https://doi.org/10.31925/farmacia.2025.6.12 **ORIGINAL ARTICLE**

APNOEA OF PREMATURITY. STUDY OF THE ACTION OF METHYLXANTHINES IN THE TREATMENT AND PREVENTION OF APNOEA CRISES IN PREMATURE PATIENTS

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Abstract

Serum methylxanthine concentrations in premature infants show marked variability due to immature hepatic metabolism and drug interactions. This study aimed to evaluate newborns diagnosed with apnoea of prematurity through a comprehensive clinicobiological analysis and to highlight the role of therapeutic drug monitoring (TDM) in preventing complications related to overaccumulation and in optimising administration strategies. A prospective observational study was conducted on 331 premature neonates divided into a study group and a control group. In the study group, 75 infants were treated with caffeine citrate and 6 with theophylline ethylenediamine. Hydroelectrolytic and metabolic imbalances occurred in infants treated with caffeine citrate. Strong correlations were observed between electrolyte imbalances and convulsions (r = -0.44), respiratory complications (r = 0.48) and hyperglycaemia with oedema (r = -0.57). High serum caffeine citrate exposure (80 µg/mL) was associated with a significantly increased risk of respiratory complications, ecchymoses and oedema. Adjustment of therapeutic doses, guided by serial monitoring of serum caffeine citrate titres, may improve clinical outcomes in critical cases, reduce adverse drug effects, and support safer, individualised therapeutic management of apnoea of prematurity.

Rezumat

Concentrațiile serice de metilxantină la sugarii prematuri prezintă o variabilitate marcată din cauza metabolismului hepatic imatur și a interacțiunilor medicamentoase. Acest studiu a avut ca scop evaluarea nou-născuților diagnosticați cu apnee de prematuritate printr-o analiză clinicobiologică cuprinzătoare și evidențierea rolului monitorizării terapeutice medicamentoase (TDM) în prevenirea complicațiilor legate de supraacumulare și posibilitatea optimizării strategiilor de administrare. A fost efectuat un studiu observațional prospectiv pe 331 de nou-născuți prematuri, împărțiți într-un grup de studiu și un grup de control. În grupul de studiu, 75 de sugari au fost tratați cu citrat de cafeină și 6 cu teofilină etilendiamină. Dezechilibrele hidroelectrolitice și metabolice au apărut la sugarii tratați cu citrat de cafeină. Au fost observate corelații puternice între dezechilibrele electrolitice și convulsii (r = -0,44), complicații respiratorii (r = 0,48) și hiperglicemie cu edeme (r = -0,57). Expunerea serică crescută la citrat de cafeină (80 µg/mL) a fost asociată cu un risc semnificativ crescut de complicații respiratorii, echimoze și edeme. Ajustarea dozelor terapeutice, ghidată de monitorizarea serială a titrului seric de citrat de cafeină, poate îmbunătăți rezultatele clinice în cazurile critice, poate reduce efectele adverse ale medicamentelor și poate susține o gestionare terapeutică mai sigură și individualizată a apneei de prematuritate.

Keywords: methylxanthines, therapeutic drug monitoring, apnoea of prematurity, individualised therapy

Introduction

Apnoea of prematurity, defined as a cessation of breathing, is a diagnosis of exclusion. A typical episode involves an interruption of airflow for at least 20 seconds and is often accompanied by hypoxemia, cyanosis, and bradycardia. In very small-for-gestational-age infants, oxygen desaturation and bradycardia may appear even before 20 seconds of apnoea, increasing the risk of neurodevelopmental impairment [1, 2]. This condition frequently results from various neonatal and maternal factors, including hematologic disorders (such as ABO/Rh incompatibility or haemolytic anaemias), maternal-foetal infections, cervical incompetence, and maternal metabolic or endocrine diseases (diabetes, obesity, thyroid disorders). Other contributors include thrombophilia, maternal neurological, cardiovascular, or pulmonary disease, and prenatal exposure to medications, narcotics, or beta-blockers transferred transplacentally [3-5]. Apnoea of prematurity is classified into three types. The absence

of inspiratory effort characterises central apnoea. Obstructive apnoea involves persistent inspiratory attempts against a blocked airway. Mixed apnoea features airway obstruction before or after a central apnoea event. Approximately half of all cases combine central and obstructive mechanisms. These events stem from immature brainstem respiratory control, exaggerated responses to peripheral vagal stimulation, and airway collapse due to underdeveloped pharyngeal dilator muscles such as the genioglossus and geniohyoid. Negative inspiratory pressure can further narrow the airway, while mucosal adhesive forces hinder airway reopening during expiration [6, 7]. Methylxanthines are the first-line pharmacologic treatment for apnoea of prematurity [8]. Their serum concentrations can vary widely among infants, making therapeutic monitoring helpful.

These drugs (caffeine, theophylline, and aminophylline) are purine-based alkaloids that bind adenosine receptors and inhibit phosphodiesterase enzymes. By blocking adenosine receptors, these medicines prevent adenosine-induced respiratory depression and increase the excitability of respiratory neurons. Phosphodiesterase inhibition increases intracellular cyclic adenosine monophosphate (cAMP) levels, enhancing protein kinase A activity and promoting cellular excitation and neurotransmitter release [9, 10]. Over the last 50 years, evidence supporting the efficacy of methylxanthines in therapy has expanded substantially [11-17]. The Caffeine for Apnoea of Prematurity (CAP) trial, published in 2006, demonstrated caffeine's efficacy not only in reducing apnoea but also in lowering the incidence of bronchopulmonary dysplasia and improving long-term neurodevelopment. The CAP trial established the standard caffeine citrate dosing regimen: a 20 mg/kg loading dose (10 mg/kg caffeine base), followed by a daily maintenance dose of 5 mg/kg, administered orally or intravenously. Therapeutic plasma concentrations range from 8 - 20 µg/mL, but levels above 50 µg/mL are considered toxic. Levels exceeding 50 µg/mL can lead to adverse effects like tachycardia, irritability, seizures and metabolic disorders [18].

Despite its extensive clinical use, pharmacokinetic data for methylxanthines are limited, and individualised therapeutic dosing for premature infants is not well defined [19]. These gaps require monitoring of methylxanthine concentration levels.

Recent methods for physicochemical characterisation [20] and high-performance liquid chromatography quantitation [21] have already been reported in the literature. Measuring serum concentrations is mandatory in premature neonates to help distinguish complications of prematurity from adverse effects caused by elevated methylxanthine levels. Quantifying serum levels and carefully adjusting doses are useful to keep concentrations below 50 µg/mL.

This study aimed to evaluate interindividual variability in caffeine citrate serum levels in premature infants treated for apnoea of prematurity and to establish clinical and biological data to optimise individualised dosing.

Materials and Methods

Study design and setting

Participants

We conducted a prospective, single-centre observational clinical study in a neonatal intensive care unit (NICU) between March 2024 and September 2025 at the Neonatology Department of the Filantropia Municipal Clinical Hospital, Craiova. The study included 331 premature newborns aged 0 - 61 days without significant pathological conditions. Upon enrolment, a comprehensive analysis was conducted for each participant, including clinical and paraclinical assessments and functional explorations performed by a multidisciplinary team. These examinations were conducted in accordance with established clinical practice guidelines for the management of prematurity and ensured that all participants met the study criteria, providing a foundation for subsequent analyses [18]. Demographic and perinatal characteristics analysed included gestational age (weeks), sex, multiple gestation (twin pregnancy), Apgar scores at 1, 5, and 10 minutes, place of residence, and birth weight (g). Participants were divided into two groups: the study group (SG) included 81 neonates diagnosed with apnoea of prematurity, who underwent pulmonary ventilation, of whom 75 received caffeine citrate, and 6 received theophylline-ethylenediamine, and the control group (CG) comprised 250 preterm neonates who underwent pulmonary ventilation and did not require methylxanthine administration. Inclusion in the GS was based on clinical criteria, the development of apnoea of prematurity crises, which were recorded in 24.47% of all premature patients.

Ethical considerations

The study was approved by the institutional ethics committee of the University of Medicine and Pharmacy of Craiova (No. 101 / 01.03.2024) and of the Municipal Hospital "Filantropia" Craiova (No. 4794/28.02.2024). During the period 01.10.2023 - 28.02.2024, the documentation for issuing the ethics committee's report approving the research project was submitted, and patient inclusion in the study began on March 1, 2024.

Inclusion criteria

Participants were eligible for inclusion in the study if they met the following criteria: (a) the mother provided verbal and written informed consent; (b) prematurity was diagnosed according to standardised criteria; (c) the preterm infant was evaluated as free of significant associated anomalies by a specialist neonatologist.

Exclusion criteria

Participants were excluded from the study if they met any of the following conditions: (a) presence of associated anomalies, such as Trisomy-21 or twin-to-twin transfusion syndrome; (b) preterm infants diagnosed with respiratory or neurological conditions that could interfere with apnoea episodes; (c) inability of the mother to understand or adhere to the recommendations provided by the medical team.

General hypoxemia treatment

The postnatal adaptation of the preterm infants included in the study group was challenging, manifesting as cyanosis of the extremities and nail beds, hypoxemia, respiratory acidosis, and hypoglycaemia. Consequently, the patients were admitted to the intensive care unit for monitoring and treatment. Hypoxemia was corrected using the Neopuff device, noninvasive nCPAP (non-invasive Continuous Positive Airway Pressure), or orotracheal intubation, with sedation provided by midazolam during mechanical ventilation. A fraction of inspired oxygen (FiO_2) up to 80% was administered as needed, with gradual reductions in ventilator parameters. Preterm infants with respiratory distress syndrome (RDS) who required orotracheal intubation (IOT) received Curosurf at 200 mg/kg, while hypoglycaemia was corrected with a 10% glucose bolus followed by nutritional infusion. Subsequently, patients were cared for in closed incubators to ensure thermal comfort, provided with incubator oxygen therapy with FiO_2 progressively reduced from 35% to 25%, and administered partial parenteral nutrition.

Methylxanthine therapy

Methylxanthine exposure was performed according to routine neonatal intensive care unit practice using caffeine citrate, a loading dose of 20 mg/kg which is equivalent to 10 mg/kg caffeine base, followed by a maintenance dose of 5 mg/kg once daily, administered intravenously, and, in a minority of cases, a loading dose of aminophylline of 5 mg/kg by slow infusion over 20 - 30 minutes, followed by 3 mg/kg/dose administered intravenously every six hours for three days, then 3 mg/kg/dose every eight hours for two more days [22-24]. Caffeine citrate was administered for 5 to 18 consecutive days or intermittently, depending on the patients' clinical condition.

Co-interventions and respiratory support

Respiratory support in the delivery room and neonatal intensive care unit consisted of: pulmonary ventilation (VPP), Neo-Puff use number of inflations, nasal CPAP use and duration, oro-tracheal intubation and its duration, and days of blended oxygen (FiO_2). Concomitant exposures to antimicrobials (meropenem, gentamicin, ampicillin, tetracycline, vancomycin, amikacin, colistin, fluconazole, metronidazole, ciprofloxacin, linezolid, hydrocortisone hemisuccinate, furosemide, phenobarbital) and combinations of antibiotics administered were recorded to contextualise the severity of the disease.

Outcomes

Apnoea of prematurity was associated with: cardio-vascular complications; perinatal asphyxia; respiratory complications; intraventricular haemorrhage (IHV); renal complications; fluid/electrolyte imbalance defined according to institutional laboratory reference ranges for sodium, potassium, chloride, and ionized calcium; ecchymoses and oedema; allergic reactions; hematologic complications; vomiting, convulsive equivalents, jaundice,; minor congenital findings (ankyloglossia, hydrocele, malposition of toes, partial syndactyly, syndactyly agenesis, Mongoloid habitus, short frenulum, haemangioma). The short-term clinical course was summarised using labelled categorical outcomes: favourable, slow favourable, severe, and death.

Methods

The study methods consisted of examining and monitoring the newborns using a specially designed clinical observation sheet. At the same time, paraclinical investigations were performed in the hospital's Medical Analysis, Radiology, Medical Imaging, and Pathological Anatomy Laboratories, as well as at the UMF Craiova Research Centre.

Caffeine and theophylline measurements

Measurement of serum caffeine and theophylline levels was performed using a previously published in-house LC-MS method developed at the University of Medicine and Pharmacy of Craiova, Romania [25]. Briefly, plasma samples (100 µL) were prepared using a simplified solid-phase extraction (SPE) procedure and analysed by high-performance liquid chromatography coupled with mass spectrometry (LC-MS). Chromatographic separation was achieved on a CORTECS C18 column with a water/ acetonitrile gradient, and detection was performed in positive electrospray ionisation mode. The method provided sensitive and accurate quantification, with a lower limit of quantification (LLOQ) of 1 µg/mL for both analytes, and included validation for accuracy, precision, and analyte stability [25]. Serum theophylline concentration was measured in six premature babies 24 hours after the loading therapeutic dose, 24 hours after the last maintenance dose, and 3 days after stopping treatment. Serum caffeine citrate concentrations were monitored dynamically in 75 premature newborns at 24 hours after the previous maintenance dose and at 14 and 30 days after treatment was stopped. Patient data include both serial measurements of methylxanthines and a binary indicator recorded as a high caffeine level (> 50 µg/mL).

Physiologic, blood gas and laboratory measurements

Physiological data and blood gas were recorded in the electronic health record (EHR) flow sheets closest to the prespecified time windows in NICU practice: oxygen saturation (SO_2), oxyhaemoglobin fraction, carboxyhaemoglobin, methaemoglobin, haemoglobin derivatives, arterial pH, pCO_2, pO_2, and bicarbonate (HCO_3). Routine laboratory variables

included glucose, lactic acid, total protein, and total/direct bilirubin. Complete blood count indices were represented by haemoglobin, haematocrit, red and white blood cell counts, platelets, erythroblasts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean concentration of corpuscular haemoglobin (MCHC) and leukocyte differentials (neutrophils, lymphocytes, eosinophils, basophils, monocytes). Electrolytes such as sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}) were recorded as both continuous values and derived categorical flags (*e.g.*, hypo-/hypernatremia, kalemia, chloremia, calcemia), according to laboratory reference ranges in effect during the study.

We monitored premature newborns to identify the onset or exacerbation of apnoea and to initiate specific treatment, given that apnoea of prematurity is a diagnosis of exclusion.

We performed a complete blood count, inflammatory markers, biochemical investigations, and microbiological cultures from biological samples collected to assess the risk of maternal-foetal infection. We ensured continuous monitoring of oxygen saturation and arterial blood gases, and chest X-rays were recommended in cases of inadequate oxygenation. We measured electrolytes, blood glucose, and serum methylxanthine levels to evaluate metabolic dysfunctions. We monitored body temperature to prevent thermal instability. We recommended a neurological examination and a cranial ultrasound to assess intracranial pathology [26].

Venous blood samples for haematological determinations were collected in vacutainers containing powdered potassium or sodium ethylenediaminetetraacetic acid (EDTA) as an additive. Haematological analyses were performed using the Mindray BC 6800 Plus automated analyser. Morphological examination of blood smears was conducted with the Nikon Eclipse Ei microscope, initially using a 40x dry objective and subsequently a 100x oil-immersion objective, enabling detailed study of the characteristics of various blood cells.

For inflammatory markers such as C-reactive protein (CRP), IL-6, PCT, and TNF-α, and for biochemical determinations, venous blood was collected in red-capped vacutainers without additives or in yellow-capped vacutainers containing a gel separator and clot activator. Serum obtained by centrifugation, immediately after complete clot formation, was frozen at -20°C. Serum analyte concentrations were measured with the MaglumiX3 automated immunology analyser, which employs chemiluminescent immuno-assay (CLIA), and the Mindray BS-1000M biochemistry analyser. Analytical methods for biochemical parameters included kinetic measurements based on changes in optical density (OD), bi-chromatic endpoint photometric measurements, and bi-chromatic two-point photometric measurements.

Statistical analysis

Definitions and coding. Complication indicators were coded 0/1. Exposure was coded 0 = normal caffeine, 1 = high caffeine. Where multiple measurements existed (*e.g.*, gases, electrolytes), values were harmonised according to the NICU data-entry convention (first measurement after admission or clinically most relevant value as documented). The data were recorded in the institutional EHR and laboratory information systems and compiled into a structured dataset. Before analysis, the data were inspected for completeness, range of values, and internal consistency. Continuous variables were screened for nonphysiologic outliers. Missing data were addressed through systematic identification and appropriate imputation strategies.

Data were analysed using Stata (version 17, StataCorp, College Station, TX, USA). Continuous variables were expressed as mean ± standard deviation, or median and interquartile range (IQR) and compared between groups using the Student's t-test. Categorical variables were reported as absolute numbers and percentages and compared using the χ^2 test or Fisher's exact test when expected counts were low. All primary analyses were focused on caffeine effects.

Statistical analyses

To explore associations between caffeine/xanthine exposure levels and clinical outcomes, we performed a multivariate analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for each neonatal complication, comparing infants with normal caffeine levels to those with high levels (> 50 µg/mL), and pre-term infants without apnoea of prematurity to those with apnoea of prematurity.

Logistic regression models were fitted to estimate adjusted odds ratios with 95% CI, using high caffeine exposure as the primary independent variable. Models were adjusted for potential confounders, including gestational age, birth weight, gender, twin status, and Apgar score at 1 minute. For outcomes with small event counts or quasi-complete separation, Firth's penalised likelihood estimation was attempted. If high caffeine perfectly predicted the outcome, the variable was omitted from the model, and the ORs were reported as 1.000 or absent, with no p-value.

Bivariate correlations (Spearman's r) were calculated between clinical and paraclinical complications to assess co-occurrence patterns. All tests were two-tailed, and p-values < 0.05 were considered statistically significant.

Results and Discussion

Infant/Preterm care/Management/Treatment

Preterm infants treated with caffeine citrate exhibited considerable variability in plasma caffeine levels at 24 hours after the last therapeutic dose,

ranging from values similar to those following the loading dose to concentrations exceeding 80 µg/mL. Serum levels measured 14 days after the previous maintenance dose indicated slow caffeine metabolism and prolonged therapeutic effect after discontinuation, suggesting that gradual dose reduction is unnecessary at treatment cessation. Given the risk of apnoea recurrence after discontinuation of caffeine citrate treatment, dynamic monitoring of serum caffeine titres could help prevent this complication. However, low serum caffeine concentrations have been detected in some infants even 1 month after cessation of therapy (Figure 1).

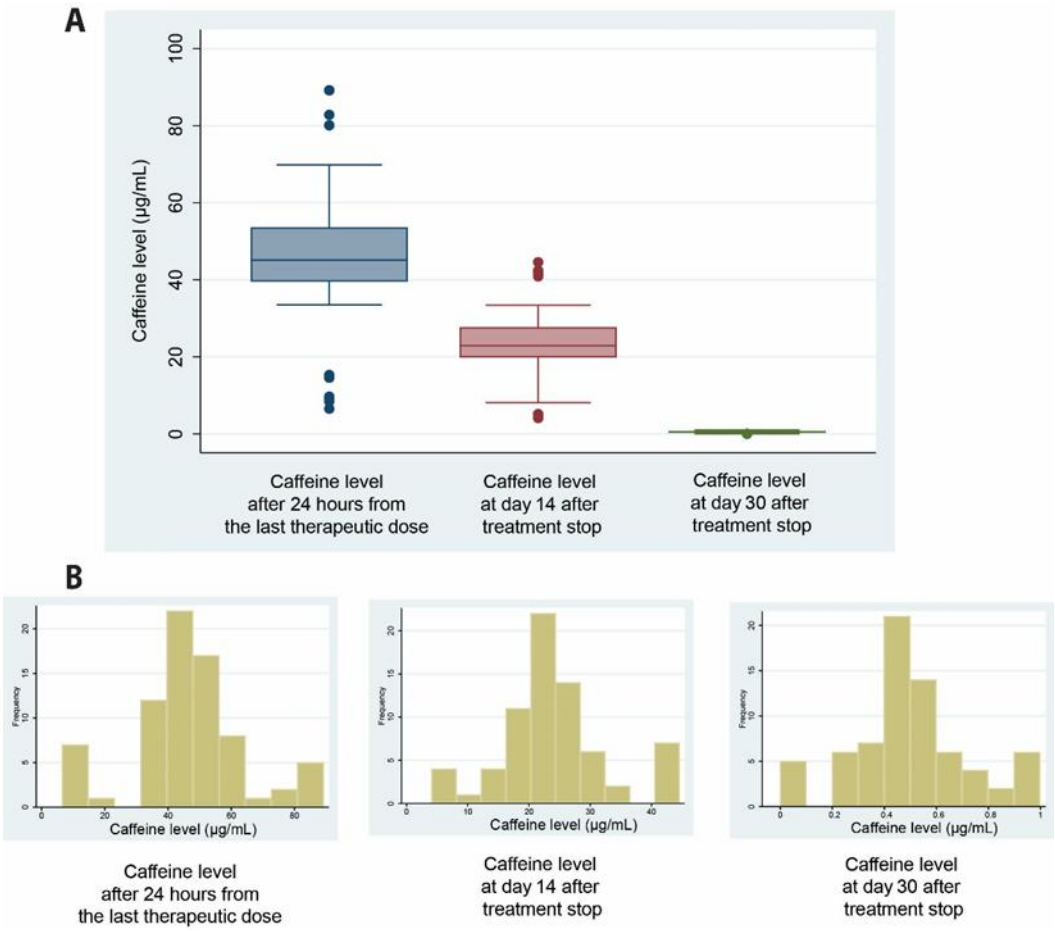

Figure 1.
Serum caffeine concentration 24 hours after the last therapeutic dose, 14 days after stopping treatment and 30 days after stopping treatment. A: boxplot representation; B: histogram representation

Of the 75 premature infants treated with caffeine citrate, four (5.33%) developed intraventricular haemorrhage, evidenced by transfontanellar ultrasound examination, due to the immaturity of the blood-brain barrier, and three (4%) developed convulsive equivalents in the first hours of life. However, they were administered a loading dose of caffeine citrate, with shock and undetectable tension, and multiorgan failure and irreversible cardiorespiratory arrest were recorded during resuscitation manoeuvres.

The effects of supratherapeutic caffeine exposure are not apparent in the literature. While higher doses may further reduce bronchopulmonary dysplasia, observational studies and some trials suggest that excessive dosing could be associated with poor tolerability and potential harm, especially in the most immature infants, and there is a need for caution due to the risk of side effects and possible complications such as intracranial bleeding [22, 27, 28]. Bronchopulmonary dysplasia (BPD), or chronic lung disease of prematurity - as defined by the 2001 National Institutes of Health conference - affects premature infants with extremely low birth weight due to inflammation and lung injury induced by oxygen administration and barotrauma caused by mechanical ventilation, which in turn arrests lung development and reduces the surface area for gas exchange [29-31]. BPD therapy includes bronchodilators, glucocorticoids, and diuretics, though no strategies are supported by robust evidence [32]. The optimal dosing and timing of caffeine therapy remain unresolved, and more research is needed to determine the safety and efficacy of high-dose regimens, particularly regarding

long-term neurodevelopmental outcomes and cardio-respiratory stability [33, 34].

Six premature infants diagnosed with mixed apnoea received a loading dose of aminophylline of 5 mg/kg by slow infusion over 20 - 30 minutes, followed by 3 mg/kg/dose (equivalent to 2.0 - 3.3 mL of diluted theophylline ethylenediamine, 1 mL of theophylline ethylenediamine 24 mg/mL with 9 mL of normal saline) administered intravenously every six hours for three days, then 3 mg/kg/dose every eight hours for two more days (Table I, Figure 2).

Table I

Theophylline levels in neonates with apnoea of prematurity treated with myofillin Miofilin®/aminophylline (n = 6)

Parameter	Theophylline level (µg/mL)
Theophylline level after 24 hours from the loading dose	3.64 (3.59 - 3.97)
Theophylline level after 24 hours from the last maintenance dose	8.79 (7.26 - 10.32)
Theophylline level on day 3 after treatment stop	1.54 (1.48 - 1.61)

Figure 2.
Serum theophylline concentration 24 hours after the loading therapeutic dose, 24 hours from the last maintenance dose and 3 days after stopping treatment

Theophylline ethylenediamine has been used in the treatment of mixed apnoea of prematurity and obstructive bronchopulmonary disease with a spasmodic component.

Plasma levels measured three days after the last dose of theophylline ethylenediamine demonstrated much faster theophylline metabolism compared to caffeine and a shorter persistence of therapeutic effect after drug withdrawal, warranting gradual tapering at the end of treatment.

In patients with hepatic or cardiac insufficiency, hypoxemia, persistent hyperthermia, viral infections, pneumonia, or thyroid dysfunction, particularly hyperthyroidism, and in those receiving certain medication, the theophylline dose should be reduced. In premature infants, the prolonged half-life of theophylline predisposes to serum concentrations above the critical threshold. The effect of theophylline-ethylenediamine may be potentiated by concomitant administration of anti-inflammatory agents, fluoroquinolones, furosemide, imipenem, calcium channel blockers, lincomycin, macrolides, mexiletine, paracetamol, pentoxifylline, ranitidine, cimetidine, allopurinol, or influenza vaccination. Ephedrine and other sympathomimetics combined with aminophylline enhance cardiac adverse reactions and central excitatory effects. Literature data further indicate that when theophylline ethylenediamine is co-administered with ciprofloxacin, the theophylline-ethylenediamine dose should be reduced by at least half, and the drug should not be administered together with milk or xanthine-containing beverages such as tea or coffee [35, 36]. Such combinations should therefore be avoided. In pregnant or breastfeeding women, theophylline-ethylenediamine should be prescribed with caution, only when maternal therapeutic benefits outweigh potential foetal risks, as theophylline crosses the placenta, is excreted in breast milk, and can induce hyperexcitability in the infant. The efficacy of theophylline-ethylenediamine may be diminished if administered concomitantly with antiepileptics or barbiturates, particularly phenobarbital. In preterm infants receiving theophylline ethylenediamine alongside one or more of these medications, serum theophylline levels must be closely monitored, and doses adjusted accordingly. Theophylline-ethylenediamine may potentiate the effects of β-agonists and diuretics, while attenuating the actions of adenosine and lithium carbonate. In patients treated with theophylline ethylenediamine, halothane

anaesthesia may induce severe cardiac arrhythmias, while ketamine increases seizure risk.

Possible adverse reactions and symptoms of theophylline ethylenediamine overdose include agitation, repeated vomiting, hyperthermia, tachycardia, ventricular fibrillation, arterial hypotension, hyperventilation followed by respiratory depression, and neurological disturbances. Seizure onset is a clear sign of theophylline intoxication, though seizures may occur earlier in some cases. Rarely, gastro-intestinal ulcerations with bleeding, obstructive syndromes, or allergic reactions may develop within 48 hours, typically presenting as cutaneous eruptions [37, 38].

Methylxanthine administration may also increase urinary catecholamine concentrations and influence serum potassium levels, which therefore require dynamic monitoring.

Differences between groups in demographic data and laboratory parameters

In an exploratory analysis, significant differences were observed between preterm infants with and without apnoea of prematurity across demographic, clinical, and paraclinical parameters (Table II). These encompassed measures of gestational maturity, respiratory physiology, gas exchange, metabolic balance, and inflammatory markers, indicating systemic immaturity and physiologic vulnerability in the apnoea of prematurity group. Reduced gestational age and birth weight were among the most pronounced differences, underscoring the role of developmental maturity in the risk of apnoea of prematurity.

Preterm infants with apnoea of prematurity exhibited significantly lower median gestational age and birth weight compared to those without apnoea (33 weeks *vs*. 36 weeks; $p < 0.001$; and 2.0 kg *vs*. 2.4 kg; $p < 0.001$). Early postnatal adaptation was also poorer, as reflected by reduced Apgar scores at 1 min: 8 (7 - 8) *vs*. 8 (8 - 8; $p < 0.001$), and at 5 min: 8 (8 - 9) *vs*. 9 (8 - 9); $p < 0.001$). These findings indicate that neonates affected by apnoea were both developmentally and physiologically less mature at birth.

Male predominance was observed in the apnoea group compared to controls (62.67% *vs*. 45.38%; $p = 0.009$). Infants with apnoea were also more frequently from urban areas (61.33% *vs*. 28.99%; $p < 0.001$). Twin pregnancies were more frequent in the apnoea group (44.00% *vs*. 26.05%, $p = 0.003$).

Oxygen saturation and oxyhaemoglobin fraction were lower in the apnoea group compared to controls (88.4% *vs*. 94.9%; 87.8% *vs*. 93.9%; both $p < 0.001$). Partial pressure of carbon dioxide was elevated (39.5 *vs*. 36.6 mmHg; $p = 0.037$), while partial pressure of oxygen was reduced (45.9 *vs*. 54.7 mmHg; $p < 0.001$). The apnoea group also exhibited lower arterial pH (7.35 *vs*. 7.39; $p = 0.065$) and higher levels of carboxy-haemoglobin and methaemoglobin (both $p < 0.001$). Lower total protein levels in the apnoea group (4.4 *vs*. 5.0 g/dL; $p < 0.001$) reflect potential nutritional or liver-maturation differences. Slightly lower total bilirubin (12.8 *vs*. 14.2 mg/dL; $p = 0.030$) could reflect metabolic or feeding differences, although both averages remain clinically unremarkable.

C-reactive protein was paradoxically slightly lower in apnoea infants, but TNF-α was elevated (8.08 *vs*. 5.59 pg/mL; $p = 0.023$), suggesting a nuanced inflammatory response that may reflect stress-mediated cytokine release.

Preterm infants with apnoea displayed notable hematologic differences compared to controls, particularly in components of the complete blood count. The apnoea group had lower white blood cell and neutrophil counts, but higher lymphocyte, eosinophil, and monocyte counts, suggesting immune system immaturity or redistribution. The elevated erythroblasts may indicate heightened erythropoietic activity.

Table II

Paraclinical parameters in preterm infants with and without apnoea of prematurity

Parameter	Control (n = 250)	Pre - term apnoea (n = 81)	p - value
Apgar score at 1 min	8 (8 - 8)	8 (7 - 8)	< 0.001
Apgar score at 5 min	9 (8 - 9)	8 (8 - 9)	< 0.001
Apgar score at 10 min	N/A	8 (8 - 8)	N/A
Gestational age (weeks)	36 (36 - 36)	33 (32 - 35)	< 0.001
Twin pregnancy	26.05%	44.00%	0.003
Birth weight (g)	2.4 (2.2 - 2.7)	2.0 (1.78 - 2.4)	< 0.001
Male gender (%)	45.38%	62.67%	0.009
Urban residence (%)	28.99%	61.33%	< 0.001
Caffeine level day 14 (µg/mL)	N/A	22.89 (19.78 - 27.71)	N/A
Caffeine level day 30 (µg/mL)	N/A	0.49 (0.38 - 0.64)	N/A
Caffeine level day 1 from the last therapeutic dose (µg/mL)	N/A	45.14 (39.52 - 53.62)	N/A
Oxygen saturation (SO_2, %)	94.9 (86.1 - 96.5)	88.4 (81.7 - 92.0)	< 0.001
pCO_2 (mmHg)	36.6 (32.5 - 41.5)	39.5 (34.9 - 46.0)	0.037
pH (arterial)	7.39 (7.28 - 7.42)	7.35 (7.30 - 7.40)	0.065
pO_2 (mmHg)	54.7 (48.4 - 67.4)	45.9 (41.3 - 48.6)	< 0.001
Carboxyhaemoglobin (%)	0.6 (0.4 - 1.1)	0.9 (0.8 - 1.3)	< 0.001

Parameter	Control (n = 250)	Pre - term apnoea (n = 81)	p - value
Methaemoglobin (%)	0.4 (0.3 - 0.6)	0.6 (0.4 - 0.8)	< 0.001
Oxyhaemoglobin fraction (%)	93.9 (84.9 - 96.2)	87.8 (82.4 - 91.7)	< 0.001
Haemoglobin H (%)	5.0 (3.1 - 13.7)	9.9 (7.0 - 15.9)	< 0.001
HCO_3 (mmol/L)	22.5 (28.4 - 23.1)	22.3 (19.5 - 24.0)	0.286
Total bilirubin (mg/dL)	14.2 (10.8 - 16.6)	12.8 (9.6 - 15.6)	0.030
Direct bilirubin (mg/dL)	0.5 (0.4 - 0.5)	0.4 (0.3 - 0.5)	0.060
Glucose (mg/dL)	69 (50 - 78)	60 (48 - 74)	0.206
Lactic acid (mmol/L)	2.20 (1.58 - 4.14)	2.25 (1.83 - 3.27)	0.689
Total protein (g/dL)	5.0 (4.4 - 5.5)	4.4 (4.2 - 4.8)	< 0.001
CRP (mg/dL)	0.29 (0.14 - 0.70)	0.29 (0.12 - 0.46)	0.146
IL-6 (pg/mL)	7.25 (6.38 - 14.90)	8.17 (8.20 - 10.40)	0.898
TNF-α (pg/mL)	5.59 (4.32 - 8.43)	8.08 (5.67 - 13.3)	0.023
PCT (ng/mL)	3.45 (0.36 - 15.4)	0.40 (0.34 - 5.90)	0.176
Haemoglobin (g/dL)	15.2 (13.3 - 16.8)	16.0 (11.4 - 18.3)	0.967
Haematocrit (%)	42.7 (36.4 - 48.1)	45.7 (32.1 - 52.5)	0.669
Red blood cells ($10^{\wedge}6$/µL)	4.10 ± 0.69	4.08 ± 1.10	0.913
MCV (fL)	105.6 (100.8 - 107.2)	104.5 (99.4 - 106.2)	0.164
MCH (pg)	37.7 (36.2 - 38.4)	36.5 (35.1 - 37.7)	< 0.001
MCHC (g/dL)	35.9 (35.6 - 36.2)	35.7 (34.9 - 36)	0.004
White blood cells ($10^{\wedge}3$/µL)	16.25 (12.45 - 18.33)	12.53 (10.02 - 17.15)	< 0.001
Neutrophils (%)	64.9 (59.4 - 71.2)	58.1 (43.0 - 70.3)	0.001
Eosinophils (%)	0.9 (0.4 - 2.1)	0.1 (0.0 - 2.2)	0.002
Basophils (%)	0.1 (0.1 - 0.2)	0.0 (0.0 - 0.2)	0.001
Lymphocytes (%)	24.4 (20.4 - 29.1)	26.7 (18.7 - 47.6)	0.015
Monocytes (%)	7.6 (6.2 - 8.5)	8.1 (6.5 - 11.5)	0.002
Platelets ($10^{\wedge}3$/µL)	277 (208 - 352)	258 (183 - 337)	0.912
Erythroblasts ($10^{\wedge}3$/µL)	2 (2 - 3)	2 (1 - 5)	0.770

Abbreviations: TNF - Tumour Necrosis Factor; IL - Interleukin; PCT - Procalcitonin; CRP - C-Reactive Protein; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

Comparison between neonates with normal versus high caffeine levels

To further explore the potential impact of pharmacologic exposure, outcomes were compared between neonates with normal and elevated caffeine levels (> 50 µg/mL). Multivariate analyses were performed to assess the relationship between drug levels and neonatal complications, while accounting for baseline clinical differences. This approach aimed to distinguish crude associations from independent effects of high caffeine exposure on respiratory, hematologic, and systemic outcomes. After adjusting for gestational age, birth weight, gender, twin status, and Apgar score at 1 minute, stronger associations emerged (Table III).

High caffeine exposure was independently associated with a significantly increased risk of respiratory complications - OR: 10.60, 95% CI: [2.01 - 23.31] with p = 0.011, ecchymoses - OR: 7.83, 95% CI: [1.36 - 45.17] with p = 0.021 and oedema OR: 9.07, 95% CI: [1.87 - 19.40] with p = 0.020. No significant associations were observed for intraventricular haemorrhage, favourable or less favourable evolution, or death.

For several outcomes (perinatal asphyxia, hematologic complications, vomiting, severe evolution), a high caffeine level perfectly predicted the outcome, and the variable was dropped from the model. In these cases, ORs are reported as absent without p-values.

The multivariate analysis revealed that elevated caffeine levels were independently associated with specific complications, particularly respiratory morbidity, ecchymoses, and oedema, while no significant associations were observed for most other outcomes. These findings highlight the complex and selective impact of high caffeine exposure, warranting further interpretation in the context of existing literature.

Electrolytic and hydric imbalances

Next, we examined differences in key electrolytes: sodium (Na), potassium (K), chloride (Cl), and calcium (Ca) between pre-term infants with apnoea and controls. Notably, only calcium levels differed significantly, while sodium, potassium, and chloride did not. The pre-term apnoea group displayed significantly lower mean calcium levels (1.18 mmol/L) compared to controls (1.23 mmol/L) (Table IV).

Other electrolytes exhibited no significant differences. Sodium levels were similar (136.16 *vs*. 136.55 mmol/L; p = 0.627), both within the normal neonatal range of ~ 135 - 145 mmol/L, including pre-term values [37]. Regarding potassium, both groups had averages within or slightly above typical neonatal values (~ 3.5 - 6.5 mmol/L); not statistically significant (p = 0.543). For chloride, values (104.9 *vs*. 105.1 mmol/L; p = 0.766) also fall within established neonatal reference limits (95 - 110 mmol/L) [39, 40].

Table III

Association between high caffeine exposure and neonatal complications: odds ratios with 95% confidence intervals (adjusted for gestational age, birthweight, gender, Apgar score at 1 minute and twin status). OR = Odds Ratio (exponentiated coefficients)

Outcome	OR	95% CI	p-value
Perinatal asphyxia	1.000	[1.000, 1.000]	-
Respiratory distress	10.598*	[2.008, 23.311]	0.011
Intraventricular haemorrhage	3.446	[0.624, 19.040]	0.156
Ecchymoses	7.827*	[1.356, 45.173]	0.021
Oedema	9.072*	[1.868, 19.403]	0.020
Allergies	0.337	[0.065, 1.743]	0.195
Haematological	1.000	[1.000, 1.000]	-
Vomiting	1.000	[1.000, 1.000]	-
Convulsions	1.000	[1.000, 1.000]	-
Ankyloglossia	7.990	[0.791, 152.760]	0.074
Hydrocele	0.446	[0.038, 5.251]	0.521
Congenital dermal melanocytosis	0.499	[0.060, 4.146]	0.520
Short frenulum	1.000	[1.000, 1.000]	-
Haemangioma	0.544	[0.141, 2.099]	0.377
Favourable evolution	0.602	[0.088, 4.125]	0.605
Lent favourable evolution	3.089	[0.408, 23.384]	0.275
Severe evolution¥	1.000	[1.000, 1.000]	-

$p < 0.05$ (statistically significant). Outcomes with OR = 1.000 and CI [1.000,1.000] indicate no events in either group. Abbreviations: OR - Odds Ratio; CI - Confidence Interval; ¥High caffeine perfectly predicted the outcome; variable omitted from model; "-" = not estimable (predicts perfectly/omitted)

Table IV

Electrolyte values (Na, K, Cl, Ca) and imbalances in preterm infants with and without apnoea of prematurity

	Patient group		
	Control group	**Pre-term apnoea**	**p value**
Electrolyte serum values			
Na (mmol/L)	136.54 ± 3.17	136.16 ± 5.96	0.627
K (mmol/L)	4.44 ± 0.56	4.37 ± 0.71	0.543
Cl (mmol/L)	105.10 ± 2.71	104.92 ± 2.99	0.766
Ca (mmol/L)	1.23 ± 0.08	1.18 ± 0.11	0.0185
Electrolyte imbalance			
Hypo Na	24.52%	52.00%	0.005
Hyper Na	4.52%	4.00%	0.907
Hypo K	7.19%	14.81%	0.181
Hyper K	30.54%	25.93%	0.627
Hypo Cl	0.00%	0.00%	-
Hyper Cl	53.89%	38.46%	0.143
Hypo Ca	20.26%	52.00%	0.181
Hyper Ca	7.84%	8.00%	0.978

Student's t-test was used to test the difference in means between the control group and the pre-term apnoea group. Chi-squared test was used to assess the difference in the prevalence of electrolyte imbalances between the two groups.

Table V summarises the strength and direction of bivariate correlations between paraclinical and clinical complications. For instance, infants with anaemia were less likely to present vomiting (r = -0.57), and neonates with anaemia are slightly more likely to have convulsive episodes. Anaemia was negatively correlated with haematemesis (probably an artefact, as only 3 infants presented haematemesis). Strong correlations were observed between electrolyte imbalances and convulsions (r = -0.44) or respiratory complications (r = 0.48).

Hypoglycaemia is modestly positively associated with convulsions (r = 0.36) and allergy (r = 0.39), but inversely associated with vomiting (r = -0.30). Hyperglycaemia shows negative correlations with oedema (r = -0.57), convulsions (r = -0.36) and ecchymosis (r = -0.41). Hidroelectrolytic imbalance has moderate positive correlations with allergy (r = 0.36), vomiting (r = 1.00), and haematemesis (r = 1.00), indicating strong co-occurrence.

Table V

Correlation between clinical and paraclinical complications in children treated with caffeine citrate. Cells contain the Spearman correlation coefficient

		Clinical complications						
Paraclinical complications		***Convulsion equivalents***	***Allergy***	***Vomiting***	***Respiratory***	***Haematemesis***	***Oedema***	***Ecchymosis***
	Hidroelectrolitic imbalance	-0.44	0.36	1.00	0.48	1.00	0.14	-0.06
	Hypoglycaemia	0.36	0.39	-0.30	0.29	1.00	0.24	0.16
	Hyperglycaemia	-0.36	0.19	1.00	-0.09	0.23	-0.57	-0.41
	Anaemia	0.20	-0.03	-0.57	0.10	-1.00	-0.09	-0.21

The effect of methylxanthines can be either potentiated or inhibited when administered concomitantly with other drugs (Table VI). Variations in serum concentration can lead to adverse reactions such as fever, tachypnoea, fasciculations, vomiting, hyperglycaemia, hypokalaemia, uraemia, allergic reactions, leukocytosis, anaemia, necrotising enterocolitis, and seizures [41-43].

Across most antibiotics, the proportion of infants receiving treatment is numerically higher in the high-caffeine group. However, for nearly all agents, these differences are not statistically significant, as reflected by p-values > 0.05. This suggests that elevated serum caffeine levels are not clearly associated with increased exposure to most antibiotics. However, Metronidazole use is higher among infants with high caffeine levels. This may imply that infants with elevated caffeine levels were more likely to have gastrointestinal pathology, such as suspected or confirmed necrotising enterocolitis, since metronidazole is commonly used in necrotising enterocolitis. This aligns with known caffeine-related risks at high serum concentrations, including gastrointestinal complications.

Table VI

Comparison of percentages of pre-term infants treated with antibiotics between infants with high and normal serum levels of caffeine

Antibiotic	**Normal caffeine levels (n = 50)**	**High caffeine levels (n = 25)**	**OR**	**p value**
Gentamicin	54.00%	76.00%	2.7	0.065
Meropenem	46.00%	64.00%	2.09	0.141
Ampicillin	68.00%	64.00%	0.84	0.729
Imipenem/Cilastatin	6.00%	16.00%	2.98	0.160
Vancomycin	2.00%	4.00%	2.04	0.612
Amikacin	14.00%	8.00%	0.53	0.451
Colistin	4.00%	2.00%	0.51	0.221
Metronidazole	2.00%	20.00%	12.25	< 0.001
Linezolid	2.00%	4.00%	2.04	0.612
Ciprofloxacin	2.00%	4.00%	2.04	0.612

The frequency of complications is much higher in the study group than in the control group. These results align with prior evidence on the vulnerability of preterm populations and provide additional insights into the systemic mechanisms underlying apnoea of prematurity [44-47]. Iranpour *et al*. [44] state that preventative caffeine can reduce the duration of nasal CPAP support in neonates with RDS. Also, caffeine significantly reduces CPAP and mechanical ventilator use [45, 46]. Authors did not find a significant correlation between apnea incidence, fatality and suggested adverse effects while taking caffeine, therefore it is more effective and safer than aminophylline. In a study by Habibi *et al*., no significant difference was observed between the caffeine and aminophylline groups regarding complete recovery (after cyanosis, bradycardia, and cessation of breathing) [47]. All findings highlight the multifactorial nature of apnoea, reflecting contributions from developmental immaturity, altered gas exchange, and immune dysregulation.

Limitations

This study is not without its limitations. Potential interferences and cross-reactions may have arisen from icteric, lipemic, or mildly haemolysed biological specimens, from concomitant medications administered during neonatal therapy, or from maternal consumption of xanthine-containing foods and beverages (such as tea and coffee) during pregnancy or breastfeeding. Furthermore, the study design was observational, precluding inferences about directionality or causality, and the reported associations should be interpreted as correlative.

Future directions

Beyond individual interventions, integrating predictive tools that combine gestational age, perinatal characteristics, and laboratory markers into clinical decision-making may improve risk stratification and enable more precise therapy tailoring. Such approaches may help to efficiently allocate resources in neonatal intensive care units, while improving the safety and quality of care for vulnerable preterm infants.

More broadly, more robust studies could support the inclusion of therapeutic drug monitoring and individualised methylxanthine dosing strategies in national and international neonatal care guidelines, ensuring that the principles of precision medicine are integrated into clinical practice.

Conclusions

The results of this study document significant interindividual variability in methylxanthine titers and clinic-biological correlates in patients treated with standard doses for apnoea of prematurity and support the possibility of personalising the therapeutic dose, either by intermittent administration of standard doses or by adjusting the therapeutic dose based on the serum titres of these drugs. Serial determinations of serum methylxanthine concentrations may ensure optimal levels and prevent the harmful effects of overaccumulation in premature patients. Therefore, monitoring plasma concentrations of methylxanthines is necessary to ensure that the therapeutic effects are exclusively in the patient's best interest.

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Conflict of interest

The authors declare no conflict of interest.

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