0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(7):3904–3907
Copyright © 2005 by The Endocrine Society
doi: 10.1210/jc.2004-2136

Cutoff Levels of 17-α-Hydroxyprogesterone in Neonatal Screening for Congenital Adrenal Hyperplasia Should Be Based on Gestational Age Rather Than on Birth Weight

Hetty J. van der Kamp, Caren G. M. Oudshoorn, Bert H. Elvers, Maja van Baarle, Barto J. Otten, Jan M. Wit, and Paul H. Verkerk

Department of Pediatrics (H.J.v.d.K., J.M.W.), Leiden University Medical Center, 2300 RC Leiden, The Netherlands; Organization for Applied Scientific Research (TNO) Prevention and Health (C.G.M.O., P.H.V.), 2301 CE Leiden, The Netherlands; Diagnostic Laboratory for Infectious Diseases and Perinatal Screening (B.H.E.), National Institute of Public Health and the Environment (RIVM), 3720 BA Bilthoven, The Netherlands; General Clinical Laboratory (M.v.B.), IJsselland Hospital, 2900 AR Capelle a/d IJssel, The Netherlands; and Department of Pediatric Endocrinology (B.J.O.), University Medical Center St. Radboud, 6500 HB Nijmegen, The Netherlands

Objective: In newborn screening programs for congenital adrenal hyperplasia, 17-α-hydroxyprogesterone (17OHP) cutoff levels are based on birth weight (BW) or on gestational age (GA). We investigated which approach would result in the greatest specificity and sensitivity.

Study design: For the determination of 17OHP, a neonatal 17OHP assay was used in filter paper blood of 9492 newborns. The relationships between 17OHP and BW and between 17OHP and GA were studied by regression analysis. Reference curves with a specificity of 99.95% were constructed with the method that summarizes the distribution by three smoothed curves representing the skewness (L curve), the median (M curve), and the coefficient of variation (S curve). Median cutoff levels for BW and for GA according to the 99.95% reference curves were calculated.

Results: Regression analysis showed that GA is a better predictor of 17OHP than BW (R^2 was 50.6 *vs.* 35.8%, respectively). At a specificity of 99.95%, the calculated median 17OHP cutoff level was lower for GA [12.6 μg/liter (38 nmol/liter)] than for BW [17.6 μg/liter (54 nmol/liter)], thus leading to a greater sensitivity.

Conclusion: This study demonstrates that GA is a better predictor of 17OHP in newborns and will result in greater specificity than BW despite the fact that the determination of GA might be less reliable than BW. **(*J Clin Endocrinol Metab* 90: 3904–3907, 2005)**

CONGENITAL ADRENAL hyperplasia (CAH) is characterized by an impaired biosynthesis of cortisol and aldosterone and an increased secretion of 17-α-hydroxyprogesterone (17OHP) and androgens. Clinical features, such as hypoglycemia, salt loss, and dehydration, are life-threatening in the first weeks of life. Therefore, newborn screening for CAH has been implemented in several countries to prevent these symptoms (1).

An increased 17OHP concentration in heel-prick blood is used to indicate patients at risk of having CAH. It is known that preterm newborns have higher 17OHP concentrations in serum (2). Therefore, cutoff levels are based on birth weight (BW) or on gestational age (GA). In the United States, Canada, and New Zealand, cutoff levels for CAH screening are based on BW, whereas in other countries, cutoff levels are based on GA (3–8). To our knowledge, no studies have been performed to compare the diagnostic performance of these two approaches. The goal of our study was, therefore, to investigate which approach would result in the greatest specificity and sensitivity in CAH screening.

First Published Online March 29, 2005

Abbreviations: BW, Birth weight; CAH, congenital adrenal hyperplasia; GA, gestational age; 17OHP, 17-α-hydroxyprogesterone.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Patients and Methods

In 1998, a 2-yr regional pilot screening for CAH was added to the routinely performed newborn screening program for phenylketonuria and congenital hypothyroidism in The Netherlands. Heel punctures were performed on d 5–7 after birth (day of birth counted as d 0). BW was always reported on the filter paper forms, whereas reporting GA on filter paper forms was only obligatory when BW was 2500 g or less. BW was measured by the midwife or gynecologist involved. GA was assessed as the time from the 1st d of the last menstrual period until birth. It was calculated using anamnestic information and cross-checked by the midwife or gynecologist, who estimated GA through physical investigation or by ultrasound.

For this study, the relationship between BW and 17OHP and between GA and 17OHP was analyzed in a sample of 9492 newborns selected from all 27,954 neonates born in the area of screening in the middle and southwest of The Netherlands in the first 4 months of 1998. The selection of the 9492 newborns was based on BW. All newborns with a BW less than 3000 g were selected and included in the study (n = 4746). Allen *et al.* (4) found that the variance in 17OHP concentrations is widest in low-BW infants. Therefore, a stratified sample was taken to include more prematures then the 6% as is normal in a newborn population (9). In addition, every newborn with a BW more than or equal to 3000 g after a newborn in the database with a BW less than 3000 g was selected (n = 4746). None of these newborns was diagnosed with CAH.

For the determination of 17OHP in dried filter paper, the AutoDELFIA Neonatal 17OHP assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) was used in the two laboratories involved. This is a solid phase time-resolved fluoroimmunoassay based on the competition between europium-labeled 17OHP and sample 17OHP for a limited number of binding sites on 17OHP-specific polyclonal antibodies (derived from rabbit). A second antibody, directed against rabbit IgG, was coated to the solid phase, giving convenient

TABLE 1. Median and range of 17OHP concentration in relation to BW in 9492 newborns

BW interval (g)	Newborns (n)	Median 17OHP [μg/liter (nmol/liter) serum]	Range 17OHP [μg/liter (nmol/liter) serum]
<1000	149	28 (86)	3.0–199 (9–603)
1000–1499	288	19 (57)	2.3–189 (7–573)
1500–1999	407	12 (37)	1.0–196 (3–594)
2000–2499	1024	8.6 (26)	0.7–82 (2–247)
2500–2999	2878	6.3 (19)	0.3–74 (1–225)
3000–3499	2049	6.0 (18)	0.7–59 (2–179)
3500–3999	1857	5.6 (17)	0.3–33 (1–99)
≥4000	840	5.6 (17)	0.3–15 (1–45)

The differences in the median of the 17OHP concentrations per subgroup were statistically significant (Kruskall-Wallis test, $P < 0.001$).

TABLE 2. Median and range of 17OHP concentration in relation to GA in 4002 newborns

GA (wk)	Newborns (n)	Median 17OHP [μg/liter (nmol/liter) serum]	Range 17OHP [μg/liter (nmol/liter) serum]
≤28	144	33 (99)	3.0–199 (9–603)
29–30	143	22 (66)	2.3–196 (7–594)
31–32	234	16 (47)	1.0–172 (3–520)
33–34	442	13 (39)	1.3–135 (4–407)
35–36	546	9.6 (29)	1.7–74 (5–225)
37–38	902	6.9 (21)	0.7–43 (2–129)
39–40	1,192	6.0 (18)	0.3–16 (1–47)
≥41	399	5.3 (16)	0.7–11 (2–32)

The differences in the median of the 17OHP concentrations per subgroup were statistically significant (Kruskall-Wallis test, $P < 0.001$).

separation of the antibody-bound and free antigen. The interassay variance was 3.2–5.9%, and the intraassay variance was 9–10,4%. Results are expressed as micrograms per liter (nanomoles per liter) serum [0.33 μg/liter (1 nmol/liter) blood = 0.66 μg/liter (2.0 nmol/liter) serum]. Heel puncture samples were assayed in duplicate.

The measured 17OHP concentrations were not normally distributed. For this reason, transformation to their natural logarithm was performed. Goodness of fit (R^2) of various linear regression models was calculated to assess which factor, GA, or BW was the best predictor of 17OHP. In these models, ln-(17OHP) was the dependent variable.

To illustrate that the factor with the highest R^2 will lead to the highest specificity and sensitivity, we used the following approach. Reference curves (99.95th percentile) for BW and 17OHP and for GA and 17OHP were constructed with the method that summarizes the distribution by three smoothed curves representing the skewness (L curve), the median (M curve), and the coefficient of variation (S curve) (10). A cutoff level

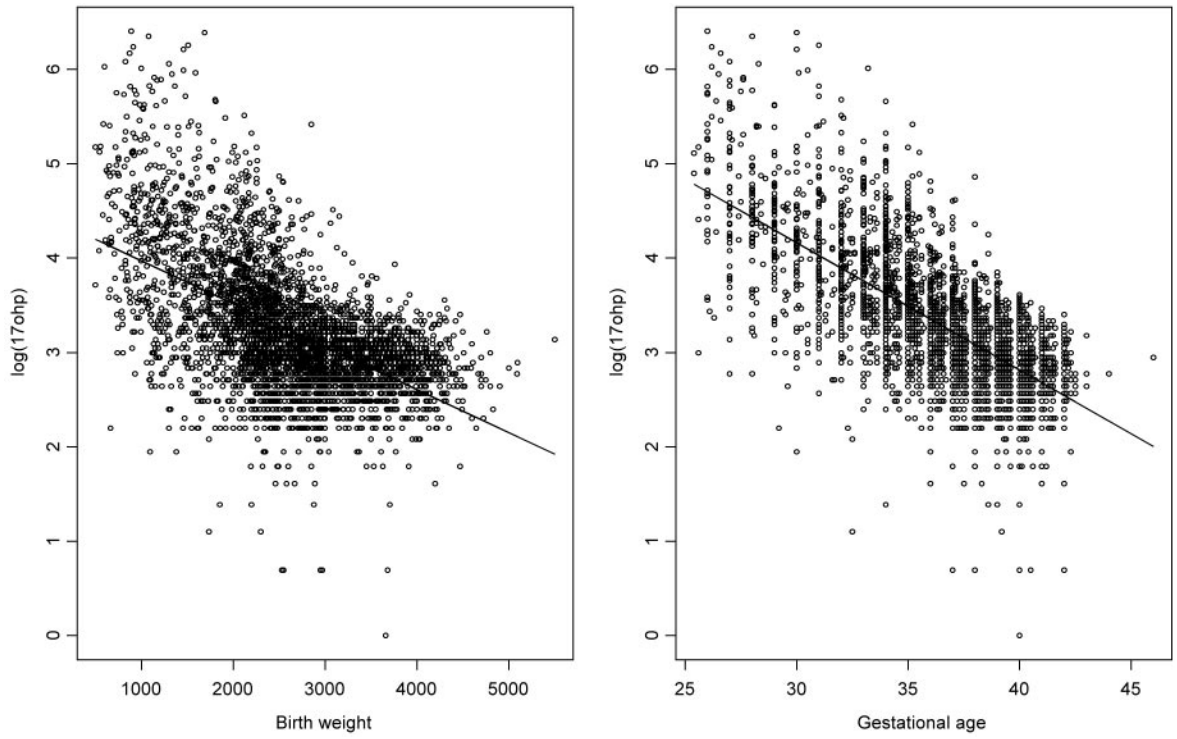

FIG. 1. The scatter gram and regression line of measured 17OHP concentrations in relation to BW and GA of the first group of newborns. Log 17OHP is the natural log of 17OHP (nanomoles per liter of serum). BW and GA are expressed in grams and weeks, respectively.

of 99.95% was chosen because it will lead to an acceptable false-positive rate in relation to the prevalence of the disease. By definition, a 99.95th percentile reference curve is equal to a specificity of 99.95%, implying that 0.05% of the healthy population has false-positive results. We then used the data of 10,000 neonates born in the first 6 wk of 1999 in the area of screening with known BW and GA (reference population). For every BW of the 10,000 newborns, the corresponding 17OHP concentration was read off in the 99.95% reference curve we made as described earlier. The same was done for GA. From these 10,000 individual 17OHP concentrations, the median 99.95% cutoff level for BW and for GA was calculated. The lower the cutoff level with a fixed specificity of 99.95%, the smaller the risk of missing a CAH patient, resulting in a greater sensitivity.

Results

The median 17OHP concentrations for BW and the median 17OHP concentrations for GA of the first group of newborns are presented in Tables 1 and 2, respectively. The median 17OHP concentrations were significantly higher in low birthweight infants and prematures ($P < 0.001$). The regression lines between ln-(17OHP) and BW and ln-(17OHP) and GA are presented in Fig. 1. The correlation coefficient r of 17OHP *vs.* BW was 0.561 (95% confidence interval 0.504–0.618) and of 17OHP *vs.* GA was 0.708 (95% confidence interval 0.553–0.863). In other words, a regression model with only BW explained 31% (R^2) of the variance of ln-(17OHP), whereas a model with only GA explained 50% (R^2). Adding BW above GA in the regression model gave a minor contribution to the explained variation (Table 3).

In the reference population, mean (SD) BW was 3414 (588) g and mean (SD) GA was 39.5 (1.9) wk. The median 17OHP cutoff level calculated from the reference population was 17.6 μg/liter (54 nmol/liter) serum for BW and 12.6 μg/liter (38 nmol/liter) serum for GA (Fig. 2). The difference between the medians was significant ($P < 0.001$).

Discussion

In screening programs, three assay techniques are used for initial screening: RIA, ELISA, and time-resolved fluoroimmunoassay (DELFIA) as was used in our study. Preterm newborns have higher 17OHP concentrations in serum than babies born at term. Therefore, cutoff levels are based on GA or on BW. Our study demonstrates that despite the fact that determination of GA may be less reliable than BW, GA is a stronger predictor of 17OHP concentration than BW. An explanation for this finding may be the fact that GA is more closely related to the development of the adrenal glands in the fetus than BW (2, 11, 12). This is comparable with the finding that mortality risk is also more dependent on GA than on BW (13). So, cutoff levels that are based on GA will lead to a higher sensitivity than cutoff levels that are based on BW at the same specificity.

TABLE 3. Goodness of fit (R^2) of various regression models with ln-(17OHP) as dependent variable

Model	R^2 (%)
BW	31.4
BW + BW^2	35.8
GA	50.1
GA + GA^2	50.6
BW + GA	50.2
BW + GA + BW^2	50.2
BW + GA + GA^2	50.7
BW + GA + GA^2 + BW^2	50.8

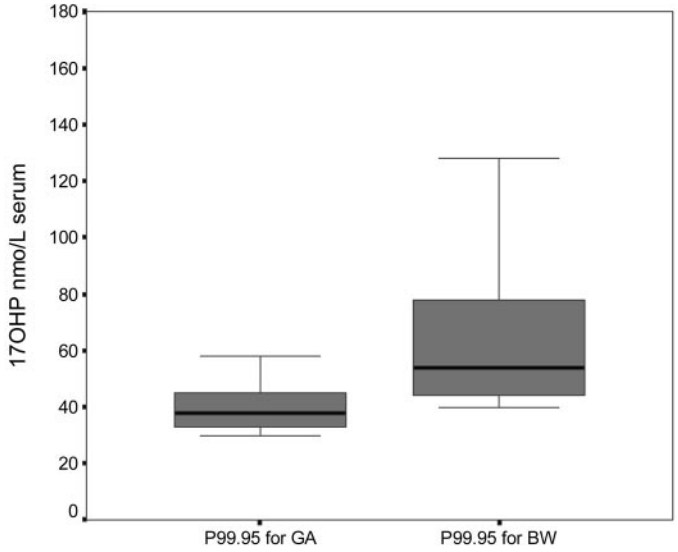

FIG. 2. The *boxes* represent the 25–75% range; the median is shown as a *line*. Presented are the 17OHP concentrations according to BW and GA of a Dutch newborn reference population. These calculated 17OHP concentrations were read off in the 99.95% reference curves made by the method that summarizes the distribution by three smoothed curves representing the skewness (L curve), the median (M curve), and the coefficient of variation (S curve). The *outer lines* indicate the most extreme observations that are within a distance of 1.5 times the interquartile range.

For the estimation of the sensitivity, we assumed that BW and GA in CAH patients are equal to the healthy newborn population. In fact, it was reported that length of gestation in CAH patients was within normal limits (14). Reports about BW in CAH patients have not been conclusive (15–17). BW has been reported as equal, higher in females, or higher in all CAH patients. However, even if BW were to be 10% higher in CAH patients, cutoff levels on the basis of GA would still result in a higher sensitivity (data not shown).

An increase in specificity can also be achieved by the elevation of cutoff levels, but this will result in loss of sensitivity. Improvement of the specificity without loss of sensitivity can be biochemically attained by additional procedures as organic extraction, chromatography, tandem mass spectrometry, or measuring the 17OHP to cortisol ratio (8, 18, 19). However, these extra procedures are complex and time consuming. Additional DNA analysis in heel puncture blood is not routinely in use because it is very expensive (20–22).

In conclusion, this study demonstrates that GA is a better predictor of 17OHP concentration in newborns than BW despite the fact that the determination of GA may be less reliable than BW. Cutoff levels based on GA result in the greatest specificity and sensitivity.

Acknowledgments

We thank Dr. J. G. Loeber and Dr. F. E. A. M. Verheul for their critical reading of the manuscript.

Received November 5, 2004. Accepted March 18, 2005.

Address all correspondence and requests for reprints to: H. J. van der Kamp, Department of Pediatrics, H-3Q, Leiden University Medical

Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. E-mail: H.J.van_der_Kamp@LUMC.nl.

This work was supported by Zorg Onderzoek Nederland (Health Research and Development Council).

References

1. **Pang S, Shook MK** 1997 Current status of neonatal screening for congenital adrenal hyperplasia. Curr Opin Pediatr 9:419–423
2. **Hingre RV, Gross SJ, Hingre KS, Mayes DM, Richman RA** 1994 Adrenal steroidogenesis in very low birth weight preterm infants. J Clin Endocrinol Metab 78:266–270
3. **Larsson A, Thilén A, Hagenfeldt L, Döbeln von U, Guthenberg C** 1992 Screening of half a million Swedish newborn infants for congenital adrenal hyperplasia. Screen 1:159–166
4. **Allen DB, Hoffman GL, Fitzpatrick P, Laessig R, Maby S, Slyper A** 1997 Improved precision of newborn screening for congenital adrenal hyperplasia using weight-adjusted criteria for 17-hydroxyprogesterone levels. J Pediatr 130:128–133
5. **Torresani T, Gruters A, Scherz R, Burckhardt JJ, Harras A, Zachmann M** 1994 Improving the efficacy of newborn screening for congenital adrenal hyperplasia by adjusting the cut-off level of 17α-hydroxyprogesterone to gestational age. Screen 3:77–84
6. **Cutfield WS, Webster D** 1995 Newborn screening for congenital adrenal hyperplasia in New Zealand. J Pediatr 126:118–121
7. **Thompson R, Seargeant L, Winter JS** 1989 Screening for congenital adrenal hyperplasia: distribution of 17 α-hydroxyprogesterone concentrations in neonatal blood spot specimens. J Pediatr 114:400–404
8. **Therrell BL, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, Gonzalez J, Gunn S** 1998 Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. Pediatrics 101:583–590
9. **Herngreen WP** 1992 SMOCC: Design of a Representative Cohort-study of Live-born Infants in the Netherlands. Eur J Public Health 2:117–122
10. **Cole TJ, Green PJ** 1992 Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 11:1305–1319
11. **Midgley PC, Russell K, Oates N, Shaw JC, Honour JW** 1996 Activity of the adrenal fetal zone in preterm infants continues to term. Endocr Res 22:729–733
12. **al Saedi S, Dean H, Dent W, Cronin C** 1995 Reference ranges for serum cortisol and 17-hydroxyprogesterone levels in preterm infants. J Pediatr 126:985–987
13. **Verloove-Vanhorick SP, Verwey RA, Brand R, Gravenhorst JB, Keirse MJ, Ruys JH** 1986 Neonatal mortality risk in relation to gestational age and birthweight. Results of a national survey of preterm and very-low-birthweight infants in the Netherlands. Lancet 1:55–57
14. **Price HV, Cone BA, Keogh M** 1971 Length of gestation in congenital adrenal hyperplasia. J Obstet Gynaecol Br Commonw 78:430–434
15. **Bergstrand CG** 1966 Growth in congenital adrenal hyperplasia. Acta Paediatr Scand 55:463–472
16. **Qazi QH, Thompson WM** 1971 Birthweight in congenital virilizing adrenal hyperplasia. Arch Dis Child 46:350–352
17. **Jaaskelainen J, Voutilainen R** 1997 Growth of patients with 21-hydroxylase deficiency: an analysis of the factors influencing adult height. Pediatr Res 41:30–33
18. **al Saedi S, Dean H, Dent W, Stockl E, Cronin C** 1996 Screening for congenital adrenal hyperplasia: the Delfia Screening Test overestimates serum 17-hydroxyprogesterone in preterm infants. Pediatrics 97:100–102
19. **Minutti CZ, Lacey JM, Magera MJ, Houn Hahn S, McCann M, Schulze A, Cheillan D, Dorche C, Chace DH, Lymp JF, Zimmerman D, Rinaldo P, Matern D** 2004 Steroid profiling by tandem mass spectrometry improves the positive predictive value of newborn screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab 89:3687–3693
20. **Nordenstrom A, Thilen A, Hagenfeldt L, Larsson A, Wedell A** 1999 Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 84:1505–1509
21. **Pang S** 2003 Newborn screening for congenital adrenal hyperplasia. Pediatr Ann 32:516–523
22. **Fitness J, Dixit N, Webster D, Torresani T, Pergolizzi R, Speiser PW, Day DJ** 1999 Genotyping of CYP21, linked chromosome 6p markers, and a sex-specific gene in neonatal screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab 84:960–966

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.