

## Serum levels of caffeine in umbilical cord and apnea of prematurity

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### Abstract

**Objective:** To determine the influence of presence of caffeine in umbilical cord blood on apnea occurrence.

**Methods:** A prospective cohort study with preterm newborns with birth weight lower than 2,000 g was undertaken. Exclusion criteria were: mothers who received opioids; mechanical ventilation during the first 4 days of life; cerebral and major cardiac malformations; perinatal asphyxia; severe periintra-ventricular hemorrhage; exchange transfusion before the fourth day of life; and those who received methylxantine prior to extubation. Neonates were divided into detectable and undetectable caffeine in umbilical cord blood. Newborns were followed for the first 4 days for occurrence of apnea spells.

**Results:** Eighty-seven newborns with and 40 without detectable caffeine in umbilical cord blood were studied. Median caffeine concentration of the 87 patients with detectable caffeine in umbilical blood was 2.3 µg/mL (0.2-9.4 µg/mL). There was no association between occurrence of apnea spells and presence of caffeine in umbilical cord blood. Neonates with detectable caffeine in umbilical blood had borderline later apnea (66.3±4.14 hours) than those with undetectable levels (54.2±6.26 hours).

**Conclusion:** Detected levels of caffeine in umbilical cord blood did not decrease occurrence of apnea of prematurity, but it had a borderline effect delaying its occurrence, suggesting that even a low level of caffeine in umbilical cord blood might delay occurrence of apnea spells.

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### Introduction

Treatment of apnea of prematurity includes pharmacological approach with caffeine, a powerful stimulant of the central nervous system that reduces occurrence of neonatal apnea, promotes consolidation of a regular pattern of breathing and increases alveolar ventilation.<sup>1,2</sup> Adequate treatment of apnea in the neonatal period is of great importance, as a higher incidence of periintra-ventricular hemorrhage (PIVH), hydrocephaly,

periventricular leukomalacia (PVL), need for ventilatory support for longer time and alterations in neurological development are observed in the first year of life when preterm infants with apnea are compared to those without apnea.<sup>3</sup>

The role of maternal consumption of caffeine and occurrence of apnea in preterm neonates have aroused general interest. As caffeine is a substance that crosses the

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This study was carried out at the Department of Pediatrics, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, and at the Newborn Section, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil.

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placental barrier, it could have an intrauterine stimulation of the fetus's respiratory center.<sup>4-6</sup>

Caffeine is probably the most frequently drug taken in the world, consumed by people of all ages. Daily caffeine consumption per capita, considering all sources, is around 3-7 mg/kg/day, approximately 200 mg/day in the general population.<sup>4</sup> Its consumption is so common that around 95% of pregnant women take some caffeine, either through diet or medication.<sup>4</sup> The role of caffeine in pregnancy and occurrence of apnea of prematurity remain controversial. This study aimed at determining the influence of presence of caffeine in umbilical cord blood on the incidence and time of occurrence of apnea of prematurity in the first days of life.

## Methods

This was a prospective cohort study, conducted at Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil. The sample was composed of preterm neonates with birth weight between 1,000 and 2,000 g and gestational age under 37 weeks, born at HCPA between March 2006 and May 2008.

The study excluded neonates whose mothers received opioids or drugs that led to respiratory center depression, neonates who remained in mechanical ventilation during the first 4 days of life, with cerebral congenital malformations, perinatal asphyxia (Apgar score under 7 at the 5th minute of life), PIVH degrees 3 and 4, major cardiac malformations, exchange transfusion before the fourth day of life, neonates who presented one isolated apnea with no need for drug or ventilatory treatment, and those that received methylxantine prior to extubation.

The variables were birth weight, gestational age, Apgar score at the 5th minute of life, gender, maternal preeclampsia, mode of delivery, small for gestational age (SGA), apnea, continuous positive air pressure (CPAP) and mechanical ventilation just after birth, respiratory distress syndrome (RDS), presence of sepsis and/or meningitis, anemia (hemoglobin < 10 g/dL), patent ductus arteriosus (PDA), PIVH, PVL, and seizures.

The method used in the gestational age assessment was preferably the obstetric gestational age obtained through ultrasonography in the first weeks of fetal life or, if such data were unavailable, the date of most recent menstruation confirmed through clinical exam of the neonate.<sup>7</sup>

Birth weight was measured using an electronic scale and the neonates were classified according to the curve of Alexander et al.<sup>8</sup> Neonates under percentile 10 were considered as SGA.<sup>8,9</sup>

For the diagnosis of early sepsis, the study considered patients that presented clinical conditions and positive blood culture.<sup>10-12</sup> In case of positive blood culture

for *Staphylococcus* coagulase-negative, patients were considered with sepsis only if presenting complete blood count and C-reactive protein with alterations and treated with adequate antibiotics to the germ with response to the therapy. All patients with diagnosis of sepsis were submitted to lumbar puncture. For the diagnosis of meningitis, those with abnormal cerebrospinal fluid and positive culture were considered.

Cerebral ultrasonography (CUS) was performed in all neonates with birth weight under 1,500 g for the diagnosis of PIVH and PVL. According to the service routine, preterm neonates whose birth weight was above 1,500 g and presented any clinical problem including apnea were submitted to CUS. Echocardiogram was performed in all preterm neonates with clinical suspicion of PDA.

Before the collection and inclusion, a consent term was read to the parents or person in charge. The study was approved by the Research Ethics Committee of HCPA (project no. 06-048).

Preterm neonates who fulfilled the inclusion criteria had their umbilical venous blood collected, immediately after birth. They were monitored during their stay at neonatal intensive care unit for occurrence of apnea spells during the first 4 days of life. Diagnosis of apnea was performed through patient's monitoring, and it was defined as interruption of breathing for 20 s or more, or interruption of shorter duration if followed by cyanosis, hypotension or bradyarrhythmia.<sup>13</sup>

The neonates were divided into two groups:

- Group 1 – preterm neonates with caffeine detected in umbilical cord blood.
- Group 2 – preterm neonates with no caffeine detected in umbilical cord blood.

Blood samples were collected from umbilical blood using syringes with heparin. After that, 500 µL of heparinized blood were centrifuged at 3,500 rpm for 5 minutes, and plasma was separated and stored at -80 °C, in Eppendorf tubes, identified with the patient's number. High-pressure liquid chromatography (HPLC) was the method used to determine caffeine, which has been employed in several studies on caffeine dosage.<sup>14-19</sup>

## Statistical analysis

There is no similar study to be used for sample size calculation; hence we assumed an apnea occurrence of 20 and 55% for the groups with detectable and undetectable caffeine levels, respectively. For a significance level of 5% and statistical power of 80%, the required sample size was 80 preterm neonates, with 40 patients in each group, considering the multifactor logistic regression and the possibility of adjustment to up to four confusion factors.

Variables were described as median and interquartile range (IQ25-75%) or mean  $\pm$  standard deviation. Group characteristics were analyzed using chi-square test, Fisher's exact test, except for the birth weight variable (Student's *t* test) and Apgar score (Mann-Whitney test). Analysis of caffeine detection in blood employed the chi-square test. Logistic regression was performed for variables with  $p \leq 0.10$  on univariable analysis. The study used the statistical program Statistical Package for Social Sciences (SPSS) 14.0 and considered the significance level of  $p < 0.05$ .

## Results

Umbilical blood was collected in 151 preterm neonates; 21 of them were subsequently excluded (seven for presenting apnea once with no need for treatment, five for remaining in mechanical ventilation until the fourth day of life, three for PIVH degrees 3 and 4, one for exchange transfusion, five for methylxantine prior to extubation), and three were lost (two due to insufficient sampled quantity for caffeine dosage and one due to family request). The study population was constituted by 127 neonates with mean gestational age of  $32.5 \pm 1.8$  weeks and mean birth weight of  $1,594 \pm 276$  g.

Group 1 was formed by 87 newborns and group 2 by 40. Median caffeine concentration of the 87 patients with detectable caffeine in umbilical blood was  $2.3 \mu\text{g/mL}$  (range  $0.2\text{-}9.4 \mu\text{g/mL}$ ; IQ25-75%  $1.5\text{-}3.5 \mu\text{g/mL}$ ).

Table 1 shows the group characteristics. Patients in group 2 had a statistically significant lower gestational age, and required more respiratory support (CPAP and mechanical ventilation just after birth) than those in group 1.

When only newborn infants with birth weight  $\leq 1,500$  g and gestational age  $\leq 34$  weeks were studied, there was no difference in birth weight and gestational age, and patients with undetectable caffeine in umbilical cord blood required more respiratory support just after birth (Table 2).

The logistic regression model employed for the whole studied population took the presence of caffeine in umbilical cord blood as a dependent factor, and gestational age, occurrence of apnea, use of CPAP and mechanical ventilation as independent factors. None of them showed significance (Table 3).

Neonates with presence of caffeine in umbilical blood had borderline later apnea: group 1 and group 2 had apnea at  $66.3 \pm 4.14$  hours and  $54.2 \pm 6.26$  hours after birth, respectively ( $p = 0.067$ ).

## Discussion

This study showed that neonates with detectable and undetectable levels of caffeine in umbilical cord blood had similar occurrence of apnea, and among the neonates who presented apnea, occurrence was later in preterm neonates

**Table 1** - Characteristics of the studied groups

	Group 1 (n = 87)	Group 2 (n = 40)	p
Birth weight (g)	1625 $\pm$ 262	1528 $\pm$ 295	0.06
Gestational age (weeks)	32.8 $\pm$ 1.6	31.98 $\pm$ 1.99	0.009
5th minute Apgar score	9.0	8.78	0.12
Male	41 (47.1%)	23 (57.5%)	0.37
Preeclampsia	25 (28.7%)	9 (22.5%)	0.60
Vaginal delivery	27 (31%)	11 (27.5%)	0.84
Small for gestational age	45 (51.7%)	24 (60%)	0.49
Apnea	34 (39.1%)	53 (60.9%)	0.08
CPAP	47 (54%)	32 (80%)	0.009
Mechanical ventilation	10 (11.5%)	15 (37.5%)	0.001
Respiratory distress syndrome	9 (10.3%)	6 (15%)	0.55
Sepsis	4 (4.6%)	0	0.30
Meningitis	0	0	1
Anemia	2 (2.3%)	0	0.84
Patent ductus arteriosus	3 (3.4%)	3 (7.5%)	0.58
Periintraventricular hemorrhage grade 1 and 2	5 (5.7%)	3 (7.5%)	1
Periventricular leukomalacia	3 (3.4%)	6 (15%)	0.14
Seizures	1 (1.1%)	0	1

CPAP = continuous positive airway pressure; IQ25-75% = interquartile range 25-75%.

Group 1 - caffeine detected in umbilical cord blood.

Group 2 - no caffeine detected in umbilical cord blood.

Values expressed as mean  $\pm$  standard deviation, median (IQ25-75%), absolute number of newborns (%), Student's *t* test, chi-square, Fisher's exact test, Mann-Whitney test.

**Table 2** - Characteristics of the newborn infants with birth weight  $\leq 1,500$  g and gestational age  $\leq 34$  weeks

	Group 1 (n = 25)	Group 2 (n = 19)	p
Birth weight (g)	1273 $\pm$ 123	1258 $\pm$ 152	0.7
Gestational age (weeks)	31.24 $\pm$ 1.5	30.53 $\pm$ 1.42	0.1
5th minute Apgar score	9 (8-9)	9 (8-9)	0.59
Male	7 (28%)	12 (63.2)	0.043
Preeclampsia	7 (28%)	7 (36.8%)	0.76
Vaginal delivery	6 (24%)	3 (15.8%)	0.71
Small for gestational age	13 (52%)	10 (52.6%)	1
Apnea	17 (68%)	16 (84.2%)	0.30
CPAP	19 (76%)	19 (100%)	0.029
Mechanical ventilation	6 (24%)	12 (63.2%)	0.021
Respiratory distress syndrome	5 (20%)	4 (21.1%)	1
Sepsis	3 (12%)	0	0.25
Meningitis	0	0	1
Anemia	0	0	1
Patent ductus arteriosus	1 (4%)	3 (15.8%)	0.30
Periintraventricular hemorrhage grade 1 and 2	5 (20%)	3 (15.8%)	1
Periventricular leukomalacia	3 (12%)	6 (31.6%)	0.14
Seizures	0	0	1

CPAP = continuous positive airway pressure; IQ25-75% = interquartile range 25-75%.

Group 1 - caffeine detected in umbilical cord blood.

Group 2 - no caffeine detected in umbilical cord blood.

Values expressed as mean  $\pm$  standard deviation, median (IQ25-75%), absolute number of newborns (%), Student's *t* test, chi-square, Fisher's exact test, Mann-Whitney test.

with detectable levels of caffeine. No significant difference was found between the groups with and without caffeine detected in umbilical cord blood in terms of birth weight, Apgar score, gender, maternal preeclampsia, mode of delivery, SGA, presence of sepsis, anemia, RDS, PDA, PIVH, PVL and seizures. In the group with no detected caffeine in umbilical cord blood, gestational age was significantly lower, and they required more respiratory support, suggesting a role for immaturity in this group of patients. Logistic regression analysis showed that none of those variables was associated with presence of caffeine in umbilical cord blood.

Some studies quantified maternal use of caffeine during pregnancy. Concentrations of caffeine and paraxanthine in saliva were measured in pregnant women in the United

Kingdom (levels of caffeine in plasma and saliva are strongly correlated), and the mean concentration of caffeine was 0.45  $\mu\text{g/mL}$ .<sup>5</sup> It was shown in the USA that, among women in the third trimester of pregnancy who reported low consumption of caffeine, mean serum level of this substance in umbilical blood was 0.48  $\mu\text{g/mL}$  (ranging between 0 and 10.49  $\mu\text{g/mL}$ ), and women who reported consumption  $\geq 300$  mg/day of caffeine in the third trimester of pregnancy had neonates with mean serum level of caffeine in umbilical cord blood of 2.1  $\mu\text{g/mL}$ .<sup>6</sup> We obtained a similar result, indicating a high consumption of caffeine by the mothers of our studied population.

McCulloch et al. quantified caffeine in umbilical blood of 79 preterm neonates.<sup>20</sup> Eleven of them (14%) had

**Table 3** - Association with presence of caffeine in umbilical cord blood

Variable	OR	95%CI	p
Gestational age	1.039	0.889-1.213	0.631
Apnea	1.109	0.632-1.945	0.719
CPAP	0.830	0.492-1.401	0.486
Mechanical ventilation	0.588	0.282-1.227	0.157

95%CI = 95% confidence interval; CPAP = continuous positive airway pressure; OR = odds ratio.

detectable levels of caffeine, while 68 had undetectable levels of caffeine, and serum levels of caffeine ranged from 1.1 to 3.7 µg/mL. No difference was observed in occurrence of apnea between both groups, but diagnosis of apnea was obtained through pneumography, which was performed only after clinical stability and in patients that did not require ventilatory support or oxygen therapy, thus excluding patients with serious conditions. In our study, caffeine detection rate and serum levels of caffeine were higher than the results of that study, probably because our population had higher caffeine ingestion.

Caffeine has been used as treatment for apnea of prematurity, and the therapeutic level is reported to be 5 to 20 µg/mL.<sup>2</sup> Umbilical cord caffeine levels detected in our study were low; hence, there was no decrease in the occurrence of apnea because our detectable levels were below therapeutic levels. One limitation of this study is the lack of comparison in the number of apnea spells between both groups. The first choice for treatment of apnea of prematurity in our unit is methylxantine, and its use would bias the result.

In a retrospective study that applied questionnaires on cigarette and caffeine consumption during and after pregnancy to 298 mothers of infants with apnea before a polysomnography as part of the patient's assessment determined that maternal caffeine and cigarette use during pregnancy was related to central apnea. For caffeine consumption during pregnancy, even after adjustment to gestational age, age at evaluation and birth weight, an increase of 1 mg/day was associated with an increase of 1 apnea/hour. It was suggested that neonates chronically exposed to intrauterine caffeine could be more sensitive to occurrences of hypoxia.<sup>21</sup> Mothers were not enquired about caffeine ingestion during pregnancy; caffeine concentration in umbilical cord blood was measured, which is not an indicator of chronic use of caffeine.

Experimental studies showed that recently born rats whose mothers had received caffeine during pregnancy had altered respiratory pattern.<sup>22</sup> Under conditions of normoxia, animals treated with caffeine presented higher breathing frequency than a control group, and under conditions of hypoxia, the resulting respiratory depression was emphasized by intrauterine exposure to caffeine.<sup>22,23</sup> Such data are in agreement with the tachypnea found in neonates of women who reported an important consumption of caffeine during pregnancy.<sup>24</sup>

Preterm infants with birth weight of 500 to 1,250 g treated with caffeine during the first 10 days have a reduced rate of bronchopulmonary dysplasia, and present a better neurodevelopmental outcome at 18 to 21 months than a control group.<sup>25,26</sup> Preterm infants with birth weight < 1,000 g were excluded from the study, and association between presence of caffeine in umbilical cord blood and occurrence of bronchopulmonary dysplasia and/or neurodevelopmental

outcome was not investigated, but such studies should be encouraged.

In our study, detected levels of caffeine in umbilical cord blood did not decrease occurrence of apnea of prematurity, but it had a borderline effect delaying its occurrence, suggesting that even a low level of caffeine in umbilical cord blood might delay occurrence of apnea spells.

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