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Congenital adrenal hyperplasia cases identified by newborn screening in one- and two-screen states☆

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ABSTRACT

There is no clear consensus among state newborn screening programs on whether routine second screening of newborns identifies clinically relevant cases of congenital adrenal hyperplasia. This retrospective study evaluated laboratory practices, along with biochemical and medical characteristics of congenital adrenal hyperplasia (CAH) cases (1) detected on the first newborn screen in one-screen compared to two-screen states, and (2) detected on the first versus the second screen in the two-screen states, to determine the effectiveness of a second screen. A total of 374 confirmed cases of CAH from 2 one-screen states and 5 two-screen states were included in this study. Demographic data and diagnostic information on each reported case were collected and analyzed. Additionally, laboratory data, including screening methodologies and algorithms, were evaluated. The one-screen states reported 99 cases of CAH out of 1,740,586 (1 in 17,500) newborns screened: 88 (89%) identified on the first screen and 5 (5%) identified on the targeted second screen. The two-screen states reported 275 cases of CAH out of 2,629,627 (1 in 9500) newborns screened: 165 (60%) identified on the first screen and 99 (36%) identified on the second screen. Using a multivariate model, the only significant predictor of whether a case was identified on the first or the second screen in the two-screen states was the type of CAH. Compared with classical salt-wasting CAH, classical simple virilizing and non-classical CAH cases were less likely to be detected on the first versus the second screen. The routine second newborn screen is important for identifying children with CAH, particularly simple virilizing and non-classical forms, which might otherwise not be captured through a single screen.

1. Introduction

Nearly all newborns in the United States receive a state-mandated newborn screen, which enables early identification and treatment of disorders that can cause intellectual disability, morbidity, and mortality. In the 1960s when newborn screening (NBS) began, it was typical that the heel stick blood specimens used for screening were collected at 48–96 h after birth. This practice enabled adequate nutritional intake for detection of metabolic disorders and allowed for the natural rise and fall of analytic marker concentrations that occur during the first day of life. In more recent years, the practice of early hospital discharge of newborns has significantly impacted NBS; many newborns have specimens collected at or before 24 h of life, and some are even collected before 12 h of life, leading to an increased chance of missing clinically significant cases [1,2]. As a response to this concern, the Maternal and Child Health Bureau recommended that (1) the NBS specimen be collected from all newborns as close as possible to the time of discharge from the nursery, and in no case later than 7 days of age; and (2) if the initial specimen is collected before 24 h of age, a second specimen should be collected before 2 weeks of age [3].

Congenital adrenal hyperplasia (CAH), caused by steroid 21-hydroxylase deficiency, was first proposed for NBS in 1977 because infants with the classical salt-wasting form have impairment of mineralocorticoid and glucocorticoid syntheses, leading to hyponatremic dehydration, shock, and eventually death, if untreated. Early identification and treatment were shown to prevent life-threatening adrenal crisis [4]. There are 2 other milder forms of CAH: classical simple virilizing and

Abbreviations: NBS, newborn screening; CAH, congenital adrenal hyperplasia; 17-OHP, 17-hydroxyprogesterone; BW, birth weight; SACHDNC, Secretary's Advisory Committee on Heritable Disorders in Newborns and Children; APHL, Association of Public Health Laboratories; NICU, neonatal intensive care unit; NHW, non-Hispanic white; NHB, non-Hispanic black; A/PI, Asian/Pacific Islander; OR, odds ratio; CI, confidence interval.

☆ Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Association of Public Health Laboratories.

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non-classical. Patients with classical simple virilizing CAH have ambiguous genitalia due to the exposure to androgens, but do not experience salt-wasting crisis. Patients with the non-classical form of CAH can be largely asymptomatic [4]. The estimated prevalence of the 2 classical forms of CAH in the United States is 1 in 16,000 to 1 in 20,000 [5].

The analytic marker used to screen for CAH cases, 17-hydroxyprogesterone (17-OHP), is typically elevated at birth and declines to stable concentrations by 1 to 3 weeks of age in healthy newborns. In contrast, 17-OHP concentrations in newborns with CAH increase over time after birth [5,6]. Milder forms of CAH may be missed on an initial screen (false-negative) because of insufficient 17-OHP elevations at the time of collection, which is typically within the first 24 to 48 h after birth [7–9]. Additionally, low birth weight (BW) and prematurity can contribute to an increased 17-OHP concentration in unaffected newborns, leading to false-positive cases; adjustments to screening cutoff values based on BW and gestational age have been used by NBS laboratories to minimize false-positive rates [10–13]. Given the disease spectrum and the fluctuations of the 17-OHP hormone concentration, especially within the first few days of life, diagnostic accuracy can be challenging.

Newborn screening programs adopt cutoff values for analytic markers that maximize detection of all true cases and minimize the number of false-positive results. The number of false-positive screening results for CAH and other disorders on the NBS panel is a concern to programs because it causes unnecessary testing of newborns, undue parental anxiety, and added costs and strain to the follow-up programs and the medical system [14,15]. However, a larger concern, and one that is much more difficult to quantify, is false-negative screen results, which can lead to missed cases or delays in identification of newborns with treatable conditions. Most states perform a single screen on term newborns that have a satisfactory specimen collected between 24–48 h after birth. For newborns that do not meet these criteria, additional screening (targeted second screen) might be recommended, based on the state-specific screening algorithm. To minimize the chance of missing clinically significant disorders on a single screen, 9 states have mandated that a routine second screen be performed on all newborns at 8–14 days of age, and 3 states have the recommended routine second screen that is obtained on ≥85% of newborns in these states. Taken together, approximately 22.5% of all U.S. newborns receive the routine second screen.

It is not uniformly agreed upon that NBS programs should detect all forms of CAH, as opposed to only the severe salt-wasting form. Additionally, evidence has been inconsistent as to the effectiveness of the routine second newborn screen to detect cases of CAH missed by the first screen. In Washington state from 1978–1992, an initial newborn screen failed to detect 21 newborns that were subsequently identified on the second screen, including 2 with CAH (5% of all identified CAH cases) [16]. In Wisconsin, where only 1 newborn screen is performed, data on newborns with false-negative results for CAH from 2000–2003 were analyzed [17]. Eight newborns during this time period were not identified by the newborn screen, and subsequently received a diagnosis of 21-hydroxylase deficiency, although none had the salt-wasting form of CAH; these results suggested that the initial screen successfully identified all newborns with the more severe form of the disorder. A study in Texas (a two-screen state) also reported that the first screen detected newborns with the salt-wasting form of CAH, while the second screen detected primarily newborns with the simple virilizing or non-classical forms of CAH [18]. In a study from Colorado, also a two-screen state, the sensitivity of the first screen was determined to be 71.8% (false-negative rate of 28.2%) for detecting classical CAH, defined as both the salt-wasting and simple virilizing forms [19]. Minnesota, a single screen state, reported 4 classical CAH cases (3 simple virilizing and 1 salt-wasting) missed over a 5 year period when using a first tier NBS protocol measuring only 17-OHP concentration in specimens (false-negative rate of 15.4%) [8,9].

To address the ongoing debate among state NBS programs regarding the utility of the routine second screen to identify clinically relevant cases of CAH missed by the first screen alone, plans for the retrospective study reported here were initiated with support from the Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC). The specific objectives of this study were to examine the effectiveness of the routine second screen for CAH by evaluating laboratory practices along with biochemical and medical characteristics of CAH cases (1) detected in the one-screen compared to two-screen states, and (2) detected on the first versus the second screen in the two-screen states.

2. Methods

A 5-year retrospective study was planned by representatives from 14 state NBS programs, endocrinologists, and representatives from the Health Resources and Services Administration, the National Newborn Screening and Genetics Resources Center, the Centers for Disease Control and Prevention, the Food and Drug Administration, the Association of Public Health Laboratories (APHL), the Health and Human Service's SACHDNC, Pediatrix Screening, and the CAH Research Education & Support Foundation. Upon execution of the study, data from confirmed cases of CAH were obtained from 2 one-screen states (CA, WI) and 5 two-screen states (AL, DE, MD, OR, TX). Data submitted to the study spanned a 3–5 year period of time (Fig. 1). Two-screen states were defined as states with a legally-mandated requirement to routinely collect a second blood specimen from all newborns, or states with a recommended second screen that results in greater than 85% of all newborns receiving a second screen at 8–14 days after birth. All participating states received Institutional Review Board approval for the study.

2.1. Screening methodologies and algorithms

All participating states quantified 17-OHP as the analytic marker for CAH using a dissociation-enhanced lanthanide fluoroimmunoassay. A fixed cutoff based on BW was used to identify newborns at risk for CAH in both of the one-screen states and in 4 of the two-screen states. One of the two-screen states used a floating cutoff that was determined daily based on a percent from the mean 17-OHP value obtained on the normal population and on low BW newborns. All states had a unique algorithm for repeat screening and reporting of abnormal results, although in general, depending upon the 17-OHP concentration, states either recommended repeating the newborn screen (by collecting the second specimen) or performing confirmatory testing and a clinical assessment. The screening algorithm for 1 of the one-screen states included collection of the targeted second screens at specific intervals after birth for newborns that had extended hospital stays due to low BW or illness.

2.2. Data elements

Individual-level anonymous data were submitted to the study coordinating center at the APHL on all confirmed cases of CAH. These data elements included newborn demographics (e.g., sex, race/ethnicity) and factors that might affect the screening result (e.g., BW, gestational age, dietary intake, exposure to medications, and whether the newborn received a blood transfusion or was in the neonatal intensive care unit (NICU) prior to collection of the NBS specimen). Laboratory factors were obtained on each case, such as assay specific information (e.g., type of assay used to measure the 17-OHP concentration, the measured 17-OHP concentration, and screening cutoff values) and timing (age of newborn at specimen collection and time from collection to analysis). Clinical characteristics pertaining to case diagnosis were also collected (prenatal treatment with steroids, how newborn sex was determined, clinical manifestations at presentation, degree of virilization, and what treatment, if any, was initiated and when). Newborn screening laboratories provided information on the type of CAH that was diagnosed and whether the newborn was identified on the first screen, routine second screen or targeted second screen (for the one-screen states),