disease case-definitions are not nationally standardized and assumed that physician subspecialists confirmed all cases reported by neonatal blood screening programs using generally agreed upon case-definitions.[21]

**Table 8 – Birth prevalence rate of detected VLCAD deficiency in newborn screening programmes**

| Country, date | Source | Total screened | Rate cases by MS/MS per 100 000 |
|---|---|---|---|
| Belgium, FWB | Annual reports from FWB reference centres.[86-88] | 652 721 | 0.6 (4 cases) |
| Belgium, PCMA | Proceedings ISNS meeting Atlanta, USA (2013)[89] | 181 246 | 1.1 (2 cases) |
| Worldwide | Orphanet | NA | 1 to 9 (>400 cases) |
| Germany | Orphanet | NA | 2.0 |
| Portugal, 2005-09 | Vilarinho et al[90] | 316 243 | 0.95 (3 cases) |
| US, whole country, 2001-10 | Therell et al[21] | 24 567 249 | 1.6 (387 cases) |
| Canada, Ontario, 2006-11 | INESS[2] | Around 870 000 | 1.3 (11 cases) |
| Australia, Victoria, 2002-05 | Boneh et al[91] | 189 000 | 3.2 (6 cases) |

## 8.3. Severity of the disease in untreated cases

VLCAD deficiency is clinically heterogeneous, with three major phenotypes depending on the residual enzyme activity. The three main variants are:[83, 84]

- Severe childhood form with early-onset, cardiac and multi-organ failure: typically presents in the first months of life with high incidence of hypertrophic or dilated cardiomyopathy, pericardial effusion, arrhythmias, as well as hypotonia, hepatomegaly, and intermittent hypoglycemia. Cardiomyopathy and arrhythmias are often lethal. Ventricular tachycardia, ventricular fibrillation, and atrioventricular block have been reported.[91] The morbidity resulting from cardiomyopathy may be severe.
- A milder childhood form, with later onset: usually presents with hypoketotic hypoglycemia and hepatomegaly as the main presenting feature, low mortality, and rare cardiomyopathy.
- An adult or later-onset episodic myopathic form, probably the most common phenotype: presents with isolated skeletal muscle involvement, intermittent rhabdomyolysis, muscle cramps and/or pain, and myoglobinuria, usually triggered by exercise or fasting. Hypoglycemia typically is not present at the time of symptoms.[92]

Because these are rare diseases the occurrence of these different variants is difficult to quantify. In one study of 54 patients, almost half of the patients (25) had the severe childhood form with early onset and 21 patients had a milder childhood form with onset in childhood. Eight patients had a myopathic adult form, with late onset.[84]

Most patients who survived the initial episode showed improvement, including normalisation of cardiac function but sudden unexpected (infant) death has occurred in several patients.

Of note is that labour and post-partum periods are catabolic states which place a mother with VLCAD deficiency at higher risk for rhabdomyolysis and subsequent myoglobinuria.[93]

## 8.4. Timely availability of the test results

In the FWB, the three screening centres use the MS/MS technique to dose Tetradecenoylcarnitine (C14:1) and the C14:1/C2 ratio (in analogy to The Netherlands where 2 cases were found during the period 2007-2012 by using the C14:1/C2 ratio but would have been missed if screening had been performed solely on basis of C14:1; personal communication of the RIVM), but implement different laboratory equipment, quality controls and cut off values which are calibrated in accordance with the observed population values. In case C14:1 shows increased values, the analysis is always repeated on the same sample and in case of strongly positive results a repeat sample is urgently requested as well.[86-88] Comparability of results is indirectly assured through good performance of each reference laboratory in international ring tests (personal communication).

Newborn screening data have affirmed that acylcarnitine analysis during periods of physiologic wellness, after recent feeding or after receiving IV glucose often fails to identify affected individuals who have the milder phenotypes. Depending on the “cut-off” limits used, initial acylcarnitine screening may on the other hand often detect heterozygotes.

Analysis of repeat samples as a second tier test after a first positive control by MS/MS (borderline or presumptive results) is reported to be unreliable as results in VLCAD deficiency cases tend to become negative after the first days of life.[91] Hence diagnostic exploration is recommended as the immediate next step, e.g. by enzyme analysis in lymphocytes or mutation analysis.[94]

VLCAD deficiency is considered a time-critical disorder because acute symptoms or potentially irreversible damage could develop in the first week of life. In a study of 54 patients, 75% of patients with the severe form (25 cases) showed onset within the first three days of life; patients with a milder childhood form (21 cases) had onset by four years of age. The eight patients with a myopathic adult form had onset after the age of thirteen years.[84] Scientific publications that report inappropriate timeliness of neonatal blood screening results for VLCAD deficiency are rare but cases have occurred in Denmark (hypoglycemia at day nine of life) and the USA (one death on day two of life).[2]

In conclusion, screening programs can provide timely results for the milder forms characterised by an onset later in life, but less likely so for the more severe cases that might develop potentially life threatening manifestations shortly after birth.

## 8.5. Efficacy of early vs late treatment

Individuals with the more severe forms are typically placed on a low-fat formula with additional calories supplied through medium-chain triglycerides oil. In addition they are recommended to avoid carefully circumstances that might trigger acute metabolic crises and to seek urgent medical help in case of manifestations such as hypoglycaemia or cardiac rhythm disturbances which are reversible by administering early supportive and symptomatic emergency care.[2, 95]

Risk for sudden death during the first months of life is not insignificant but could be contained with treatment once the condition is diagnosed as avoidance of metabolic crises would prevent development of cardiomyopathy.[2]

A complete neuropsychologic assessment of seven children (aged four to ten years, on low fat diet supplemented with medium chain triglyceride oil and diagnosed through newborn screening with VLCAD deficiency) and one additional child with partial assessment concluded that VLCAD deficiency does not have a significant impact on cognitive or motor skills but that some children may be vulnerable to speech, social and behavioural problems.[96]

Even with dietary treatment, 10 to 20% of patients will experience episodes of rhabdomyolyses while others will never show symptoms.[97]

The phenotypic variation within the group of FAO disorders makes it difficult to assess the efficacy of current therapy,[98] but VLCAD deficiency recognized in neonatal screening has frequently a less severe disease course and dietary restrictions in many patients may be loosened.[99] It is presumed that acute metabolic crises and late/long term complications can be prevented and prognosis can be ameliorated but there is a paucity of data regarding which infants are at risk for neonatal or childhood symptoms, the extent to which symptoms can be prevented by early diagnosis and treatment, or which treatments are likely to be most efficacious. A review of the literature resulted mainly in retrieval of case reports or expert opinion without consensus on all issues, particularly concerning the dietary management of asymptomatic infants diagnosed through newborn screening.[95]

## 8.6. Probability and impact of false positive results

In Belgium in two screening centres, false positives for VLCAD deficiency were not separately reported. The third centre requested in 2013 three repeat samples for VLCAD deficiency on a total of 20 845 tests (first tier borderline or positive test rate of 14.4 / 100 000) but finally withheld none of them as positive.[87]

In other countries, the MS/MS technique is known for a high incidence of false positive cases for VLCAD deficiency on newborn screening.[100] Overall, out of 2 802 504 children screened in California, Oregon, Washington, and Hawai from 2005–2008 through 2009, there were 242 cases screen-positive for VLCAD deficiency. There were 34 symptomatic true positive cases, 18 asymptomatic true positives, 112 false positives, 55 heterozygotes, 11 lost to follow-up, and 12 other disorders. As a result, 8.6 / 100 000 newborns (1.9 and 6.8 / 100 000 for true and false positives respectively) had an abnormal neonatal blood screening for suspected VLCADD. This corresponds to a PPV of 21.5%.[101] In Denmark (including Greenland and the Faroe Islands) on the other hand, 504 049 neonates were screened from 2002 till 2009. During the study 3 true positives, 9 false positives and no false negatives were identified for VLCAD deficiency, resulting in a true positive rate of 0.6 / 100 000, a false positive rate of 1.8 / 100 000 and a predictive positive value of 25%.[10]

Based on the Danish study, false positive screening results would involve approximately two newborns per year in Belgium with approximately one true positive case every three year (0,7 cases / year, assuming 125 000 births annually).[10] For comparison, the ULB and UCL screening centre in Belgium together reported a true positive test rate of 0.72 / 100 000 on 557 833 newborns screened during the years 2000-2013 (or 0.9 true cases yearly for the whole of Belgium). In contrast, using the figures of Merritt et al., approximately 11 abnormal test results per year might be anticipated in Belgium of which eight false positives and two true cases.[101].

**Table 9 – Performance of the VLCAD screening test**

| | Disease positive | Disease negative | Total | Predictive values | Belgium Disease positive | Disease negative |
|---|---|---|---|---|---|---|
| Test positive | 1.9 | 6.8 | 8.6 | 21.5% | 2 | 8 |
| Test negative | 0.0 | 99 991.4 | 99 991.3 | Cannot be calculated | 0 | 124 989 |
| Total | 1.5 | 99 997.8 | 100 000 | | 2 | 124 997 |
| In Belgium births | 125 000 | | | | | |

*Source: Merritt et al.*[101]

False positive results after screening likely result in anxiety of parents when they are contacted for confirmatory testing, as well as additional costs and burden on the health care system associated with confirmatory tests. No study describing this impact was found.

## 8.7. Probability and impact of false negative results

False negatives are only very rarely reported in the literature for VLCAD. Moreover, studies generally rely on passive case finding which may not be entirely exhaustive.[62]

Positive test results in VLCAD deficiency cases tend to be transitory and correct timing of the sample taking is important to avoid false negative results. It is therefore recommended to collect the blood specimen between the first 36 to 72 hours of life.[94] It is also reported that the test can be negative in the absence of stress factors, during periods of physiologic wellness, after recent feeding or after receiving IV glucose, especially in affected individuals who have the milder phenotypes.

The consequence of a false negative result could be valuable time loss in the event of an acute metabolic crisis and possibly death as the urgent need for symptomatic treatment might be underestimated in the absence of a diagnosis.

## 8.8. Impact on the health care system of screening

Several reports appear to support the cost-effective use of tandem MS in a neonatal screening programme for PKU and MCAD deficiency (and probably GA-I) combined in terms of reduced mortality and morbidity.[102] The impact of adding screening for VLCAD to the program would therefore be minimal as the cost of MS/MS screening remains virtually unchanged irrespective of the number of conditions being screened and the necessary components (laboratory equipment, IT-technology, trained personal) are already in place in Belgium. However, costs for other screening components, e.g., patient retrieval, verification of diagnosis, treatment, etc., could increase.

Health economic data are not readily available in Belgium but in view of low anticipated number of positive screening test results, the effect on financial cost and capacity of the health system can be assumed to be low.

Impact of the treatment on health related quality of life is difficult to estimate and seems not to have been studied (no data found) but patients with the severe form are subjected to a diet, strict avoidance of fasting and heavy exercise and may experience episodic muscle pain.

## 8.9. Key points for VLCAD deficiency

- **The frequency of VLCAD deficiency is very low as one case every three years would be expected in Belgium. The birth prevalence was 0.6 / 100 000 in Wallonia-Brussels over the years 2000-2013 with four detected cases only. In other industrialised countries, it varied between 0.95 to 3.2 per 100 000 births.**
- **Three forms of this disease are known. The most severe form, which is also the most frequent one, often starts with a metabolic crisis during the first days of life marked by disturbances of the heart rythm and function, among other problems. This can result in death when not treated. Most newborns who survive this initial episode show improvement, including normalisation of cardiac function but sudden death has occurred in several patients. Heart problems and mortality are rare in the less severe forms of VLCAD deficiency but affected individuals may suffer repeated episodes of too low blood sugar or muscle damage when exercising which can cause potentially severe renal problems.**
- **A positive NBS result would not always be available in time to prevent early life metabolic crises in the most severely affected newborns. For the less severe forms, timeliness of NBS results does not present a problem.**
- **Early treatment aims at preventing metabolic crises. It consists of a diet in which normal fat is replaced by types of fat that can be metabolised by the patient's body and avoidance of fasting and heavy exercise. This approach is mainly based on theoretical arguments. Evidence of its efficacity still lacks due to poor knowlegde of the course of VLCAD deficiency in the absence of preventive therapy. When metabolic crises do occur, urgent admission is required to treat blood sugar problems, heart rhythm disturbances and renal complications.**
- **A number of false positive results are found after the first test (two false positives per year expected in Belgium) but the samples with positive results are systematically retested. If the result of the first test is strongly positive, retesting of both the first sample and a repeat sample is performed.**
- **No false negative cases have been identified in Belgium so far. Especialy in milder forms of the disease, in the absence of fasting or health threatening circumstances or when the sample was not taken soon enough after birth, cases can be missed by NBS.**
- **The impact on the health care system is expected to be low. The cost of adding the detection of VLCAD deficiency to the current NBS would be marginal as it is performed by MS/MS. Around two cases every year would be referred to paediatricians and require confirmatory testing. Cost of treatment comprises mainly dietary measures and emergency admissions to treat metabolic crises symptomatically.**

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