ABSTRACT.  

Objectives.  The aim of this study was to evaluate the benefits of neonatal screening for congenital adrenal hyperplasia (CAH).

Methods.  All children with CAH born in Sweden from January 1989 to December 1994 were subjected to a systematic follow-up. Clinical symptoms were recorded and laboratory data collected. The clinical diagnosis versus diagnosis by screening was investigated. The results were compared with those of a retrospective study of all patients diagnosed during 1969–1986 (before the introduction of neonatal screening).

Results.  The prevalence of CAH in Sweden was 1/9800 with screening. Patients with CAH were identified earlier by screening. Half of the infants (47%) were not diagnosed at the time of recall, which was 8 days (median). In the study population, 25% of the girls and 73% of the boys were diagnosed by screening alone. The median age at the time of the definitive diagnosis in boys was 21 days before screening as compared with 9 days (median) during the last part of the screening period. During the screening period, only 1 boy had a severe salt loss crisis, which occurred at the age of 8 days. Before screening, (1969–1986) 2 boys had died in the neonatal period because of an adrenal crisis. The lowest serum sodium recorded at the time of diagnosis was 124 mmol/L (median; range, 93–148) before, as compared with 134 mmol/L (median; range, 115–148) after the introduction of screening.

The number of girls who were initially considered to be boys was not reduced by screening (17% vs 18%). The period of uncertainty regarding gender attributable to virilization was shortened considerably, as well as the time it took to make a correct gender assignment: 23 days (median) before screening versus 3 days (median) with screening. The maximum time it took to make the correct gender assignment was 960 days before screening and 14 days with screening. The number of patients diagnosed late, ie, after the first year of life, decreased considerably after the introduction of screening. The false-positive rate (when a new filter paper blood sample was requested or when a child was referred to a pediatrician for follow-up) was <0.05% and in about 60% of the cases, it was attributable to preterm infants. The cost of screening was US dollar 2.70 per screened infant.

Conclusion.  The main benefits of screening were avoidance of serious salt loss crises, earlier correct gender assignment in virilized girls, and detection of patients who would have otherwise been missed in the neonatal period. Deaths in the neonatal period were prevented by screening. The aim of the screening program was to identify patients with the severe forms of CAH. Nevertheless, it must be considered a distinct benefit that a number of patients with milder forms of CAH were detected earlier, because earlier therapy results in decreased virilization, normalized growth and puberty, and, in all probability, an improved psychosocial situation for these children.

We conclude that, in the Swedish health care system, the benefits of screening for CAH outweigh the costs.

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C ongenital adrenal hyperplasia (CAH) comprises a group of inborn errors in the synthesis of adrenal corticoid hormones. More than 95% of the patients have a 21-hydroxylase deficiency. The 21-hydroxylase gene has been cloned and localized in chromosome 6. Traditionally, patients with CAH have been classified as classic (salt-losing or simple virilizing), late-onset, and cryptic variants of the disorder. This has been shown to reflect the severity of the 21-hydroxylase deficiency rather than different entities of the disease. More than 19 different mutant alleles have now been identified.

Life-threatening neonatal complications of CAH, such as salt loss and hypoglycemia as well as the consequences during childhood and adolescence involving virilization, early puberty, and short stature prompted the development of neonatal laboratory screening. The technique of analyzing 17-hydroxyprogesterone (17-OHP) in filter paper blood samples was developed by Pang et al in 1977. Since then, nationwide and regional screening for CAH has been introduced in several countries. Doubt has been expressed, however, about the benefits of CAH screening. It has been claimed that the majority of CAH patients will be identified clinically at birth and the result of the screening will not be available before the development of a serious adrenal crisis.

Neonatal screening for CAH was started as a nationwide program in Sweden in 1986. From the start of screening to the end of 1996, the program involved 1.22 million infants and identified 106 pa-
tients with CAH, all with 21-hydroxylase deficiency. To avoid a high false-positive recall rate, the initial cut-off level was deliberately set high. During 1986–1988, 8 out of 31 (26%) patients with CAH were not detected by screening. The cut-off limit was therefore lowered for full-term babies from 200 nmol/L of plasma to 150 nmol/L plasma (assuming a 50% hematocrit). The currently used 75 nmol/L plasma was introduced at the same time as the Delphia method in 1991. Gestational age-related recall levels were used.* In all pregnancies in Sweden gestational age is determined by ultrasound, which makes this parameter reliable. Since 1986, the recall level for infants born before gestational week 37 was 200 nmol/L plasma after ether extraction (see Table 1).

The purpose of the study was to evaluate the benefits of screening to provide the basis for a decision as to whether CAH should be included in the neonatal screening program in Sweden.

MATERIALS AND METHODS

Filter paper blood samples were used. They were collected on days 3 to 5 after birth and analyzed for 17-OHP. Initially, 17-OHP was determined by radioimmunoassay. Since 1991, the Delphia method (Wallac, Turku, Finland) has been used. Gestational age-related cut-off levels were used, as described earlier (see Table 1). All recalls were communicated to the physician-in-charge by telephone to shorten the recall time.

A prospective study of all Swedish infants born from January 1989 to December 1994, was undertaken. All CAH patients diagnosed during this period were examined by one of us (A.T.). We collected clinical and laboratory information about these patients continuously and also recorded the number of false-positive, false-negative, true-positive and true-negative cases. We specifically asked if the diagnosis was suspected on clinical grounds before the screening result was available. The 44 departments of pediatrics in Sweden were repeatedly asked for reports on CAH cases missed by the screening.

The outcome of CAH screening was compared with a retrospective analysis of all Swedish CAH patients detected by clinical symptoms during 1969–1986 before the screening started.2 Regression analysis and the Mann-Whitney U test were used for statistical comparisons.

RESULTS

Characteristics of the population are shown in Tables 2 and 3. The screening identified 66 of 73 (90%) infants as true-positive cases.

The 7 false-negative cases were diagnosed by clinical signs and symptoms. Four of these would not have been detected with our current cut-off level. One boy had a screening 17-OHP of 41 nmol/L plasma. Because he had a sister with simple virilizing CAH, he was investigated and diagnosed at 7 days of age. He had a subnormal serum sodium (S-Na) value (131 mmol/L, reference 133–146 mmol/L) and elevated P-renin (43 ng/mL/h, reference <10 ng/mL/h) levels. Another boy, born in gestational week 34, had a screening level of 157 nmol/L after ether extraction. When he was diagnosed at the age of 6 he had pseudopubertas praecox and accelerated growth with a height of +5 standard deviations. Two girls were diagnosed late with signs of precocious puberty at 6 and 7 years of age. Their screening values were 66 and <50 nmol/L, respectively. All of the false-negative cases had mutations in the 21-hydroxylase gene, yielding mild to moderately severe forms of CAH.3

The overall rate of false alarms was low. It ranged between 0.006 and 0.044% during different years, ie, <0.05%. About 60% of the cases involved preterm infants.

One boy had a false-positive screening test. He was born in the 37th week of gestation. The initial screening test on day 3 showed a 17-OHP of 97 nmol/L plasma and the second sample at 2 weeks showed 154 nmol/L in a direct screening assay and 53 nmol/L after ether extraction. Treatment was started although a separate serum sample showed only slightly elevated 17-OHP (14 nmol/L). A subsequent DNA analysis failed to reveal any known mutation in the 21-hydroxylase gene and an adrenocorticotropic hormone stimulation test was normal. Therapy was stopped and the child has remained healthy.

The 66 true-positive cases are described further in Table 3. Thirty-five CAH cases (53%; 27 girls and 8 boys) were suspected or diagnosed before recall, while the remaining 31 (47%; 9 girls and 22 boys) were detected by screening. Fourteen of 73 patients (19%) had at least one older sibling with CAH. Eleven of these children were diagnosed prenatally and were monitored as described elsewhere.10

The incidence of CAH was 1/10 200 and the prevalence 1.9800 after the introduction of screening.

TABLE 1. Recall Levels

<table>
<thead>
<tr>
<th>Gestational Age (Weeks)</th>
<th>17-OHP (nmol/L Plasma)</th>
<th>17-OHP (nmol/L Plasma)</th>
<th>Ether Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥37</td>
<td>75</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>33–36</td>
<td>400</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>&lt;33</td>
<td>600</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

* The recall level for ether extraction is not relevant to full-term infants, first recall; it is used to confirm the direct assay.


<table>
<thead>
<tr>
<th>Number of CAH Patients</th>
<th>Study Population</th>
<th>Girls</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Totals</td>
<td>73</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>True-positive screening test</td>
<td>66</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>False-negative screening test</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Neonatal Symptoms

Neonatal problems of a nonspecific nature occurred in 31 of the 66 CAH patients (45%). The most common problems were reduced alertness (6 patients) and hyperbilirubinemia (6 patients). In addition, respiratory problems, infections, poor weight gain and/or feeding problems were observed. Eight infants had hypoglycemia (defined as B-glucose <2 mmol/L). In 13 girls with CAH, gender assignment was uncertain for 1 to 6 days (median, 4 days) and 6 were assigned the wrong gender for 2 hours to 14 days (median, 3 days). Twenty-one girls were classified as Prader type III-IV, ie, 59% of the early diagnosed girls. Twelve patients (3 girls and 9 boys, 18%) had no recorded clinical symptoms character-
Reaching 10 to 20 times the upper normal limit. The additional costs of CAH screening were calculated as the costs in addition to the preexistent routine neonatal screening program, which, in Sweden, includes phenylketonuria, congenital hypothyroidism, and galactosemia. The costs of CAH screening including reagents, personnel, rent, and equipment in the national neonatal screening laboratory covering about 100,000 newborns per year was US dollar (USD) 2.60 per newborn screened. The additional costs of follow-up for false-positive cases were USD 150 for full-term infants (return visit and laboratory costs) and USD 2.60 for preterm infants (one additional filter paper). 21-Hydroxylase mutation analysis costs USD 500, this is performed for about 10 children per year and adds USD 5000 to the total cost. At the same time, it decreases the follow-up costs for doubtful cases that would otherwise have to be followed clinically for a long period of time. It also decreases the psychological trauma by shortening the period of uncertainty of the diagnosis. These follow-up costs add USD 0.07 per screened infant.

The benefits of screening for CAH are, to a large extent, dependent on the health care system available. The costs are also determined by local conditions. We have, therefore, evaluated CAH screening in the Swedish context. We compared the outcome for CAH patients before and after the introduction of nationwide neonatal CAH screening with respect to certain critical indicators. We decided to allow for an initial period of nationwide screening before conducting the study so that a number of starting-up problems could be solved. Nevertheless, the efficiency of the screening system, ie, the turnaround time, continued to improve during the study period.

The prevalence of CAH was 1:9800 after the introduction of screening, which is not a statistically significant difference from the prevalence before screening, 1/11 500.9 This indicates that the screening is not overdiagnosing patients even if a larger number of patients seem to be identified.

For the screening program to be able to prevent neonatal salt crisis, it is necessary to have an early

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>All Cases</th>
<th>Diagnosed by Screening</th>
<th>Suspicion of Diagnosis Before Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Totals</td>
<td>66 (36F, 30M)</td>
<td>31 (9F, 22M)</td>
<td>35 (27F, 8M)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>12 (3F, 9M)</td>
<td>8 (3F, 5M)</td>
<td>4 (4M)</td>
</tr>
<tr>
<td>Virilization alone</td>
<td>18 (F)</td>
<td>2 (F)</td>
<td>16 (F)</td>
</tr>
<tr>
<td>Virilization and salt loss†</td>
<td>13 (F)</td>
<td>3 (F)</td>
<td>10 (F)</td>
</tr>
<tr>
<td>Salt loss alone†</td>
<td>12 (M)</td>
<td>10 (M)</td>
<td>2 (M)</td>
</tr>
<tr>
<td>Nonspecific symptoms alone</td>
<td>11 (2F, 9M)</td>
<td>8 (1F, 7M)</td>
<td>3 (1F, 2M)</td>
</tr>
<tr>
<td>Wrong gender assignment</td>
<td>7 (F)</td>
<td>1 (F)</td>
<td>6 (F)</td>
</tr>
</tbody>
</table>

F, female; M, male.

* S-Na ≥130 mmol/L, no general symptoms.
† Salt loss, S-Na ≤129 mmol/L.

**DISCUSSION**

The economic costs of CAH screening were calculated as the costs in addition to the preexistent routine neonatal screening program, which, in Sweden, includes phenylketonuria, congenital hypothyroidism, and galactosemia. The costs of CAH screening including reagents, personnel, rent, and equipment in the national neonatal screening laboratory covering about 100,000 newborns per year was US dollar (USD) 2.60 per newborn screened. The additional costs of follow-up for false-positive cases were USD 150 for full-term infants (return visit and laboratory costs) and USD 2.60 for preterm infants (one additional filter paper). 21-Hydroxylase mutation analysis costs USD 500, this is performed for about 10 children per year and adds USD 5000 to the total cost. At the same time, it decreases the follow-up costs for doubtful cases that would otherwise have to be followed clinically for a long period of time. It also decreases the psychological trauma by shortening the period of uncertainty of the diagnosis. These follow-up costs add USD 0.07 per screened infant.

The cost per CAH patient in the population was USD 26,700 and per patient who was diagnosed earlier because of the screening, USD 53,400. It is impossible to evaluate the psychological burden of false-positive tests in economic terms but, nevertheless, it is very important to minimize it.12

**Turnaround Time**

The time between sampling (median, day 4) and the time for recall decreased from 7 days (median) at the beginning of the study period to 4 days (median) after 1991. This was attributable to greater efficiency in all the steps in the screening procedure and to the introduction of the Delphia method, which shortened the time for analysis by one day. The age for recall in the whole population (1989–1994) was 8 days (median) while it was 9 days (median) for the boys.

**Costs of CAH Screening**

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The benefits of screening for CAH are, to a large extent, dependent on the health care system available. The costs are also determined by local conditions. We have, therefore, evaluated CAH screening in the Swedish context. We compared the outcome for CAH patients before and after the introduction of nationwide neonatal CAH screening with respect to certain critical indicators. We decided to allow for an initial period of nationwide screening before conducting the study so that a number of starting-up problems could be solved. Nevertheless, the efficiency of the screening system, ie, the turnaround time, continued to improve during the study period.

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Before screening the prevalence of CAH was higher for girls than for boys. This difference has evened out with the introduction of screening, indicating that a number of boys were not diagnosed before. Some boys might have died in the neonatal period but it is also possible that a number of boys with milder forms of CAH escaped diagnosis before screening was started. The economical gain of saved lives is always very difficult to evaluate.13

For the screening program to be able to prevent neonatal salt crisis, it is necessary to have an early...
time for recall. Because salt loss often occurs at 2 to 3 weeks of age, it is essential to pursue a screening program in which the test results are available before that time. The amount of time elapsed before the diagnosis of unsuspected cases is a very critical parameter in this context. The median age at the definite diagnosis in boys was 21 days before screening as compared with 9 days (median) during the last part of the screening period. During our study period, only 1 boy had a severe salt loss crisis, at 8 days of age, although hyponatremia (S-Na ≤129 mmol/L) was observed in 25 (36%) of the patients at the time of the diagnose. During the period 1969–1986, ie, before the screening, 2 boys died in the neonatal period because of an adrenal crisis with salt loss and at least 5 to 10 children had been critically ill during the neonatal period. The lowest S-Na recorded at the time of the diagnosis was 124 mmol/L (median; range, 93–148). With screening, the median S-Na at the time of the diagnosis was 134 mmol/L (range, 115–148). The difference in S-Na at the time of the diagnose was statistically significant between the two groups, P < .0001. This indicates that the patients were more seriously ill at diagnosis before screening was introduced.

Before screening, the gender was uncertain for some time for 23 of the 93 (25%) girls studied. In addition, 16 girls (17%) were considered to be boys for 23 days (median), with a range of 2 hours to 960 days. In the present study, there was uncertainly about the gender of 16 girls (42%) and 7 girls (18%) were assigned the wrong gender for 3 days (median) with a range of a few hours to 14 days. The decrease in the period of incorrect gender assignment was statistically significant, P = .01. The number of girls who were considered to be boys was not reduced, but the maximum time it took to make the correct gender assignment was shortened considerably (960 vs 14 days), which we consider to be an important achievement.

Hypoglycemic episodes were noted in 8 children during the neonatal period. Hypoglycemia is an early symptom/complication of CAH that is difficult to evaluate. In our study, no hypoglycemic episodes were reported in the neonatal period after the first week of life, which means that they occurred before the time of recall and, therefore, were not affected by the screening.

We found that 47% of the CAH patients did indeed benefit from screening. The other 53% had already been diagnosed or were suspected to have CAH when the screening result became available (Table 3). The clinically diagnosed patients were identified or suspected usually because of virilization in girls or previous siblings with the disorder. It is not surprising that boys are most likely to benefit from CAH screening, but, interestingly enough, about 25% of the girls were also detected by screening. The fact that there is a screening program for CAH might possibly increase the awareness and general knowledge of the disease among pediatricians. This would also contribute to an earlier clinical diagnosis, before the screening result is available.

It is important to keep in mind that the aim of most screening programs is to identify patients with the severe forms of CAH. No screening program can detect all milder forms of CAH without having an unacceptably high false-positive rate. All the false-negative cases in our study had milder forms of CAH, as shown by genotyping. Nevertheless, a large number of patients with less severe forms are detected earlier by screening, as determined by 21-hydroxylase mutation analysis. Earlier therapy results in decreased virilization, normalized growth and puberty, and presumably an improved psychosocial situation for these children. These must be considered benefits of screening. Also in areas with adequate neonatal and endocrinologic services the clinical diagnosis of classic and late-onset CAH is often delayed.14 Our experience in Sweden is that growth acceleration as a sign of hyperandrogenism in the preschool period was frequently overlooked. The median diagnostic delay from the start of symptoms was 17 months (range, 6–44 months).9

The costs and risks of CAH screening are the economic expenditure and the risk of overdiagnosis and treatment, as well as the psychosocial side effects of false alarms. The overall rate of false alarms was, also in an international perspective, considered to be low, <0.05%, and in about 60% of the cases occur in preterm infants. The false alarms for preterm infants with the Delphia screening procedure has recently been discussed by Saedi et al.15 The problem is similar to that in radioimmunoassay procedures. Our strategy to minimize this problem is to use gestational age-related cut-off limits and ether extraction procedures for samples from preterm infants with elevated levels of 17-OHP.8 In the Swedish health care system, it is easy to obtain a second sample before the child leaves the hospital. When the follow-up level of 17-OHP had decreased considerably, this was taken as evidence that the initial screening test was false-positive. We are currently reevaluating the 17-OHP cut-off levels for premature infants in an effort to minimize the false-positive and false-negative rate.

The introduction of 21-hydroxylase mutation analysis has simplified the verification of a doubtful diagnosis.3,16 The genotype-phenotype correlation is a valuable tool for estimating the prognosis and deciding on the treatment. This was previously based on clinical symptoms and chemical laboratory tests, including adrenocorticotropic hormone stimulation analysis. For children with true and suspected true-positive screening tests, we now recommend a follow-up with 21-hydroxylase mutation analysis.

CONCLUSIONS

We conclude that the benefits of screening for CAH outweigh the costs in the Swedish health care system. On the basis of these results, the Swedish National Board of Health and Welfare has recently ruled that CAH screening shall be a part of the nationwide routine neonatal screening program.
ACKNOWLEDGMENTS

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Benefits of Neonatal Screening for Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency) in Sweden

Astrid Thilén, Anna Nordenström, Lars Hagenfeldt, Ulrika von Döbeln, Claes Guthenberg and Agne Larsson

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