Procalcitonin is a Prognosis Biomarker in Late Onset Neonatal Sepsis: A Pilot Study

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Research Article

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Abstract

Background. Neonatal sepsis contributes substantially to neonatal morbidity and mortality. Procalcitonin (PCT) is a recognized biomarker for the diagnosis of late-onset neonatal sepsis (LONS), however, little is known about the prognosis value of PCT in LONS. This study aims at assessing PCT value as a prognosis biomarker in preterm infants with LONS.

Methods. Retrospective single center observational cohort. All premature infants (less than 32 weeks of gestational age) with LONS admitted in a tertiary neonatal intensive care unit.

Discussion. Among the 59 preterm infants included in the analysis, 48 survived (81.4%, 48/59). Deceased patients had a significantly lower gestational age ($p=0,025$) and weight ($p=0,016$) at the time of LONS diagnosis. Although PCT values were not different between both groups at the time of LONS diagnosis, it was more elevated during the first 24 hours in deceased patients ($p=0,041$). Accuracy of PCT for LONS prognosis ranged from 0.70 to 0.82 of area under the curve on receiver operating characteristics curves. Optimal PCT cut-off values at LONS diagnosis was 8,92 µg/L (Youden's J index 0.53), 15.75 µg/L for PCT values during the first 24 hours (J index 0.56), and 6.74 µg/L between 24 and 48 hours after diagnosis (J index 0.54). The estimated survival probability at day 60 was above 95% for patient with a PCT value at sepsis diagnosis under 8,9 µg/L and less than 45% if higher ($p<0.0001$).

Conclusion. A PCT value > 8.92 µg/L obtained at LONS diagnosis suspicion seems to be a good prognosis biomarker.

What Is Known:

- Procalcitonin (PCT) is a recognized biomarkers of 28-days mortality in critically ill adults with septic shock and trauma.
- Failure to have decreased in PCT in the first days of critical care is associated with increase mortality.

What is new:

- Hereby, we show that PCT has a prognosis value in premature infants with late-onset neonatal sepsis.
- Procalcitonin value >8.92 µg/L at LONS diagnosis is associated with an increase 60-days mortality.

Introduction

Neonatal sepsis is defined as a systemic infection occurring in the first 28 days of life, with early-onset neonatal sepsis (EONS) occurring within 72 hours after birth and late-onset neonatal sepsis (LONS) thereafter. The incidence of LONS ranges between 0.6 to 27.6% according to birth weight (BW) and gestational age (GA) [1–4]. Several other neonatal factors such as the presence of congenital

malformations, mechanical ventilation, administration of total parental nutrition, use of central venous catheter are identified additional risk factors for LONS [5–6]. Gram-positive bacteria, dominated by coagulase-negative staphylococci (CoNS), are being the most frequently encountered pathogen, followed by Gram-negative enterobacteria and fungi which are known to induce more severe clinical manifestations, with higher mortality [7–8]. Due to the poor diagnosis specificity of clinical symptoms, biological biomarkers of sepsis diagnosis are increasingly used [9–10]. Procalcitonin (PCT) is a recognized biomarker with high sensitivity and specificity for the diagnosis of LONS [11]. Currently, PCT has the highest reported negative predictive value (87–100%) of all established biomarkers for severe invasive bacterial infections in neonates [12–13]. Prognostic value of PCT is recognized in adults with sepsis and septic shock but has not been assessed in neonatal sepsis [14] The aim of this study is to assess PCT as a prognosis biomarker in preterm with LONS admitted to NICU.

Methods

Retrospective study conducted between January 1st, 2015 and December 31st, 2018 in the neonatal intensive care unit of the AP-HP Paris-Saclay University, Bicêtre Hospital, Le Kremlin-Bicêtre, France. Patients were identified in a prospectively maintained healthcare-associated infection (HAI) surveillance program database. Data were retrieved from the patients' electronic medical records. All neonates under 32 weeks of GA hospitalized with LONS were eligible. Infection was defined according to the Center for Disease Control criterion, as presence of pathogenic organism isolated from one blood culture or clinical infection with two commensal-germ positive blood culture [26]. Neonates who underwent surgery, neonates with necrotizing enterocolitis, and those who previously received antibiotics for EONS were excluded. In order to reduce the survivor bias, infants deceased before day 5 after LONS diagnosis were excluded. The primary endpoint was 60-days mortality. The study (French study classification: MR004) was approved by the local IRB and French Data Protection Authority (CNIL number: 271813) waiving the need of written consent. All legal representatives were informed and could refuse to participate at any time. Patients' records were reviewed to collect the following data on a standardized form: GA, gender, birth weight, mode of delivery, antenatal steroids, postnatal age, and weight at the time of LONS diagnosis. Clinical parameters, respiratory support modalities, use of vasopressors, vascular filling, serum lactate, microbiologic identification, antimicrobial therapy were obtained. Procalcitonin was measured (Elecys→ BRAHMS Procalcitonin (PCT) test, Roche Diagnostics, Indianapolis, IN) at sepsis diagnosis time as part of the standard sepsis workup (incl. blood cell count, hemoculture, PCT), and during the following three days (H0 to H24, H24 to H48, H48 to H72). Sepsis diagnosis time was considered as the time LONS diagnosis suspicion was established and initial biological workup performed. If no PCT assay was available within 48 hours, patients were excluded from the analysis.

Statistical analysis was performed using Easymedstat (EasyMedStat, Levallois-Perret, France). Quantitative variables were described as median and quartiles. Qualitative variables were expressed as percentages and 95% confidence interval (95% CI). The unpaired Student's *t*-test and the Mann-Whitney test were used to compare age and laboratory variables. Categorical variables were compared with the chi-square (χ^2) or the Fisher exact test. The predictive value of PCT for mortality was assessed using

Receiver-Operating Characteristics (ROC) curves, area under the curve (AUC) calculated. Youden's J index was calculated to find the best discriminatory cut-off value. Kaplan-Meier survival curves were plotted, and log rank test performed. Hazard ratio (HR) were subsequently calculated. A p value < 0.05 was considered as significant.

Results

Out of 689 premature infants hospitalized during the study period, 251 were identified in the HAI surveillance database (36.4%, 251/689) (Fig. 1). One hundred and sixty-one patients were excluded. Among the remaining 90 premature infants with LONS, 31 were excluded for missing data on PCT kinetics. A total of 59 preterm were included in the analysis, of whom 48 were alive at day 60 (81.4%, 48/59). Patients' characteristics are displayed in Table 1. Deceased patients had a significantly lower GA (28 weeks of GA [27–30] vs. 30 [29–32], $p = 0.025$) and weight (820 grams [730–1065] vs. 1072.5 [850–1320], $p = 0.016$) at the time of LONS diagnosis than survivors. No statistically significant difference was found for clinical symptoms nor vascular filling. Deceased patients required more invasive mechanical ventilation ($p < 0.001$) and vasopressors such as norepinephrine ($p < 0.001$) than survivors. Gram-positive bacteria were identified in 43 patients (73%, 43/59) with CoNS found in 32 (74%, 32/43) (Table 1). The proportion of LONS caused by CoNS were significantly higher in survivors (63% vs. 18% $p = 0.016$) whereas LONS caused by *Enterobacter cloacae* complex was more frequently found in deceased ones (46% vs. 12%, $p = 0.023$).

Table 1
Patients' characteristics according to 60-days mortality.

	Survivors (n = 48)	Deceased (n = 11)	<i>p</i> value
Gender Male, n (%)	26 (54)	5 (45)	0.74
Term of birth , weeks	28 [26–30]	27 [26–29]	0,053
Birthweight , grams	965 [830–1230]	790 [760–1040]	0,042
Mode of delivery			
Spontaneous vaginal	16 (33)	6 (55)	0,23
Primary caesarean section	3 (6)	0	0,08
Emergency caesarean	29 (60)	5 (45)	0,4
Time of LONS			
days	8 [5–11]	8 [6–14]	0,78
Postmenstrual age, weeks	30 [29–32]	28 [27–30]	0,025
Pathogens			
CoNS	30 (63)	2 (18)	0,016
<i>Staphylococcus aureus</i>	9 (19)	3 (27)	0,54
<i>Escherichia coli</i>	3 (6)	1 (9)	0,75
<i>Enterobacter cloacae</i> complex	6 (12)	5 (46)	0,023
<i>Klebsiella pneumoniae</i>	0 (0)	1 (9)	-
Clinical symptoms			
Bradycardia	42 (87)	9 (82)	0,63
Respiratory distress or apnea	39 (81)	11 (100)	0,18
Invasive mechanical ventilation	13 (27)	9 (82)	< 0.0001
Hyperglycemia	8,8 [6, 7–11, 6]	10,2 [7, 6–14, 8]	0,19
Vascular Filling	6/48 (12,5)	8/11 (72,7)	NA
Use of vasopressor	2	11	< 0.001
Dobutamine	0 (0)	1 (%)	-
LONS, late onset neonatal sepsis; CoNS, coagulase-negative staphylococcus; WBC, white blood cell.			
Values are expressed as number (%) or median (IQR). A <i>p</i> value < 0.05 is considered as significant.			

	Survivors (n = 48)	Deceased (n = 11)	<i>p value</i>
Norepinephrine	2 (4)	9 (81,8)	< 0.001
Epinephrine	0 (0)	2 (18)	-
Laboratory findings			
Time to positive blood culture (hours)	11 [8–15]	7 [5–12]	0,012
Serum lactate (mmol/L) [n = 23/8]	2 [1,8 – 2,8]	2,25 [1,6 – 2,8]	0,37
WBC (/mm ³) [n = 39/11]	8550 [6180–13530]	13090 [8290–22500]	0,39
Platelets count (/mm ³) [n = 39/11]	182 [128–220]	104 [39–214]	0,11
LONS, late onset neonatal sepsis; CoNS, coagulase-negative staphylococcus; WBC, white blood cell.			
Values are expressed as number (%) or median (IQR). A <i>p value</i> < 0.05 is considered as significant.			

Procalcitonin values were significantly higher in deceased patients at H0-H24 and H48-H72 (Fig. 1). Accuracy of PCT prognostic value was assessed by calculating AUC-ROC curves. Due to limited number of samples available between H48-H72, only the initial three time points (up to H48) were plotted. At diagnosis (H0), AUC was 0,70 [95% CI: 0,49 – 0,91] (*p* = 0,0413) for PCT to predict mortality. Optimal threshold value was 8,92 µg/L and was associated with a 63,6% [95%CI: 30,79–89,07] sensitivity and a 90,9% [95%CI: 78,33–97,47] specificity (Youden J index 0.53). The proportion of patients with PCT > 8,92 µg/L in the deceased group was significantly higher (63,6%, 7/11) than in survivors (9%,4/44) (Log rank test *p* < 0.0001; HR 7.66, 95% CI 1.36–43.09) (Fig. 2A). At H0-H24, the AUC was 0,79 [95% CI: 0,56 – 1,03] (*p* = 0,0647), and optimal cut-off was 15,75 µg/L with a sensitivity of 75% [95%CI:19,41–99,37] and a specificity of 81,8% [95%CI: 59,72–94,81] for the 60 days mortality (Youden J index 0.56) (Fig. 2B). At H24-H48, the AUC was 0,8276 [95% CI: 0,64 – 0,1,01] (*p* = 0,0361), with an optimal threshold value at 6.74 µg/L with a 75% [95% CI: 19,41–99,37] sensitivity and a 79,3% [95% CI: 60,28–92,01] specificity (Youden J index 0.54) (Fig. 2C).

Discussion

Hereby, we show that PCT has a prognosis value in premature infants with LONS. A PCT value at sepsis diagnostic suspicion higher than 8.92 µg/L has a sensitivity of 63.9% and specificity of 90.9% for 60-days mortality. In addition, deceased infants show a significant increase in PCT value from diagnosis to H24-H48.

Late-onset neonatal sepsis is an important cause of death in premature infants and the PCT serum concentration is a valuable tool for early detection of LONS [14]. Majority of LONS occurred primarily in extremely low birth weight (ELBW) infants with a median occurrence time around the third week of

life [2,6,7,10]. In our series, LONS occurred earlier (median post-natal age of 8 days). Similarly, to reported series, CoNS was the predominant pathogen, but Gram-negative bacteria were not rare, with *Escherichia coli*, *Klebsiella spp.*, *Enterobacter cloacae* complex. The later was the most frequently encountered pathogen in lethal LONS and may be related to specific virulence of *E. cloacae* complex [15]. Impact of causative agents on PCT value was not assessed in our study although suggested by others [14].

To our knowledge, no studies have focused on PCT kinetics in LONS. In our study, we showed that PCT values are significantly higher 24 hours after the first clinical symptoms in deceased infants. Multiple studies have shown that the lack of decrease in PCT is associated with mortality in critically ill septic and trauma adults and septic children [14, 16,17,18]. Cui *et al.* showed in adults with sepsis and septic shock that on the 2nd, 3rd and 5th days after diagnosis PCT values were higher in deceased patients compared to survivors [21]. Sakran *et al.* demonstrated significantly increased mortality among critically ill adults with PCT level $\geq 5 \mu\text{g/L}$ (odds ratio of 3.65 (95% CI 1.23–4.61) [24]. In our study, PCT cut-off value at diagnosis was 8.92 $\mu\text{g/L}$ which is slightly higher. Nevertheless, 9/59 infants (15%) had PCT level $<0.5 \mu\text{g/L}$ that progressively increased in the following 24 hours. Those “erroneously normal” PCT values may be considered as false negative as all patients of our cohort had microbiologically proven LONS and are certainly resulting from premature sampling. Similarly, Beltempo *et al.* suggested that optimal timing for biomarkers measurement in VLBW infants with suspected LONS may range between the first 24 hours after diagnosis suspicion [22].

This study has several limitations. First, a lack of power may have occurred due to the small sample size. We have intentionally excluded a significant number of patients (eg. Surgery, necrotizing enterocolitis) with conditions that may significantly affect PCT values and kinetics. Of significance, 21 patients who died within the first five days of LONS were excluded to limit the effect of a survival bias. Second, this study was limited to 60-days mortality and no other prognosis outcome were evaluated. This primary endpoint was set on purpose as we could not exclude delayed mortality considering that LONS are identified risk for long term mortality and morbidity. Duration of antibiotherapy is another recognized prognosis outcome as its impact on the development of multidrug resistance bacteria may strongly impact patients’ outcome. Third, as a retrospective study, our results may need to be confirmed in prospective multi-centers cohort to ascertain both the prognostic value of PCT in LONS as well as the identified cut-off values.

In conclusion, we showed that PCT has a prognosis value in premature infants with LONS, with a PCT value $>8.9 \mu\text{g/L}$ associated with 60-days mortality. In addition, we showed that deceased patients had a significant increase in PCT value between diagnosis and 24-48 hours, suggesting that PCT sampling may be optimal at the time of diagnosis and 24 hours later.

Abbreviations

CoNS, Coagulase negative Staphylococcus; EONS: Early onset neonatal sepsis; ELBW: Extremely low birth weight; LONS: Late onset neonatal sepsis; NICU: Neonatal intensive care unit; VLBW: Very low birth

weight.

Declarations

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Availability of data and materials: All materials are available upon request to Pr. Pierre Tissires at pierre.tissieres@aphp.fr

Code availability: none

Author's contribution: Design of the work: VR, PT. Acquisition, analysis, or interpretation of data for the work: VR, PT. Drafting the manuscript: VR, PT. Revising it critically for important intellectual content: all authors. All authors approved the final version submitted for publication and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval: The study (French study classification: MR004) was approved by the local IRB and French Data Protection Authority (CNIL registration number: 271813) waiving the need of written consent.

Consent to participate: All legal representatives were informed and agreed to participate.

Consent for publication: All authors agreed for publication of this study.

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Figures

Figure 1

Flow Chart

Figure 2

Procalcitonin (PCT) kinetics during the first 72 hours following late onset neonatal sepsis (LONS) diagnosis. PCT values at diagnosis (H0), during the first day (H0-H24), during the second day (H24-H48) and third day (H48-H72) in survivors (black) and deceased infants (white). * $p < 0,05$; ns, non significant.

Figure 3

Kaplan-Meier Survival curve