clinical settings, but calculated estimates of GFR appear to provide equally good information at much lower cost. In keeping with current best practice guidelines (12), we recommend that calculated estimates of GFR should supplement serum creatinine measurement alone and replace measured creatinine clearance.

We thank Wendy Van Der Steen of the Nuclear Medicine Department for the $^{51}$Cr-EDTA measurements. We are grateful to Drs. Y. Williams, A. Borr, R. Gale, and C. Pocock, from the Department of Haematology, for assistance with patient recruitment. We would also like to thank the staff of the Clinical Biochemistry Department, Kent and Canterbury Hospitals, for their cooperation and help. We are grateful to Dr. J. Sheldon from the Protein Reference Unit, St. George’s Hospital, London, for the β₂-microglobulin measurements. This work was funded by the South East Regional NHS Project Grant Scheme (Grant Reference SEO 150).

References


Previously published online at DOI: 10.1373/clinchem.2004.036947

Circulating Corticotropin-Releasing Hormone mRNA in Maternal Plasma: Relationship with Gestational Age and Severity of Preeclampsia, Antonio Farina,1* Carol W.M. Chan,1 Rossa W.K. Chiu,2 Nancy B.Y. Tsui,2 Paolo Carinci,1 Manuela Concu,1 Irina Banzola,1 Nicola Rizzo,1 and Y.M. Dennis Lo2 (1 Department of Histology, Medical Embryology, Obstetrics and Gynecology, University of Bologna, Bologna, Italy; 2 Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China; address correspondence to this author at: Department of Histology, Medical Embryology, Obstetrics and Gynecology, University of Bologna, Policlinico S. Orsola Malpighi, Bologna, Italy; fax 39-051-2094110, e-mail antonio.farina@unibo.it)

Higher concentrations of circulating nucleic acids (DNA and RNA) in maternal plasma/serum have been reported in pregnancies complicated by preeclampsia (PE) compared with controls matched for gestational age (1–5). Fetal and total DNA concentrations were also demonstrated to be directly correlated to both gestational age and to the severity of PE (6). Again, both hypertension and proteinuria (the two main symptoms for PE classifi-
PCR was performed with the EZ r.

Applied Biosystems 7700 Sequence detector. Each sample was analyzed in duplicate, and the corresponding calibration curve was run in parallel. Several negative water controls were also included in the analysis. The thermal profile used for the CRH gene was as follows: the reaction was initiated at 50 °C for 2 min, followed by 60 °C for 30 min. After denaturation at 95 °C for 5 min, 40 cycles of PCR were carried out at 94 °C for 20 s and 58 °C for 1 min.

The absolute concentrations of CRH mRNA were expressed as copies/mL of plasma based on the following calculation:

\[
c = Q \times \frac{V_{RNA}}{V_{ext}}
\]

where \( c \) is the concentration of CRH mRNA in plasma (copies/mL); \( Q \) is the quantity (copies/µL) of CRH mRNA detected by the sequence detector; \( V_{RNA} \) is the total volume of RNA obtained after extraction, i.e., 30 µL; and \( V_{ext} \) is the volume of plasma extracted, i.e., 1.6 mL.

Sample analysis was performed without knowledge of the gestational age and disease status (affected or not affected). Median CRH mRNA concentrations as a function of increasing gestational age (expressed in days) were calculated by weighted log-linear regression. In addition, PE (PE absent/present) was added to the regression as a “dummy” variable. Thus, this variable could assume a value of either 0 (absent) or 1 (present). All mRNA values in both cases and controls were expressed as multiples of the median (MoM). The MoM values were illustrated in a box-and-whisker plot, and the comparisons across generated groups was performed with nonparametric statistics.

The unadjusted median mRNA concentrations for CRH in controls and in the PE cases were 100 (0–568) copies/mL and 962 (284–5896) copies/mL, respectively. The CRH mRNA concentration directly correlated with gestational age (\( P = 0.045; t = 2.096 \)) and to PE (\( P < 0.001; t = 6.482 \); Table 1 and Fig. 1A). The mean (SD) MoM values were 1.00 (1.48) and 9.35 (11.18) for controls and PE cases, respectively (\( P < 0.001 \)). For cases affected by mild PE, the mean MoM was 8.30 (3.68); for cases affected by severe PE the mean MoM was 10.38 (14.24). The difference was not significant (Fig. 1B). The median (minimum–maximum) gestational ages were 256 (209–274) and 256 (181–279) days for those pregnancies affected by mild and severe PE, respectively. Because of the small sample size, no definitive conclusions can be reached about the estimated MoM values in mild and severe PE, but it is worth mentioning that the net difference for severe PE when compared with mild PE was +2 MoM.

A correlation between placental hormones and their respective maternal plasma mRNAs has been described previously (4). Such a result is important because the abnormal hormonal profiles detected in numerous patho-

<table>
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<tr>
<th>Variable</th>
<th>Table 1. Bivariate log-linear regression analysis for the log estimation of CRH mRNA concentrations.</th>
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</thead>
<tbody>
<tr>
<td>Gestational age (days)</td>
<td>0.056</td>
</tr>
<tr>
<td>PE (absent vs present)</td>
<td>0.938</td>
</tr>
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logic conditions would allow a preliminary evaluation of plasma mRNA products having a potential discriminant ability (especially for monitoring and screening) for a specific disease. Placental CRH plays a major role controlling those mechanisms for the maintenance of pregnancy. Plasma CRH is significantly higher in women with preterm deliveries and significantly lower in women who delivered post term, when compared with those who delivered at term (8). Such a mechanism, which begins at the first trimester, is a placental clock that triggers the onset of labor. CRH participates in the control of vascular tone in the human placenta, and it has been found in higher than expected concentrations in those conditions associated with vascular damage as well as PE (9), a disease that affects ~3-5% of pregnancies (10).

The pathogenesis of PE, although poorly understood, has been associated with apoptotic change of the villous trophoblasts (11), possibly secondary to inadequate oxygenation of blood within the intervillous spaces (10). The increases in circulating fetal DNA and RNA observed in PE (2) are likely to be epiphenomena of the apoptosis of such placental tissues, although 5-fold (6) and 10-fold (5) increases, respectively, were shown for fetal DNA and CRH mRNA even after adjustment for possible confounding factors (6). Such increases in CRH mRNA concentrations, if also demonstrated for women who will eventually develop PE but are asymptomatic at the time of blood collection, would make CRH mRNA a promising tool for possible use in a screening program. It must be emphasized that in women who will eventually develop PE, a 2.5-fold increase in circulating fetal DNA has been observed (12). Furthermore, for those women who screen positive for anomalies of the uterine arteries based on Doppler waveform (a current method for PE and fetal growth restriction screening), circulating fetal nucleic acid quantification could be used as an adjunct for better differentiation of those women who will develop the complications (13). CRH mRNA, as described here, is an attractive candidate because of its gender- and polymorphism-independent nature.

This work was supported by an Earmarked Research Grant (CUHK4474/03M) from the Research Grants Council of the Hong Kong Special Administrative Region, China, and by Fondazione CARISBO Progetto Triennale-Molecular Genetics of Fetal DNA, and by Fondi ex-60 A.F., Italy.

References
Automated Measurement of Cerebrospinal Fluid Bilirubin in Suspected Subarachnoid Hemorrhage. Jacobus Petrus Johannes Ungerer,* Sandra Jayne Southby, Christopher Michael Florkowski, and Peter Myles George (Canterbury Health Laboratories, PO Box 151, Christchurch, Canterbury, New Zealand; * author for correspondence: fax 64-3-364-0750, e-mail ungerer@mweb.co.za)

Early detection of subarachnoid hemorrhage (SAH) can improve clinical outcome (1). After hemorrhage into the cerebrospinal fluid (CSF), erythrocytes lyse, releasing oxyhemoglobin, which is metabolized to bilirubin. The detection of increased CSF bilirubin is the basis for the laboratory identification of in vivo hemorrhage (2). Guidelines for the detection of CSF bilirubin in suspected SAH have recently been published (2). In the guidelines, the preferred method for detection of bilirubin is spectrophotometric scanning, with the net bilirubin absorbance (NBA) calculated according to Chalmers' modification (3, 4). The procedure is not necessarily ideally suited for a clinical laboratory environment, and most hospital laboratories in the United States still use subjective visual inspection to identify CSF xanthochromia (5). The visual interpretation of spectrophotometric spectra is also subjective (which complicates clinical interpretation), although an iterative model has been proposed to circumvent this problem (6). We evaluated the measurement of CSF bilirubin on an automated instrument, using the Jendrassik–Gröf method, calibrated to measure lower concentrations. We report our experience with this method and compare the results with those of spectrophotometric scanning.

Spectrophotometric scanning was performed according to the guidelines (2), and a cutoff value of >0.007 for NBA was defined as positive. In all positive cases, a corrected value was calculated using the CSF and serum protein concentrations and the serum bilirubin concentration (2). Bilirubin was measured by a modification of our routine serum bilirubin assay, which consists of an in-house method on an Aeroset analyzer (Abbott Laboratories).

The method is based on the Jendrassik–Gröf principle as described by Doumas et al. (7), with a modification of the diazo reagent according to Chan et al. (8). To measure bilirubin at the concentrations typically found in CSF, we increased the ratio of sample to reagent volume from 0.043 to 0.150. The calibrator was diluted to give a 7-point calibration curve ranging from 0 to 10 550 nmol/L. We tested assay linearity by use of polynomial regression according to NCCLS document EP6-A. Oxyhemoglobin interference on CSF bilirubin and NBA determinations were investigated in samples prepared from purified bilirubin to concentrations of 340 and 6000 nmol/L. Hemoglobin was prepared from patient erythrocytes, and the concentration was verified by measurement on a Coulter Stack S analyzer. The hemoglobin was added to the two pure bilirubin solutions to give concentrations of 500, 1000, 2500, and 5000 mg/L. A 1:10 dilution of our serum bilirubin quality-control sample, Multiqual Level 1 (Bio-Rad) was used for continuous internal quality control.

The CSF bilirubin method had an intraassay CV of 20% (n = 20) at 100 nmol/L, based on repeat analysis of dilutions of Multiqual Level 1 (Bio-Rad). At 200 nmol/L, the CV was 11% (n = 20), and at 400 nmol/L it was 7% (n = 20). The detection limit was 35 nmol/L, based on replicate analysis (n = 25) of saline within batch and calculation of the mean plus 2 SD. The diluted serum quality control had mean value of 800 nmol/L and a CV of 6% (n = 140 days). The response was linear from 100 to 2400 nmol/L, encompassing the clinically relevant interval. We found no significant interference at a hemoglobin concentration ≤100 mg/dL (10 mg/dL). Higher hemoglobin was associated with increasing negative interference.

To estimate a reference interval, we measured CSF bilirubin on samples from patients in which SAH was not suspected and which were visually clear and had normal CSF protein (≤400 mg/L). For 172 samples, the mean (SD) CSF bilirubin was 234 (62) nmol/L.

We studied 144 CSF samples for which xanthochromia testing had been requested (Fig. 1A). Results were positive in 23 by spectrophotometric analysis. The ability of CSF bilirubin measurements to correctly identify the presence of xanthochromia assigned in this way was investigated by ROC curve analysis.

ROC curve analysis gave an area under the curve of 0.99 (95% confidence interval, 0.95–1.00). At our upper reference limit for CSF bilirubin (359 nmol/L), the sensitivity was 100% and specificity was 92%. The sensitivity and specificity were calculated at various cutoffs (Table 1).

In 5 of the 23 positive cases, the NBA corrected to <0.007. The five samples had initial NBA results ranging from 0.007 to 0.218, and all had CSF bilirubin concentrations (391–6037 nmol/L) above the reference interval. When corrected, four of the five CSF bilirubin results returned to below the upper reference limit. The fifth result had a value of 1219 nmol/L and corrected to 431 nmol/L. These findings suggest that the principle of correcting for the NBA that would be expected if no