Umbilical blood biomarkers for predicting early-onset neonatal sepsis

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Background: **Since the 1990s, finding the most efficient markers or combinations as predictors of early-onset neonatal sepsis has been the hot topic of studies. But there is no review of such biomarkers detected in umbilical blood at birth. By comparing clinical values of common inflammatory markers detected in cord blood shortly after birth, in this study we tried to find the most performing one or the most efficient combination that might be potentially used in birth room, as the earliest predictor of early-onset neonatal sepsis.**

Data sources: **We searched PubMed and Elsevier's Web of Science for studies evaluating cord blood inflammatory markers in relation to early-onset neonatal sepsis.**

Results: **Among C-reactive protein (CRP), procalcitonin (PCT), IL-6, IL-8, TNF-α and IL-1β, none of them could be used individually to establish or exclude the diagnosis of early-onset neonatal sepsis, but PCT, IL-6 and IL-8 have great superiority to CRP, TNF-α and IL-1β. When combined with other hematological markers and clinical observation, the clinical reliability of PCT, IL-6 and IL-8 could be improved. Prolonging the sample collection time window seems to have a positive effect on the clinical utility of IL-6 and IL-8.**

Conclusions: **More researches focusing on the combination of different umbilical cord biomarkers in different clinical settings are needed to achieve clearer conclusions. Multi-center, large-sized analysis, especially examining groups of cytokines, is also expected.**

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Review article

Introduction

According to the 2002 International Pediatric Sepsis Consensus Conference, pediatric sepsis is defined as systemic inflammatory response syndrome in the presence of or as a result of suspected or proven infection.[1] But so far, a worldwide agreement on the definition of early-onset neonatal sepsis (EONS) has not been reached. In this review, we aim to integrate the previous literatures investigating biomarkers in cord blood at birth for the immediate diagnosis of EONS. Cord blood is the earliest hematologic sample from the object, which could guide the clinicians to carry out effective therapeutic strategy as soon as possible. Besides, a painless and non-invasive manipulation avoids iatrogenic stress source to vulnerable newborns, which could cause deterioration and possible anemia.

Theoretically, the ideal markers for detecting EONS should have the highest sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and optimal likelihood ratio (LR), which means a LR(+)>10 and LR(-)<0.1, but these requests cannot be met at the same time. In consideration of the possible devastating sequele of missed diagnosis, a maximum sensitivity and NPV should be considered prior to meeting high specificity and PPV. But in terms of the value of performing a diagnostic test, the likelihood ratios, independent of prevalence, are recognized as the best index to determine whether a test result changes the probability that a condition (here, infection) exists.

For each biomarker, we described its kinetic features in response to infection, origin cells, relationship between its initial cord blood concentration and gestational age, its permeable ability to cross the placenta, as well as the statistical value. We also evaluated possible combination of different umbilical blood markers.

Blood biomarkers

C-reactive protein (CRP)

This acute phase protein is often used as a routine infectious marker in the past decade,[2,3] Serum CRP level increases within 6-10 hours in neonates after exposure to infection and peaks at 2-3 days followed by a decrease with favorable evolution.[2,4,5] It is suggested that fetal CRP independent production.[6] Gestational age has a positive effect on CRP concentration at birth and its response extents after birth.[7]

When investigating inflammatory mediators in umbilical plasma drawn immediately after delivery, Døllner et al[5] found that CRP levels were undetectable in nearly all of the neonates, both in infectious and control groups. In another similar investigation, Santana et al,[4] using a more sensitive method allowing a detection limit of 1 mg/dL, found that cord blood CRP levels were low (0.076-0.63 mg/dL) and of no significant difference among infectious, non-infectious and healthy groups.

But in a prospective test run by 197 samples, Joram et al[8] reported the limitation of cord blood CRP as a diagnostic marker for EONS. Irrespective of gestational age, with the cut-off value of 5 mg/L, they received a performance as follows: sensitivity 50%, specificity 97%, PPV 67%, NPV 94%, LR(+) 16.7, and LR(-) 0.51.

Infection may be initiated relatively close to delivery, resulting in low levels of umbilical plasma CRP concentration. In infected infants born with extremely prematurity, CRP responses could be undetectable several days after birth.[9] Cord blood CRP may not increase in presence of umbilical vasculitis which often reflects severe chorioamnionitis with neonatal diseases whereas many other inflammatory markers do.[10] In contrast, elevated cord blood CRP levels were observed in absence of infection with several intrapartum risk factors for infection,[11] and in case of prenatal corticosteroid use,[7] meconium inhalation syndrome and higher birth weight in preterm infants.[12]

CRP has been proved as a "specific" but "late" marker of neonatal infection. Cord blood CRP concentration alone has little utility in EONS diagnosis.

Procalcitonin (PCT)

Firstly demonstrated to increase at the onset of bacterial infection and sepsis by Assicot et al[13] in 1993, this acute phase reactant has the characters of acute phase proteins, hormones and cytokines.[14] Serum PCT concentration raises 2-4 hours after endotoxin injection, reaches its peak level right after 6 hours, maintains a plateau through 8 to 24 hours[15] and decreases to its normal level if the infection stimulus stops. Its half-life time is about 25-30 hours.[3] In an investigation on postnatal physiological fluctuation of PCT in term and preterm cohorts, the serum PCT level increased rapidly after birth, peaked at 24 hours in term babies and a little earlier in preterm ones, decreased gradually by 48 hours until a minimum value appeared at about 80 hours for term ones and 5 days for preterm ones.[7] However, this is contradictory to another investigation suggesting that PCT concentrations decreased with prematurity.[16]

Antenatal inflammation, intracranial hemorrhage, birth asphyxia, respiratory distress syndrome, hypoxemia, hemodynamic failure, pneumothorax, neonatal resuscitation and gestational diabetes can also cause an increased circulating PCT concentration.[17-20] It was demonstrated that maternal venous PCT levels do not correlate with umbilical cord blood concentration in the infected neonates.[21] But in non-infected babies, whether umbilical PCT levels are affected by maternal venous PCT levels remains controversial.[21,22] Reference value in healthy neonates was established for the first time by time-resolved amplified cryptate emission (TRACE) technology in 2007, ranging from 0.04 to 0.43 mg/L.[23] Considering that gestational age has a negative effect on PCT levels at delivery, clinical utility of PCT in the diagnosis of EONS requires the establishment of reference covering a range of both gestational and postnatal ages.[7]

In 2006, Joram et al[8] reported that umbilical blood PCT concentration could serve as a predictive marker for early diagnosis of very early onset neonatal sepsis. In this prospective study, the sample size was relatively small (197 neonates) and the result was inspiring. With the cut-off point being 0.5 μg/L, the sensitivity (0.875), specificity (0.987), PPV (0.875), NPV (0.987), LR(+) (67.3) and LR(-) (0.13) were all high.

However, in a recent report, a monocenter retrospective analysis[17] has shown a different conclusion. This analysis was conduced on a large cohort of newborns with risk factors for EONS. When a cut-off value was 0.6 ng/mL, the sensitivity, specificity, NPV, LR(+), and LR(-) were all high while PPV was only 0.28. In the preterm subgroup, the sensitivity and NPV reached 1.0 but the PPV was still low. The authors verified the ability of PCT to detect non-infected patients among those presenting risk factors but a more certain conclusion claims prospective studies. Its low PPV was explained as the result of impact of perinatal asphyxia and antenatal inflammation which promoted PCT production. Indeed, in the former report, preterm babies occupied only 18% of the objects, but in the latter, premature babies' proportion was 38%. The increased proportion of premature neonates might enhance the bias caused by perinatal asphyxia and antenatal inflammation. Besides, the lower incidence of EONS in the latter study (1.2%) also influenced the

PPV compared with 9.6% in the former study. In the second study, they used TRACE technology assay with the KRYPTOR automated analyzer. Fifty μL of plasma was needed and the whole process from blood-drawn to turn-over of result was about 1.5 hours.

In a prospective study investigating new approaches to better predict EONS, with a sample-size of 286, when the authors used cord blood PCT as the only predictive factor, the result was similar to that in Joram's study (Table 1).[8,17,24] Kordek et al[24] attempted to create a model, considering the complete set of information about maternal and perinatal factors, physical exam results, postpartum adaptation, neonatal clinical status and laboratory tests results. The model was anticipated to reach the most satisfactory performance. But the final model was not ideal: based on cord blood PCT and CRP concentration, the composite of 7 biochemical and clinical variables including tocolysis, nutritional status of newborns, Apgar score, neutrophil ratio and red blood cell count in neonatal venous blood, has shown a sensitivity of 0.91, a specificity of 0.90, PPV 0.64, NPV 0.98, LR(+) 9.13, and LR(-) 0.1.[24] However, this method seems to be able to compensate the low PPV.

A mono-center prospective test, as part of a randomized-controlled international multi-center intervention study, showed that a seriated PCT-based algorithm allowed to shorten the duration of antibiotic therapy in term and near-term newborns with suspected EONS, but the blood sources were not limited to cord blood and the collecting time of the first sample of each object was not enforced immediately after birth when the suspicion of EONS was established.[20,25]

PCT has been demonstrated as a more sensitive marker than CRP and cytokines for EONS patients. Focusing on its concentration in umbilical cord blood before physiological increase makes interpretation of its diagnostic value easier. Combination with other hematological markers and clinical observation improves its clinical reliability. Its eventual ability of discriminating non-infected infants from those presenting infection risk factors may contribute to reducing unnecessary antibiotic use although there is another article that precluded the use of PCT concentration in the decision of whether to start antibiotic therapy at birth.[16] Serial PCT measurements might serve as a reference index of antibiotic use.

Table 1. Diagnostic value of cord blood procalcitonin in early-onset neonatal sepsis

Cut-off point	Sample size	Sensitivity	Specificity	PPV	NPV	LR(+)	LR(-)	Methods	Authors
0.5 μg/L	197	0.88	0.99	0.88	0.99	67.32	0.13	I	Joram et al, 2006[8]
0.6 ng/mL	2151	0.92	0.97	0.28	0.99	32	0.08	T	Joram et al, 2011[17]
1.22 ng/mL	286	0.80	0.71	0.35	0.95	2.84	0.28	Q	Kordek et al, 2008[24]

I: immuno-chromatographic semiquantitative test; T: time-resolved amplified cryptate emission technology; Q: quantitative immunolunometric method. PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

Table 2. Diagnostic value of cord blood cytokines in early-onset neonatal sepsis

Cytokines	Sample size	Sensitivity	Specificity	PPV	NPV	LR(+)	LR(-)	Methods	Author
TNF-α (13 pg/mL)	94	0.75	0.88	0.67	0.51	6.25	0.28	D	Berner et al, 1998[26]
TNF-α	31	No significant relationship between I/NI/NIH groups						ELISA	Santana et al, 2001[4]
TNF-α (15 pg/mL)	110	0.78	0.41	-	-	1.32	0.53	RIA	Kowalik et al, 2003[27]
IL-1β (10 pg/mL)	94	0.83	0.86	0.71	0.94	5.92	0.20	D	Berner et al, 1998[26]
IL-1β (6.2 pg/mL)	24	0.74	0.70	-	-	2.47	0.37	ELISA	Døllner et al, 2001[5]
IL-1β	31	No significant relationship between I/NI/NIH groups						ELISA	Santana et al, 2001[4]
IL-6 (100 pg/mL)	94	0.87	0.93	0.76	0.97	12.42	0.14	D	Berner et al, 1998[26]
IL-6 (33.0 pg/mL)	24	0.84	0.70	-	-	2.80	0.23	ELISA	Døllner et al, 2001[5]
IL-6 (100.8 pg/mL)	31	0.50	0.87	0.31	0.66	3.85	0.57	Cs	Santana et al, 2001[4]
IL-6 (80 pg/mL)	100	0.96	0.95	-	-	19.20	0.04	C	Krueger et al, 2001[28]
IL-6 (39 pg/mL)	70	1.00	0.81	0.37	1.00	5.26	0.00	E	Tasci et al, 2005[29]
IL-8 (300 pg/mL)	94	0.91	0.93	0.91	0.97	13.00	0.10	D	Berner et al, 1998[26]
IL-8 (50.7 pg/mL)	24	0.81	0.77	-	-	3.52	0.25	ELISA	Døllner et al, 2001[5]
IL-8 (111.7 pg/mL)	31	0.78	0.91	1.00	0.84	8.67	0.24	Cs	Santana et al, 2001[4]
IL-8 (90 pg/mL)	100	0.87	0.94			14.50	0.14	C	Krueger et al, 2001[28]

The values in bracket in the first column are the cut-off values considered in the study. I/NI/NIH: infection/non-infectious/non-infectious healthy; D: double sandwich enzyme immunoassay technique; Cs: chemiluminescent enzyme immunoassay in solid phase; C: random access chemiluminescence assay; E: ensyme-linked immunosorbent assay.

Cytokines

Studies focusing on the diagnostic value of cord blood cytokines are listed in Table 2.[4,5,26-29] All these studies are prospective.

IL-6

IL-6 is the most often studied cytokine in neonatal population. IL-6 is a cytokine of the early host response to infection, preceding the increase of CRP and after the release of TNF-α. It is synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, deciduas, chorion, amnion and trophoblastic cells shortly after stimulus of microbial products.[29] *In vivo* experiments have shown that the release, peak response and declination to baseline of IL-6 was observed at 1-2 hours, 3 hours and 8 hours, respectively, after lipopolysaccharide (LPS) injection.[30] The half-life time is considered to be around 100 minutes[31] and its circulating concentration can drop precipitously following antimicrobial treatment.[18]

Both maternal and fetal contributions are involved in increased umbilical cord blood IL-6 concentration.[32] IL-6 levels are negatively correlated with gestational age and the kinetics over the first 48 hours after delivery is different between healthy term and near-term neonates.[33] Delivery mode has no impact on its initial concentration in cord blood.[34]

Cord blood IL-6 levels as a diagnostic marker for EONS have been investigated in the past decade. For premature babies, with cord blood drawn immediately after birth, Døllner et al[5] found IL-6's significant diagnostic value but the number of analyzed babies was limited. When the sample collection time was prolonged within 48 hours after birth, a much more inspiring result was obtained in a later research with a larger sample size.[28] When preterm and term neonates were analyzed together, IL-6 detected in cord blood obtained at birth[4] has a poorer performance than postponing the collection time within 15 minutes after birth.[26] When detected in cord blood in term infants without risk factors, IL-6 did not show significant clinical utility to differentiate infected and healthy newborns,[5] but in neonates born with premature rupture of membranes, IL-6 showed high sensitivity and specificity in predicting positive cord blood culture and funisitis.[29]

The undetectable cytokine response in term infected babies in Døllner et al's test was considered as a result of different infection time from preterm individuals: the former may be exposed to bacteria possibly during the passage of the birth canal, later than the latter, often pendant intrauterine life.[5] This is not contradictory with Tasci et al's investigation, in which the term newborns were exposed to extra-membrane environment for a relatively long time.[29] The difference between experiments carried out on cord blood at birth and cord blood drawn at a relatively long time after birth is possibly because of IL-6's kinetics. Another study proved that sample collection timing is an important factor for detection of high plasma IL-6 level in newborns with EONS.[35] Unfortunately, both the investigations of Santana[4] and Døllner[5] were small sized, so we could not tell whether the better results were caused by postponing the collection time. An investigation suggested that the association of cord blood IL-6 at birth and CRP at 24 hours of life could exclude neonatal bacterial infection,[36] but we did not find this combination reported in any research for EONS.

Many other physiological and pathological factors, such as perinatal asphyxia, meconium aspiration syndrome, even the delivery itself, as a source of inflammation, can augment umbilical plasma IL-6 level in the absence of neonatal infection.[37] This characteristic is responsible for its low PPV when detected in cord blood drawn at delivery. When we used IL-6 with clinical risk factors such as prematurity and premature rupture of membrane, its utility could be improved.

The needed plasma volume is more than 50 μL, and the turn-over time was about 80 minutes.[4,26,36]

IL-8

IL-8 belongs to the class of proinflammatory chemokines. Placental cells, fetal monocytes/macrophages and endothelial cells are able to produce IL-8 after an infectious process originated in the uterus. After endotoxin injection, serum IL-8 level increases at about 90 minutes, and peaks at around 120 minutes.[38] For septic neonates, the circulating concentration decreases significantly 48 hours after birth with regard to that in the initial cord blood, similar to the kinetics of IL-6.[26]

Gestational age has little effect on the cord blood IL-8 concentration. Only in very preterm (≤32 weeks' gestation) neonates it can increase IL-8 level.[39,40] Delivery modes have independent effect on its concentration in whole blood detection but not in serum.[40] Postnatal "physiological" increase, as part of stress response, is not evident for IL-8, which was in accordance with the observation of Dembinski et al,[40] which reported that IL-8 was undetectable in cord blood by investigating a large proportion of healthy newborns.

IL-8 is also widely studied over the past years as a predictive biomarker for EONS (Table 2).[4,5,26,28] Døllner et al[5] reported that a significant initial elevation of IL-8 in cord blood collected immediately at birth was observed in infected preterm neonates but not in term infants, with the same explanation as for IL-6. Similarly to IL-6, when postponing the sample collection time

by 48 hours after birth, Krueger et al[28] got a better result for premature babies than in Døllner et al's study. Comparison between investigations of Santana[4] and Berner et al[26] also suggested that prolonging sample collection time window may contribute to a better clinical value for IL-8 in predicting EONS. Its PPV is obviously higher than PCT and other cytokines discussed in this review. IL-8 combined with CRP detected in blood obtained in initial suspicion of infection appeared to be able to decrease unnecessary antibiotic treatment for newborns;[41] however, when detecting cord blood, Santana et al[4] concluded that the combined use of IL-8 with other cytokines or CRP or leucocyte values could not improve its diagnostic capability. But as mentioned above, Santana's investigation was small sized. More convictive studies are hence needed.

To realize a detection of IL-8, 50 to 100 μL of plasma is needed and the turn-over time was about 40 minutes.[26,36]

TNF-α

TNF-α is a proximal mediator in response to infection, initiating the inflammatory cascade. Macrophages and monocytes are believed to be the main cellular source of TNF-α.[31] The release of TNF-α could take place as early as 30 minutes after LPS injection and the circulating concentration reaches the peak level at around 1.5 hours.[30] Its half-life time is estimated to be around 70 minutes, relatively short.[31]

TNF-α levels are demonstrated to decrease with advancing gestational age,[39] but there are also contradictory results which proved no difference with gestational age in healthy preterm infants.[42] Its initial concentration is affected neither by mode of delivery nor by maternal serum level.[34] This confirms that TNF-α does not traverse the placenta tissue during delivery.[43]

Berner et al[26] have shown its low sensitivity and specificity to distinguish EONS patients from healthy ones, which accorded with the result of Kowalik et al's[27] prospective survey of 110 newborns (Table 2). Even worse, in a report published in 2001, Santana et al[4] demonstrated that TNF-α was not different significantly between sick and healthy babies.

The disparity of studies could be due to its kinetics which has not been completely understood in the early period of life and the inhibitory power of IL-6.[4] Besides, because of its short half-life time, short-term concentration and its conjunction with the soluble receptor, TNF-α is sometimes hard to be precisely detected.[32] Thus, its reliability as a diagnostic marker is limited. Although the review of Pickler et al[44] revealed that TNF-α, as well as the IL-6 and IL-1 family, is most frequently linked with neonatal sepsis, they also confirmed its poor sensitivity and specificity for differentiating sepsis in infected neonates.

IL-1β

In the cascade of inflammatory response, IL-1β as well as TNF-α induces the release of IL-6 by endothelial cells.[31] Other cytokines such as TNF-α can also mediate the production of IL-1β, thus it is hypothesized that two phases of release of IL-1β exist.[45] The central nervous system, especially the hypothalamus, is a site of IL-1β production. It was reported that bacterial endotoxin, administered *in vivo*, evoked increases in hypothalamic IL-1β levels which were significant within 1 hour, and reached maximum levels at 5-10 hours.[46,47] Its initial values in cord blood are dependent on the mode of delivery[34] and whether the gestational age has impact on its concentrations in cord blood remains controversial.[4,39,48] Although Aaltonen et al[43] found no evidence of its passage across term healthy placenta, there is a significant correlation between maternal serum and umbilical cord levels.[34]

Cord blood L-1β showed a low clinical value in predicting EONS in the studies carried out either on premature babies[5] or symptomatic term babies, even when the sample collection time was postponed to 15 minutes after birth.[26] In Santana et al's study,[4] IL-1β, similarly to TNF-α, had no statistical signification between infected/non-infected sick/non-infected healthy groups. Of note, Atici et al[48] in 1996 reported decreasing concentrations of IL-1β were in association with neonatal sepsis.

Because of its kinetics, not completely understood during the early period of life, its susceptibility to maternal circulating levels in parturition and environmental changes, its interference with TNF-α, and IL-6's inhibitory power on IL-1β,[4,45] IL-1β has limited value in diagnosing EONS.

Other biomarkers

Cord blood levels of mid-regional pro-atrial natriuretic peptide (MR-proANP), mid-regional pro-adrenomedullin (MR-proADM) and C-terminal pro-endothelin-1 (CT-proET-1), as important vasoactive and natriuretic mediators in circulatory transition from fetal to neonatal life, have shown promising values in distinguishing patients with EONS from those with risk factors but without real sepsis evolution. Their reference values for term healthy newborns were also established as a starting point for further investigations.[49,50]

Umbilical levels of thiobarbituric acid reactive substances (TBARS), as an oxidative parameter, could significantly increase in infants with neonatal sepsis. In

the same report, compared with IL-6, IL-10 and another oxidative stress marker, only TBARS levels were independently related to the development of neonatal sepsis.[51] More detailed studies are expected.

Among cell surface markers, neutrophil CD11b and CD64 are shown to be the most promising markers in diagnosing both early and late-onset neonatal sepsis.[52,53] Investigations on their concentration in cord blood at birth with relevance to the diagnose of EONS are needed.

The level of inter-alpha inhibitor proteins (IaIp) is also able to predict neonatal sepsis.[54] Besides, IaIp may offer potential benefits to the treatment of neonatal sepsis.[55] But its diagnostic value in umbilical cord blood has not been explored.

Conclusion

Cord blood CRP alone has little utility in EONS diagnosis. As for PCT, IL-6, IL-8, TNF-α, and IL-1β, none of them could be used individually to establish or exclude the diagnosis of EONS but PCT, IL-6 and IL-8 have great superiority to TNF-α and IL-1β. Both PCT and IL-6 have low PPV while IL-8 seems not to have this inferiority. PCT has a stable high sensitivity with regard to IL-6 and IL-8.

When combined with other hematological markers and clinical observation, the clinical reliability of PCT could be improved. Serial PCT measurements in the first days of life might serve as a reference index of antibiotic use.

For both IL-6 and IL-8, combined use with clinical risk factors such as prematurity and premature rupture of membrane could improve their diagnostic value. Prolonging the sample collection time window seems to have a positive effect on their clinical utility. CRP combined with either of them appears to reduce unnecessary antibiotic use in the first period of life. Whether IL-8's diagnostic capacity can be enhanced by combining CRP or other cytokines remains controversial.

With regard to blood sample volume, PCT, IL-6 and IL-8 have almost an equal demand. The turn-over time of IL-6 is the shortest.

Since each inflammatory marker has its own triggering time, peak time and half-life, the optimal obtaining time window for each marker is different. Besides, gestational age dependent immune maturity degree characterizes different gestational age categories, with various kinetics patterns after birth. These elements decide that single infectious marker can hardly be of perfect diagnostic value.

Hence, more studies focusing on the combination of different umbilical cord biomarkers in different clinical settings are needed to achieve a clearer conclusion. For cytokines, all of the studies on this topic we reviewed are small sized. Multi-center, large sized analysis, especially examining groups of cytokines, is also expected. Meanwhile, gestational age dependent references for each marker are also claimed.

In addition, new umbilical biomarkers such as MR-proANP, MR-proADM, CT-proET-1 and TBARS showed potential ability to diagnose EONS. Cell surface markers and IaIp detected in cord blood could also be investigated in further studies.

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