

Reference range for serum cortisol in well preterm infants

Matthias Heckmann, Stefan A Wudy, Doris Haack, Frank Pohlandt

Abstract

Aim—To establish a reference range for serum cortisol concentrations in preterm infants with a gestational age of less than 30 weeks during the first two weeks of life. **Methods**—Infants were prospectively classified by the following exclusion criteria: surfactant administration, arterial hypotension, acute or uncontrolled infection, ventricular haemorrhage II° or above, serum glucose < 2.2 mmol/l, exchange transfusion, stress as a result of any kind of examination or nursing for at least 4 hours before blood sampling. The cortisol value was measured once using radioimmunoassay in each infant.

Results—In appropriate for gestational age (AGA) infants (n = 37, median gestational age 27.7 weeks, median birthweight 1030 g) the distribution of the cortisol concentrations was non-Gaussian. These had a nearly normal distribution, when log₁₀ values of the data were used. The points determined by mean (2 SD) on the logarithmic scale were transformed back to the original units to provide a reference range: 73–562 nmol/l. Gestational age was significantly (p = 0.033) associated with cortisol values (log₁₀) with a regression coefficient (standard error) of -0.045 (0.020). Small for gestational age (SGA) infants (n = 8) had significantly higher cortisol values (median 357 nmol/l) than AGA infants (median 199 nmol/l) (p=0.028).

Conclusions—There is a strictly defined reference range of serum cortisol concentrations in AGA preterm infants.

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Keywords: reference range; serum cortisol; preterm

Critically ill preterm infants may have inadequately low serum cortisol concentrations.¹ The lowest cortisol values were found in those preterm infants who had the highest ventilatory requirements, had received surfactant, or who had been treated with inotropes.² Arterial hypotension in preterm infants, which might reflect adrenal insufficiency, was successfully treated with dexamethasone or hydrocortisone.³⁻⁵ So far, reference values for cortisol have not been established in healthy preterm infants with a gestational age of under 30 weeks. This prospective study aimed to measure cortisol in healthy preterm infants during the first two weeks of life.

Methods

All infants under 30 weeks of gestational age who had been admitted to our newborn intensive care unit were eligible for study. Gestational age was determined using the expanded Ballard score⁶ and/or the date of the mother's last menstrual period. Small for gestational age (SGA) was defined as a birthweight below the 10th percentile.⁷ The infants had no family history of adrenal illnesses, no congenital anomalies, surgery, or postnatal corticosteroid treatment before blood sampling. Prenatal betamethasone treatment, which consisted of two doses (12 mg) betamethasone for a minimum of 24 hours before birth, was not an exclusion criterion. After birth the infants were randomly assigned to one day of blood sampling during the first 14 days. In each infant one sample was drawn between 0400 and 0800 hours by venepuncture or via an indwelling catheter. For the interval 48 hours before to 24 hours after blood sampling the following exclusion criteria were applied: surfactant administration, arterial hypotension, acute or uncontrolled infection, acute or ventricular haemorrhage II° or more,⁸ serum glucose <2.2 mmol/l (40 mg/dl), exchange transfusion, stress induced by medical measures such as ultrasound scans, x-ray picture, blood sampling, intubation or major nursing for at least 4 hours before blood sampling. Arterial hypotension was defined as a mean arterial blood pressure of less than 23–24 mm Hg (birthweight 500–750 g), 25–26 mm Hg (750–1000 g), 27–28 mm Hg (1000–1250 g) and 30 mm Hg (1250–2000 g) and the necessity of treatment (volume expansion, catecholamines).

The study was approved by the Ethics Committee of the University of Ulm and written informed consent was obtained from the parents.

Blood samples for cortisol measurement were immediately centrifuged and serum was stored at -20°C until assayed, as described before.⁹ In brief, precipitation of proteins with ethanol was followed by direct radioimmunoassay using a high specific antibody. The intra-assay and interassay variabilities were 4.0% and 10%, respectively. The limit of detection of the assay calibration curve was 2.5 pg.

All data were analysed using the Statistica (version 5) statistical package (StatSoft, Inc., Tulsa, USA). The Mann-Whitney U test was used for non-parametric comparisons, the χ^2 test to analyse differences in proportions, and a multiple regression analysis was done to investigate the effect of gestational age, postnatal

Division of
Neonatology and
Paediatric Critical
Care
Department of
Paediatrics
University of Ulm
Germany
M Heckmann
S A Wudy
F Pohlandt

Department of
Pharmacology
University of
Heidelberg
Germany
D Haack

Correspondence to:
Professor Frank Pohlandt.

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Table 1 Clinical characteristics of well preterm infants and serum cortisol values

	AGA (n=37)	SGA (n=8)	p Value
Gestational age (weeks)*	27.7 (24.7–29.9)	27.6 (25.6–29.3)	0.95†
Birthweight (g)*	1030 (690–1580)	680 (500–895)	0.00012‡
Sex (females/males)	20/17	8/0	0.015‡
Apgar scores* at 5 mins	8.5 (5–10)	9 (6–10)	0.72†
Apgar scores* at 10 mins	9 (7–10)	9 (8–10)	0.82†
Umbilical cord pH*	7.30 (7.03–7.42)	7.19 (6.68–7.36)	0.0089†
Prenatal steroids	31 (84%)	6 (75%)	0.75‡
Caesarean section	29 (78%)	8 (100%)	0.15‡
Cortisol (nmol/l)*	199 (58–475)	359 (130–519)	0.028†

*Values presented as median (minimum–maximum); †Mann-Whitney U test; ‡ χ^2 test.

Table 2 Reference range for serum cortisol in well preterm infants

Gestational age (weeks)	Cortisol (nmol/l)
24	110–744
25	100–671
26	90–605
27	81–545
28	73–491
29	66–443

Based on the regression equation ($\log(\text{cortisol}) = 3.5373 - 0.045 \times \text{GA}$) and the residual standard deviation (0.207098) about the regression line, a reference range for each week of GA is given by the points determined by mean ± 2 residual standard deviations on the logarithmic scale, which were transformed back to the original units.

age, and birthweight on cortisol values. The level of significance was set at $p = 0.05$.

Results

Between August 1996 and January 1998, 99 infants were enrolled in the study. Forty seven fulfilled our criteria for good health. Table 1 shows the demographic characteristics. Only one infant was being mechanically ventilated at the time of blood sampling. No infant was treated with phenobarbital or phenytoin before or at the time of blood sampling.

Before further analysis of the cortisol pattern two outliers (1233 and 2050 nmol/l), both measured on the first day of life, were excluded by visual inspection.¹⁰ SGA infants had significantly higher cortisol values than appropriate for gestational age (AGA) infants (table 1). The distribution of cortisol values among the AGA infants was non-Gaussian and skewed to the right. They had a nearly normal distribution however, when \log_{10} transformed.¹¹ The points determined by mean (2 SD) on the logarithmic scale were transformed back to the original units to provide a reference range: 73–562 nmol/l. Taking into account, gestational age, birthweight, and postnatal age as independent variables, and cortisol (\log_{10}) as the dependent variable in a stepwise multiple

regression model, gestational age and birthweight had a significant effect ($p = 0.0041$ and $p = 0.032$, respectively). Birthweight alone was not significantly correlated with cortisol values (regression coefficient = -0.0001 , $p = 0.363$), but after including birthweight in the model, r^2 increased from 0.123 to 0.236. In this model the regression coefficient (standard error) of birth weight was 0.00066 (0.00030) and the regression coefficient of gestational age was 0.125 (0.041). However, the combination of birthweight and gestational age to predict cortisol values seemed to be cumbersome in clinical practice and birthweight was omitted on grounds of simplicity. The regression coefficient (standard error) of gestation alone was -0.045 (0.020) ($p = 0.033$) and the regression equation was $\log(\text{cortisol}) = 3.5373 - 0.045 \times \text{gestational age}$. Based on this equation and the residual standard deviation about the regression line, a reference range for each week of gestational age was derived (table 2). Again, the points determined by mean (2 SD) on the logarithmic scale were transformed back to the original units to provide a reference range. Furthermore, in AGA infants, no significant differences in cortisol values were found between girls and boys ($p = 0.82$) as well as between those infants whose mothers had or had not received a complete course of prenatal steroids ($p = 0.94$) (Mann-Whitney U test).

Discussion

Table 3 summarises the published data on well preterm infants, most of whom were 30 weeks or more of gestational age.^{1 12 13 14} The definition of “well” varied from a single criterion to very detailed criteria.^{1 15} In only one study was serum glucose concentration included.¹⁴ Finally, none of the studies stipulated the time interval before blood sampling during which the patient remained undisturbed. This is mandatory in intensive care patients, who have a busy programme of nursery care and medical activity. Healthy term infants show an up to 11-fold rise in cortisol half an hour after a painful venepuncture.¹⁶ The 48 hour interval was based on the observation that cortisol values in term neonates returned to baseline within 48 hours of surgery.¹⁷ Our strict criteria meant that only 47% of all eligible infants could be included in the study.

Compared with previously published cortisol values in a more mature population (median cortisol 137, 140, and 104 nmol/l for

Table 3 Gestational age and criteria of wellbeing on serum cortisol concentrations in preterm infants

Reference	Gestational age (weeks)	n=	Criteria of “wellbeing”
1	33.5; 31–35 (mean, range)	13	Mild or transient respiratory distress
12	32.8; 28–36 (mean, range)	15	Not seriously ill
13	33–36 (range)	8	Healthy mothers, uneventful peri- and postnatal course normal serum electrolytes, no respiratory distress
18	28; 24–33 (mean, range)	25	No major congenital malformations, receiving less than 25% inspired oxygen not requiring assisted ventilation, tolerating full gastric feed
14	33.5 (1.5) (mean SD)	15	pH 7.3–7.4, PaO ₂ 50–70 Torr, PaCO ₂ <55 Torr, serum glucose 2.5–7.0 mmol/l, serum calcium ≥ 2.0 mmol/l, haematocrit >40%, no respiratory distress
15	29 (1.5) (mean SD)	39	No congenital anomalies, no family history of adrenal disorders, no current major acute illness, had not received inotropes or hormone treatment within 48 hours. Infants with mild hyaline membrane disease and apnoea of prematurity were included. Samples taken within 48 hours before onset of sepsis were excluded.

< 30, 30, and 31 weeks postconceptional age, respectively), our values are higher (median cortisol 199 nmol/l).¹⁵ This agrees with our finding of a significantly negative correlation between gestational age and cortisol concentrations and published data.² In this study, the effect of gestational age on cortisol values is more pronounced, if birthweight is included together with gestational age in a multiple regression model, whereas birthweight alone did not have a significant effect on cortisol values. In a recent report cortisol values decreased significantly with advancing postnatal age between 1 and 8 weeks,¹⁸ but we found only a weak negative correlation between cortisol concentration and postnatal age in the first two weeks of life. This may be explained by the high rate of administration of prenatal steroids in our study group (table 1).

However, the data on adrenal suppression after prenatal administration of corticoids for fetal lung maturation are contradictory. Two groups found postnatal normalisation of suppressed cortisol values within two hours or two days, respectively.^{19, 20} Others found that serum cortisol concentrations were maximally suppressed by 55% within the first four to seven days of life^{18, 21, 22} but not after the first week.^{18, 23} In our study the cortisol values, which were obtained during the first week, were solely derived from infants whose mothers received prenatal steroids. Only six of 37 (16%) AGA infants, whose cortisol values were measured on days 7 to 13, were not under the influence of prenatal steroids. Thus the presented reference range applies to preterm infants after prenatal administrations of betamethasone. This is important, because the generally accepted benefit of prenatal steroids leads to an increasing rate of prenatal administration of steroids in at risk pregnancies.²⁴ Among the published findings on serum cortisol, the administration of prenatal steroids was taken into consideration by only one author for the analysis of the data.¹⁸

Little is known about cortisol concentrations in SGA infants. The concentrations of several glucocorticoids, including cortisol, were lower in term SGA infants than in AGA infants at 12 hours of age, but the cortisol values at 24 hours of age were higher, and after the first day of life no differences were found in cortisol values between term SGA and AGA infants.²⁵ These findings suggest a different adrenocortical response in SGA infants and we therefore separated out the SGA infants before further analysis. In this study preterm SGA infants had significantly higher concentrations compared with preterm AGA infants of the same gestational age.

Ten of 47 (21%) of our subjects had cortisol concentrations below 138 nmol/l, the threshold of adrenal insufficiency.²⁶ However, they had no clinical signs of adrenal insufficiency. We do not know whether preterm infants under 30 weeks of gestational age with cortisol concentrations < 138 nmol/l are at greater risk of developing clinical signs of adrenal insufficiency in stressful situations. Our study does not give any information on the function of the hypothalamic-pituitary-adrenal axis in these patients.

Although newborn infants lack a circadian rhythm for cortisol,²⁷ there is pulsatility of cortisol secretion.²⁸ However, only small changes in serum cortisol within ill extremely low birthweight infants were shown by Jett *et al.*²⁹ The standard deviations ranged between 10 and 114 nmol/l. The authors concluded that a single random cortisol measurement adequately reflected the adrenal status of an extremely low birthweight infant. We can therefore fill a gap by supplying a strictly defined reference range for serum cortisol concentrations in well AGA preterm infants with under 30 weeks of gestational age during the first two weeks of life, a period of highest critical morbidity in this population.

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