Review

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Perinatal presepsin assessment: a new sepsis diagnostic tool?

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Abstract: Perinatal sepsis constitutes a medical emergency and is still one of the major causes of mortality and morbidity. The possibility of an early diagnosis of sepsis is still debated and controversial. In particular, clinical symptoms can be hidden by the association of sepsis with other perinatal diseases and/or by therapeutic strategies performed. In this context, there is evidence that the accuracy of standard of care diagnostic parameters (i.e. blood culture, C-reactive protein, procalcitonin) can be biased by additional confounding factors (gestational age, birthweight, acute-chronic hypoxia). Therefore, the inclusion in clinical daily practice of new biomarkers of sepsis is of utmost importance. Of a panel of biomarkers, Presepsin (P-SEP) plays an important role in the development and response of the immune system and as an early marker of sepsis both in adult and pediatric patients. Therefore, in the present review we aim to offer an overview of the role of P-SEP in the early detection of perinatal sepsis as a trustworthy marker according to actual statements of official international institutions. Future perspectives regard the possibility of a longitudinal non-invasive biological fluids P-SEP assessment thus limiting the sample stress in high risk newborns.

Keywords: newborns; presepsin; sCD14; sepsis.

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Introduction

Sepsis is an overwhelming and life-threatening inflammatory response to invading pathogens in the bloodstream [1]. In the perinatal period, it is one of the major contributors to worldwide mortality and morbidity [2]. Infants, especially preterm ones (PT), have an immature immune system and they can be infected by many microorganisms [3]. If the infection is not promptly managed, the disease will rapidly evolve causing septic shock and even multiple organ failure with a high fatality rate [3].

Neonatal sepsis is categorized into early (EOS) and late onset sepsis (LOS). EOS is defined as the appearance of typical clinical and laboratory signs within the first 72 h of life and is mainly due to bacteria acquired before and/or during delivery [4]. Conversely, LOS comes after that period and is due to bacteria acquired after delivery like nosocomial ones [5].

Today standards of care for the diagnosis of neonatal sepsis are based on blood culture, C-reactive protein (CRP) and procalcitonin (PCT) blood measurement. The former remains the 'gold standard' for the diagnosis of sepsis in newborns, although at least three principal factors influence its sensitivity: the timing of collection, the number of cultures and the blood volume [6]. The latter (i.e. PCT, CRP) lack accuracy in reflecting inflammation and infection and can be affected by several biases, for example gestational age (GA), hypoxia etc. [6, 7].

Among the several biomarkers identified as potential diagnostic tools for neonatal sepsis, the soluble cluster of

differentiation CD14 sub-type (sCD14), also called Presepsin (P-SEP), seems to be promising [7]. Membrane CD14 (mCD14) is a multifunctional glycoprotein expressed in the surface of various cells, including monocytes, macrophages and neutrophils [8]. Indeed, CD14 is a recognition receptor for Gram-negative and Gram-positive bacteria lipopolysaccharides [8]. In adults, in children and in infants P-SEP levels have been shown to increase in response to infections and constitute an early biomarker of sepsis (within 2–3 h) [9–11].

Therefore, in the present review we offer an overview of P-SEP and the recent advances in its use as a biomarker for the diagnosis of sepsis.

Research strategy

In the present review, we (D.G, E.D'A, V.B) analyzed data from the Literature, presented as original articles covering the period 1992 to 2021 about P-SEP as a biomarker for the diagnosis of neonatal sepsis. A PubMed and Science direct search of literature was performed using the following key words: "Newborns" and "sCD14" and "Presepsin" and "Sepsis". During the research process bibliographic updates were performed (Figure 1). We found 38 articles in whom perinatal P-SEP was investigated as a biomarker of sepsis diagnosis. Moreover, 4 out of 38 articles provided P-SEP reference curves in arterial cord and peripheral blood.

Content

Epidemiology

Neonatal sepsis is still an important cause of mortality and morbidity in developed and developing countries [12]. A recent meta-analysis comprising 2,797,879 live births in 14 countries reported an incidence of neonatal sepsis of 2,824 cases per 100,000 live births with a mortality rate of 17.6%. PT have the highest incidence of sepsis and EOS occurred less than LOS [13, 14]. Overall, between 2009 and 2018, the former has an incidence of 3.12% with a mortality rate of 16.4%, and the latter has an incidence of 0.7% with a mortality rate of 9.1% [13]. Moreover, incidence of perinatal sepsis has been found to be inversely correlated with GA and weight at birth (BW) [15].

Diagnosis of sepsis

In clinical practice, the diagnosis of perinatal sepsis is still a challenge, mainly due to the non-specific clinical symptoms at a stage when standard monitoring procedures can be silent or unavailable. In this regard, standard of care laboratory testing for neonatal sepsis includes blood culture, CRP and PCT blood measurements. Although blood culture remains the 'gold standard' for the diagnosis of perinatal sepsis, several limitations can affect its accuracy. The main ones regard: (i) timing of collection, (ii) number of cultures, (iii) blood volume, (iv) higher contaminant rate, and (v) long wait for result output, requiring at least 36–48 h of incubation [16].

CRP is still the most commonly used laboratory test in the diagnosis and monitoring of perinatal sepsis. Its release into the blood increases at 4–6 h after stimulation and peaks at 48 h. In healthy infants, CRP levels physiologically increase in the first three days after birth, and they are GA, hypoxia and asphyxia dependent [15, 17]. Thus, the main limitation of this marker regards its low specificity for infection in the presence of one of the aforementioned conditions.

PCT is the fastest emerging analyte in the diagnosis and monitoring of perinatal sepsis. Its blood levels increase at 4 h and peak at 6–8 h after stimulation. Although its sensitivity is slightly greater than CRP, in both PT and term infants (T) it peaks at 1–2 days after birth, followed by a progressive decrease, making this marker less specific [17]. On the basis of the aforementioned findings the identification of new non-invasive early biomarkers of perinatal sepsis still constitutes an eagerly awaited and fundamental development.

Current standards for biomarkers validation

According to recent recommendations from the Food and Drug Administration (FDA), European Medicine Agency (EMA), and the National Institute of Health (NIH), several conditions must be satisfied before a biomarker may be included in clinical daily practice, such as: (i) clearly identified and characterized, (ii) its source material or matrix, (iii) measurement technique of high reproducibility and specificity, (iv) reference ranges and cut-off values, (v) highly specific and accurate in terms of high sensitivity, specificity and positive and negative predictive values (PPV, NPV), and (vi) limits of detection and prognostic value intended as pharmacokinetic/pharmacodynamic [18–20].

Presepsin

What is P-SEP?

P-SEP is a molecule that originates from the mCD14 receptor, a membrane glycoprotein expressed in monocytes,

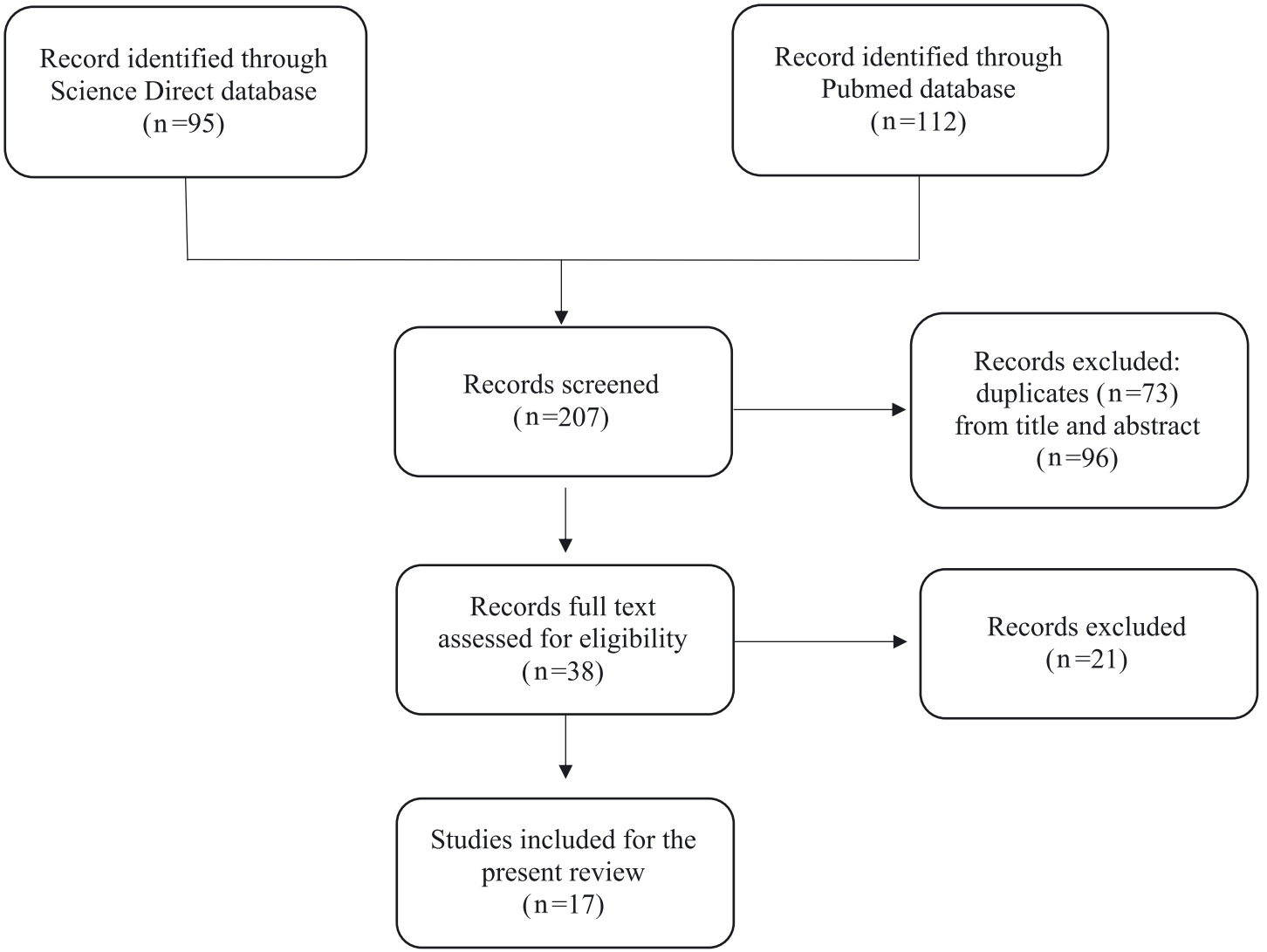

Figure 1: Study flow chart.

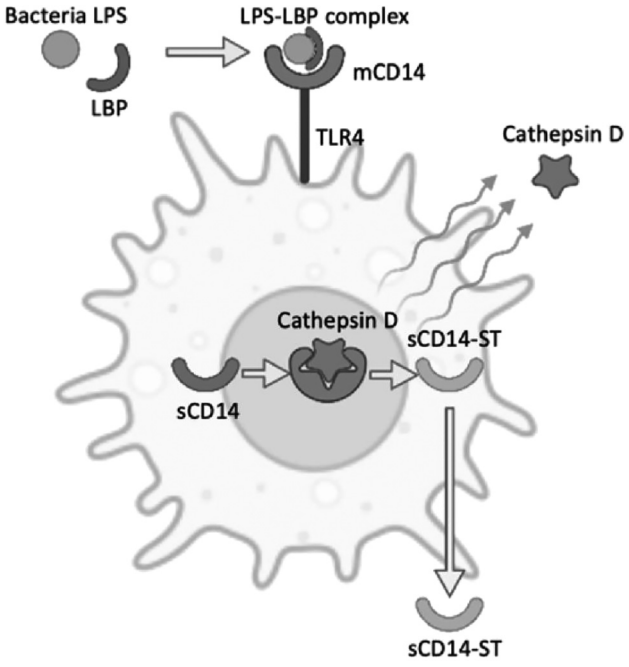

Figure 2: Presepsin activation and releasing processes.
LBP, lipoprotein binding protein; LPS, bacterial lipopolysaccharide; TLR4, Toll-like receptor 4; mCD14, membrane CD14; sCD14, soluble CD14; sCD14-ST, presepsin.

macrophages and neutrophils cells [21]. The mCD14 receptor has the ability to interact with bacterial lipopolysaccharide binding protein [21]. The resulting complex activates the toll-like receptor-4 specific proinflammatory signaling cascade against invading bacteria during which mCD14 soluble part is cut and released (i.e. sCD14). During the proinflammatory process sCD14 undergoes a further cut by cathepsin D (a lysosomal protease), which detaches its N-terminal part. The resulting molecule made up of 64 aminoacidic residues is called P-SEP [22] (Figure 2).

P-SEP measurement and method reproducibility

P-SEP can be quantitatively determined by means of the following main methods: an enzyme-linked immunosorbent assay (ELISA) and a non-competitive chemiluminescent enzyme immunoassay (CLEIA) [23–25]. Briefly, the ELISA assay (Merck KGaA, Darmstadt, Germany) is designed to measure P-SEP in plasma samples. It is a plate-based assay able to detect and quantify soluble substances such as peptides, proteins, antibodies, and hormones in a complex mixture. Colorimetric detection is accomplished via a highly specific antibody-antigen interaction. This method has low linearity and several endogenous (i.e. rheumatoid factor, complement, cross-reactive substances) and exogenous (i.e. specimen hemolysis, bacteria and iatrogenic tube contamination) interfering factors [23, 24].

The CLEIA method (Mitsubishi PATHFAST ® by LSI Medience Corporation) is the most common P-SEP measurement technique requiring a sample volume ranging from 50 µL to 5 mL. The assay is a single point-of-care tool analyzing plasma and the whole blood through a micro method. The CLEIA method is the one that paved the way for P-SEP quantitative detection [25].

P-SEP reference ranges and cut-off

The P-SEP cut-off references values in infants are reported in Table 1. In detail, P-SEP levels have been: (i) detected in arterial cord and peripheral blood of PT singly or in both PT and T populations, (ii) found identical or slightly increased in healthy PT as opposed to T infants according to different monitoring time-points [15, 26–28]. In this regard, P-SEP has been measured from birth up to 7 days of post-natal life except for one observation involving prolonging longitudinal monitoring to the first 28 days from birth [28], and (iii) inversely correlated to GA [27, 28]. Altogether, on the basis of the aforementioned findings it is possible to argue for the wide heterogeneity in P-SEP monitoring time-points, limiting its accuracy as a diagnostic test for sepsis. Further investigations in this regard are therefore required.

P-SEP accuracy and prognostic value

The P-SEP accuracy as an early diagnostic biomarker of sepsis in infants has been recently evaluated in a cohort of PT and T infants and results are reported in Table 2. A wide heterogeneity in the recruitment of cases admitted into the studies has been found. In particular: T singly in 2 [2, 35], PT singly in 6 [30–34, 40], and both T and PT in 5 studies [1, 8, 9, 36, 37]. Ten out of the 13 series, identified by the research strategy, performed longitudinal P-SEP monitoring from birth up to the first week of life. Peripheral blood was the only biological fluid collected, CLEIA assay was performed in 9 out of 13 studies [1, 8, 31–35, 37, 40] and ELISA assay in 4 out of 13 reports [2, 9, 30, 36].

Among perinatal outcomes, EOS singly in 4 [2, 31, 32, 35], LOS singly in 3 [30, 33, 40], systemic inflammatory response syndrome (SIRS) in 2 [1, 8], and both EOS and LOS in 5 studies [8, 9, 34, 36, 37] were the main ones considered.

The sensitivity, specificity, PPV and NPV of P-SEP as a diagnostic test for sepsis are reported in Table 2. According to the population and outcome investigated, we found a wide heterogeneity in P-SEP accuracy as a predictor of

Table 1: Presepsin (P-SEP) cut-off levels (pg/mL) expressed as medians (minimum-maximum) in healthy control infants.

P-SEP time-points	PT cut-off, ng/L	T cut-off, ng/mL	Ref.
	0–24 h		
0–6	583 (405.0–800.0)	NA	[27]
12–15	614 (450.0–812.0)	NA	[27]
24	NA	386.0 (147.0–640.0)	[28]
24	406.0[a] (181.0–1,046.0)	NA	[28]
24	494.5[b] (219.0–1,052.0)	NA	[28]
	25–71 h		
24–27	604 (445.0–825.0)	NA	[27]
48–54	513 (371.0–734.0)	NA	[27]
	3–7 d		
3	NA	295.5 (148.0–788.0)	[28]
3	319.0[a] (99.2–795.0)	NA	[28]
3	377.5[b] (154.0–1,024.0)	NA	[28]
3–4	NA	603.5 (466.5–791)	[15]
3–7	620.0 (503.0–864.0)	NA	[15]
5	NA	255.0 (116.0–806.0)	[28]
5	348.5[a] (133.0–876.0)	NA	[28]
5	404.5[b] (228.0–947.0)	NA	[28]
2–7	584[a] (453.0–813.0)	NA	[26]
2–7	521[b] (414.7–603.7)	NA	[26]
7	NA	256.0 (101.0–575.0)	[28]
7	358.0[a] (121.0–947.0)	NA	[28]
7	382.0[b] (209.0–933.0)	NA	[28]
	>7 d		
14	NA	225.0 (116.0–806.0)	[28]
14	262.5[a] (116.0–778.0)	NA	[28]
14	431.0[b] (171.0–799.0)	NA	[28]
21	NA	226.0 (83.6–772.0)	[28]
21	246.5[a] (101.0–457.0)	NA	[28]
21	399.0[b] (159.0–787.0)	NA	[28]
28	NA	224.0 (89.4–802.0)	[28]
28	211.0[a] (99.6–581.0)	NA	[28]
28	376.0[b] (127.0–873)	NA	[28]

[a]Late preterm infants. [b]Very preterm infants. P-SEP, presepsin; PT, preterm infants; T, term infants; GA, gestational age; NA, not available; Ref, reference.

sepsis (EOS, LOS, SIRS). In particular, sensitivity (67–100%) [8, 33, 36], specificity (75–100%) [8, 32, 33, 35, 40], PPV (57–100%) [32, 33, 37, 40], NPV (59–100%) [35, 36] significantly varied between PT and T. Notably, in 3 out of the 13 studies PPV and NPV analysis was not reported [1, 8, 9].

P-SEP accuracy compared with CRP and PCT

In order to fulfill the requirements stated by official institutions on P-SEP accuracy as a diagnostic test for perinatal sepsis, a comparison with standard of care parameters such as CRP and PCT is provided in Table 3. The research strategy allowed for the selection of 7 studies in which P-SEP, CRP and PCT accuracy were investigated according to different outcomes [1, 9, 30, 32, 34–36]. At this stage, results are still controversial and a matter for debate, showing differences both from a clinical point of view and in the design of the trials themselves. Although on the one hand, peripheral blood constituted the only biological fluid collected in all studies, on the other the differences in the outcomes analyzed and the measurement techniques require further consideration. In fact, CLEIA and ELISA

Table 2: Presepsin (P-SEP) accuracy as a valuable predictor of sepsis in preterm (PT) and term (T) infants.

Population	LM	BF	Assay	Outcome	P-SEP cut-off, ng/L	SE, %	SP, %	PPV, %	NPV, %	Ref.
PT, T	N	PB	C	EOS, LOS	548.0	100.0	81.20	NA	NA	[8]
PT, T	N	PB	C	SIRS	548.0	96.70	81.20	NA	NA	[8]
PT, T	N	PB	C	EOS, LOS	600.0	97.50	100.0	NA	NA	[8]
PT, T	N	PB	C	SIRS	600.0	96.70	100.0	NA	NA	[8]
PT, T	Y	PB	C	EOS, LOS	987.5	72.00	87.00	57.00	93.00	[37]
PT, T	Y	PB	E	EOS, LOS	485.0	97.80	94.10	NA	NA	[9]
T	N	PB	E	EOS	480.0	96.80	95.00	96.80	95.00	[2]
PT	Y	PB	E	LOS	823.0	88.90	88.90	72.70	87.50	[30]
PT	Y	PB	C	EOS	795.0	85.00	89.00	85.00	89.00	[31]
PT	Y	PB	C	EOS	788.0	93.00	100.0	100.0	94.00	[32]
PT	Y	PB	C	LOS	885.0	94.00	100.0	100.0	95.00	[40]
PT	Y	PB	C	LOS	800.5	67.00	100.0	100.0	74.00	[33]
PT	N	PB	C	EOS, LOS	686.0	82.70	95.50	95.40	83.10	[34]
PT, T	Y	PB	C	EOS	304.5	95.80	84.90	NA	NA	[1]
PT, T	Y	PB	C	EOS, SIRS	300.5	95.80	88.60	NA	NA	[1]
T	Y	PB	C	EOS	539.0	80.00	75.00	91.00	59.00	[35]
PT, T	Y	PB	E	EOS, LOS	0.722	100.0	97.50	98.70	100.0	[36]

LM, longitudinal measurement; BF, biological fluid; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; Ref, reference; Y, yes; N, no; PB, peripheral blood; C, CLEIA (chemiluminescent enzyme immunoassay); E, ELisa assay; EOS, early onset sepsis; LOS, late onset sepsis; SIRS, systemic inflammatory response syndrome; NA, not available.

Table 3: Presepsin (P-SEP), C-reactive protein (CRP) and Procalcitonin (PCT) sensitivity (SE), specificity (SP), positive and negative predictive values (PPV, PPV), according to different neonatal outcomes. Data are given in percentages.

Outcome	CRP				PCT				P-SEP			Ref.
	SE, %	SP, %	PPV, %	NPV, %	SE, %	SP, %	PPV, %	NPV, %	SE, %	SP, %	PPV, %	
EOS	83.0	75.0	97.0	75.0	67.0	67.0	84.0	59.0	80.0	75.0	91.0	[35]
Death	71.0	94.1	93.9	71.6	NA	NA	NA	NA	82.7	95.5	95.4	[34]
S	82.0	77.5	88.0	68.9	NA	NA	NA	NA	100	97.5	98.7	[36]
LOS	81.0	72.2	73.6	81.4	NA	NA	NA	NA	88.9	88.9	72.7	[30]
EOS	66.0	90.0	89.0	83.0	83.0	81.0	79.0	83.0	93.0	100	100	[32]
S	NA	NA	NA	NA	93.5	79.4	NA	NA	97.8	94.1	NA	[9]
EOS	78.1	81.1	NA	NA	82.3	83.0	NA	NA	95.8	84.9	NA	[1]

NA, not available; Ref, reference; S, sepsis; LOS, late onset sepsis; EOS, early onset sepsis.

assay were performed in 4 [1, 32, 34, 35] and 3 [9, 30, 36] studies respectively. Furthermore, the main outcomes considered were EOS in 3 [1, 32, 35], LOS in 1 [30] and both EOS and LOS in 3 studies [9, 34, 36]. In detail, P-SEP was found to be: (i) a valuable diagnostic test for sepsis (EOS, LOS) with higher sensitivity and specificity values than PCT and CRP [9, 35, 36], (ii) similarly accurate as a diagnostic test of EOS to CRP and PCT, respectively [1, 34], (iii) the best predictor of ominous sepsis dependent outcome [34], and (iv) a reliable indicator of treatment efficacy [1].

P-SEP limit of detection

Representative performance data on the PATHFAST P-SEP based on CLEIA technology reports a limit of detection (LOD) <20.0 ng/L. Literature data showed a LOD of 13.4 ng/L [8, 26, 38] when P-SEP was quantitatively determined using CLEIA method. No LOD values were reported when ELISA method was performed.

Discussion

Perinatal sepsis still constitutes one of the main causes of mortality and morbidity in newborns with an incidence of 2.82% and a mortality rate of 17.6% [13, 39]. Its incidence is inversely correlated with GA and BW with the highest occurrence (up to 30%) in very low BW infants [29]. The incidence of EOS and LOS have been reported to be 12.7 and 17.6% respectively, with a mortality rate that can reach up to 56% for EOS and 37.6% for LOS at very low BW [4, 5, 29].

The current standards of care used in clinical practice (blood culture, CRP and PCT) show limitations in the early diagnosis of neonatal sepsis leading to a high percentage of bias in the timely identification of EOS and LOS [15–17]. Thus, a biomarker able to early diagnose the occurrence of sepsis is still eagerly awaited [36]. Within this context, the promising results herein reported corroborate the inclusion of P-SEP in daily clinical practice. Literature data showed that P-SEP fulfilled the majority of FDA, EMA and NIH requirements (i.e. identification, characterization; material source or matrix; reproducibility and specificity; available diagnostic test and LOD). Another potential advantage resides in P-SEP measurability in the so-called non-invasive biological fluids (i.e. urine, saliva) as well as tracheal aspirate for adults, children and infants [41–46]. The issue is noteworthy bearing in mind that anemia due to repeated blood sampling is a common pathology in high-risk newborns [47]. Preliminary data in urine supports the hypothesis that in the near future the early detection, the longitudinal monitoring and the effectiveness of therapeutic strategies performed could be evaluated by the assessment of P-SEP and/or other biomarkers in non-invasive biological fluids [48].

Despite P-SEP represents a promising biomarker, there are several criticisms that require further elucidation. The major points refer to: (i) P-SEP measurement technique (CLEIA, ELISA) that can provide in the same sample different results. Therefore, a close multidisciplinary collaboration (biologists, biochemists, pharmacologists, neonatologists etc.) is of the utmost importance in order to provide a factor of conversion of P-SEP levels according to different measurement strategies. The issue is of relevance especially for out-born infant management in whom the possibility to compare, at the hub center, laboratory results performed at the spoke centers, will significantly improve the timing of early diagnosis and subsequent therapeutic strategies, (ii) lack of reference curves in arterial cord and peripheral blood samples measured by ELISA instead of CLEIA technique showing P-SEP levels being GA dependent. In this regard, further studies aimed at investigating whether the conversion of P-SEP levels from pg/mL to multiple of medians, today considered a better standard measurement unit, will empower its role as an early predictor of perinatal sepsis, are more than justified, (iii) the absence of a general consensus regarding P-SEP longitudinal monitoring, bearing in mind its short half-life (about 6 h). Notably, P-SEP assessment varied from soon after birth up to 28 days of age and patient recruitment was limited to PT and/or T population. Additional discrepancies have been also found on the timing to decide when to begin P-SEP monitoring in high-risk infants. For example, there is data reporting P-SEP assessments before, after and at 7 days from antibiotic treatment whilst others started P-SEP measurement at admission into the NICU as well as at birth and/or at sepsis onset (Table 4). On the basis of the present findings further multicenter studies are therefore justified in order to provide frontline physicians with a reliable time-dependent and homogenous reference curve of a diagnostic test for sepsis. In this regard, it is mandatory that the reference population should include both PT and T infants to fill the gaps identified in previous observations, (iv) inclusion and exclusion criteria need further elucidation. Briefly, discrepancies have been observed among several studies, especially occasional exclusion of risk factors such as the occurrence of maternal fever and maternal immunosuppressant treatment, which has been taken into account as inclusion criteria in only one observation [37]. Moreover, no consensus on infants complicated by intrauterine infections, as well as those having undergone surgery or antibiotic therapy has been reached [2, 8, 34, 35]. Another issue regards the choice of primary endpoint. Some Authors have investigated P-SEP, singly or combined, as a predictor of a series of primary outcomes such as EOS and/or LOS and SIRS syndrome, while others have added the occurrence of ominous outcome to the list of primary end-points (Table 3). Lastly, additional confounding factors such as pneumonia, biliary enzymes alteration, cholestasis and interspecies differences in Gram-negative vs. Gram-positive bacteria infections response known to

Table 4: Presepsin assessment time-points in peripheral blood of preterm and term infants complicated by early or late onset sepsis.

Monitoring time-points	Ref.
Before any medical treatment and at 3 d after antibiotic treatment	[8]
At birth and after sepsis onset	[9]
At admission, 2 and 6 d after admission	[36]
Before antibiotic treatment; 3, 5 d after treatment beginning	[1]
Before antibiotic treatment, at 72 h; at 7 d	[35]
At sepsis onset; at 12, 24, 36, 48 h from first sampling; at the end of antibiotic treatment	[37]
Before antibiotic treatment; at 48, 120 h from first sampling	[30]
At birth; during the first 3 d after sepsis onset	[31]
At admission; before antibiotic treatment; at 12, 24, 48 h of life	[32]
At admission; at 1, 3, 5 d after first sampling	[40]
At sepsis onset before antibiotic treatment; at 3, 7 d from sepsis onset.	[33]
At first signs and symptoms of sepsis	[34]
At 1, 3 d in septic infants	[2]

Ref, reference; d, day; h, hours.

affect P-SEP availability as sepsis diagnostic test have to be taken into account [49, 50]. Altogether, it is possible to conclude that, although P-SEP usefulness as an early reliable biomarker of sepsis has been documented in adults, only the resolution of the aforementioned issues will empower its accuracy in PT and T infants. Notably, at this stage P-SEP shows both a valuable diagnostic accuracy when compared with current standard of care analytes and the possibility to get the results to the bedside within 15 min. Another advantage resides in the small volume (50 µL) required for P-SEP measurement. Finally, it has to be highlighted that the cost per sample is superimposable between P-SEP and standard of care biomarkers.

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