Calcemia and Inflammatory Markers in Early-Onset Neonatal Infection

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ABSTRACT

Introduction: Ionised hypocalcemia (S-Ca^{2+}) has been repeatedly observed in neonates with sepsis. Our aim was to evaluate total calcemia (S-Ca) and its relationship to laboratory markers of infection.

Methods: We retrospectively evaluated total calcemia (S-Ca) and its relationship to laboratory markers of sepsis/infection (serum levels of C-reactive protein – S-CRP and procalcitonin – S-PCT) in 29 full-term neonates with early-onset neonatal infection hospitalized at our neonatology ward between 2012 and 2016. The control group consisted of 705 neonates without infection.

Results: In neonates with early-onset infection , the S-Ca on day 1, 2 and 3 was significantly lower ($p < 0.0001$; $p < 0.0001$; $p = 0.05$ versus controls) same as the pooled S-Ca ($p < 0.0001$ versus controls). There was a weak negative correlation between pooled S-Ca and S-PCT, or pooled S-Ca and S-CRP ($r = -0.22$, $p = 0.06$; $r = -0.19$, $p = 0.09$).

Conclusion: S-Ca was decreased in neonates with early-onset infection and did show a slight tendency to inverse correlation with S-CRP and S-PCT. Pediatricians must be aware of the fact that a drop in total S-Ca should alert their attention to the risk of neonatal infection, and, likewise, that the children with neonatal infection are at a higher risk of hypocalcemia with all its consequences.

KEYWORDS

calcium; early-onset neonatal infection; neonate

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INTRODUCTION

Neonatal hypocalcemia can occur in premature or hypotrophic children, other risk factors include infection, maternal diabetes, perinatal asphyxia, low calcium intake, maternal hyperparathyroidism, phosphate overload, transient or primary hypoparathyroidism, hypomagnesemia, hepatopathy or end organ resistance to the biological actions of parathyroid hormone (PTH) (1–7). Hypocalcemia, in particular low level of serum ionized calcium (S-Ca^{2+}), has been repeatedly reported in neonates with sepsis (1–7).

Newborns with hypocalcemia are either asymptomatic, or may present with hypotonia, apnea, poor feeding, jitteriness, seizures, cardiac failure. Signs of hypocalcemia rarely occur unless S-Ca drops below 1.75 mmol/l (7).

The pathogenesis of hypocalcemia in sepsis is explained by the increased secretion of procalcitonin, the precursor of calcitonin, with consequent hypocalcemic effects (8, 9).

The upregulation of calcium-sensing receptor (CaSR) by cytokines, in particular tumor-necrosis factor alpha (TNF-alpha), interleukin-1 beta (IL-1beta) and IL-6 may also play a significant role in the pathogenesis of hypocalcemia in sepsis. The upregulation of CaSR results in decreased serum PTH and 1,25-dihydroxyvitamin D and calcium levels (10–11).

Administration of aminoglycosides, in particular gentamicin, to neonates, is also associated with a drop in calcemia (12–13). This could be explained by the fact that aminoglycoside antibiotics are CaSR agonists (14–15).

Ionised hypocalcemia is considered a negative prognostic factor in the neonatal sepsis, together with calcitoninemia and serum levels of PTH (5, 6, 8). Furthermore, ionised hypocalcemia is associated with organ dysfunction in children admitted to intensive care unit (4).

Our aim was to retrospectively evaluate total calcemia (S-Ca) and its relationship to laboratory markers of sepsis/infection (serum levels of C-reactive protein – S-CRP and procalcitonin – S-PCT) in neonates.

PATIENTS, MATERIALS, METHODS

Between the years 2012 and 2016, 3441 neonates were hospitalized at our neonatology ward. Total calcemia was assessed in 988 samples drawn from 828 babies. Out of those, neonatal infection was diagnosed in 29 full-term children (Table 1), based on Töllner scores (abnormal skin color, prolonged capillary refill time, muscular hypotonia, bradycardia, apnea, respiratory distress, hepatomegaly, gastrointestinal symptoms, number of leukocytes, increased number of immature neutrophils, thrombocytopenia, metabolic acidosis) >5 points (16) and S-CRP or S-PCT elevation. All 29 children were treated by intravenously administered gentamicin and ampicillin for seven days, with a favorable outcome. The control assessments of inflammatory markers and S-Ca were based on the clinical situation of the patients The blood samples were collected on days 1, 2 and 3 of the infection, however all three parameters (S-Ca, CRP, PCT) were not always assessed in each obtained sample (vide infra).

Tab. 1 Patient data.

Total number of patients	**29**
Boys : girls ratio	16 : 13
Mean and median gestational age ± SD (weeks)	39.4; 39.5 ± 1.9
Mean and median birthweight ± SD (grams)	3310; 3355 ± 537
Mean and median body length ± SD (cm)	50; 50 ± 2.5
Mean and median age at onset of infection ± SD (days)	1.6; 1.0 ± 1.4
Number of blood draws in children with early-onset infection within the first three days of illness	87
Mean and median age at the time of blood draws ± SD (days)	1.8; 2.0 ± 0.9
Control group – total number	705
Boys : girls ratio	413 : 292
Total number of blood draws in control group	800
Mean and median age at the time of blood draws ± SD (days)	2.2; 2.0 ± 1.8

SD: standard deviation

Total calcemia (kit CalciumC – Abbott, method Arsenazo III; analyser Architect) was assessed on day 1 of the infection in 29 patients (n = 29; 100%); on day 2 (n = 14; 48.3%) and on day 3 (n = 17; 58.6%).

C-reactive protein (kit CRP Vario, method turbidimetry/imunoturbidimetry; analyser Architect) was assessed on day 1 of the infection (n = 29; 100%), on day 2 (n = 27; 93.1%) and on day 3 (n = 24; 82.8%).

Procalcitonin (kit Liaison Brahms PCT II GEN, chemiluminiscence analysis CLIA, analyser Liaison XL) was assessed on day 1 of the infection (n = 19; 65.5%), on day 2 (n = 21; 72.4%) and on day 3 (n = 15; 51.7%).

As mentioned above, out of 828 neonates, where S-Ca was assessed, 29 were diagnosed with early-onset infection. Therefore, 799 neonates (424 boys and 375 girls) were considered as free of infection/sepsis. Due to the fact, that all 29 patients suffered from early-onset infection within the first three days of life and we evaluated their biochemical data (S-Ca, CRP, PCT) in the following three days, the age-matched control group was selected to include S-Ca results from full-term neonates under 6 days of age (n = 705; 413 boys and 292 girls) (Table 1). The reasons for blood draws and biochemical assessments (including S-Ca) in these children were: hypotonia, tachypnea, jaundice, body temperature changes, vomiting, maternal risk factors (diabetes, hypertension, nicotinism/drug addiction). None of the controls presented with infection or severe metabolic disorder.

Unpaired t-test and Pearson's correlation were used for statistical analysis (SigmaPlot software). The values were expressed as mean and median ± standard deviation (SD).

RESULTS

The mean value of S-Ca in 799 children without infection was 2.36 ± 0.19 mmol/l. The mean value of S-Ca in 705 children (considered as a control group) was 2.38

Tab. 2 Correlations between S-Ca and S-CRP and S-PCT, respectively.

Parameter	S-CRP day 1	S-CRP day 2	S-CRP day 3	S-PCT day 1	S-PCT day 2	S-PCT day 3
S-Ca day 1	-0.07	-0.1	-0.1	-0.08	-0.09	-0.1
S-Ca day 2	-0.08	-0.1	-0.08	-0.08	-0.1	-0.09
S-Ca day 3	-0.1	0.03	-0.08	-0.09	-0.08	-0.09

± 0.21 mmol/l. In neonates with infection , the mean value of S-Ca on day 1 was 2.16 ± 0.30 mmol/l, median 2.13 mmol/l; 2.12 ± 0.18 mmol/l, median 2.03 mmol/l on day 2 ($p < 0.0001$ versus controls; unpaired t-test) and 2.27 ± 0.18 mmol/l, median 2.32 mmol/l on day 3 ($p = 0.05$ versus controls; unpaired t-test). The mean value of the pooled S-Ca in neonates with infection was 2.18 ± 0.24 mmol/l, median 2.19 mmol/l ($p < 0.0001$ versus controls; unpaired t-test).

The mean values of S-CRP were 24.63 ± 29.58 mg/l, 34.63 ± 31.46 mg/l, and 22.02 ± 18.64 mg/l (normal < 5 mg/l) on days 1, 2 and 3, respectively. The median values of S-CRP were 12.55 mmol/l, 23.65 mmol/l and 19.25 mmol/l on days 1, 2 and 3, respectively. The mean value of the pooled S-CRP in neonates with sepsis was 27.16 ± 27.73 mg/l; median 19.0 mg/l.

The mean values of S-PCT were 20.85 ± 23.30 µg/l, 23.44 ± 36.12 µg/l and 8.05 ± 16.71 µg/l (normal < 0.5 µg/l) on days 1, 2 and 3, respectively. The median values of S-PCT were 7.12 µg/l, 6.0 µg/l and 2.77 µg/l on days 1, 2 and 3, respectively. The mean value of the pooled S-PCT in neonates with sepsis was 18.35 ± 27.64 µg/l; median 6.0 µg/l.

There were no mutual correlations between S-Ca and S-CRP or S-PCT on days 1, 2 and 3. (Table 2).

We found weak inverse correlations with tendency to statistical significance between S-Ca and S-PCT ($r = -0.22$; $p = 0.06$), and S-Ca and S-CRP ($r = -0.19$; $p = 0.09$), respectively, once the data were pooled.

DISCUSSION

Our paper gives evidence about a transient drop in total serum calcium in the course of early-onset neonatal infection. Furthermore, we also found a tendency to slight inverse relationship between pooled total serum calcium levels and biochemical markers of inflammation (CRP and PCT), with tendency to statistical significance.

Regarding our patients, the relationship between S-PCT and S-Ca, in particular the hypocalcemic effect of PCT, was also considered (8, 9, 17). The relationship between PCT and serum levels of calcium remains unclear and rather controversial, as there is no evidence of PCT binding to the calcitonin cellular receptors (18). In a study with healthy volunteers, infusion of calcium gluconate physiologically stimulated the release of mature calcitonin with only minimal effects on the S-PCT levels (19). Furthermore, septic patients with ionised hypocalcemia were reported as having low serum 25(OH)vitamin D levels which were inversely correlated with S-PCT (20). In another study in patients with septic shock, S-PCT levels were correlated with the severity of disease and S-CRP, but not with $S\text{-}Ca^{2+}$ levels (21). In adult patients with sepsis, the low $S\text{-}Ca^{2+}$ concentrations were inversely related to S-PCT, TNF-alpha and IL-6 (17). Therefore, in patients with sepsis/infection, the combined hypocalcemic effect of PCT together with TNF-alpha, IL-1, IL-6, and aminoglycoside up-regulation of CaSR can not be ruled out (10–15, 17).

All our patients with early-onset neonatal infection received appropriate antibiotic treatment and the clinical course and outcome was favorable. None developed organ dysfunction. The S-Ca was not found to be a predictor of further changes in markers of inflammation, as there were no mutual correlations between S-Ca and S-CRP or S-PCT on days 1, 2 and 3, respectively (Table 2).

We are well aware of the limitations of our paper as $S\text{-}Ca^{2+}$, albumin-adjusted-Ca, serum levels of 25-OH-vitamin D, S-PTH, TNF-alpha, IL-1 and IL-6 were not assessed, together with the fact that all three observed parameters (S-Ca, CRP, PCT) were not always measured in each obtained sample in the course of infection. Furthermore, we analysed only full-term neonates with early-onset infection and our results are probably not fully applicable to pre-term neonates.

In conclusion, total S-Ca was decreased in neonates with early-onset infection and did not show any strong or significant correlation with S-CRP and S-PCT, however, there was a tendency to inverse relationship with these parameters once the data were pooled. Pediatricians should be aware of the fact that low total S-Ca should alert their attention to the risk of neonatal infection/sepsis, and, likewise, that the children with neonatal infection/sepsis are at a higher risk of hypocalcemia with all its consequences.

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