Clinical value of lactate measurement and nucleated red blood cell counts in the placental segment of the umbilical vein of premature newborns for diagnosis of hypoxia-ischemia

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Abstract

Objective: To evaluate the clinical value of lactate measurement and nucleated red blood cell (NRBC) counts when compared to base excess (BE) in the blood collected from the placental segment of the umbilical vein.

Methods: 25 umbilical cords from premature babies were sampled after placental delivery and cord clamping. Babies were followed until discharge. Statistics involved linear regression, Spearman's correlation, ROC curves, and Fisher's exact test.

Results: The relationship between lactate in the umbilical vein blood and pH and BE was significant (p < 0.0001). A 4.04 mmol/L lactate level showed a sensitivity of 62.5% and a specificity of 94.1% in detecting pH <7.2 and BE < -10 mmol/L. NRBC counts were related to BE (p = 0.0095), but with a sensitivity of 37.5% and specificity of 82.4% in detecting BE < -10 mmol/L.

Conclusions: Lactate is a valuable marker of fetal hypoxia when sampled from placental segment veins. NRBC counts demonstrated low sensitivity for the detection of acidosis.

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Introduction

Biochemical markers are valuable tools for the detection of hypoxic-ischemic events. Historically, fetal umbilical pH and base excess (BE) have been used as markers of hypoxic-ischemic insult, reflecting the cellular production of metabolic acids in consequence of hypoxemia. Lactate has been proposed as a useful method for the detection of fetal hypoxia.[1] Nucleated red blood cell (NRBC) counts are increased in association with higher levels of erythropoietin in response to tissue hypoxia, and studies have demonstrated the relationship between this marker and perinatal hypoxia-ischemia.[2,3] Both lactate and NRBC counts have been investigated as predictors of short and long-term prognosis, with conflicting results.[1,4-6]

The aim of this study was to evaluate lactate levels and NRBC counts in the blood from the placental segment of the umbilical vein of premature babies in relation to BE values. We also investigated correlations of lactate and nucleated red blood cells to Apgar scores and maternal complications, and

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prospectively followed these babies until hospital discharge, to assess the prognostic value of the markers with respect to the short-term outcome.

Methods

Twenty-five premature newborns, born at a tertiary hospital in Brazil, from April 2004 to January 2005, were assessed. Inclusion criteria were gestational age < 37 weeks, weight < 2,000 g, and a written informed consent signed by the mother. The study was approved by the local Research Ethics Committee. Congenital malformations, maternal diabetes and blood group incompatibility were exclusion criteria. Immediately after placental delivery, blood was sampled (0.5 mL) from the umbilical vein in the segment close to the placenta, using 5-mL heparinized plastic syringes, and then placed on ice. Blood was also sampled into sodium fluoride vials for lactate measurements, and into K3-EDTA vials for blood cell counts. Analysis of blood gases was performed within 30 minutes after sampling, using a Rapid Lab 865 (Bayer) analyzer. Plasma lactate was analyzed by an enzymatic method (Ortho-Clinical Diagnostics – Vitros 750, Johnson & Johnson). NRBC and leukocytes were counted through manual microscopy of the blood smear, and confirmed by a second technician. NRBC were calculated as the number of NRBC per 100 leukocytes.

Classically, acidosis at birth has been defined as BE in umbilical arterial blood lower than -12 mmol/L, which is approximately 2 standard deviations (SD) below the mean for normal newborns.[7-9] Nodwell et al. demonstrated that values of blood gases are different in placental and umbilical segments after cord clamping and placental delivery. The values observed for BE in the placental segment were -3.4 (mean) ± 2.2 (SD).[10] We defined our endpoint for acidosis as BE equal to or lower than -10 mmol/L, which corresponds to 3 SD below the mean, based on these values.

For the short-term outcome we prospectively collected information about the occurrence of perinatal infection (defined by clinical signs and symptoms or positive blood culture, or both), periventricular or intraventricular hemorrhage (assessed by cranial ultrasound), necrotizing enterocolitis, need for vasoactive drugs, parenteral nutrition, blood products, oxygen, mechanical ventilation (invasive or non-invasive). Clinical information was also collected to calculate the Clinical Risk Index for Babies (CRIB) score in the first 12 hours of life.[11] Lactate levels and NRBC correlations were investigated for each one of these factors. Apgar scores and maternal complications (infection, hypertensive disease, premature rupture of membranes more than 18 hours before birth) were also analyzed for relationships with the markers. Maternal infection was defined as the presence of fever, leukocytosis, or use of antibiotics for reasons such as urinary

tract infection or pneumonia at birth. As neurological examination of premature babies is uncertain, this criterion was not included.

Statistical significance of correlations between investigated markers and pH, BE, PCO_2, O_2 saturation and oxygen blood content was determined by linear regression analysis with 99% confidence intervals. For comparison between scores (Apgar and CRIB) and the markers, Spearman's non-parametric correlation was used. Through receiver operating characteristic (ROC) curves and contingency tables, we determined the value of the markers for identifying acidosis, and established the cutoff values for this parameter. We also tested the presence of a value higher than the defined cutoff values for the occurrence of perinatal outcome factors with Fisher's exact test. The statistical analysis was performed using the Analyse-it software (www.analyse-it. com).

Results

Clinical characteristics, perinatal events and their relationships to the investigated markers are shown in Table 1. Means and standard deviations were determined as follows: lactate 4.26 ± 3.78 mmol/L; NRBC counts 20.52 ± 37.96 cells/100 leukocytes; BE, -9.35 ± 5.99 mmol/L; pH, 7.22 ± 0.17; CO_2, 46.06 ± 17.74 mmHg. Figure 1 shows the correlation between umbilical vein blood values of lactate and BE (R^2 = 0.72, p < 0.0001). For the identification of acidosis (BE < -10 mmol/L), the area under the ROC curve was 0.842. The sensitivity of a lactate cutoff level equal to 4.04 mmol/L in relation to BE lower than or equal to -10 mmol/L was 62.5%, and specificity was 94.1%, with a positive predictive value equal to 83.3%, and negative predictive value of 84.2%. The same values were observed for pH. Although pH and CO_2 were not endpoints, because of their variability caused by placental metabolism and gas exchange after cord clamping, we observed a strong relationship between lactate and pH (R^2 = 0.82, p < 0.0001), and between lactate and PCO_2 (R^2 = 0.6, p < 0.0001). There was a weak correlation between lactate and bicarbonate (R^2 = 0.23, p = 0.014), and no correlation was found between lactate and O_2 blood content, PO_2 and hemoglobin oxygen saturation, and Apgar scores. Apgar scores were also not related to pH and BE.

NRBC counts were related to BE (R^2 = 0.26, p = 0.009) and to pH (R^2 = 0.38, p = 0.009). A cutoff value of 10 NRBC/100 leukocytes showed poor sensitivity (40%) and specificity of 80% for more severe acidosis (BE lower than -12 mmol/L). For our endpoint (BE lower than -10 mmol/L), NRBC count was not an adequate test, with an area under the ROC curve equal to 0.577 (p = 0.26), sensitivity of 37.5% and specificity of 82.4%, positive predictive value equal to 50% and negative predictive value equal to 73.6%. The area under the ROC curve was 0.87 for the determination of pH < 7.2, for

the cutoff value of NRBC, with sensitivity of 50% and specificity of 88.2%. NRBC were not correlated to blood oxygen content and PO_2 and showed a weak correlation to oxygen saturation ($R^2 = 0.07$, $p = 0.018$) and PCO_2 ($R^2 = 0.33$, $p = 0.0027$). NRBC count and lactate levels were related to each other ($R^2 = 0.4$, $p = 0.0008$), and NRBC count also showed weak correlation to Apgar scores ($p = 0.03$ at 1 minute and $p = 0.02$ at 5 minutes).

Both lactate levels and NRBC count were unable to predict short-term perinatal complications, with an exception: from the seven babies with NRBC counts equal to or higher than 10/100 leukocytes, three developed necrotizing enterocolitis

(42%, $p = 0.026$), with one intestinal perforation. Lactate levels and NRBC counts were not related to maternal complications.

Discussion

The diagnosis of hypoxia-ischemia at birth is critical, and combining clinical markers with laboratory data would be useful to identify premature infants with higher risk for neurological damage.[4]

Samples from the umbilical artery may be limited by small volume and by the difficulty in collecting them, especially in thin umbilical cords. The umbilical vein, close to the site of

Table 1 - Characteristics of patients, perinatal events and their relationships to lactate levels and NRBC counts

		Relationship to lactate	Relationship to NRBC
Gestational age (weeks, mean ± SD, minimum, maximum)	33.57±1.98 (29-36.8)	NS*	NS*
Gender (male/ female ratio)	15/10	NS†	NS†
Apgar score at 1 min (median, min, max)	8 (1-9)	NS*	p = 0.029 (RS = -0.41)
Apgar score at 5 min (median, min, max)	9 (7-10)	NS*	p = 0.002 (RS = -0.53)
Birth weight (kg, mean ±SD, min, max)	1.701±0.233 (1.075-2.000)	NS*	NS*
CRIB score (median, min, max)	4 (1-12)	NS*	NS*
Maternal infection (ratio and %)	9/25 (36%)	NS†	NS†
Maternal hypertensive disease (ratio and %)	6/25 (24%)	NS†	NS†
Premature rupture of membranes more than 18 hours (ratio, %)	10/25 (40%)	NS†	NS†
Perinatal infection (ratio and %)	13/25 (52%)	NS†	NS†
Need for blood products	10/25 (40%)	NS†	NS†
Need for invasive mechanical ventilation	9/25 (36%)	NS†	NS†
Need for parenteral nutrition	5/26 (19.2%)	NS†	NS†
Need for vasoactive drugs (ratio and %)	10/25 (40%)	NS†	NS†
Necrotizing enterocolitis (grade 2 or higher according to Bell's classification, ratio and %)	3/25 (12%)	NS†	p = 0.026†
Periventricular or ventricular hemorrhage (grade I to IV, ratio and %)	5/25 (20%)	NS†	NS†

NS = non-significant; RS = Spearman's correlation coefficient.
* Spearman's non-parametric correlation.
† Fisher's exact test.

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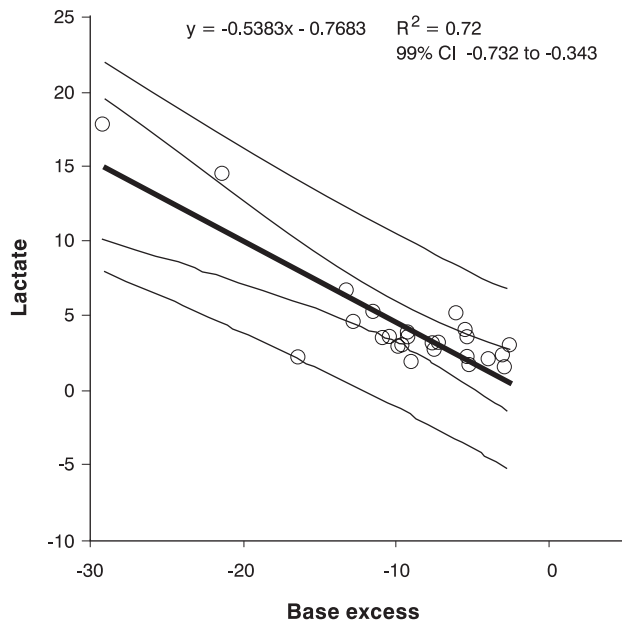Jornal de Pediatria - Vol. 83, No.2, 2007 **189**

Figure 1 - Correlation between lactate and BE
CI = confidence interval; R^2 = linear regression corre-
lation coefficient; y = slope coefficient

placental insertion, can provide an alternative source for sampling, but only after placental delivery. Placental metabolism and gas exchange proceed after delivery and cord clamping, affecting measurements in vein blood. This is particularly true for oxygen measurements and PCO_2, but Nodwel et al. demonstrated that the agreement between BE in blood from the placental segment of the umbilical vein, and in blood from the umbilical segment of the umbilical vein or artery, is acceptable for clinical purposes.[10] We observed that in blood from the placental segment of the umbilical vein after placental delivery, lactate maintains the same good correlation to BE previously reported for arterial measurements.[1] Thus, lactate has a potential as a simple and inexpensive tool for the diagnosis of metabolic acidosis associated with hypoxia-ischemia in premature infants. A whole blood gas analysis costs nearly three times more than a single lactate measurement.

Elevated NRBC counts in umbilical venous blood have been correlated to acute and chronic antepartum asphyxia.[12] Maternal nucleated blood cells can transfer into fetal circulation when uteroplacental perfusion is impaired, but this is not significant for counts (one maternal cell to at least 100 fetal cells).[13] NRBC counts in placental circulation are also correlated to counts in umbilical blood.[14] These facts, coupled with the assumption that NRBC count in umbilical vein blood is not influenced by placental metabolism and cord clamping, make it attractive as one more tool for monitoring the condition of premature newborns. We found that NRBC counts are weakly related to BE and pH, with low sensitivity

and positive predictive values. NRBC count cannot be recommended for routine clinical diagnosis of perinatal acidosis, since classical markers and lactate measurements are more adequate.

Neither lactate nor NRBC counts were useful to predict the short-term outcome as single markers. Cases of necrotizing enterocolitis with elevated NRBC counts have been previously reported,[15] suggesting that NRBC counts in premature infants can help to identify risk groups. More prospective studies are needed.

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