Supplementary Data Materials & Methods

The study was conducted at the Prince of Wales Hospital, teaching hospital to the Chinese University of Hong Kong; at The First Affiliation Hospital of Medical College of Jinan University; and at The Women’s & Children’s Hospital of Guangdong Province, teaching hospital to the Guangzhou Medical College. Ethical approval was received from the clinical research ethics committees/authorities of each of the three teaching hospitals.

STUDY PARTICIPANT SELECTION

Pregnancies at risk of poor outcome were identified during labour (bad obstetric history, meconium stained liquor/oligohydraminos, or fetal heart rate decelerations). Cord arterial blood samples were collected immediately after delivery. Infants who had developed signs of fetal distress during labour or showed signs of respiratory depression at the time of birth were recruited if the attending neonatologist considered that the infant was born in poor condition and required immediate resuscitation, and direct admission to the NICU was indicated for respiratory support.

The principal indications for direct admission to the NICU from the delivery suite are: 1) ‘Fetal distress’ defined as either a pH of < 7.20 on fetal scalp blood sampling performed in response to an abnormal CTG, or a prolonged fetal bradycardia requiring immediate operative intervention. An abnormal CTG tracing was defined as one either containing recurrent late decelerations with no variability; severe variable decelerations with atypia and baseline changes; late/variable decelerations associated with absent baseline variability; or prolonged decelerations without recovery; 2) prematurity; 3) any condition where there may have been long term hypoxia such as antepartum haemorrhage or intra-uterine growth retardation; 4) traumatic delivery: ventouse, forceps or assisted breech delivery or following shoulder dystocia; 5) suspected neonatal sepsis.

Informed and written consent was obtained from the mother prior to the infant becoming a study participant. Major errors of morphogenesis in fetus or medical complications in mother were excluded.
from the study. Normal control patients (cared for in post-natal ward with mother) were obtained from a study of the ultrasonic prediction of cord entanglement, which was running concurrently with this project (1). Informed consent was obtained from the mothers.

NEONATAL CARE

Neonates were transferred to the NICU after appropriate resuscitation at birth, where they received routine neonatal care.

CLASSIFICATION OF POOR OUTCOME

The principal perinatal outcomes recorded in this study were the primary reasons for admission to the neonatal intensive care unit (NICU). Neonates were further classified as having a good perinatal outcome unless they died or developed seizures within 7 days of delivery (poor outcome). Perinatal death (PND) refers to either stillbirths or death within 28 days after birth of an infant weighing 500g or more. HIE was diagnosed according to the criteria described by Badawi and colleagues (2).

UMBILICAL CORD BLOOD SAMPLING

After delivery a segment of umbilical cord was isolated with two clamps and blood drawn from both the umbilical artery and vein, avoiding the withdrawal of placental blood.

ACID-BASE STUDIES

Cord blood samples were analysed within 5 minutes of collection using a Ciba Corning 288 blood gas analyzer (Medfield, MA) for pH, carbon dioxide (pCO₂), oxygen (pO₂), and base excess (BE). Acid base parameters were compared between the paired cord arterial and venous samples using a computer program designed to identify double venous samples (3). Only cases where a cord blood sample could be confirmed as arterial were used for analysis.

LIPID PEROXIDATION

Cord arterial plasma was obtained from cord arterial samples by refrigerated centrifugation at 1000g for 10 minutes, and stored at -80°C in 0.005% BHT-treated cryovials for less than 3 months prior to
blinded isoprostane analysis. We have developed a rapid and robust methodology for assay of 8-isoprostanes from plasma, using single step ethanol extraction and de-proteinization, pentafluorobenzyl bromide and bis(trimethylsilyl)trifluoracetamide (Sigma, St. Louis) derivatization, followed by gas chromatography (Agilent 6890 plus, CA) negative chemical ionization mass spectrometry (Agilent 5973N, CA). This methodology achieved absolute recovery of 83% (±1.9%), analytical accuracy of 99.0% (± 0.4%), linearity of 0.9985 over the concentration range of 10-5000 pg/ml 8-isoprostane, and a method detection limit of 21 pg/ml (4). The coefficient of variation in determination of 8-isoprostane was 5.7% within assays and 8.1% between assays.

STATISTICS

All statistical analyses were performed on SPSS version 11.01 for Windows, or on software written in the Dept. Obstetrics and Gynaecology, CUHK (http://www.obg.cuhk.edu.hk). The triangular test (PEST4) is a group sequential model with integrated automatic stopping rules which come into play if a significant difference is achieved or if a significant result is unlikely to be achieved for the predetermined effect size (5). Patient allocation under the group sequential model minimizes the number of cases required to come to a firm conclusion, although it does not necessarily reduce the numbers in comparison to a fixed sample size based on power analysis (6). We explored sequential methodology in an earlier study that investigated the treatment effect of intrapartum amnioinfusion on concentrations of organic hydro-peroxides and malondyaldehyde in cord arterial blood and found it to be robust (7). Its major advantage is in dealing with measurements of unknown variance (8), which was the case for isoprostanes in cord blood: the distribution being unknown amongst infants with poor outcome. With an estimated effect size of 0.5 standard deviations a maximum number of 589 cases could have been required if the sequential plot reached the extreme right of the triangle. In fact a larger effect size of almost 2 standard deviations was observed, triggering the automatic stopping rule after recruiting only 297 cases.
**Hypothesis 1:** As the delivery of infants with significant levels of morbidity is uncommon and sporadic, the group comparisons (testing hypothesis 1) were made using a group sequential model formulated to identify a critical difference of 0.5 standard deviations in isoprostane concentration between neonates requiring immediate admission to NICU and controls, with a significance level set at 0.01 and power of 0.95.

**Hypothesis 2:** Receiver operating characteristic (ROC) curves were created for those cases admitted to NICU on clinical grounds, and for each variable’s ability to predict HIE/PND for all cases. Paired comparisons between ROC curves were made using the DeLong statistic (9).

**References**