

ORIGINAL ARTICLE

WILEY

Corticotrophin-releasing hormone stimulation tests for the infants with relative adrenal insufficiency

Kougoro Iwanaga  | Akane Yamamoto | Takashi Matsukura | Fusako Niwa | Masahiko Kawai

Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Correspondence

Masahiko Kawai, Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan.
Email: masahiko@kuhp.kyoto-u.ac.jp

Summary

Background: Very low birthweight (VLBW) infants are considered to be vulnerable to relative adrenal insufficiency (RAI); however, diagnosis is difficult in some clinical settings. Considering this background, it is necessary to establish a diagnosis of RAI in preterm infants.

Objective: In this study, we attempted to clarify the difference in response to CRH stimulation tests for preterm infants with or without RAI.

Methods: Between June 2009 and December 2015, we performed CRH stimulation tests for preterm infants born at a gestational age of <30 weeks at around 2 weeks of age. Retrospectively, subjects were classified into two groups: infants with RAI ($n = 9$) or without RAI ($n = 17$) based on the clinical symptoms and responsiveness to hydrocortisone.

Results: We found no difference in base or peak serum cortisol levels related to CRH stimulation tests between the two groups; however, delta cortisol levels and responsive ratio (peak-to-base ratio) were significantly reduced in infants with RAI. 140 nmol/L for delta cortisol or 1.5 times for peak-to-base ratio may be cut-off levels in preterm infants.

Conclusion: This study provides evidence that base cortisol levels of preterm infants with RAI were not different from those without RAI; however, CRH stimulation tests may be a useful tool for the diagnosis of RAI in preterm infants.

KEYWORDS

bronchopulmonary dysplasia, hypotension, preterm infants, relative adrenal insufficiency

1 | INTRODUCTION

Advances in neonatal medicine have enabled a reduction in neonatal mortality for the past several decades. In particular, a marked change in the mortality of extremely low birthweight infants has been observed.¹ However, neurobehavioural impairment has become a recent focus.² Apart from the long-term problems, short-term morbidity has also changed in accordance with the survival of more immature infants. Significant increases are reported in morbidities of bronchopulmonary dysplasia (BPD),³ necrotizing enterocolitis, symptomatic patent ductus

arteriosus (PDA) and late-onset circulatory collapse (LCC).¹ Some of these conditions were considered to be strongly associated with relative adrenal insufficiency (RAI). Among them, the association between RAI and BPD⁴ or LCC^{5,6} has been discussed intensively.

RAI is considered as an aetiology for respiratory or hemodynamic instability, not only for adults but also for preterm infants.^{7,8} As a concept, most neonatologists agree on RAI but it's difficult to diagnose. In adults, Cooper described a flow chart to diagnose corticoid insufficiency;⁹ however, there are no reliable tools for diagnosis in preterm infants.

In 2012, we reported the results of corticotrophin-releasing hormone (CRH) stimulation tests at 2 weeks of age in healthy preterm infants born at a gestational age (GA) of <30 weeks.¹⁰ In this study, we performed CRH stimulation tests with or without RAI. Based on the results obtained, we propose a new diagnostic tool for RAI in preterm infants born at a GA of <30 weeks.

2 | PATIENTS AND METHODS

2.1 | Subjects and study design

Ninety-six VLBW infants, who were born at a GA of <30 weeks at Kyoto University Hospital between June 2009 and December 2015, were included in this study. After obtaining an informed consent, CRH stimulation tests were performed on these preterm infants with or without symptoms at around 2 weeks of age, to examine the function of the naive hypothalamic-pituitary-adrenal (HPA) axis. For this purpose, infants who were administered glucocorticoid before performing the CRH stimulation tests were excluded. Infants who were transferred to other hospitals or died during the NICU stay were excluded. In addition, infants whose parents did not consent to participate in this study were also excluded. As shown in Figure 1, 41 infants were applicable for CRH stimulation tests at about 2 weeks of postnatal age.

After 7 infants were excluded by technical problems, the subjects were divided into two groups. Infants who met the following criteria (Table 1) at the time of the examination, were defined as "critical illness." Thirteen infants were grouped as critical illness, and glucocorticoid therapy was started immediately after CRH stimulation tests. Nine infants responded to 1-2 mg/kg/d of hydrocortisone dramatically; clinical symptoms such as hypotension, oliguria, oedema, respiratory deterioration, or serum electrolyte abnormalities were disappeared within a few days after the hydrocortisone therapy, and were diagnosed as "clinical RAI."

However, 4 infants who did not respond to hydrocortisone well and for whom a subsequent administration of dexamethasone was needed were excluded from further analysis because they were not

clearly diagnosed with RAI clinically. Twenty-one infants were considered as noncritical illness on the day of the CRH tests, but 4 of them deteriorated within 2 weeks after the CRH tests and were administered hydrocortisone. These infants were also excluded from further analysis because it was difficult to diagnose them as RAI or non-RAI at the time of the CRH tests. Finally, 9 infants with RAI and 17 infants without RAI were analysed in this study.

Ethical approval was obtained from Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and written informed parental consent was obtained before each procedure.

2.2 | CRH stimulation tests and sample collection

We performed the CRH stimulation tests between 0900 and 1200 at 2 weeks of life. One hundred micrograms of synthetic human CRH (hCRH; Mitsubishi-Tanabe Pharma, Kyoto, Japan) were diluted using 0.9% NaCl to a final concentration of 5 µg/mL and then administered at a dose of 1 µg/kg via a peripheral intravenous route. The dose of hCRH was selected based on previous studies of premature infants.¹⁰⁻¹² In the previous studies, cortisol levels peaked at around 30-min post-CRH stimulation, thus, blood samples for measuring serum cortisol were collected at 0 and 30 minutes after the administration of hCRH. The blood samples were immediately cooled on ice and transported to a laboratory in the hospital.

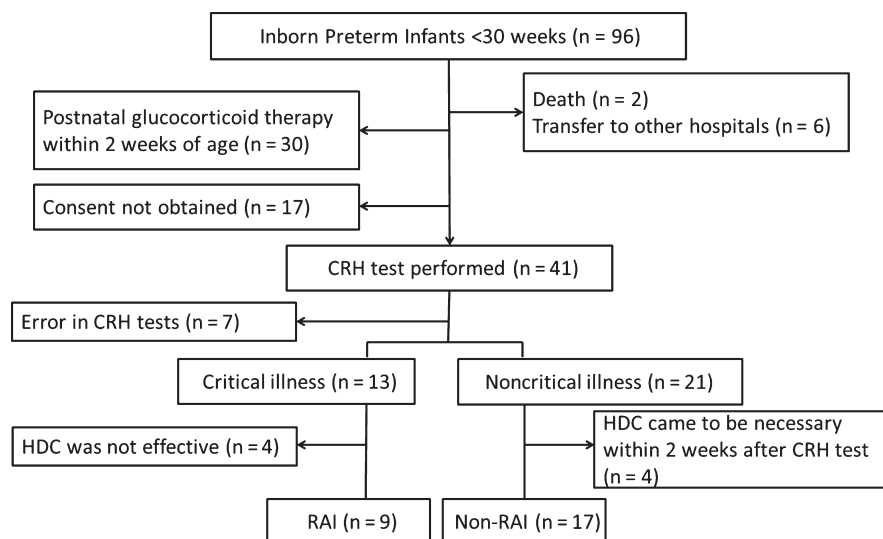
2.3 | Serum total cortisol assay

The total cortisol concentrations of patients' serum were analysed using an enzyme immunoassay (TOSOH Corporation, Tokyo, Japan). The detection range of the cortisol assay was 5.5-16.55 nmol/L, and the intra-assay coefficient of variation was less than 1.5%.

2.4 | Statistical analysis

SPSS (version 11.0; SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses. To assess the differences between groups, two-sample

FIGURE 1 Infant enrollment flow chart. Ninety-six VLBW infants, born at a GA of <30 wk at Kyoto University Hospital, were enrolled. Based on the exclusion criteria described in the manuscript, 9 infants with RAI and 17 non-RAI infants were analysed in this study. HDC, hydrocortisone; CRH, corticotrophin-releasing hormone; RAI, relative adrenal insufficiency



t test or Fisher's exact test was performed. Significant difference was defined as $P < .05$.

3 | RESULTS

3.1 | Profiles of the subjects

Nine infants with RAI and 17 infants without RAI were analysed in this study. The profiles of each group are shown in Table 2. Briefly, the GA of infants with RAI was significantly shorter, but birth-weight and birth-weight SDS were not different between the two groups. The rate of infants receiving antenatal steroids and the incidence of respiratory distress syndrome were not different. Concerning the incidence of patent ductus arteriosus (PDA), the requirement for indomethacin and surgical ligation seemed to be higher in the RAI group, but no statistical difference was found. Concerning chronic lung disease (CLD), the incidence of CLD defined as oxygen requirement at 36 weeks of postmenstrual age looks higher in the RAI group, but was not significant; however, the duration of artificial ventilation was significantly longer in infants with RAI. In addition, grade 3 for bronchopulmonary dysplasia (BPD) was significantly more frequent in the RAI group.

3.2 | Response to CRH stimulation tests

CRH stimulation tests were performed at 13.0 ± 2.7 days of life. The date of the CRH tests was not different between the two groups as shown in Table 3. Concerning the base and peak serum cortisol levels, we found no difference between the two groups; however, there were significant differences in delta cortisol levels and peak-to-base ratio as shown in Table 3. These two parameters were significantly reduced in infants with RAI compared to the non-RAI group.

TABLE 1 Criteria for critically ill infants in this study

Hypotension	Mean arterial pressure decreased below 80% of that of the previous few days
Oliguria	Urine output during the last 8 h decreased below half, or urine output decreased less than 1 mL/kg/h
Oedema	Inappropriate body weight increase of more than 15 g/kg/d
Acute deterioration of bronchopulmonary dysplasia	Oxygen requirement increased more than 10% compared to the previous few days, or hazy lung appearance shown on X-ray
Abnormal serum electrolyte	Serum sodium concentration <130 mEq/L, or serum potassium concentration >5.5 mEq/L

Infants who met more than two criteria were defined as "critical illness". Criteria for suspecting relative adrenal insufficiency in critically ill infants were postulated. These are modified based on the criteria for late-onset circulatory collapse of prematurity in Japan.⁶

Based on the analysis of the ROC curve, we defined the cut-off value for delta cortisol in response to CRH tests as 140 nmol/L. With this cut-off value, we obtain a sensitivity of 88.9% and a specificity of 70.6% for diagnosing RAI in preterm infants. We also defined the cut-off value for peak-to-base ratio of cortisol in response to CRH tests as 1.5 times. With this cut-off value, we obtain a sensitivity of 77.8% and a specificity of 82.4% for diagnosing RAI in preterm infants.

4 | DISCUSSION

Respiratory or hemodynamic instability could be a trigger for intra-ventricular haemorrhage and periventricular leukomalacia. Relative adrenal insufficiency is postulated to be at least partly an aetiology for such conditions.

The effectiveness of glucocorticoid therapy for respiratory or circulatory dysfunction has been reported repeatedly. Apart from that, short-term adverse events, such as intestinal perforation were

TABLE 2 Subject profiles

	RAI (n = 9)	Non-RAI (n = 17)
Gestational age (week)	26.8 $\pm$ 1.4*	28.0 $\pm$ 1.15
Birth-weight (gram)	837 $\pm$ 264	956 $\pm$ 254
Birth-weight SDS	-0.71 $\pm$ 2.28	-0.98 $\pm$ 1.36
Antenatal steroid (%)	9/9 (100%)	12/17 (71%)
RDS (%)	7/9 (78%)	11/17 (65%)
Indomethacin for PDA (%)	7/9 (78%)	8/17 (47%)
PDA ligation(%)	3/9 (33%)	1/17 (6%)
CLD ₃₆ (%)	6/9 (67%)	6/17 (35%)
BPD grade 3 (%)	9/9 (100%)*	5/17 (29%)
Duration of artificial ventilation (days)	28.8 $\pm$ 14.2*	11.2 $\pm$ 10.8

SDS, standard deviation score; RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; CLD, chronic lung disease; BPD, bronchopulmonary dysplasia.

The profiles of each group were described.

*Indicate a statistically significant difference between the RAI and non-RAI groups ($P < .05$).

TABLE 3 CRH stimulation tests

	RAI (n = 9)	Non-RAI (n = 17)
Postnatal days of CRH test (days)	13.4 $\pm$ 2.9	12.8 $\pm$ 2.7
Basal cortisol levels (nmol/L)	249.8 $\pm$ 86.7	218.8 $\pm$ 71.5
Peak cortisol levels (nmol/L)	341.8 $\pm$ 102.6	416.9 $\pm$ 97.4
Delta cortisol levels (nmol/L)	92.0 $\pm$ 54.6*	198.2 $\pm$ 72.1
Peak-to-base ratio	1.41 $\pm$ 0.30*	2.02 $\pm$ 0.52

CRH, corticotrophin-releasing hormone.

Results of the CRH tests are described.

*Indicate a statistically significant difference between the RAI and non-RAI groups ($P < .05$).

reported.¹³ In addition, long-term adverse events on psychomotor development have also come to be a major concern for neonatologists.^{14,15} As a result, currently glucocorticoid therapy requires special attention to balance efficiency and potential adverse events.¹⁶

Based on this background, some neonatologists are trying to validate safer use of glucocorticoid. Rademaker et al reported the safety of hydrocortisone therapy for preterm infants.¹⁷⁻²⁰ In addition, Tanney reported that low-dose dexamethasone therapy is also safe for preterm infants.²¹ Such reports encourage us to use glucocorticoid for preterm infants at risk for RAI; however, we still need to be aware of the risk caused by glucocorticoid therapy. Taking this into consideration, it is important to minimize the use of glucocorticoid. From this perspective, we considered it important to diagnose RAI in preterm infants.

Diagnosis of RAI is difficult. In adults, a diagnostic tool based on the basal cortisol levels and response to ACTH stimulation tests have been reported.⁹ However, there are no such diagnostic tools available for preterm infants. In preterm infants, there are several reports in which CRH or ACTH stimulation tests were investigated.¹⁰⁻¹² Yamada reported that secretion of ACTH was decreased in some infants with refractory hypotension,²² and that pituitary dysfunction may play a role in RAI in preterm infants, at least partly. Considering this, we chose CRH stimulation tests instead of ACTH tests for the evaluation of the HPA axis in preterm infants.¹⁰

In this study, infants with RAI had a higher incidence of longer duration of artificial ventilation. In addition, the incidence of grade 3 BPD was higher in the RAI group. These facts suggested that RAI is related to the development of severe BPD. The incidence of refractory PDA was also high in the RAI group although no statistical difference was found. These findings suggested that RAI might obstruct closure of the ductus arteriosus, however this requires further analysis.

Concerning the cortisol measuring, we found that basal serum cortisol levels were not changed in infants with RAI; however, the response to CRH tests was markedly suppressed in infants with RAI. These results clearly suggested that infants clinically diagnosed as RAI showed lower response to CRH stimulation tests. In other words, using CRH stimulation tests, we can diagnose RAI in preterm infants. Basal cortisol levels are not a useful marker for the diagnosis of RAI, but the response to CRH is useful. From the ROC curve, less than 140 nmol/L increase in serum cortisol concentration, or lower than 1.5 times increase in peak-to-base ratio suggests RAI in preterm infants born at a GA of <30 weeks. This study is the first report concerning the diagnostic criteria of RAI in critically ill preterm infants.

There are several limitations in this study. First, the number of participants was small because we had to exclude infants who had already received glucocorticoid by the date of the CRH stimulation test. This is because preterm infants born at a GA of <30 weeks have a high risk for refractory hypotension during the early neonatal period. Secondly, we examined only serum cortisol, but not ACTH, even though we performed CRH stimulation tests. This is because blood volume is limited in tiny subjects. We needed to decrease the amount of blood sampling to as little as possible in this study to reduce the burden on the preterm infants.

In conclusion, basal cortisol levels are not a useful marker for the diagnosis of RAI, but the lower response to CRH is useful for the diagnosis of RAI in preterm infants born at a GA of <30 weeks.

CONFLICT OF INTEREST

There are no potential conflicts of interest to disclose.

ORCID

Kougoro Iwanaga  <http://orcid.org/0000-0002-6087-7539>

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How to cite this article: Iwanaga K, Yamamoto A, Matsukura T, Niwa F, Kawai M. Corticotrophin-releasing hormone stimulation tests for the infants with relative adrenal insufficiency. *Clin Endocrinol.* 2017;87:660–664.

<https://doi.org/10.1111/cen.13446>