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Perinatal asphyxia partly affects presepsin urine levels in non-infected term infants

Abstract

Objectives: Standard of care sepsis biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) can be affected by several perinatal factors, among which perinatal asphyxia (PA) has a significant role. In this light, new early sepsis biomarkers such as presepsin (P-SEP) are needed to enact therapeutic strategies at a stage when clinical and laboratory patterns are still silent or unavailable. We aimed at investigating the potential effects of PA on longitudinal P-SEP urine levels.
Methods: We conducted an observational case-control study in 76 term infants, 38 with PA and 38 controls. Standard clinical, laboratory, radiological monitoring procedures and P-SEP urine measurement were performed at four time-points (first void, 24, 48, 96 h) after birth.
Results: Higher (p<0.05) CRP and PCT blood levels at T1–T3 were observed in PA than control infants whilst no differences (p>0.05, for all) at T0 were observed between groups. P-SEP urine levels were higher (p<0.05) in PA at first void and at 24 h while no differences (p>0.05) at 48 and 96 h were observed. No significant correlations were found (p>0.05) between P-SEP and urea (R=0.11) and creatinine (R=0.02) blood levels, respectively.
Conclusions: The present results, showed that PA effects on P-SEP were limited up to the first 24 h following birth in absence of any kidney function bias. Data open the way to further investigations aimed at validating P-SEP assessment in non-invasive biological fluids as a reliable tool for early EOS and LOS detection in high-risk infants.

Keywords: kidney; newborn; perinatal asphyxia (PA); presepsin (P-SEP); 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, early and late onset sepsis (EOS, LOS) are one of the main contributors to morbidity and mortality worldwide, accounting for up to 50% for EOS and 20% for LOS, respectively [1–3].

An accurate diagnosis is the main difficulty as sepsis shares a similar clinical presentation with other common conditions in newborns [1–3]. Standard of care procedures for sepsis diagnosis include laboratory tests such as blood culture, complete blood cell count and proinflammatory markers [3]. Blood culture procedure is commonly used but is time consuming and lacks sensitivity [2, 4]. Sepsis blood level biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) can have an accuracy of 86% and 96% respectively [5, 6]. However, several perinatal factors affecting CRP and PCT as reliable sepsis diagnostic tests

have to be taken into account. These include gestational age (GA), postnatal age, type of delivery, birth weight (BW) and, last but not least, acute and chronic hypoxia insults, which can trigger an inflammatory non-infectious systemic response [6–8]. In this light, new early sepsis biomarkers are needed in order to enact therapeutic strategies at a stage when clinical and laboratory patterns are still silent or unavailable.

Among different sepsis biomarkers currently investigated, the soluble cluster of differentiation CD14 sub-type (sCD14), also called presepsin (P-SEP), seems to be promising. P-SEP is a protein that originates from cleavage of the CD14 receptor – a membrane glycoprotein expressed in monocyte, macrophage, and neutrophil cells – in response to bacterial infections [9]. P-SEP can be useful in sepsis diagnosis because of the measurability, singly or as a part of CD14, in biological fluids (i.e., cerebrospinal, blood, urine), rapid activation and kinetics, early peak of concentration, and quick result output [10–12]. P-SEP has been shown to be a reliable biomarker of sepsis in adults, infants, and more recently in newborns complicated by EOS and LOS, showing higher accuracy than CRP and PCT [13–15]. However, data regarding the effects of acute neonatal hypoxia on P-SEP levels as for PCT and CRP are still lacking.

Therefore, the aim of the present study was to investigate whether longitudinal P-SEP urine levels measured in newborns complicated by perinatal asphyxia (PA) changed in the absence of any clinical and laboratory sign of infectious disease.

Materials and methods

From April 2019 to December 2020, we conducted a prospective study at our third level referral centers for neonatal intensive care (NICU) in Chieti, Cuneo, Alessandria, Rome, Brescia and Turin, Italy and Maastricht, the Netherlands.

The study was approved by local Ethics Committees and parents of the subjects admitted in the study gave informed and signed consent (Presap.ASO.Neonat.19.02/23.05.19).

For sample size calculation, we used changes in P-SEP as the main parameter [16]. As no basic data are available regarding PA populations, we assumed an increase of 0.5 standard deviation (SD) in P-SEP to be clinically significant. Considering an $\alpha=0.05$ and using a two-sided test, we estimated a power of 0.95, recruiting 28 PA infants. We added n=10 per group to allow for dropouts, crossover, EOS, LOS and mortality [17]. The study population therefore consisted of 38 PA infants and 38 healthy controls matched for GA and weight at birth (BW) (Figure 1).

GA was determined by clinical data and by first trimester ultrasound scan. Appropriate growth was defined when ultrasonography showed a biparietal diameter and abdominal circumference between the 10th and 90th centiles, according to our population standards, and by postnatal confirmation of a BW between the 10th
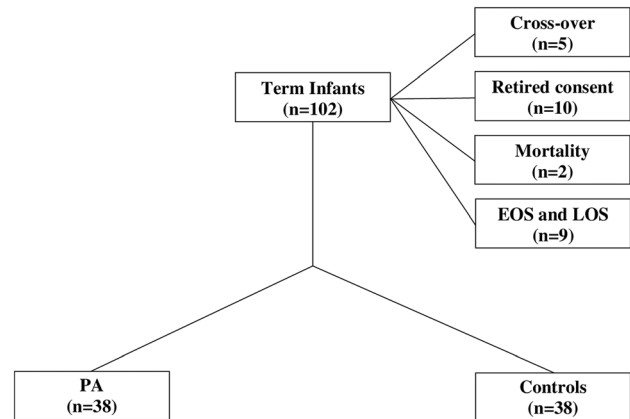

Figure 1: Patients' recruitment flow chart.

and 90th centiles, corrected according to the mother's height, weight, parity, and the sex of the newborn [18].

At birth, both term and preterm newborns showed a normal postnatal neurologic outcome at the 7th day of age and at discharge from hospital fulfilled all of the following criteria: no maternal illness; no signs of fetal distress; pH>7.2 in cord or venous blood; and Apgar scores >7 at 1 and 5 min.

All asphyxiated infants were delivered by emergency cesarean section because of acute fetal distress classified according to the criteria of the American College of Obstetricians and Gynecologists [19]. Asphyxia was defined using the following criteria: Apgar score <5 at 5 min, pH<7.0, base excess ≤−12 mmol/L in cord or venous blood taken within 60 min of birth, the need for resuscitation at birth and/or for positive pressure ventilation (>3 min), and the occurrence of multiorgan failure [19].

Infants fulfilling three or more of the above criteria were included in the asphyxia group, received mechanical ventilation, and were sedated by means of fentanyl citrate (Fentanest; Pharmacia & Upjohn International, Milan, Italy), 0.5–2.5 µg/kg per h, and midazolam hydrochloride (Ipnovel; Roche SA, Fontenay-sous-Bois, France), 50–400 µg/kg per h.

Exclusion criteria were: central nervous system (CNS) malformations, chromosomal abnormalities, congenital heart diseases, multiple pregnancies, congenital infections, chorioamnionitis, maternal drug addiction, hypertension, diabetes, and EOS. Infants with any malformation, cardiac or hemolytic disease were also excluded from the study.

Clinical and laboratory parameters (red blood cell count RBC; hemoglobin blood concentrations, Hb; hematocrit rate, Ht; venous blood pH; partial carbon dioxide venous pressure, pCO_2; partial oxygen venous pressure, pO_2; base excess, BE; CRP, PCT, blood culture, ions, glucose, urea, and creatinine levels) were recorded in all infants on admission to NICU and at 24 h, 72 h, and 96 h from birth. For each enrolled subject at least 1 mL of whole blood was collected in a blood culture bottle (Bactec Pediatrics; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA).

Cerebral monitoring

All infants included in the study underwent neurological examination [20, 21], cerebral ultrasound recordings (CUS) (Acuson 128SP5,

Mountain View, CA, USA), according to standard of care protocols. PA infants underwent cerebral function monitoring recordings (CFM) (CFM Olympic Brainz Monitor, Natus Medical Incorporated, CA, USA) and active cooling according to international guidelines.

P-SEP measurement

Urine samples (100 μL) were collected by a standard urine collector (Pennine Heathcare, London, UK) and measured at first void (time 0, T0), 24 (time 1, T1), 48 (time 2, T2), 96 (time 3, T3) h from birth. In the asphyxiated infants, a catheter was inserted into the bladder for urine sampling because of their critical clinical conditions and the effects of sedative drugs. The studied groups included only infants in whom urination occurred at least three times during the collection times. Cord blood and urine samples were collected at T0 and measured in 52 patients (PA: n=26; controls: n=26) selected without conscious bias.

After collection, urine samples were immediately centrifuged at 900 g for 10 min and stored at −70 °C until assay.

P-SEP urine levels were measured using a chemiluminescent enzyme immunoassay with the point-of-care automated analyzer PATHFAST-TM (Gepa Diagnostics, Milan, Italy) according to the manufacturer's instructions.

Table 1: Perinatal characteristics in term infants complicated by perinatal asphyxia (PA) and healthy controls.

Parameter	PA (n=38)	Controls (n=38)
Maternal age, years	32 (2)	33 (2)
Mode of delivery, n (%)		
Caesarean	38 (100)	8 (21)[a]
Vaginal	0 (0)	30 (79)[a]
GA, weeks	38 (1)	40 (1)
BW, g	3,415 (274)	3,412 (307)
Gende (male/female)	16/22	18/20
Apgar score <7 n (%)		
At 1 min	38 (100)	0 (100)
At 5 min	38 (100)	0 (100)
Respiratory distress syndrome, n (total)	25 (38)	0 (38)[a]
Mechanical ventilation support, n (total)	25 (38)	0 (38)[a]
Inotrope therapy, n (total)	13 (38)	0 (38)[a]
aEEG		
Normal, n (total)	0 (38)	NP
Moderate abnormality, n (total)	7 (38)	NP
Severe abnormality, n (total)	31 (38)	NP
Sarnat		
Stage I, n (total)	0 (38)	NP
Stage II, n (total)	18 (38)	NP
Stage III, n (total)	20 (38)	NP
Neurological examination		
Normal/suspect/abnormal	0/11/27	36/2/0[a]
Cerebral ultrasound (normal/total)	22 (38)	0 (38)[a]

Values are expressed as mean ± SD. [a]p<0.05. n, number; GA, gestational age; BW, birthweight; aEEG, electroencephalography; NP, not performed; PA, perinatal asphyxia.

Statistical analysis

When the Kolmogorov–Smirnov test showed that values were not normally distributed, P-SEP concentrations were expressed as medians and interquartile ranges, and statistical significance of differences evaluated using a non-parametric test. Data for neonatal outcomes and laboratory parameters were analyzed according to Tukey's one-way ANOVA and the two-sided Mann–Whitney U-test. Comparison between proportions was performed using Fisher's exact test. Correlations between P-SEP and kidney function parameters were performed by linear regression analysis. Statistical significance was set at a p<0.05.

Results

In Table 1 perinatal characteristics of the studied groups are reported. As expected, no significant differences (p>0.05, for all) were observed in the groups regarding BW, GA as well as gender. Cesarean section incidence, Apgar scores at 1 and 5 min, the incidence of respiratory distress syndrome, and need for mechanical ventilation and inotropic therapy significantly differed (p<0.05, for all) between groups. Neurological examination, Sarnat score significantly differed (p<0.05, for both). As expected, venous blood pH, pCO_2, pO_2, BE were significantly different (p<0.05, for all) in the two studied groups. No significant differences (p>0.05, for all) were found at birth in RBC, Hb, Ht, blood ion, glucose, CRP and PCT levels, and blood culture (Table 2). Higher urea (p=0.64) and creatinine blood levels (p<0.05) were observed in PA infants although only the latter statistically significant.

Table 2: Laboratory parameters recorded at birth in PA term infants and healthy controls.

Parameter	PA (n=38)	Controls (n=38)
RBC count, ×10^{12}/L	4.0 ± 0.1	3.9 ± 0.2
Hemoglobin, g/L	14.1 ± 0.01	14.0 ± 0.01
Hematocrit rate, %	42.2 ± 0.03	41.9 ± 0.04
Venous blood pH	6.99 ± 0.01	7.34 ± 0.04[a]
pCO_2, mmHg	62.6 ± 1.7	42.3 ± 1.8[a]
pO_2, mmHg	29.1 ± 2.8	46.1 ± 0.8[a]
BE	−14.4 ± 0.1	−0.2 ± 1.3
Positive blood culture, n/total	0/38	0/38
Na^+, mmol/L	136 ± 0.2	138 ± 0.3
K^+, mmol/L	4.1 ± 0.1	4.1 ± 0.2
Ca^{++}, mmol/L	1.13 ± 0.01	1.14 ± 0.02
Urea, mmol/L	16.1 ± 10.3	12.9 ± 2.5
Creatinine, mmol/L	0.1 ± 0.02	0.08 ± 0.01[a]

Values are expressed as mean ± SD. [a]p<0.05. RBC, red cells blood count; pCO_2, partial carbon dioxide venous pressure; pO_2, partial oxygen venous pressure; BE, base excess; PA, perinatal asphyxia.

CRP and PCT measurements

In healthy infants, CRP pattern was characterized by a significant increase from first void (T0) at 24 h (T1), peaking at 48 h (T2) and decreasing at 96 h (T3) from birth (p<0.05, for all).
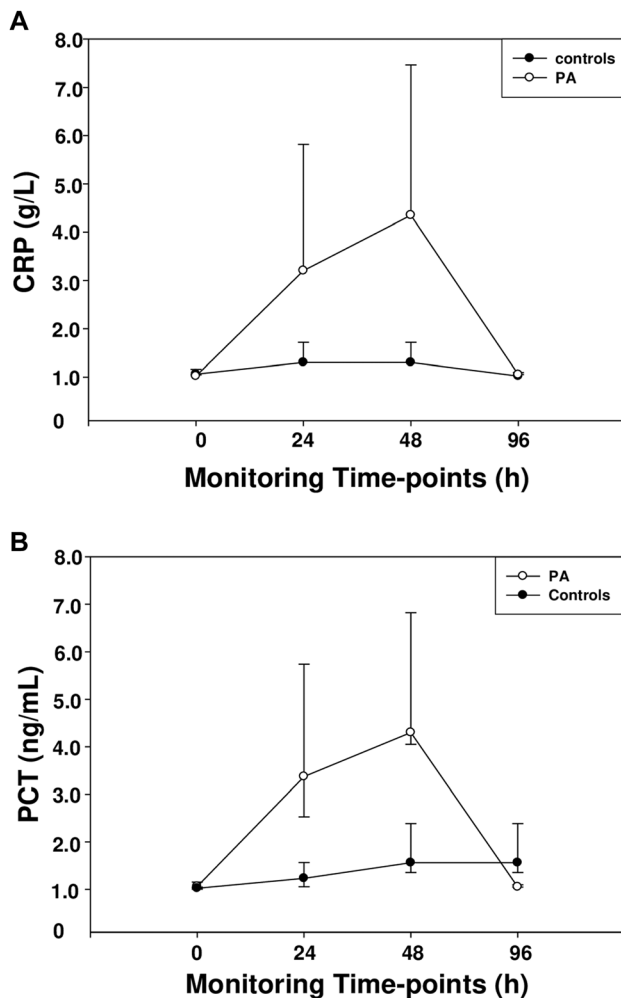

Figure 2: Early onset sepsis (EOS) biomarkers blood levels at different monitoring time-points.
(A) C-reactive protein (CRP) blood levels (g/L) measured at different monitoring time-points at admission to unit, 24, 48, and 96 h from birth in perinatal asphyxia term infants (PA, O) and healthy controls (●). Values are expressed as mean ± SD. CRP was significantly higher (p<0.01, for both) in PA group at 24 and 48 h time-points. No differences (p>0.05, for both) were observed at admission to the unit and at 96 h, respectively. (B) Procalcitonin (PCT) blood levels (ng/mL) measured at different monitoring time-points at admission to unit, 24, 48, and 96 h up to 96 h from birth in perinatal asphyxia term infants (PA, O) and healthy controls (●). Values are expressed as mean ± SD. PCT was significantly higher (p<0.01, for both) in PA group at 24 and 48 h time-points. No differences (p>0.05, for both) were observed at admission to the unit and at 96 h, respectively.

In PA infants, the CRP pattern was characterized by a significant increase at T1, reaching the highest peak at T2 hours and remaining at high levels at T3 (p<0.05, for all). When CRP blood levels were compared between studied groups, higher (p<0.05, for all) CRP levels at T1–T3 and no differences (p>0.05, for all) at T0 were observed, respectively (Figure 2A).

In healthy infants, PCT pattern was characterized by a significant increase from T0 peaking at T1 and decreasing from T2 to T3.

In PA infants, PCT pattern was characterized by a significant increase from T0 reaching the highest peak at T1 hours and remaining at high levels at T3 (p<0.05, for all).

When PCT blood levels were compared between studied groups, higher (p<0.05, for all) PCT levels at T1–T3 and no differences (p>0.05) at T0 were observed, respectively (Figure 2B).

P-SEP measurements

P-SEP levels were measurable in all samples collected. Moreover, in the same 52 patients selected without a conscious bias, a superimposable pattern at T0 (p>0.05, for both) was found when P-SEP cord blood and urine concentrations were measured in PA (PA blood T0: median 971.53 ng/L; 25° centile: 525.12 ng/L; 75° centile 2,200.25 ng/L vs. PA urine T0: median 979.61 ng/L; 25° centile: 531.44 ng/L; 75° centile 2,212.03 ng/L) and healthy infants (controls blood T0: median 613.02 ng/L; 25° centile: 433.27 ng/L; 75° centile 717.11 ng/L vs. controls urine T0: median 619.22 ng/L; 25° centile: 439.37 ng/L; 75° centile 721.04 ng/L), respectively.

In healthy infants, P-SEP pattern was characterized by a decrease from T0, reaching its lowest point at T1 and remaining lower at T2, T3.

In PA infants, P-SEP pattern was characterized by higher (p<0.05, for both) levels at T0 and T1 and by a progressive decrease at T2 and T3 when returned within reference ranges (p>0.05).

When P-SEP was compared between studied groups, higher (p<0.05, for all) P-SEP levels at T0 and T1 and no differences (p>0.05) at T2, T3 were observed, respectively (Figure 3).

To analyze whether in PA infants multiorgan failure, especially of the kidney, could somewhat affect P-SEP levels, we analyzed the correlation between P-SEP and urea and creatinine blood levels at T0 and T1. No significant correlations were found between P-SEP and urea (T0: R=0.11; T1: R=0.14; p>0.05 for both) and creatinine (T0: R=0.02; T1: R=0.12; p>0.05, for both) blood levels, respectively. Furthermore, according to the occurrence or not of
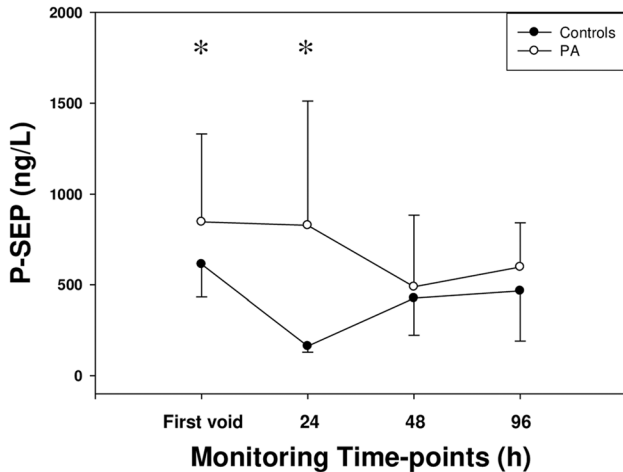
Figure 3: Presepsin (P-SEP) urine levels (ng/L) measured at different monitoring time-points at first void, 24, 48, and 96 h from birth in perinatal asphyxia term infants (PA, O) and healthy controls (●). Values are expressed as mean ± SD. P-SEP was significantly higher (p<0.01, for both) in PA group at first void and at 24 h time-points. No differences (p>0.05, for both) were observed at 48 and 96 h, respectively.

neonatal kidney failure and its main symptoms, we also calculated the P-SEP/urea (P-I) and P-SEP/creatinine (P-C) ratios at T1. No significant differences (p>0.05, for both) were found between groups in P-I and P-C ratio values (data not shown).

Discussion

PA still remains one of the major causes of early neonatal death, multiorgan failure, and short/long-term neurological handicap [22]. Among different PA pathophysiological cascade of events leading to multiorgan failure, to date still controversial and a matter of investigation, the activation of aberrant inflammatory mediators (i.e., cytokines, vascular endothelial growth factors) and innate immunity response appear particularly interesting [23]. Notably, there is evidence that PA can limit the diagnostic accuracy of some biomarkers of sepsis (i.e., CRP, PCT) with a considerable rate of false-positive cases [8, 24]. Therefore, data on the potentially confusing effects of PA on other biomarkers of sepsis such as P-SEP is still lacking.

In the present study we have found that the urine concentrations of a promising biomarker of sepsis, namely P-SEP, increased early in PA infants compared to healthy controls in the absence of any infectious disease. The same pattern was observed in CRP and PCT blood levels although biomarkers concentration remained at high levels up to 72 h from birth. Furthermore, no correlations were shown

between P-SEP and kidney function parameters such as urea and creatinine blood levels.

Results on CRP and PCT are in agreement with previous observations confirming the high rate of false-positives for EOS in PA infants [6–8].

The finding of an early P-SEP increase at first void followed by a return to normal ranges from 24 h onwards deserves further consideration. In particular: i) P-SEP activation to hypoxic insult suggests its involvement in the inflammatory cascade following PA [25]. The issue is corroborated by data *in-vitro* and *in-vivo* showing monocyte/macrophage CD14+ production triggered after a hypoxic-ischemic insult in both animals and humans [26–29]; ii) the fast decline in P-SEP urine levels to normal ranges suggests a more limited PA influence on biomarker levels in biological fluids than CRP and PCT. The explanation lies in a shorter P-SEP half-life (6 h), allowing an early detection of further EOS and LOS in this high-risk group. Although PA infants underwent prophylactic antibiotic treatment, further usefulness of P-SEP measurement may regard the possibility of early treatment suspension once P-SEP levels reached normal ranges; iii) P-SEP urine levels were superimposable to those previously reported in the blood by several authors [30–32]. Results are in agreement with those obtained from other biomarkers of brain development and damage, supporting the notion that multiorgan monitoring in non-invasive biological fluids of high-risk infants is becoming feasible [33, 34].

Overall, it is possible to argue that P-SEP *per-se* and its assessment in a non-invasive biological fluid such as urine is not dependent on PA effects from 24 h of life onwards and constitutes a promising point-of-care biomarker in the management of EOS and LOS. However, as for other biomarkers, several issues will have to be ironed out before P-SEP can be included in daily clinical practice. The main one regards the need for a reference curve in the urine as for blood taking into account other potential bias parameters such as GA, gender, delivery mode etc. [30].

In the present study we have also found that P-SEP urine levels in PA and healthy newborns, as well as for other biomarkers, were not kidney function-dependent, as they were in adults complicated by organ failure [34–38]. In fact, no correlations were found between P-SEP urine levels and laboratory parameters, to date considered to be the standard of care, such as urea and creatinine blood levels, respectively. Of course, further studies correlating trustable parameters of kidney function, such as N-Gal, will be able to empower the present data.

The findings are also corroborated by the absence of any differences between studied groups when data was analyzed after correction for P-SEP/urea or P-SEP/

creatinine ratios. The explanation may lie in different stages of kidney maturation between infants and adult patients as well as in the severity of the disease (PA vs. chronic kidney insufficiency). Another issue regards P-SEP low molecular weight (13 kDa), suggesting that it could be transported from systemic circulation into the urinary tract *via* a passive mechanism like other biomarkers with a low molecular weight [9, 12, 34]. Thus, complications rendering the collection of urine samples more difficult, such as oliguria and renal failure, were not met in our series partly because of the small amount of urine needed for P-SEP measurements (50 µL). Importantly, it must be highlighted that urine samples are much more easily collected than blood or CSF and are thus more convenient in view of the need for repeated sampling when monitoring risky neuroprotective strategies (e.g., mild/deep body core hypothermia): at the same time, improved care of critical newborns is possible, bearing in mind that anemia due to repeated blood sampling is a common pathology in high-risk newborns [39]. In addition, PATHFAST assays for the quantification in the urine of P-SEP are rapid (about 15 min), inexpensive, and simple to perform; they can even be automated.

Last but not least, the present study has the following main limitations: i) the small sample size suggesting the need of a multicenter study in a wider population, and ii) the lack of a P-SEP reference curve in urine fluid. Further investigations in this respect are so justified.

In conclusion, the present data shows that PA effects on P-SEP are limited up to the first 24 h from birth and biomarker levels are not kidney function-dependent. Results open the way to further investigations aimed at validating P-SEP assessment in non-invasive biological fluids as a reliable tool for early EOS and LOS detection in high-risk infants.

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