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ABSTRACT

Background In 2002, a nationwide screening for congenital adrenal hyperplasia (CAH) was introduced in the Netherlands. The aim of our study is to evaluate the validity of the neonatal screening for CAH and to assess how many newborns with salt-wasting (SW) CAH have already been clinically diagnosed before the screening result was known.

Methods Retrospective, descriptive study. The following data of patients with positive screening results since implementation of the screening programme were collected (1 January 2002 up until 31 December 2013): gestational age, sex, diagnosis, clinical presentation and contribution of screening to the diagnosis.

Results In the evaluated period, 2 235 931 newborns were screened. 479 children had an abnormal screening result, 133 children were diagnosed with CAH (114 SW, 14 simple virilizing (SV)), five non-classic CAH. During this period, no patients with SW CAH were missed by neonatal screening (sensitivity was 100%). After exclusion of 17 cases with missing information on diagnosis, specificity was 99.98% and positive predictive value was 24.7%. Most false positives (30%) were attributable to prematurity. Of patients with SW CAH, 68% (71/104) patients were detected by neonatal screening and 33 (33/104) were clinically diagnosed. Of girls with SW CAH, 38% (14/37) were detected by neonatal screening and 62% (23/37) were clinically diagnosed.

Conclusion The Dutch neonatal screening has an excellent sensitivity and high specificity. Both boys and girls can benefit from neonatal screening.

What is already known on this topic?

- Neonatal screening programs can contribute to early diagnosis of CAH patients, before severe symptoms occur.
- Neonatal screening mainly has benefits for boys, since girls with classic CAH are likely detected immediately after birth due to virilization of the external genitalia.

What this study adds?

- The Dutch newborn screening program for CAH achieved excellent sensitivity and a high specificity and good positive predictive value.
- Contrary to expectation almost 40% of girls with SW CAH were detected by screening.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of conditions that affects 1:10 000 to 1:20 000 newborns. Most cases are due to mutations in the *CYP21A2* gene, resulting in 21α-hydroxylase deficiency. 21α-hydroxylase catalyses the conversion of 17α-hydroxyprogestrone (17-OHP) to 11-deoxycortisol. Due to the enzymatic defect, the synthesis of cortisol and in most cases also synthesis of mineralocorticoids is affected with overproduction of adrenal precursors before the enzymatic block. The presence of these elevated adrenal steroid precursors such as 17-OHP is an important marker in the diagnosis of CAH. Salt wasting (SW) CAH is a potentially life-threatening disease in newborns.[1 2] As clinically manifest hyponatremia mostly develops after the first week of life, neonatal screening may help to detect CAH before life-threatening SW crisis develops. To facilitate early detection and treatment, neonatal screening programmes were introduced in many countries all over the world.[3–11] There is, however, ongoing discussion as to whether the advantages of neonatal screening for CAH outweigh the disadvantages.[10–15] Some critics state that screening might only be useful for male patients as it is expected that girls with classic forms of CAH are detected after birth due to virilisation of the external genitalia.[16] Furthermore, screening for CAH is accompanied by a high rate of false positive results within hindsight unnecessary diagnostic procedures and parental anxiety.[11 17 18] Additionally, also false negative classic CAH cases are reported.[3]

The estimated mortality rate without neonatal screening during infancy ranges from 0% to 4%.[4 19] In the past, reduction of mortality has been established, probably mainly due to improved healthcare. Several reports claim that in recent decades, there were no deaths caused by CAH in unscreened populations.[20–22]

The main goal of the Dutch neonatal screening is to prevent life-threatening SW and adrenal crisis in patients with SW CAH (www.draaiboekhielprikscreening.rivm.nl).[4 13] Aim of our study is to evaluate the validity of the Dutch neonatal CAH screening

Table 1 Cut-off levels

	17 OHP (nmol/L blood)				
(A) First screening result Dutch neonatal CAH screening					
Gestational age (weeks)					
	0–24	25–54	55–104	105–199	≥200
≤33+0	Negative	Negative	Negative	Negative	Inconclusive
33+1–35+0	Negative	Negative	Negative	Inconclusive	Positive
35+1–36+0	Negative	Negative	Inconclusive	Positive	Positve
≥ 36+1	Negative	Inconclusive	Positive	Positive	Positive
Birth weight (g)					
	0–24	25–54	55–104	105–199	>=200
≤2100	Negative	Negative	Negative	Negative	Inconclusive
2101–2500	Negative	Negative	Negative	Inconclusive	Positive
2501–2700	Negative	Negative	Inconclusive	Positive	Positve
≥2701 or unknown	Negative	Inconclusive	Positive	Positive	Positive
(B) Second screening result Dutch neonatal CAH screening					
Gestational age (weeks)					
	0–24	25–54	55–104	≥105	
≤33+0	Negative	Negative	Negative	Positive	
33+1–35+0	Negative	Negative	Positive	Positive	
35+1–36+0	Negative	Positive	Positive	Positive	
≥36+1	Negative	Positive	Positive	Positive	
Birth weight (g)					
	0–24	25–54	55–104	>=105	
≤2100	Negative	Negative	Negative	Negative	
2101–2500	Negative	Negative	Negative	Positive	
2501–2700	Negative	Positive	Positive	Positive	
≥2701 or unknown	Negative	Positive	Positive	Positive	

OHP, hydroxyprogestrone.

programme and to assess how many newborns with SW CAH have already been clinically diagnosed before the screening result was known.

METHODS

Organisation of the neonatal screening in the Netherlands

The organisation of the programme is assigned to the Centre for Population screening of the Dutch National Institute for Public Health and the Environment (www.rivm.nl). Currently, the neonatal screening programme covers 19 disorders, mostly inborn errors of metabolism. In 1998–1999, a pilot screening programme for CAH was performed in the middle and south-western part of the Netherlands. This pilot was extended to a national pilot programme in 2000–2001 and officially implemented in Dutch routine neonatal screening on 1 January 2002.[4 13] Participation in the neonatal screening programme is voluntary.

Screening and diagnostic procedure

For the vast majority of newborns, the screening is performed between 3 and 7 days after birth. Guthrie filter papers with the blood samples are sent by regular postal service to one of the five regional designated screening laboratories. 17-OHP concentrations are determined using automated fluorescence immunoassay (AutoDelfia or GSP-instruments; PerkinElmer, Turku, Finland). Cut-off levels of 17-OHP related to gestational age (GA) are applied (table 1A and B).[23] When GA is not known, birth weight categories are used.

The laboratories report the results to the Department for Vaccine Supply and Prevention Programmes (RIVM-DVP). In case of an inconclusive result (table 1a), a second blood sample is collected within 7 days after the first blood sample. Children with a positive screening result for CAH after the first heel prick are directly referred to a paediatric endocrinology centre. After an inconclusive result on the first heel prick, a second heel prick is performed. After referral, the child is seen by a paediatric endocrinologist for thorough physical examination (eg, signs of virilisation in girls) and diagnostic workup according to national protocols. Diagnostic workup consists of sodium and potassium levels in serum and urine, glucose, renin levels or plasma renin activitiy and blood gas analysis, adrenocorticotropic hormone, steroid profile on liquid chromatography with tandem mass spectrometry (LC-MS)/mass spectrometry (17-OHP, androstenedione and 21-deoxycortisol level). An ultrasound of the abdomen for evaluation of the adrenal glands and kidneys is performed. If indicated, the uterus and presence and appearance of gonads are checked. In case of elevated 17-OHP level, genetic analysis for CYP21A2 mutations is performed. Referrals and screening results are registered in a national registry (www.neorah.nl). After careful physical examination and first results of diagnostic workup (sodium, potassium, renin) children are diagnosed with CAH or as false positive. Afterwards, when also results of genetic analysis is known, patients with CAH can be classified as SW, simple virilising (SV) or non-classic (NC) or having other enzymatic defects or as a false-positive screening result. The paediatric endocrinologist provides additional information in the national registry after diagnostic evaluation. An independent research institute (the Netherlands Organisation for applied scientific research, TNO; www.tno.nl) is currently assigned to annually evaluate the neonatal screening programme. All Dutch

Table 2 Results from the neonatal screening for congenital adrenal hyperplasia (CAH) in the Netherlands from 1 January 2002 to 31 December 2013

	n (%)
Screened neonates (participation rate)	2 235 931 (99.7)
Positive screening	479
SW CAH	114 (73 boys/41 girls)
SV CAH	14 (10 boys/4 girls)
Non-classic	5 (4 girls/1 boy)
Other enzyme deficiency*	2
Missing CAH classification	4
No CAH	327
Missing diagnosis†	13
True negatives† (no SW CAH)	2 235 452
False negatives (SW CAH)	0
Sensitivity (SW CAH)†	(100)
Specificity (SW CAH)†	(99.98)
Positive predictive value (SW CAH)†	(24.7)

*Other enzyme deficiencies: one child with suspected P450 oxidoreductase deficiency; no mutation analysis has been performed; one child with compound heterozygous 3β-hydroxysteroid dehydrogenase.
†Excluding 17 children without available information on diagnosis (n=13) or CAH classification (n=4).
CAH, congenital adrenal hyperplasia; SW, salt wasting; SV, simple virilising.

paediatricians are asked to report false-negative screening results to the Dutch Paediatric Surveillance System (www.nsck.nl).

Patients

For the current study, all children with a positive CAH screening result born in the period between 1 January 2002 until 31 December 2013 were included. Data on sex, birth weight, GA, 17-OHP levels, classification of CAH and genetic mutation analyses reported by the paediatric endocrinologist were collected from the national registry. Patients are classified as SW, SV or NC by the local paediatric endocrinologist based on clinical presentation including lowest sodium levels before treatment in combination with results of genetic mutation analysis afterwards. Prematurity is defined as GA ≤ 36^{+0} weeks or birth weight ≤2500 g.

Statistical analysis

Data were summarised and analysed by descriptive statistics (frequency tables and crosstabs). Analyses were performed in IBM SPSS Statistics V.24.

RESULTS

Diagnosis and prevalence of CAH in the Netherlands

In the period from 1 January 2002 until 31 December 2013, neonatal screening on CAH was performed in 2 235 931 children with a participation rate of 99.7%.

Four hundred seventy-nine neonates had a positive CAH screening result (table 2). In 114 patients (73 boys and 41 girls), the diagnosis SW CAH was confirmed. In addition, 14 patients had SV (10 boys, four girls) and five patients NC CAH (four boys, one girl). There have been no reports on children with SW CAH missed by screening in the Dutch Paediatric Surveillance System and also no reports from paediatric endocrinologists in the Netherlands outside the surveillance systems. Therefore, no false negative screening results are currently known. In the evaluated period four children who had a negative screening result, were diagnosed with SV CAH later in life.

Table 3 Characterisation of patients with salt-wasting congenital adrenal hyperplasia (SW CAH) detected in the Dutch neonatal screening programme from 1 January 2002 to 31 December 2013

	Male n (%)	Female n (%)	Total n (%)
Total SW CAH	73 (64)	41 (36)	114 (100)
Detected by screening	57	14	71
Detected clinically	10	23	33
Ambiguous genitalia	0	20	20
Prenatal diagnosis	2	3	5
Salt wasting crisis	2	0	3
Family history of CAH	3	0	3
Pigmented scrotum	1	0	1
Unknown	2	0	2
Missing data*	6	4	10*(9%)

*Ten cases (9%) had missing data on whether or not diagnoses was made due to screening or clinically.

In 1727 cases, a second heel prick was necessary after an inconclusive result on the first heel prick.

The prevalence of classic CAH (SW and SV) is 1:16939 (0.59 per 10 000). The prevalence of SW CAH is 1:19 613 (0.51 per 10 000).

Sensitivity, specificity and positive predictive value of neonatal CAH screening

Sensitivity of the Dutch screening for SW CAH was 100%. After exclusion of 17 children with missing data on diagnosis specificity was 99.98% and positive predictive value (PPV) was 24.7% for SW CAH. For classic CAH (SW and SV), PPV was 27.7%.

Contribution of the neonatal screening in diagnosing SW CAH

Most boys with SW CAH were detected by neonatal screening; 10 boys (15%) had already been clinically diagnosed before screening (table 3). Most of the girls with SW CAH were diagnosed before the neonatal screening result was known. Fourteen girls (38%), in whom virilisation was not present or missed at physical examination, were detected only by neonatal screening. Thirty-three children were clinically diagnosed (ambiguous genitalia, SW crises, prenatal diagnosis). In 10 patients with SW CAH, no additional data about the diagnosis were available on whether the diagnosis was made clinically or after an abnormal screening result (table 3).

In two children, confirmation of the diagnosis was delayed because of logistic problems in the screening process (delay in dried blood spot hormone measurement) and delay in reporting the abnormal result.

Two boys with SW CAH were admitted to the hospital due to hyponatremia before the neonatal screening results were known; one boy was admitted at 9 days of age with a lowest sodium concentration of 115 nmol/L (screening was performed on day 5), the other boy had a lowest sodium concentration of 125 nmol/L (there is no further information on age at clinical presentation and admission and timing of neonatal screening).

Fourteen patients (10 boys; four girls) with SV and five patients (four boys; one girl) with NC CAH were detected by neonatal screening. There was no clear correlation between the severity of the disease and the 17 OHP levels (data not shown).

We found a skewed sex distribution in the patients with SW CAH with a male:female ratio of 1.8 (64% boys; $p<0.01$).

False positive screening results and premature neonates

In 327 (67%) neonates with a positive CAH screening, CAH was not confirmed. Thirty per cent of them were premature born children (n=106; GA $\leq 36^{+0}$ weeks). Only in four premature patients (3.8%), the diagnosis CAH was confirmed (two SW, two SV).

Other enzyme deficiencies

Two patients with other adrenal enzyme deficiencies were detected. One child is suspected of having P450 oxidoreductase deficiency; no mutation analysis has been performed. This child presented with ambiguous genitalia directly after birth; 17-OHP in the neonatal screening was 600 nmol/L. In the other child, a compound heterozygous 3 β-hydroxysteroid dehydrogenase deficiency was found. This child also presented with ambiguous genitalia directly after birth; 17-OHP was 140 nmol/L in neonatal screening.

DISCUSSION

The Dutch neonatal screening programme for CAH was introduced in 2002 to prevent morbidity and mortality due to CAH-related SW crisis in SW CAH.[4 13] We were able to detect two-thirds of all Dutch patients with SW CAH by neonatal screening. The other one-third of patients with SW CAH and a positive screening result had already been diagnosed before screening results were known. Prevalence rates of SW and SV CAH in the Netherlands are comparable to those in other European countries.[9 11 17] Hyponatremia could be prevented in all but two neonates (table 3).

Interestingly, 38% of the girls with SW CAH were detected by the neonatal screening programme. In these patients, signs of virilisation, which could have led to an earlier diagnosis, were not noticed at physical examination after birth. This means that girls with SW CAH can also benefit from the Dutch neonatal screening programme. This is in agreement with the results of an earlier study by Van der Kamp in which CAH was not suspected clinically in three out of eight girls in the Netherlands.[4] To improve detection of virilisation after birth, paediatricians and midwives should be trained to also detect mild and moderate virilisation of female external genitalia.

We found a statistically significant skewed male to female ratio of 1.8 for patients with SW CAH detected by neonatal screening. Other studies have reported a more equally distributed sex ratio in neonatal screening, generally around 1.0.[2 4 23] It can be speculated that early treatment in girls detected before the screening is performed, may lower 17-OHP values to a level below the cut-off level used for screening, leading to a negative screening result. However, as far as we know, no false negative CAH girls were reported in this evaluation period. Therefore, other unknown causes may play a role.

A valid neonatal screening programme detects patients before they become sick and ideally has as little false positive results as possible. Early diagnosis of CAH to prevent SW crisis requires a well-functioning national infrastructure for the screening programme. Performing the screening before the age of 3 days will increase the false positive rate due to physiological higher 17-OHP levels within the first 72 hours after birth.[13]

Seventy-five per cent of newborns with abnormal CAH screening results were not diagnosed with SW CAH. Reducing false positive screening results is an important goal for improving the screening programme. Several clinical conditions such as neonatal sepsis, seizures, birth asphyxia or hydronephrosis may increase the concentration of 17-OHP leading to false positive CAH screening result.[24 25] The most important cause of false positive CAH screening is prematurity. In our cohort, 106 premature newborns had a positive screening, but only in four of them, the diagnosis classic CAH was confirmed. It is well known that premature newborns have higher 17-OHP levels due to immature adrenal cortex enzymes, especially 11β-hydroxylase,[26] leading to false positive results in neonatal screening programmes. Therefore, in the Netherlands, as in most other countries, adapted cut-off levels are used for premature neonates to reduce false positive screening results in this group. Van der Kamp found that GA has a better correlation with 17-OHP than birth weight.[27] Several additional methods have been described in the literature to lower false positive results.[13 28] Few papers report additional measurements, such as a second tier with LC-MS/MS or consecutive measurements of 17-OHP in the first weeks, especially for the extreme and/or low birthweight premature newborns.[29]

Additional measurement of 21-deoxycortisol (21 DF) has been reported as an effective method to decrease false positive screening results.[30–32] 21 DF is the 11-hydroxylated product of 17-OHP. In unaffected neonates, 21 DF is barely detectable. The additional measurement of 21 DF is therefore a promising method to reduce false positive screening results.[30–32] Furthermore, LC-MS/MS takes quite some time before results are known. For CAH, this amount of time is not acceptable since timely diagnosis is important.

In our cohort, 14 patients with SV CAH have been found. Although the screening programme is primarily aimed to find patients with SW CAH to prevent SW crisis, 17-OHP levels are similar and the levels of 17-OHP alone cannot discriminate between SW and SV CAH.[33] Presentation of SV CAH and SW CAH have overlapping symptoms, in both girls present with virilisation.[34] This implies that there is also a risk for SW and Addisonian crisis in several circumstances (warm weather, strenuous exercise, severe illness) in patient with SV CAH. Furthermore, in untreated patients with SV CAH, there is an increased risk of early puberty and impaired final adult height due to chronic exposure of adrenal androgens. Treating SV CAH from the first weeks of life is therefore beneficial.

In our cohort, five patients with NC CAH were detected by the neonatal screening programme. A positive screening in NC CAH is considered as an unintended finding without the need to treat these children at an early age. We also found two patients with adrenal enzyme deficiencies other than 21α-hydroxylase deficiency. It is known that in 11β-hydroxylase deficiency, 17-OHP is also slightly elevated leading to abnormal positive neonatal screening result in some cases.[35] In 3β-hydroxysteroid dehydrogenase deficiency (3βHSD2), 17-OHP can be slightly elevated due to conversion of elevated concentration of dehydroepiandrosterone (DHEA), and positive screening is anecdotally reported.[36] In infants with a positive screening result, 11-deoxycortisol and DHEA should be measured to exclude 11-hydroxylase deficiency and 3βHSD2 deficiency. Cytochrome P450 oxidoreductase deficiency is a rare disorder. Most patients present with ambiguous genitalia at birth and have elevated 17-OHP, 17-OH-pregnenolone, pregnenolone and progesterone. These patients can also have adrenal insufficiency.[37 38] These cases are unintended findings of neonatal screening for CAH.[38]

CONCLUSION

The Dutch neonatal screening programme for CAH has an excellent sensitivity and high specificity. Boys and girls with SW

CAH can benefit from neonatal screening. Two-thirds of patients with SW CAH are detected by neonatal screening. There is a high false positive rate mostly in prematurity. Additional diagnostic tools, such as the introduction of routine measurement of 21 DF might be helpful to improve the neonatal screening in upcoming years.

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Contributors AvdL, HCvdG and PV drafted the initial manuscript and reviewed and revised the manuscript. YS carried out the statistical analyses and reviewed and revised the manuscript. All authors are members of the Dutch Advisory board on neonatal screening for CAH. The paediatric endocrinologists in this advisory board, HvdK, EvdA, MvA, MF, SH, GH-N, RO, SS, PvT provided data of the children with abnormal screening results in the national registry and reviewed and revised the manuscript. AB and PS critically reviewed the manuscript for intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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