Early activation of the hypothalamic-pituitary-adrenal axis in very-low-birth-weight infants with small thymus at birth

C. DE FELICE1, P. TOTI4, M. MUSARÒ2, L. PERUZZI2, P. PAFFETTI2, L. PASQUI3, R. MAGALDI5, F. BAGNOLI1, M. RINALDI5, G. RINALDI5, G. GRILLI6, G. TONNI7, & G. LATINI8,9

1Neonatal Intensive Care Unit, 2Pediatric Laboratory, & 3Endocrinology Laboratory Azienda Ospedaliera Universitaria Senese, Siena, 4Department of Human Pathology and Oncology, University of Siena, 5Neonatal Intensive Care Unit & 6Radiology Unit, Azienda Ospedaliera Universitaria, Foggia, 7Division of Obstetrics and Gynecology, Guastalla Provincial Hospital, AUSL Reggio Emilia, 8Division of Neonatology, Perrino Hospital, Brindisi, Italy, and 9Clinical Physiology Institute, National Research Council of Italy (IFC-CNR), Lecce Section, Italy

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Abstract

Background. An acute thymic involution in human fetuses and newborns has been described in very-low-birth-weight (VLBW) infants with histological chorioamnionitis. However, the mechanisms of thymic involution remain to be clarified. Here, we tested the hypothesis that an activation of the hypothalamic-pituitary-adrenals (HPA) axis occurs in VLBW infants with acute thymic involution at birth.

Methods. A total of 180 randomly selected VLBW newborns (28.8 ± 3.15 wk gestation; 1093 ± 305 g) entered the study. Thymic size was measured on standard chest radiographs at birth, and expressed as the ratio between the transverse diameter of the cardiothymic image at the level of the carina (CT) and that of the thorax (T). CT/T ≤ 0.28 was considered to indicate a small thymic size. Plasma cortisol and adrenocorticotropic hormone (ACTH) concentrations were determined on days 1 (d-1) and 7 (d-7), and at 1 month (mo-1).

Results. A total of 66 (36.7%) newborns had CT/T ≤ 0.28. Infants with small thymus had significantly increased cortisol on d-1 (5.2-folds) [median: 18.95 (95% CI: 11.20–39.4) μg/dl vs. 3.66 (1.94–6.82) μg/dl, p = 0.0001] and d-7 (1.7-folds) [12.0 (4.39–22.97) μg/dl vs. 7.8 (3.63–12.8) μg/dl, p = 0.0384], while no significant differences for cortisol at mo-1 or ACTH concentrations on d-7 and mo-1 were evidenced (p > 0.50). From a multivariate logistic regression analysis, a small thymus at birth was a significant independent predictor of plasma cortisol concentrations in the top-quartile (OR = 14.4; 95% CI: 6.079–34.11), and plasma ACTH concentrations in the top-quartile (OR = 4.40 (95% CI: 1.99–9.74) on d-1 (results adjusted for variables significant at univariate analysis).

Conclusions. Our data indicated the presence of a previously unrecognized, early activation of the HPA axis in VLBW newborns with a small thymus at birth.

Keywords: Thymus, very-low-birth-weight infants, hypothalamic-pituitary-adrenals axis, cortisol, adrenocorticotropic hormone, chorioamnionitis

Introduction

An acute thymic involution in human fetuses and newborns has been described in very-low-birth-weight (VLBW) infants with various different perinatal pathological conditions, including chorioamnionitis (CA) [1–5]. However, the mechanisms underlying acute thymic involution in VLBW infants remain to be clarified. It is emerging opinion that the placenta actually takes part in a stress syndrome, either acute or chronic, by activating the hypothalamic-pituitary-adrenals (HPA) axis and inducing the release of corticotropin-releasing hormone (CRH). This activation, in turn, leads to a prompt
increase in adrenocorticotropic hormone (ACTH) production, followed by secretion of cortisol, trying to protect the fetus from a hostile environment. As a consequence, the HPA axis is considered to be a key component of the stress reaction [6,7].

We have previously suggested that acute thymic involution following a placental inflammatory process, could be the result of a possible stress-related activation of the fetal and/or maternal HPA axis [4,5].

Here, we tested whether an activation of the hypothalamic-pituitary-adrenals (HPA) axis occurs in VLBW infants with acute thymic involution at birth.

**Patients and methods**

A total of 180 (M: 87, F: 93), VLBW newborns (28.8 ± 3.15 wk gestation; 1093 ± 305 g), admitted to the NICUs of Siena and Foggia, Italy, were evaluated in a cohort study. This infant population was selected from a larger population study aimed at measuring HPA axis hormones in view of a possible need for hydrocortisone supplementation to prevent or reduce the risk for bronchopulmonary dysplasia. Our sub-population was selected on the basis of an available chest X-ray on the first 3 hours of life. All X-ray films were obtained on the basis of clinical grounds, independently from the aim of the present study. Blood samples were taken from umbilical cord on day 1 and from peripheral sites for the following tests (all performed at 09.00 am.). All assays were performed in a single Research Laboratory. Samples were immediately centrifuged at 1200 g at 4°C for 20 min, and the obtained plasma was either stored at −80°C until analysis, or stored at −20°C and subsequently sent on ice to the laboratory. Plasma cortisol (units: µg/dl) and adrenocorticotropic hormone (ACTH) (units: pg/ml) concentrations were determined on days 1 (d-1) and 7 (d-7), and at 1 month (mo-1), using commercially available kits (Cortisol Reagent pack, Ortho-Clinical Diagnostics, Inc. Raritan, NJ, USA; and Immunolyte 2000 ACTH, Diagnostic Products Corporation, Los Angeles, CA, USA). All assays were performed at least in duplicate and mean values were used for data analysis.

Thymic size was measured on standard chest radiographs at birth, and expressed as the ratio between the transverse diameter of the cardiothymic image at the level of the carina (CT) and that of the thorax (T). Cases with nonvaluable cardio-thymic images were excluded from the study. The parameters of interest were measured on the digital images of the chest radiogram, using an automated procedure (Image Pro-Plus version 1.3 image analysis software), and a CT/T < 0.28 was considered to indicate a small thymus, as previously reported [4].

The study was approved by the local institutional review boards and ethical committees, and written informed consent was obtained from the parents of all the enrolled infants prior to the study.

**Data analysis**

Data were evaluated by the D’Agostino-Pearson test for normal distribution. Results were expressed as means ± SD for continuous normally distributed data and medians with inter-quartile range [25th and 75th percentiles] for the non-normal distribution of ACTH and cortisol. Comparisons between categorical variables were performed by using the analysis of variance or the Kruskal-Wallis H test as appropriate. Discrimination was tested using receiver-operating characteristic (ROC) curves, using the area under the curve (AUC) as a measure of predictor accuracy. An ROC is a graphical plot of the number of true positives versus the number of false positives for a binary classifier system as its discrimination threshold is varied [8,9].

An AUC value above 0.5 is accepted to indicate a statistically significant discrimination. To evaluate the effects of possible confounders on the HPA axis early activation/small thymus relationship, stepwise multiple logistic regression models were used with either top-quartile cortisol concentrations or top-quartile ACTH concentrations as the dependent variable. Predictors statistically significant at the univariate analysis were tested in the models. The MedCalc version 9.2 statistical software package (MedCalc Software, Mariakerke, Belgium) was used. Two-tailed p values < 0.05 were considered to be statistically significant.

**Results**

Plasma cortisol and ACTH concentrations distributions were non-gaussian at all the examined ages (p = 0.0021). A total of 36.7 % (66/180; M:32, F:34) of the examined newborns showed a small thymus (i.e., CT/T ratio < 0.28). Univariate analysis (Table I) indicated that the infants with a small thymus showed a significantly higher frequency of spontaneous delivery (p = 0.0002), lower gestation age (p = 0.012), higher frequency of endotracheal intubation on day-1 (p = 0.0096) and during the first week (p = 0.0252), and higher frequency of PDA (p = 0.0095), as compared to those with a normal thymic size at birth. Infants with small thymus had significantly increased cortisol concentrations on d-1 (~5.2-folds) [median: 18.95 (95% CI:11.20–39.4) µg/dl vs. 3.66 (1.94–6.82) µg/dl, p < 0.0001] and d-7 (~1.7-folds) [12.0 (4.39–22.97) µg/dl vs. 7.8(3.63–12.8) µg/dl, p = 0.0384] as compared with
those with normal thymic size, together with higher ACTH concentrations on d-1 (~1.9-folds) [28 (15.6–61.07) pg/ml vs. 14.9 (9.0–23.42) pg/ml, $p = 0.0005$]) (Figures 1 and 2). No significant differences for cortisol at mo-1 ($p = 0.9485$), or ACTH concentrations on d-7 ($p = 0.6713$) and mo-1 ($p = 0.5006$) were evidenced.

The results of a stepwise multivariable logistic regression analysis (entered variables: small thymus, gestational age, mode of delivery, endotracheal intubation on d-1, and PDA) indicated that a CT/T ratio $\leq 0.28$ at birth was a significant independent predictor of cortisol concentrations in the top-quartile at life-day 1 (i.e., $>16.85 \mu$g/dl; 95% CI: 13.14–22.66), with OR $= 14.4$ (95% CI: 6.079–34.11), null model -2 Log likelihood $= 178.08$; full model -2 Log likelihood $= 132.79$; $\chi^2 = 45.35$; df $= 1$; $p < 0.0001$). In the same model, a small thymic size at birth was an independent predictor of ACTH concentrations in the top-quartile at life day 1 (i.e., $>30.97$ pg/ml; 95% CI: 26.72–53.72), with OR $= 4.40$ (95% CI: 1.99–9.74), null model-2 Log likelihood $= 158.19$; full model-2 Log likelihood $= 144.12$; $\chi^2 = 14.065$; df $= 1$; $p = 0.0002$).

### Discussion

Our findings show a previously unrecognized activation of the HPA axis early in life in VLBW newborns with a small thymus at birth. High ACTH and cortisol concentrations during the first day of life, together with a lack of significant changes in ACTH concentrations on d-7 and in ACTH and cortisol concentrations on mo-1, suggest that the observed hormonal activation can occur during intrauterine life or during delivery.

Even after birth, activation of the HPA axis is critical to maintain homeostasis and to respond to stress [10]. It is well known that the thymus becomes involuted during the response to stress and it is dramatically affected by the acute phase response, a systemic reaction to tissue injury and/or infection. The acute phase response comprises production and release of cytokines, particularly interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-alpha, and glucocorticoids. The stimulation of acute phase response activates the HPA axis, that it is well documented to influence immunological responses to stress while IL-1 is a potent stimulator of this axis (11). As a part of an infection-induced stress

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yes (n = 66)</th>
<th>No (n = 114)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-to-Female ratio</td>
<td>0.94</td>
<td>0.93</td>
<td>0.9015</td>
</tr>
<tr>
<td>SD: C-section</td>
<td>18 (27.27%)</td>
<td>7 (6.14%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>27.9 ± 3.6</td>
<td>29.1 ± 2.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1048 ± 345</td>
<td>1113 ± 284</td>
<td>0.1969</td>
</tr>
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<td>IUGR/SGA</td>
<td>21 (31.81%)</td>
<td>44 (38.59%)</td>
<td>0.4524</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>38 (57.57%)</td>
<td>71 (62.28%)</td>
<td>0.6425</td>
</tr>
<tr>
<td>ET intubation (day 1)</td>
<td>49 (74.24%)</td>
<td>61 (53.5%)</td>
<td>0.0096</td>
</tr>
<tr>
<td>ET intubation (day 7)</td>
<td>10 (74.2%)</td>
<td>5 (33.5%)</td>
<td>0.0219</td>
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<tr>
<td>Surfactant (Rx)</td>
<td>33 (15.15%)</td>
<td>42 (36.84%)</td>
<td>0.1167</td>
</tr>
<tr>
<td>Ventilation (days)</td>
<td>7 (4–8)</td>
<td>5 (4–5)</td>
<td>0.0192</td>
</tr>
<tr>
<td>PDA</td>
<td>9 (13.64%)</td>
<td>3 (2.63%)</td>
<td>0.0095</td>
</tr>
</tbody>
</table>

**Figure 1.** Trends of plasma cortisol concentrations as a function of postnatal life-days (the values are medians with 95% CI). 0 defines infants with normally sized thymus, while 1 indicates infants with a small thymus.

**Figure 2.** Trends of plasma ACTH concentrations as a function of postnatal life-days (the values are Medians with 95% CI). 0 defines infants with normally sized thymus, while 1 indicates infants with small thymus.
response during critical periods of development, IL-1 has been shown to induce functional ‘malprogramming’ of the HPA axis together with a thymic involution and serum glucocorticoids elevation in animal models [12–14]. The mechanism for increased fetal cortisol production in HCA is well established and likely related to fetal adrenal development, as larger adrenal glands have been reported in infants who had been exposed to HCA, as compared to those not exposed [15]. Fetal inflammation most likely stimulates fetal cortisol production through the action of proinflammatory cytokines such as IL-1-beta and IL-6 [16].

It is therefore reasonable to postulate that activation of the HPA axis is the underlying cause of the acute thymic involution in VLBW infants.

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References
