Cytokine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Review Article

Fetal and early neonatal interleukin-6 response

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ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form 23 March 2015
Accepted 24 March 2015
Available online xxxx

Keywords:
Interleukin-6
Fetal inflammatory response syndrome
Early-onset neonatal sepsis
Reference intervals
Diagnostic accuracy

ABSTRACT

In 1998, a systemic fetal cytokine response, defined as a plasma interleukin-6 (IL-6) value above 11 pg/mL, was reported to be a major independent risk factor for the subsequent development of neonatal morbid events even after adjustments for gestational age and other confounders. Since then, the body of literature investigating the use of blood concentrations of IL-6 as a hallmark of the fetal inflammatory response syndrome (FIRS), a diagnostic marker of early-onset neonatal sepsis (EONS) and a risk predictor of white matter injury (WMI), has grown rapidly. In this article, we critically review: IL-6 biological functions; current evidence on the association between IL-6, preterm birth, FIRS and EONS; IL-6 reference intervals and dynamics in the early neonatal period; IL-6 response during the immediate postnatal period and perinatal confounders; accuracy and completeness of IL-6 diagnostic studies for EONS (according to the Standards for Reporting of Diagnostic Accuracy statement); and recent breakthroughs in the association between fetal blood IL-6, EONS, and WMI.

1. Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine that is produced by a variety of cells in response to infection and tissue injury. In the last two decades, the body of literature concerning the use of blood concentrations of IL-6 as a hallmark of the fetal inflammatory response syndrome (FIRS), a diagnostic marker of early-onset neonatal sepsis (EONS) [1] and a risk predictor of white matter injury (WMI) has grown rapidly, leading to improved understanding as well as new questions about the role of this cytokine in the perinatal period. In this article, we comprehensively review: (1) IL-6 biological functions; (2) current evidence on the association between IL-6, preterm birth, FIRS and EONS; (3) IL-6 reference intervals and dynamics in the early neonatal period; (4) IL-6 response during the immediate postnatal period and perinatal confounders; (5) accuracy and completeness of IL-6 diagnostic studies for EONS [including the design, conduct, analysis and results of such studies according to the Standards for Reporting of Diagnostic Accuracy (STARD) statement]; and (6) recent breakthroughs in the association between fetal blood IL-6, EONS, and brain injury.

2. IL-6 biological functions and signaling pathways

Systemic inflammatory response syndrome, which is characterized by an excessive proinflammatory response, is a hallmark of sepsis [2]. Many proinflammatory cytokines including IL-6 have been implicated in the pathogenesis of sepsis [2]. Until recently, it was not known how the cytokine-driven inflammatory process is initiated. It is now known that microorganisms interact with a family of toll-like receptors (TLRs) belonging to the innate immune system, which trigger intracellular activation of nuclear factor kappa B and various kinases, leading to the production and release of cytokines [3]. Among these, IL-6 has been reported to be produced in the early phase of infectious inflammation by monocytes and macrophages immediately after the stimulation of TLRs, with distinct pathogen-associated molecular patterns [4]. In

Abbreviations: AUC, area under the curve; CI, confidence interval; CRP, C reactive protein; EONS, early-onset neonatal sepsis; FIRS, fetal inflammatory response syndrome; gp130, glycoprotein 130; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; NICU, neonatal intensive care unit; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PPROM, preterm premature rupture of the membranes; PVL, periventricular leukomalacia; sgp130, soluble glycoprotein 130; sIL6-R, soluble interleukin-6 receptor; STARD, Standards for Reporting of Diagnostic Accuracy; TLRs, Toll-like receptors; WMI, white matter injury.

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http://dx.doi.org/10.1016/j.cyto.2015.03.015
1043-4666/

noninfectious inflammatory conditions, such as tissue injury, damage-associated molecular patterns from damaged or dying cells stimulate TLRs to produce IL-6 [5]. This acute IL-6 expression plays a central role in the body's defense against infection or injury by stimulating various cell populations [6].

IL-6 is produced by various cells, such as T-cells, B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, adipocytes, and some tumour cells [7]. While serum concentrations of IL-6 in healthy donors are hardly detectable or in the low pg/mL range [8,9], during an inflammatory episode IL-6 is highly expressed, and circulating levels can rise dramatically from 1–5 pg/mL to several μg/mL in extreme cases [10,11]. Consequently, IL-6 is one of the most highly expressed mediators of inflammation [11].

IL-6 is a multifunctional cytokine involved in regulating the immune response, hematopoiesis, the acute phase response and inflammation. Its many biologic activities are at the root of its pathogenic properties. IL-6 engages in two distinct downstream signaling pathways to achieve these activities: classic and trans-signaling.

In classic signaling, IL-6 first binds to its specific membrane-bound α–receptor (IL-6R), which in turn associates with and activates the signal-transducing β-receptor chain gp130. Whereas gp130 is expressed by most, if not all, cells in the body [12], IL-6R is only expressed on a limited number of cell types including epathocytes, megakaryocytes and some leukocytes, namely monocytes, macrophages, B cells and subtypes of T cells [12,13]. In hepatocytes, IL-6R expression is essential for the production of acute phase response proteins including C reactive protein(CRP) and fibrinogen. Being transiently expressed on lymphocytes, macrophages, and megakariocytes, the IL-6R also orchestrates phases of the immune response [12]. All other cells, which do not express membrane-bound IL-6R, obtain IL-6 signals by trans-signaling. IL-6 binds to the soluble form of IL-6R (sIL-6R), and this complex not only protects IL-6 and prolongs its circulating half-life [14], but also acts as an agonist capable of directly activating many cell types via the ubiquitously expressed gp130 in a process termed trans-signaling [11]. It has been shown that sIL-6R strongly sensitizes target cells [14]. Embryonic stem cells, early hematopoietic progenitor cells, T cells, many neural cells, smooth muscle cells, mesothelial cells, and endothelial cells, among others, are only responsive to IL-6 in the presence of sIL-6R [12]. sIL-6R is present in the sera of healthy subjects at high concentrations (25–145 ng/mL), and these levels increase during inflammation [15–17]. sIL-6R in humans is generated through differential mRNA splicing but primarily through proteolytic cleavage and subsequent shedding of membrane-bound IL-6R [18,19]. In contrast to classic signaling, comprising the anti-inflammatory or regenerative activities of IL-6, evidence suggests that IL-6 trans-signaling via soluble IL-6R accounts for the proinflammatory properties of IL-6 [20]. In fact, while IL-6 classic signaling plays a role in developmental processes, tissue homeostasis, and acute phase response [21], trans-signaling is involved in many processes that are important in sepsis: activation of endothelial cells and smooth muscle cells [22,23], mononuclear cell recruitment [24], and apoptosis of neutrophils and T cells as well as the expression of chemokines [12,25,26].

Since the cellular responses to the IL-6/sIL-6R complex can be dramatic, ranging from the induction of hepatocyte proliferation to a massive increase in hematopoiesis [27,28], there must be a "buffer" acting as a control mechanism to prevent excessive IL-6 trans-signaling [8]. The soluble form of gp130 (sgp130), which is found at concentrations between 100 and 400 ng/mL in the sera of healthy humans [17], has been suggested to be the natural antagonist of the IL-6/sIL-6R complex *in vivo* [21], probably to prevent systemic IL-6 trans-signaling during inflammatory diseases [29].

The concentrations of IL-6, sIL-6R and sgp130 have to be considered. Under steady state conditions, levels of sIL-6R and sgp130 are roughly 1000 times higher than IL-6 levels [8]. However, during inflammatory conditions, IL-6 levels can increase up to a million-fold [10], whereas serum concentrations of sIL-6R and sgp130 show much smaller differences between healthy and diseased and in most cases do not rise by more than a factor of 2 [15]. These concentrations imply that IL-6, once secreted, will bind to sIL-6R in the serum and this complex will associate with sgp130, and thereby be neutralized [8]. Only when IL-6 levels exceed the levels of sIL-6R and sgp130, IL-6 can act systematically-as seen under septic conditions [10]. Because IL-6 alone does not interact with sgp130, with a molar excess of IL-6 over sIL-6R, sgp130 would only be able to block trans-signaling because free IL-6 will not, or will only, be partially trapped in IL-6/sIL-6R/sgp130 complexes. In contrast, under physiologic conditions, IL-6 is thought to act in a paracrine fashion [30]. Physiological conditions are a molar excess of serum sIL-6R over IL-6 characterized by free IL-6 and IL-6 found in IL-6/sIL-6R complexes. Under these conditions, the sgp130 protein can block both classic and trans-signaling because sgp130 would be able to trap all the free IL-6 molecules in IL-6/sIL-6R/sgp130 complexes [21].

3. Preterm birth and interleukin-6

Subclinical intrauterine infection and/or inflammation, represented by chorioamnionitis, is the most firmly established trigger of preterm delivery [31–33]. The etiology of preterm birth is complex but previous data indicate that chorioamnionitis induces an intra-amniotic inflammatory response involving the activation of a number of cytokines and chemokines which, in turn, may trigger preterm contractions, cervical ripening and rupture of the membranes [34]. Numerous studies have found that IL-6 is the most predictive and best candidate as a diagnostic tool for detecting pre-clinical chrioamnion inflammation and intra-amniotic inflammation leading to preterm birth [35–40]. Elevated levels of IL-6 in mid-trimester amniotic fluid have been associated with increased risk for spontaneous preterm birth (before 32 weeks), acute chorioamnionitis and funisitis [41]. In the recent Pregnancy Outcomes and Community Health cohort study, IL-6 levels in vaginal fluid at midtrimester had the greatest sensitivity for detecting spontaneous delivery at <35 weeks' gestation and preterm delivery accompanied by chorioamnionitis [42]. Studies in tissue extracts from term and preterm deliveries have shown that IL-6 concentrations in the extraplacental membranes are modestly elevated with spontaneous term labor, but are dramatically elevated in preterm deliveries [43].

IL-6 is a proinflammatory cytokine known to be expressed by stimulated leukocytes [44]. In the tissues from preterm deliveries (with and without intrauterine infection), IL-6 concentrations correlate with the extent of leukocyte infiltration [45]. At the inflammatory site, leukocytes (predominantly neutrophils and macrophages) are recruited into the gestational membranes and activated, and eventually secrete proinflammatory cytokines including IL-6 [45,46]. Neutrophil infiltration to the chorionic plate, choriodecidua, and umbilical cord can be measured through IL-6 concentrations of the amniotic fluid and cord blood [47]. The association between high IL-6 levels in the amniotic fluid and pregnancies complicated by premature rupture of membranes is especially strong when infection is present [48,49]. Elevated maternal serum IL-6 concentrations have been reported to be diagnostic for intrauterine infection-associated preterm labor, giving indirect evidence of a placental involvement in some instances of infection-associated preterm deliveries [45]. However, a decidual, or even cervical, source of this cytokine cannot be discounted [45].

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The human amnion, choriodecidua, and fetus have been proposed as potential sites of IL-6 synthesis and probable sources of IL-6 in the amniotic fluid [38,50,51]. Therefore, a relevant question is whether targeting amniotic fluid IL-6 signaling could be a promising option for the prevention of preterm delivery [31,38]. In a recent study, Lee et al. suggested that the IL-6 trans-signaling pathway plays a critical role in preterm birth and premature rupture of membranes by demonstrating an increase in amniotic fluid concentration of IL-6 and sIL-6R in women with intra-amniotic inflammation, and a decrease in the amniotic fluid concentration of sgp130 in women with preterm premature rupture of membranes (PPROM) [38]. In the same article, Lee et al. also showed that in physiologic gestations, amniotic fluid levels of sgp130 decline with gestational age [38]. These findings suggest that targeting the IL-6 trans-signaling pathway may prevent complications such as premature rupture of membranes and preterm birth.

4. Fetal inflammatory response, early-onset neonatal sepsis, and interleukin-6

FIRS is a systemic activation of the fetal innate immune system and was originally reported in pregnancies complicated by preterm labor (with intact membranes) and PPROM [52,53]. Affected fetuses had evidence of multisystemic involvement; they had a higher rate of adverse neonatal morbidity after adjustment for gestational age, and had a higher risk of subsequent spontaneous preterm delivery in cases of PPROM [53,54].

FIRS was originally defined as a level of IL-6 >11 pg/mL in samples of fetal plasma obtained by cordocentesis [52,53]. Following this precept, Hofer et al. have recently demonstrated that in preterm neonates FIRS is an independent predictor of adverse neonatal outcome and some primary outcome parameters including EONS [55]. In an observational study of 176 consecutive preterm neonates admitted to the neonatal intensive care unit (NICU) [62 (35%) with FIRS (cord blood IL-6 > 11 pg/mL), and 114 without], Hofer et al. [55] demonstrated that FIRS was significantly associated with EONS [odds ratio 10.26, 95% confidence interval (CI) 2.95–35.68; $P < 0.001$)] independently of gestational age and idiopathic respiratory distress syndrome. The receiver operating characteristic curve analysis for cord blood IL-6 showed an area under the curve (AUC) 0.75 (95% CI 0.68–0.84; $P < 0.001$) for the prediction of overall adverse neonatal outcome, and specifically an AUC 0.80 (95% CI 0.70–0.90; $P < 0.001$) for the prediction of EONS [55].

In view of the clinical importance of FIRS (defined by a fetal blood IL-6 concentration) as a predictor for morbidity in preterm neonates, a major topic to be addressed is whether the level of IL-6 depends on the severity of intra-amniotic inflammation. Also the paper by Buhimschi et al. [56] may be of great interest. This addressed the issue in a prospective observational cohort involving 132 consecutive mothers [median gestational age (interquartile range): 29.6 (24.1–33.1) weeks] who had clinically indicated amniocentesis to rule out infection. The intensity of intra-amniotic inflammation was graded on the presence of four inflammatory proteomic biomarkers (neutrophil defensins 2 and 1 and calgranulins C and A) in the amniotic fluid that have been previously [57,58] reported as highly predictive of histologic chorioamnionitis, funisitis and EONS. The three grades used were: none of the 4 biomarkers, "no inflammation"; 1-2 biomarkers, "minimal inflammation"; and 3-4 biomarkers," severe inflammation". Results showed that neonates with severe intra-amniotic inflammation had significantly elevated cord blood IL-6 levels but not those delivered by women with no or minimal inflammation. Because the level of IL-6 was highly associated with the degree of placental histological chorioamnionitis, one can conclude that at the stage of no or minimal amniotic fluid inflammation, the fetus is usually protected. However, detection of a positive blood culture and umbilical cord IL-6 levels well above the mean in several neonates delivered by women with no or minimal intra-amniotic inflammation [56], suggested that the hematogenic infection of the human fetus and increasing fetal inflammatory response, in the absence of concurrent amniotic fluid colonization and inflammation, are possible. Thus, the early and accurate recognition of neonates who have already had *in utero* a robust inflammatory response in the context of minimal intra-amniotic inflammation remains a major clinical concern. Additional studies of the genetic and perinatal factors accounting for the differential maternal and fetal inflammatory response are needed.

5. IL-6 reference intervals and dynamics in the early postnatal period

As a prerequisite for analyzing the IL-6 neonatal response associated with early-onset sepsis, there is a need to establish the IL-6 reference intervals and dynamics in the healthy neonate during the early postnatal period, counterbalanced by a greater awareness of the potential confounding factors that may affect them.

In a longitudinal study to assess upper reference limits for IL-6 in 148 healthy babies (113 term, 35 near-term) during the early postnatal period, Chiesa et al. [59] showed that gestational age was negatively associated with IL-6 values obtained at three fixed neonatal ages (0, 24, and 48 h after birth). In fact, the geometric mean IL-6 concentration in the healthy near-term babies at birth and at 24 and 48 h of life was 6.40 (95% CI 3.61–11.50; $P < 0.0001$), 2.38 (95% CI 1.41–3.99; $P = 0.001$), and 2.40 (95% CI 1.49 –3.88; $P < 0.001$) times higher, respectively, than the concentration observed in the healthy term babies [59]. They also showed that the kinetics of IL-6 during the first 48 h of life in healthy infants, are different in the near-term infant compared with the term neonate, suggesting different physiologic processes [59]. Among the term babies, the geometric mean IL-6 values were significantly lower at birth [1.69 pg/mL (95% CI 1.28–2.23)] than at 24 h [4.09 (3.13–5.33)] of life, with no significant change from 24 to 48 h [3.45(2.70–4.43)] of life. In the term neonate, the surge of IL-6 at 24 h of age probably reflects a physiological stress reaction induced at birth. Similar data regarding IL-6 dynamics in healthy term neonates have also been reported by other investigators [60]. In contrast, within the subgroup of near-term babies, the geometric mean IL-6 concentrations were already increased at the time of birth [10.9 (6.53–18.4)] being not significantly different from those found at 24 [9.3 (6.2–14.1)] and 48 h [8.4 (5.97–11.9)] of life, therefore suggesting that a physiological stress reaction had already begun before birth. Taken together, these data demonstrate the effects of development on IL-6 reference intervals and dynamics during the 48-h period after birth. Different gestational age- and postnatal-dependent physiologic processes may be potential confounding factors when interpreting IL-6 reference intervals and dynamics throughout the early postnatal period.

In view of the latest advances in neonatal care, these reference intervals derived from only 35 near-term and 113 term newborns need additional refinement in a larger sample of healthy neonates including, in particular, those born before 35 weeks of gestation, that is, those that now populate most NICUs [61].

6. Studies of IL-6 diagnostic accuracy for early-onset neonatal sepsis: quality of reporting

6.1. Data sources

We systematically reviewed PubMed, Scopus, and the Cochrane Library databases up to June 1, 2014. The PubMed combined search

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term used was: (Interleukin-6 OR IL-6) AND (neonatal sepsis OR neonatal infection OR sepsis). The search terms applied to the Scopus and the Cochrane Library were "Interleukin-6 and sepsis" and "Interleukin-6", respectively.

6.2. Study selection criteria

Articles were eligible for inclusion in our review if they provided measures of IL-6 accuracy for diagnosing EONS, defined by the National Institute of Child Health and Human Development and Vermont Oxford Network, as sepsis with onset at $\leqslant$3 days of age. We excluded studies that used IL-6 measurements that were made only on maternal blood samples. We also excluded duplicate articles. Conference abstracts or studies written in languages other than English were also excluded.

6.3. Data extraction

Data extraction included the country of the research, year of publication, journal, reference standard employed, the type of study design, the number and specific characteristics of the patients in the septic and non-septic groups (Table 1), and items related to the quality of the methods and reporting (Table 2). Specific data regarding the IL-6 cut-off level used, the sensitivity, specificity, and AUC for the diagnosis of EONS were also extracted (Table 1). In cases in which estimates of uncertainties around the observed values of sensitivities and specificities were not calculated using Wilson's method [62], we calculated them.

6.4. Quality assessment of the included studies

In 2003, the STARD statement was published in 13 biomedical journals [63–65]. The STARD initiative was developed in response to accumulating evidence of poor methodological quality. Therefore, a data extraction form based on the STARD checklist and adapted for neonates with EONS was used to appraise the overall quality of the included studies. All articles were closely examined on the extent to which they adhered to the STARD checklist by assigning a yes or no response to each item.

Twenty-five items make-up the STARD checklist. When the papers considered in this study were assessed, only those STARD items that have been empirically shown to have a potentially biasing effect on diagnostic accuracy [66–69] and those items we deemed may account for variation between estimates of diagnostic accuracy for neonatal sepsis, were evaluated.

The checklist of STARD items we used is shown in Table 2. Adequate reporting of three key domains, that is, descriptions of participant recruitment, reference standard and index test, and study population, was considered essential for capturing the global and integrative quality of the estimated accuracy of the diagnostic tests. As these were used for ruling in or for ruling out a multifaceted clinical entity such as EONS, they were split into various complementary items. Two investigators (CC, BR) independently assessed the methodological quality of all eligible studies.

6.5. Methodological quality of the included studies

We found 19 articles which were eligible for inclusion in our review [61,70–87]. Papers were published between 1995 and 2014. All were studies of diagnostic accuracy, and stated this as the research objective in the introduction. Most studies were performed in single perinatal centers. The IL-6 accuracy for the diagnosis of EONS yielded discrepant results (Table 1). Potential sources of the wide variation in IL-6 sensitivity and specificity include the lack of consistent IL-6 cut-off values regardless of the time of sampling; and differences in study sample sizes, patient demographic and clinical characteristics, and participant recruitment.

We therefore evaluated the extent to which IL-6 accuracy studies adhered to the STARD initiative. Agreement between the two reviewers was almost perfect with an overall agreement percentage of 98.8%. The kappa statistic had a value of 0.98 (95% CI 0.96–0.99), indicating very good agreement.

Overall, the quality of IL-6 diagnostic accuracy studies on EONS over the last two decades was sub-optimal. Information on the key elements of design, conduct, analysis, and interpretation of test accuracy were frequently missing. The importance of describing how eligible subjects were identified cannot be overemphasized. It is crucial to describe the populations from which patients and patient controls originated, as it allows an assessment of the "spectrum of disease", which is likely to influence the diagnostic performance of the test. Reported estimates of diagnostic accuracy may have limited clinical applicability (generalizability) if the spectrum of tested patients is not similar to the patients for whom the test will be used in practice. While some IL-6 accuracy studies enrolled neonates who were suspected of having the disease because of presenting symptoms, other studies recruited neonates who were (initially asymptomatic and) only at risk of developing the disease because of a history of maternal risk factors. Other studies included neonates already diagnosed with sepsis and those in whom sepsis had been already excluded. Finally, there were also designs starting with: two separate selection processes to sample patients with sepsis and patients without sepsis (a case-control design with limited spectrum [66,88]); non-consecutive sampling of patients (a method leading to the "limited challenge bias" [89]); retrospective data collection; and identification of patients by searching hospital records. These alternative study designs are likely to influence the spectrum of disease. Spectrum bias also results from differences in the severity of sepsis/EONS between populations. Clinical and birth characteristics (as proxy measures of morbidity) of the septic and non-septic neonatal populations were reported in only half of the IL-6 diagnostic accuracy studies. As individual clinical and demographic attributes, such as gestational age and birth weight, do not capture the overall morbidity status, it is of greater concern that reporting of the distribution of scores of the major measures of illness severity was remarkably poor in neonates with and without sepsis.

Differential verification bias is a key issue in any diagnostic accuracy study. It occurs if some patients receive a different reference standard. Studies that relied on 2 or more reference standards to verify the results of the index test, have reported estimates of diagnostic accuracy on average 60% higher than those found in studies that used a single reference standard [68]. The origin of this difference probably resides in differences between the reference standards in how they define sepsis, or in their quality. Reference standards are not interchangeable. They may not have the same degree of error, and may not identify the same segment of the disease spectrum. Thus it is worrying that in most of the included IL-6 diagnostic accuracy studies, different reference standards were used to diagnose (or exclude) EONS and verify index test results.

A further step in the critical appraisal of the reference standard is whether the index test or the comparator of the index test formed part of the reference standard [90,91]. Unfortunately, in six of the 19 included studies the comparator of the index test such as CRP test was also a component of the reference standard (incorporation bias). In such situations it is likely that the person interpreting the results of the comparator will have knowledge of the results of the other test (index test and reference standard).

In order to make a valid comparison between the index test and the standard test, it is essential that the criteria (cut-off values etc) are defined before the start of the study. If the cut-offs are decided after the results are obtained, the likelihood that another study will

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Table 1
Characteristics of IL-6 accuracy studies (1995-2014) for diagnosing early (⩽ 72 h) -onset neonatal infection.

Country, year [reference]	Recruitment	Reference standard in infected neonates	Reference standard in control neonates	Sample studied	IL-6 Cutoff (pg/mL)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC
Germany, 1995 [72]	46 NICU preterm and term babies: 13, infected; 33, uninfected	1) ⩾3 categories of clinical signs, and positive blood culture; or 2) ⩾3 categories of clinical signs, and ⩾ 2 abnormal laboratory results in the first 48 h of life	NA	Cord blood	150	69 (42–87)	91 (76–97)	NA
Switzerland, 1996 [73]	199 preterm and term babies (enrolled from the Obstetric Unit, or the NICU): 18, infected; 181, unifected	1) Positive culture of blood and/or CSF, abnormal WBC and CRP, and clinical signs; or 2) ⩾3 categories of clinical signs	Neither clinical nor biological signs of infection	⩽1 h	100	100 (82–100)	92.3 (87–95)	NA
USA, 1997 [71]	23 preterm and term babies with suspected EONS: 8, infected; 15, uninfected	1) Positive blood or CSF culture; or 2) clinical signs and positive laboratory (WBC, I:T ratio, or spinal tap) results	NA	UA	>7	87.5 (52.9–97.8)	66.7 (41.7–84.8)	NA
				UV	>7	87.5 (52.9–97.8)	93.3 (70.2–98.8)	NA
Italy, 1997 [74]	60 NICU preterm and term babies: 13, infected; 47, uninfected	Positive blood culture, clinical signs, and/or radiographic evidence of pneumonia	Babies with various types of distress who were well within 48–72 h	⩽24 h	70	69 (42–87)	36(24–51)	NA
				>24 h to ⩽48 h	50	92 (67–99)	98 (89–100)	NA
USA, 1999 [75]	28 preterm babies with either spontaneous preterm labor or PPRM: 14, infected; 14, uninfected	1) Autopsy; or 2) clinical signs and ⩾2 laboratory (WBC, I:T ratio, PC, spinal fuid) abnormalities	NA	UV blood	25	92.9 (68.5–98.7)	92.9 (68.5–98.7)	NA
USA, 2000 [76]	43 NICU singleton, very preterm babies: 21, infected; 22, uninfected	1) Positive culture of blood and/or CSF; or 2) ⩾3 maternal/neonatal risk factors for infection, clinical signs, and abnormal hematologic findings	Negative blood culture, and <3 maternal/neonatal risk factors for infection	UV blood	100	81 (60–92.3)	90.9 (72.2–97.5)	NA
Norway, 2001 [77]	24 NICU preterm neonates: 11, infected; 13, uninfected	1) Clinical signs, and a positive blood culture; or 2) ⩾3 categories of clinical signs, and CRP ⩾3 mg/dL; or 3) radiographic evidence of pneumonia and CRP ⩾3 mg/dL	Clinical conditions apparently noninfectious	Cord blood	33	82 (52–95)	69.2 (42–87)	0.86 (0.66 –0.96)
Sweden, 2001 [78]	32 NICU preterm and term babies with suspected sepsis: 20, infected; 12, uninfected	1) Positive culture of blood and/or CSF; or 2) abnormal CRP, WBC, and ⩾1 category of clinical signs (i.e. oliguria, metabolic acidosis, or hypoxemia)	Clinical conditions apparently noninfectious	⩽24 h	>160	100 (84–100)	69(42–87)	NA
Spain, 2001 [79]	31 preterm and term babies: 10, infected; 11, uninfected; 10, healthy controls	⩾2 categories of clinical signs, ⩾1 abnormal laboratory finding, and a positive blood culture	Clinical conditions apparently noninfectious; and normal postnatal course through the 1st month of life	Cord blood	100.8	50 (24–76)	91 (62–98) 90 (60–98)	~0.5

(*continued on next page*)

Table 1 (*continued*)

Country, year [reference]	Recruitment	Reference standard in infected neonates	Reference standard in control neonates	Sample studied	IL-6 Cutoff (pg/mL)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC
Germany, 2001 [80]	136 preterm and term babies: 68, infected;68, uninfected. Of the 136 babies, 77 were preterm:40, infected;37, uninfected	1) Clinical signs and positive blood culture; or	Noninfectious clinical conditions	Cord blood	80	87(77–93)	90(80–95)	NA
		2) clinical signs, and abnormal laboratory (CRP, I:T ratio) results, biological fluids positive for bacteria, or signs of inflammation in placenta		Cord blood	80	95(84–99)	95(82–99)	NA
Italy, 2003 [81]	134 NICU preterm and term babies:19, infected; 115, uninfected	1) Positive blood culture and clinical signs; or	Symptomatic babies who had negative body fluid cultures, and were apparently well within 24–48 h	At birth	⩾200	74 (51–88)	89 (82–93)	NA
		2) ⩾3 clinical signs prompting ⩾5 d of antibiotic therapy, and historical and clinical risk factors for EONS		24 h	⩾30	63 (41–81)	71 (63–79)	NA
				48 h	⩾30	53 (32–73)	70 (62–78)	NA
Austria, 2003 [82]	68 NICU preterm and term babies with suspected sepsis:41, infected;27, uninfected	1) Positive blood culture; or	Negative blood culture, negative sepsis screen, and antibiotic therapy ⩽3 d	⩽12 h	⩾10	71(56–82)	67(48–81)	NA
		2) ⩾3 categories of clinical signs, positive sepsis screen and/or risk factors, and antibiotic therapy ⩾7 d			⩾60	54(39–68)	100(88–100)	NA
					⩾150	46(32–61)	100(88–100)	NA
Denmark, 2008 [83]	123 NICU babies with at least 1 clinical sign suggesting EONS:29, infected;94, uninfected	1) Positive blood culture; or	Clinical signs, CRP ⩽5 mg/dL, and antibiotic therapy for 3 days; or clinical signs, but no antibiotic therapy	0 h [a]	250	59(41–75)	94(87–97)	0.77
		2) clinical signs and CRP >5 mg/dL						
Brazil, 2010 [84]	144 NICU preterm babies presenting RDS during the first 24 h of life:44, infected;100, uninfected	In addition to RDS,	No clinical signs, and a haematologic sepsis score <3	⩽36 h	>36	82(68–91)	44(35–54)	0.72 (0.62 –0.83)
		1) ⩾2 categories of clinical signs, or clinical chorioamnionitis, and positive blood or CSF culture; or 2) ⩾2 categories of clinical signs, or clinical chorioamnionitis, and a haematologic sepsis score >3; or 3) radiographic evidence of pneumonia and a haematologic sepsis score >3						

France, 2011 [61]	213 NICU preterm babies with a presumptive diagnosis of EONS:31, infected;182, uninfected	1) Positive culture of blood or CSF, and clinical signs; or	Positive superficial culture without abnormal CRP; alternative diagnosis (obstetrical trauma, perinatal asphyxia, meconium aspiration syndrome, pneumothorax, etc); neither positive superficial culture nor abnormal CRP	⩽6 h	300	87.1(71.1–94.9)	81.9(75.6–86.8)	0.89 (0.84–0.95)
		2) clinical signs, CRP >10 mg/L, positive superficial or placental cultures, and no alternative diagnosis						
Iran, 2012 [85]	65 NICU preterm and term babies with clinical signs of sepsis or maternal risk factors for EONS:49, infected;16, uninfected	1) Positive blood culture; or	Negative blood culture, <3 categories of clinical signs, and a negative sepsis screen	⩽12 h	⩾60	53 (39–66)	100 (81–100)	NA
		2) ⩾3 categories of clinical signs, and positive sepsis screen or maternal risk factors		24 h	⩾10	71 (58–82)	62.5 (39–82)	
				36 h	⩾150	46.9 (34–61)	100 (81–100)	
Spain, 2012 [86]	128 preterm and term babies with prenatal risk factors for EONS (77% asymptomatic at birth):10, infected;118, uninfected	1) Positive blood culture and clinical signs; or	NA	Cord blood	255.87	90	87.3	0.88
		2) ⩾3 categories of clinical signs				(94–96)	(80.1–92.3)	(0.70 –1.06)(sic)
Spain, 2013 [70]	176 preterm babies born to mothers with PPROM:12, infected;164, uninfected	1) Positive blood culture; or	NA	Cord blood	38	83 (55–95)	82 (75–87)	0.91 (0.85–0.97)
		2) clinical signs and ⩾2 abnormal hematological laboratory (WBC, PC, I:T ratio) results						
Austria, 2014 [87]	218 NICU preterm babies with risk factors for EONS:30, infected;188, uninfected	1) Positive blood culture; 2) ⩾3 categories of clinical signs; or	NA	Cord blood	15.85	73.3 (56–86)	84 (78–89)	0.81 (0.67–0.95)
		3) ⩾1 category of clinical signs, and ⩾2 laboratory (WBC, I:T ratio, CRP) abnormalities						

AUC = Area under the curve; CSF = Cerebrospinal Fluid; CRP = C reactive protein; EONS = Early-onset neonatal sepsis; I:T = Immature-to-total neutrophil; NA = Not available; NICU = Neonatal Intensive Care Unit; PC = Platelet count; PPROM = Preterm Premature Rupture of Membranes; RDS = Respiratory Distress Syndrome; UA = Umbilical artery; UV = Umbilical Vein; WBC = White Blood Cells.

[a] At the time of suspicion of EONS.

Table 2
Quality of reporting of IL-6 accuracy studies (1995–2014) for diagnosing early (⩽72 h)-onset neonatal infection.

Category and Item No.	YES	NO
METHODS-PARTICIPANTS		
Describe the study population:		
1A. the inclusion and exclusion criteria,	13	6
1B. setting, and locations where data were collected	14	5
Describe participant recruitment:		
2A. Was enrollment of patients based only on clinical signs suggesting infection?	6	13
2B. Were such patients consecutively enrolled?	4	2
2C. Was enrollment of patients based only on maternal risk factors for infection?	4	15
2D. Were such patients consecutively enrolled?	0	4
2E. Were patients identified by searching hospital records?	1	18
2F. Did the study include both patients already diagnosed with sepsis and participants in whom sepsis had been excluded?	4	15
Describe data collection:		
3. Was data collection planned before the index test and reference standard were performed (prospective study)?	6	13
TEST METHODS		
Methods pertaining to the reference standard and the index test:		
4A. Was a composite reference standard used to identify all newborns with sepsis, and verify index test results in infected babies?	18	1
4B. Was a reference standard used to exclude sepsis?	13	6
4C. Was a composite reference standard used to identify all newborns without sepsis, and verify index test results in uninfected babies?	6	7
4D. Did the index test or its comparator form part of the reference standard?	6	13
5. Were categories of results of the index test (including cut-offs) and the reference standard defined after obtaining the results?	19	0
6. Did the study report the number, training and expertise of the persons executing and reading the index tests and the reference standard?	1	18
7. Was there blinding to results of the index test and the reference standard?	4	15
STATISTICAL METHODS		
8. Describe the statistical methods used to quantify uncertainty (i.e. 95% confidence intervals)?	7	12
9. Describe methods for calculating test reproducibility	2	17
RESULTS-PARTICIPANTS AND TEST RESULTS		
10A. Describe when the study was done, including beginning and ending dates of recruitment	16	3
10B. Did the study report clinical and demographic (postnatal hours or days, gestational age, birth weight, gender) features in those with and without sepsis?	10	9
10C. Did the study report distribution of illness severity scores in those with and without sepsis?	3	16
11. Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test	11	8
12. Report a cross-tabulation of the results (including indeterminate and missing results) by the results of the reference standard; for continuous results report the distribution of the test results by the results of the reference standard	10	9
RESULTS-ESTIMATES		
13. Report measures of statistical uncertainty (i.e. 95% confidence intervals)	7	12
14. Report how indeterminate results, missing responses and outliers of index tests were handled	2	17
15. Report estimates of test reproducibility	10	9

replicate the findings is reduced. Apparently all studies defined cut-offs *post hoc*.

We also determined whether the interpretation of the index test or reference standard was influenced by knowledge of the results of the other test. Review biases, including test review bias (knowledge of the outcome of the reference standard when reviewing the index test) and diagnostic review bias (knowledge of the outcome of the index test when reviewing reference standard) can lead to inflation of the measures of diagnostic accuracy. Information about masking was reported in a minority of reports (4/19). Information regarding methods for calculating IL-6 test reproducibility, as well as the number, training and expertise of the persons executing and reading the index test and the reference standard were among the least commonly reported items from the STARD guidelines. There may be a lack of understanding of the effects of poor reproducibility or low level of expertise on the final outcome of the diagnostic accuracy of a test.

Measures of diagnostic accuracy will be biased if the result of the index test influences the decision to order the reference standard [90]. It is therefore important to state how many participants satisfying inclusion criteria failed to undergo the index tests and/or the reference standard and the reasons for failing to do so. This item was reported in over half of the publications included (11/19). A flow diagram is highly recommended to illustrate the design of the study and provide the exact number of participants at each stage of the study [63,64]; none of the 19 IL-6 diagnostic accuracy studies had a flow diagram.

As the absolute values of diagnostic accuracy are only estimates, when evaluations of diagnostic accuracy are reported the precision of the sensitivity and specificity should be reported. Reporting of CIs is critical to allow a physician to know the range within which the true values are likely to lie [92]. Only 7 (37%) of the 19 included studies reported CIs.

Intermediate, indeterminate, and uninterpretable results may not always be included in final assessment of the diagnostic accuracy of a test. The frequency of these results, by itself, is an important pointer of the overall usefulness of the test. Only two of the 19 included studies reported this item.

Since the technology for existing tests is rapidly improving, it is important to report the actual dates when the study was performed. This will allow the reader to consider any technologic advances since the study was done. Fortunately, most publications provided this information.

In general, the results of our analysis show that over the last two decades the quality of reporting on IL-6 diagnostic accuracy studies for EONS was sub-optimal leaving ample room for improvement. Studies of the same test can produce different estimates of diagnostic accuracy depending on the design, conduct and data analysis. Authors should be aware of the STARD criteria before starting a study in this field.

7. IL-6 response in the early postnatal period and perinatal confounders

Previous studies have suggested that interpretation of the IL-6 response in early life may be affected and confounded by concurrent factors associated with stress, systemic injury and infection [74,79,93]. Absent from most publications on IL-6 accuracy studies for the diagnosis of EONS, however, are data on how the interpretation of the IL-6 response in the infected and uninfected neonates might have been hampered by the severity of the underlying illnesses (and their extent of inflammatory reaction). Clinical severity was described in only half of these publications, and most of them used gestational age and birth weight as simple proxies for more elaborate measures of illness severity (Table 2). Although birth weight and gestational age may be adequate for some purposes, they do not completely account for variations in the severity of illness. Previous reports have emphasized apparent variations in morbidity and mortality among NICUs despite controlling for birth weight and gestational age. Substantial residual variation is still evident after such adjustments [94]. Hence, it is worrying to think that Cobo et al. have considered prematurity and related factors, such as the use of maternal steroids and antibiotics, as the only

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possible confounding factors to be adjusted for when assessing the independence of umbilical cord IL-6 in predicting EONS [70]. Moreover, risk factors such as antenatal corticosteroid and antimicrobial use are influenced by the interval between maternal presentation and delivery and by access to care [95]. In a few IL-6 accuracy studies, Apgar scores have also been used as simple proxies for measuring illness severity in neonates with and without early-onset sepsis [76,77,80,83,86]. However, Apgar scores are known to be dependent on gestational age [96], and may actually reflect intrapartum obstetric or anesthesia practices. Low Apgar scores may also suggest a pattern of suboptimal resuscitation or of greater antecedent fetal sedation or compromise [95]. Thus the use of appropriate measures of illness severity is essential for assessing clinical severity in the comparison of results of diagnostic accuracy studies [94,97,98]. To this end, in a study on IL-6 accuracy in diagnosing sepsis in critically ill neonates admitted to the NICU during the first 48 h of life [81], Chiesa et al. used two objective, validated measures of neonatal illness severity, the Score for Neonatal Acute Physiology and its perinatal extension [94,97]. They found that IL-6 levels increase at birth and at 24 and 48 h of life in the presence of bacterial infection, and that the increases are independent of illness severity [81]. They also found that illness severity has the potential to confound IL-6 concentrations in that, among newborns without infection, the higher the illness severity and risk indices, the greater the IL-6 response at birth.

Previous studies have sought to identify which prenatal and perinatal variables would mimic or mask alterations in the IL-6 response caused by infection. Prolonged exercise has been associated with increased production of IL-6 from the stimulated blood monocytes [99]. This observation can possibly explain the higher IL-6 levels during vaginal delivery, where the parturient makes a stronger effort than that during an elective cesarean section. Thus, IL-6 has been consistently found to increase in the fetal circulation in response to labor or other labor-related events both with and without histologic chorioamnionitis [100–104].

Other noninfectious causes can lead to an increase in IL-6. Cord serum or cerebrospinal fluid levels of IL-6 have been reported to be elevated during the immediate postnatal period in infants with perinatal asphyxia in the presence as well as absence of infection [81,105–110], and have been correlated to the degree of encephalopathy and neurodevelopmental outcome [106]. In a group of 20 infants with fetal acidemia, respiratory depression, and low Apgar scores, cerebrospinal fluid levels of IL-6 were higher among infants with more severe neonatal encephalopathy [105]. This elevation of IL-6 levels could be attributable to brain damage manifesting as neonatal encephalopathy and/or antecedents (including chorioamnionitis) of encephalopathy [111]. The association between elevated IL-6 levels and encephalopathy was found even among infants born to mothers without the diagnosis of chorioamnionitis [112]. Thus, antecedents of neonatal encephalopathy other than chorioamnionitis (that is, hypoxia-ischemia) might lead to elevated levels of IL-6 in the neonatal blood [113].

8. Association of FIRS and EONS with brain injury

Brain injury in the premature infant consists of multiple lesions, principally germinal matrix/intraventricular hemorrhage(IVH), posthemorrhagic hydrocephalus, and periventricular leukomalacia (PVL) [114]. The last of these now appears to be the most important determinant of the neurologic morbidity observed in survivors with birth weight <1500 g, of whom about 10% later exhibit cerebral palsy, and about 50%, suffer cognitive and behavioral deficits [115,116].

The pathogenesis of PVL is multifactorial and probably involves damage, either related to ischemia/reperfusion injury in the critically ill premature infant with impaired regulation of cerebral blood flow, or to inflammation-induced brain injury associated with infection [117]. Relating to the latter, a recent study has shown that maternal intrauterine infection and fetal systemic inflammation play a role in the pathogenesis of PVL [118]. An association between WMI and/or cerebral palsy and maternal, placental, or fetal infections [119–121] with high levels of IL-6 in the amniotic fluid and cord blood, has been demonstrated [122]. Martinez et al. showed that mothers of neonates who developed PVL and IVH within the first week of life had higher median amniotic fluid IL-6 levels (42,795 pg/ml versus 8,020 pg/mL; $P = 0.009$), more positive amniotic fluid cultures (64% versus 21%; $P < 0.003$), and a shorter median amniocentesis-to-delivery interval (16 h versus 24 h; $P = 0.045$) than women who delivered neonates without PVL or IVH [123]. The hypothesis is that the fetal systemic inflammatory response leads to WMI. Indeed, neuropathologic examination has shown that an inflammatory reaction is detected at the early stage of PVL in the brain that persists until the late phase of cystic cavitation [124]. This process is characterized by the presence of active astroglial and microglial proliferation in the brains of infants with PVL and cytokine production [124,125]. The intensity of cytokine production observed in the brains of infants with PVL is higher than in anoxic lesions without PVL, particularly at the early stage of PVL [124]. Whether this brain inflammatory response is secondary to systemic inflammation, hypoxia-ischemia, or both, remains unclear.

Surprisingly, what is emerging is that a significant increase of WMI, including a progressive form of WMI that is more readily evident on magnetic resonance imaging scans at term-equivalent age [126], may arise from postnatal infections through a systemic inflammatory response [127–129]. Postnatal infections have been linked to altered development of white matter pathways [130] and widespread impairments in brain development. The increased risk of early WMI with postnatal infection is consistent with the observation that recurrent postnatal sepsis is a risk factor for progressive WMI [126,129]. A few studies have observed that during the immediate postnatal period WMI in preterm infants is associated with an altered equilibrium in the inflammatory IL-6 response to early-onset sepsis and that proinflammatory cytokines such as IL-6 act as part of a larger systemic response. In a prospective cohort study examining the association between cytokines including IL-6 and WMI in 84 very-low-birth weight infants with early-onset sepsis and necrotizing enterocolitis (NEC), Procianoy and Silveira [131] showed that neonates with proven early-onset sepsis, NEC, and high plasma levels of IL-6 were at high risk for WMI. Proven EONS and NEC carried a high risk for WMI even after adjustment for gestational age and birth weight [relative risk, 3.04 (95% CI 1.93-4.80) and relative risk, 2.2 (95% CI 1.31-3.74), respectively]. IL-6 levels obtained during the first 24 h of life were higher in infants with WMI than in control subjects. Using ROC analysis, a plasma level of IL-6 $\geqslant$ 116 pg/mL was a good risk predictor for WMI with sensitivity, specificity and negative predictive value being 96%, 77%, and 98%, respectively. Thus these data support the hypothesis that neonatal sepsis leads to an extracerebral inflammatory stimulus and WMI in the immature brain, without bacterial antigen gaining access to the brain. More recently, in an observational study of 176 preterm infants, Hofer et al. [55] showed that elevated cord blood IL-6 concentrations were significantly associated with EONS as well as neonatal mortality, respiratory morbidity, and neurologic morbidity including IVH and PVL.

9. Conclusions

IL-6 has consistently been found to be the best candidate as a diagnostic tool for detecting pre-clinical chrioamnion inflammation and intra-amniotic inflammation leading to preterm birth.

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Determination of FIRS (and fetal blood IL-6 concentration) provides a means to recognize neonates who are at risk for adverse neonatal outcome, in particular EONS. Physiologic processes and perinatal confounders in the early life should be taken into account to optimize use of IL-6 in the diagnosis of EONS. The reliability of this cytokine for the differential diagnosis of infectious versus noninfectious systemic inflammatory response during the immediate postnatal period has yielded variable results. Some of this variation reflects differences in reporting quality of IL-6 diagnostic accuracy for EONS. Future well-reported studies showing information on key elements of design, conduct, and analysis would be useful resources to guide decisions on the use and interpretations of IL-6 test results in the diagnosis of neonates with early-onset sepsis. Future studies are also needed to clarify how systemic inflammatory IL-6 response *in utero* and postnatally, may increase the risk of WMI in postnatal sepsis.

References

[1] Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004;50:279–87.
[2] Kurdowska A, Krupa A. Blockade of interleukin-6 in murine sepsis revisited: is there an indication for a new therapy in human patients? Crit Care Med 2011;39:1582–3.
[3] Ulevitch RJ. Therapeutics targeting the innate immune system. Nature Rev Immunol 2004;4:512–20.
[4] Janeway Jr CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197–216.
[5] Matzinger P. The danger model: a renewed sense of self. Science 2002;296:301–5.
[6] Tanaka T, Kishimoto T. Targeting interleukin-6: all the way to treat autoimmune and inflammatory diseases. Int J Biol Sci 2012;8:1227–36.
[7] Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci 2012;122:143–59.
[8] Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 2012;8:1237–47.
[9] Kallen KJ. The role of transsignalling via the agonistic soluble IL-6 receptor in human diseases. Biochim Biophys Acta 2002;1592:323–43.
[10] Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 1989;169:333–8.
[11] Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest 2011;121:3375–83.
[12] Rose-John S, Scheller J, Elson G, Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. J Leukoc Biol 2006;80:227–36.
[13] Scheller J, Rose-John S. Interleukin-6 and its receptor: from bench to bedside. Med Microbiol Immunol 2006;195:173–83.
[14] Peters M, Jacobs S, Ehlers M, Vollmer P, Müllberg J, Wolf E, et al. The function of the soluble interleukin 6 (IL-6) receptor in vivo: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. J Exp Med 1996;183:1399–406.
[15] Montero-Julian FA. The soluble IL-6 receptors: serum levels and biological function. Cell Mol Biol 2001;47:583–97.
[16] Mitsuyama K, Toyonaga A, Sasaki E, Ishida O, Ikeda H, Tsuruta O, et al. Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating interleukin-6. Gut 1995;36:45–9.
[17] Gaillard JP, Bataille R, Brailly H, Zuber C, Yasukawa K, Attal M, et al. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gammopathy. Eur J Immunol 1993;23:820–4.
[18] Rose-John S, Heinrich PC. Soluble receptors for cytokines and growth factors: generation and biological function. Biochem J 1994;300(Pt 2):281–90.
[19] Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J 2001;15:43–58.
[20] Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: a highly regulated and dynamic system. Cytokine 2014;70:11–20.
[21] Garbers C, Thaiss W, Jones GW, Waetzig GH, Lorenzen I, Guilhot F, et al. Inhibition of classic signaling is a novel function of soluble glycoprotein 130 (sgp130), which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor. J Biol Chem 2011;286:42959–70.
[22] Klouche M, Bhakdi S, Hemmes M, Rose-John S. Novel path to activation of vascular smooth muscle cells: up-regulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. J Immunol 1999;163:4583–9.
[23] Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 1997;6:315–25.
[24] Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, et al. Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. Immunity 2001;14:705–14.
[25] Meszaros AJ, Reichner JS, Albina JE. Macrophage-induced neutrophil apoptosis. J Immunol 2000;165:435–41.
[26] Teague TK, Marrack P, Kappler JW, Vella AT. IL-6 rescues resting mouse T cells from apoptosis. J Immunol 1997;158:5791–6.
[27] Peters M, Schirmacher P, Goldschmitt J, Odenthal M, Peschel C, Fattori E, et al. Extramedullary expansion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice. J Exp Med 1997;185:755–66.
[28] Peters M, Müller AM, Rose-John S. Interleukin-6 and soluble interleukin-6 receptor: direct stimulation of gp130 and hematopoiesis. Blood 1998;92:3495–504.
[29] Jostock T, Müllberg J, Ozbek S, Atreya R, Blinn G, Voltz N, et al. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur J Biochem 2001;268:160–7.
[30] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011;1813:878–88.
[31] Wakabayashi A, Sawada K, Nakayama M, Toda A, Kimoto A, Mabuchi S, et al. Targeting interleukin-6 receptor inhibits preterm delivery induced by inflammation. Mol Hum Reprod 2013;19:718–26.
[32] Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. BJOG 2006;113(Suppl 3):S17–42.
[33] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75–84.
[34] Hagberg H, Mallard C, Jacobsson B. Role of cytokines in preterm labour and brain injury. BJOG 2005;112(Suppl 1). S16–8.
[35] El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. Amniotic fluid interleukin-6 and preterm delivery: a review. Obstet Gynecol 2000;95(6 Pt 2):1056–64.
[36] Harirah H, Donia SE, Hsu CD. Amniotic fluid matrix metalloproteinase-9 and interleukin-6 in predicting intra-amniotic infection. Obstet Gynecol 2002;99:80–4.
[37] Krupa FG, Faltin D, Cecatti JG, Surita FG, Souza JP. Predictors of preterm birth. Int J Gynaecol Obstet 2006;94:5–11.
[38] Lee SY, Buhimschi IA, Dulay AT, Ali UA, Zhao G, Abdel-Razeq SS, et al. IL-6 trans-signaling system in intra-amniotic inflammation, preterm birth, and preterm premature rupture of the membranes. J Immunol 2011;186:3226–36.
[39] Thomakos N, Daskalakis G, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: relationship to intra-amniotic microbial invasion and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2010;148:147–51.
[40] Vrachnis N, Karavolos S, Iliodromiti Z, Sifakis S, Siristatidis C, Mastorakos G, et al. Review: impact of mediators present in amniotic fluid on preterm labour. In Vivo 2012;26:799–812.
[41] Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. J Perinat Med 2012;40:329–43.
[42] Taylor BD, Holzman CB, Fichorova RN, Tian Y, Jones NM, Fu W, et al. Inflammation biomarkers in vaginal fluid and preterm delivery. Hum Reprod 2013;28:942–52.
[43] Keelan JA, Marvin KW, Sato TA, Coleman M, McCowan LM, Mitchell MD. Cytokine abundance in placental tissues: evidence of inflammatory activation in gestational membranes with term and preterm parturition. Am J Obstet Gynecol 1999;181:1530–6.
[44] Young A, Thomson AJ, Ledingham M, Jordan F, Greer IA, Norman JE. Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term. Biol Reprod 2002;66:445–9.
[45] Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition-a review. Placenta 2003;24(Suppl A):S33–46.
[46] Gomez-Lopez N, Laresgoiti-Servitje E, Olson DM, Estrada-Gutiérrez G, Vadillo-Ortega F. The role of chemokines in term and premature rupture of the fetal membranes: a review. Biol Reprod 2010;82:809–14.
[47] Geer LA, Pycke BF, Sherer DM, Abulafia O, Halden RU. Use of amniotic fluid for determining pregnancies at risk of preterm birth and for studying diseases of potential environmental etiology. Environ Res 2015;136:470–81.
[48] Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, et al. Infection in the pathogenesis of preterm labor. Semin Perinatol 1988;12:262–79.
[49] Romero R, Baumann P, Gomez R, Salafia C, Rittenhouse L, Barberio D, et al. The relationship between spontaneous rupture of membranes, labor, and microbial invasion of the amniotic cavity and amniotic fluid concentrations of prostaglandins and thromboxane B2 in term pregnancy. Am J Obstet Gynecol 1993;168(6 Pt 1):1654–64.
[50] Lockwood CJ, Murk WK, Kayisli UA, Buchwalder LF, Huang SJ, Arcuri F, et al. Regulation of interleukin-6 expression in human decidual cells and its potential role in chorioamnionitis. Am J Pathol 2010;177:1755–64.
[51] Keelan JA, Sato T, Mitchell MD. Interleukin (IL)-6 and IL-8 production by human amnion: regulation by cytokines, growth factors, glucocorticoids, phorbol esters, and bacterial lipopolysaccharide. Biol Reprod 1997;57:1438–44.

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[52] Romero R, Gomez R, Ghezzi F, Yoon BH, Mazor M, Edwin SS, et al. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol 1998;179:186–93.
[53] Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol 1998;179:194–202.
[54] Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O, et al. The fetal inflammatory response syndrome. Clin Obstet Gynecol 2007;50:652–83.
[55] Hofer N, Kothari R, Morris N, Müller W, Resch B. The fetal inflammatory response syndrome is a risk factor for morbidity in preterm neonates. Am J Obstet Gynecol 2013;209. 542.e1-11.
[56] Buhimschi CS, Dulay AT, Abdel-Razeq S, Zhao G, Lee S, Hodgson EJ, et al. Fetal inflammatory response in women with proteomic biomarkers characteristic of intra-amniotic inflammation and preterm birth. BJOG 2009;116:257–67.
[57] Buhimschi CS, Buhimschi IA, Abdel-Razeq S, Rosenberg VA, Thung SF, Zhao G, et al. Proteomic biomarkers of intra-amniotic inflammation: relationship with funisitis and early-onset sepsis in the premature neonate. Pediatr Res 2007;61:318–24.
[58] Buhimschi IA, Zambrano E, Pettker CM, Bahtiyar MO, Paidas M, Rosenberg VA, et al. Using proteomic analysis of the human amniotic fluid to identify histologic chorioamnionitis. Obstet Gynecol 2008;111:403–12.
[59] Chiesa C, Signore F, Assumma M, Buffone E, Tramontozzi P, Osborn JF, et al. Serial measurements of C-reactive protein and interleukin-6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders. Clin Chem 2001;47:1016–22.
[60] Marchini G, Berggren V, Djilali-Merzoug R, Hansson L-O. The birth process initiates an acute phase reaction in the fetus-newborn infant. Acta Paediatr 2000;89:1082–6.
[61] Labenne M, Lizard G, Ferdynus C, Montange T, Iacobelli S, Bonsante F, et al. A clinic-biological score for diagnosing early-onset neonatal infection in critically ill preterm infants. Pediatr Crit Care Med 2011;12:203–9.
[62] Newcombe RG, Altman DG. Proportions and their differences. In: Altman DG, Machin D, Bryant TN, Gardner MJ, editors. Statistics with Confidence. BMJ Books, 2nd ed. 2005, p. 45–56. [chapter 6].
[63] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. Clin Chem 2003;49:1–6.
[64] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Clin Chem 2003;49:7–18.
[65] Korevaar DA, van Enst WA, Spijker R, Bossuyt PM, Hooft L. Reporting quality of diagnostic accuracy studies: a systematic review and meta-analysis of investigations on adherence to STARD. Evid Based Med 2014;19:47–54.
[66] Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, et al. Empirical evidence of design-related bias in studies of diagnostic tests. JAMA 1999;282:1061–6.
[67] Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. Ann Intern Med 2004;140:189–202.
[68] Rutjes AW, Reitsma JB, Di Nisio M, Smidt N, van Rijn JC, Bossuyt PM. Evidence of bias and variation in diagnostic accuracy studies. CMAJ 2006;174:469–76.
[69] Wilczynski NL. Quality of reporting of diagnostic accuracy studies: no change since STARD statement publication–before-and-after study. Radiology 2008;248:817–23.
[70] Cobo T, Kacerovsky M, Andrys C, Drahosova M, Musilova I, Hornychova H, et al. Umbilical cord blood IL-6 as predictor of early-onset neonatal sepsis in women with preterm prelabour rupture of membranes. PLoS One 2013;8: e69341.
[71] Smulian JC, Bhandari V, Campbell WA, Rodis JF, Vintzileos AM. Value of umbilical artery and vein levels of interleukin-6 and soluble intracellular adhesion molecule-1 as predictors of neonatal hematologic indices and suspected early sepsis. J Matern Fetal Med 1997;6:254–9.
[72] Lehrnbecher T, Schrod L, Kraus D, Roos T, Martius J, von Stockhausen HB. Interleukin-6 and soluble interleukin-6 receptor in cord blood in the diagnosis of early onset sepsis in neonates. Acta Paediatr 1995;84:806–8.
[73] Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U. Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996;129:574–80.
[74] Panero A, Pacifico L, Rossi N, Mancuso G, Stegagno M, Chiesa C. Interleukin 6 in neonates with early and late onset infection. Pediatr Infect Dis J 1997;16:370–5.
[75] Smulian JC, Vintzileos AM, Lai YL, Santiago J, Shen-Schwarz S, Campbell WA. Maternal chorioamnionitis and umbilical vein interleukin-6 levels for identifying early neonatal sepsis. J Matern Fetal Med 1999;8:88–94.
[76] Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T. Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. Pediatr Infect Dis J 2000;19:238–43.
[77] Døllner H, Vatten L, Linnebo I, Zanussi GF, Laerdal A, Austgulen R. Inflammatory mediators in umbilical plasma from neonates who develop early-onset sepsis. Biol Neonate 2001;80:41–7.
[78] Martin H, Olander B, Norman M. Reactive hyperemia and interleukin 6, interleukin 8, and tumor necrosis factor-alpha in the diagnosis of early-onset neonatal sepsis. Pediatrics 2001;108:e61.
[79] Santana C, Guindeo MC, González G, García-Muñoz F, Saavedra P, Doménech E. Cord blood levels of cytokines as predictors of early neonatal sepsis. Acta Paediatr 2001;90:1176–81.
[80] Krueger M, Nauck MS, Sang S, Hentschel R, Wieland H, Berner R. Cord blood levels of interleukin-6 and interleukin-8 for the immediate diagnosis of early-onset infection in premature infants. Biol Neonate 2001;80:118–23.
[81] Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, et al. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem 2003;49:60–8.
[82] Resch B, Gusenleitner W, Müller WD. Procalcitonin and interleukin-6 in the diagnosis of early-onset sepsis of the neonate. Acta Paediatr 2003;92:243–5.
[83] Bender L, Thaarup J, Varming K, Krarup H, Ellermann-Eriksen S, Ebbesen F. Early and late markers for the detection of early-onset neonatal sepsis. Dan Med Bull 2008;55:219–23.
[84] Rego MA, Martinez FE, Elias J, Mussi-Pinhata MM. Diagnostic value of interleukin-6 and C-reactive protein on early onset bacterial infection in preterm neonates with respiratory distress. J Perinat Med 2010;38:527–33.
[85] Abdollahi A, Shoar S, Nayyeri F, Shariat M. Diagnostic value of simultaneous measurement of procalcitonin, interleukin-6 and hs-CRP in prediction of early-onset neonatal sepsis. Mediterr J Hematol Infect Dis 2012;4:e2012028.
[86] Cernada M, Badía N, Modesto V, Alonso R, Mejías A, Golombek S, et al. Cord blood interleukin-6 as a predictor of early-onset neonatal sepsis. Acta Paediatr 2012;101:203–7.
[87] Steinberger E, Hofer N, Resch B. Cord blood procalcitonin and interleukin-6 are highly sensitive and specific in the prediction of early-onset sepsis in preterm infants. Scand J Clin Lab Invest 2014;74:432–6.
[88] Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PM. Case-control and two-gate designs in diagnostic accuracy studies. Clin Chem 2005;51: 1335–41.
[89] Philbrick JT, Horwitz RI, Feinstein AR, Langou RA, Chandler JP. The limited spectrum of patients studied in exercise test research. Analyzing the tip of the iceberg. JAMA 1982;248:2467–70.
[90] Ransohoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. N Engl J Med 1978;299:926–30.
[91] Worster A, Carpenter C. Incorporation bias in studies of diagnostic tests: how to avoid being biased about bias. CJEM 2008;10:174–5.
[92] Harper R, Reeves B. Reporting of precision of estimates for diagnostic accuracy: a review. BMJ 1999;318:1322–3.
[93] Weeks JW, Reynolds L, Taylor D, Lewis J, Wan T, Gall SA. Umbilical cord blood interleukin-6 levels and neonatal morbidity. Obstet Gynecol 1997;90:815–8.
[94] Richardson DK, Gray JE, McCormick MC, Workman K, Goldmann DA. Score for Neonatal Acute Physiology: a physiologic severity index for neonatal intensive care. Pediatrics 1993;91:617–23.
[95] Richardson DK, Shah BL, Frantz 3rd ID, Bednarek F, Rubin LP, McCormick MC. Perinatal risk and severity of illness in newborns at 6 neonatal intensive care units. Am J Public Health 1999;89:511–6.
[96] Catlin EA, Carpenter MW, Brann 4th BS, Mayfield SR, Shaul PW, Goldstein M, et al. The Apgar score revisited: influence of gestational age. J Pediatr 1986;109:865–8.
[97] Richardson DK, Phibbs CS, Gray JE, McCormick MC, Workman-Daniels K, Goldmann DA. Birth weight and illness severity: independent predictors of neonatal mortality. Pediatrics 1993;91:969–75.
[98] Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: simplified newborn illness severity and mortality risk scores. J Pediatr 2001;138:92–100.
[99] Haahr PM, Pedersen BK, Fomsgaard A, Tvede N, Diamant M, Klarlund K, et al. Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, tumour necrosis factor-alpha, interleukin 2 and interferon-gamma. Int J Sports Med 1991;12:223–7.
[100] Chan CJ, Summers KL, Chan NG, Hardy DB. Cytokines in umbilical cord blood and the impact of labor events in low-risk term pregnancies. Early Hum Dev 2013;89:1005–10.
[101] Jokic M, Guillois B, Cauquelin B, Giroux JD, Bessis JL, Morello R, et al. Fetal distress increases interleukin-6 and interleukin-8 and decreases tumour necrosis factor-alpha cord blood levels in noninfected full-term neonates. BJOG 2000;107:420–5.
[102] Malamitsi-Puchner A, Protonotariou E, Boutsikou T, Makrakis E, Sarandakou A, Creatsas G, et al. The influence of the mode of delivery on circulating cytokine concentrations in the perinatal period. Early Hum Dev 2005;81: 387–92.
[103] Rogers BB, Alexander JM, Head J, McIntire D, Leveno KJ. Umbilical vein interleukin-6 levels correlate with the severity of placental inflammation and gestational age. Hum Pathol 2002;33:335–40.
[104] Mestan K, Yu Y, Thorsen P, Skogstrand K, Matoba N, Liu X, et al. Cord blood biomarkers of the fetal inflammatory response. J Matern Fetal Neonatal Med 2009;22:379–87.
[105] Martín-Ancel A, García-Alix A, Pascual-Salcedo D, Cabañas F, Valcarce M. Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. Pediatrics 1997;100:789–94.
[106] Sävman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res 1998;43: 746–51.
[107] Chiesa C, Pellegrini G, Panero A, De Luca T, Assumma M, Signore F, et al. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest 2003;33:352–8.
[108] Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev 2006;28:178–82.

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[109] Walsh BH, Boylan GB, Livingstone V, Kenny LC, Dempsey EM, Murray DM. Cord blood proteins and multichannel-electroencephalography in hypoxic-ischemic encephalopathy. Pediatr Crit Care Med 2013;14:621–30.
[110] Shalak LF, Perlman JM. Infection markers and early signs of neonatal encephalopathy in the term infant. Ment Retard Dev Disabil Res Rev 2002;8:14–9.
[111] Whitaker AH, Feldman JF, Van Rossem R, Schonfeld IS, Pinto-Martin JA, Torre C, et al. Neonatal cranial ultrasound abnormalities in low birth weight infants: relation to cognitive outcomes at six years of age. Pediatrics 1996;98(4 Pt 1):719–29.
[112] Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, et al. Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. Pediatr Res 2004;56:960–6.
[113] Dammann O, O'Shea TM. Cytokines and perinatal brain damage. Clin Perinatol 2008;35:643–63.
[114] Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. Pediatrics 2003;112:176–80.
[115] Wood NS, Marlow N, Costeloe K, Gibson AT, Wilkinson AR. Neurologic and developmental disability after extremely preterm birth. EPICure Study Group. N Engl J Med 2000;343:378–84.
[116] Hack M, Wilson-Costello D, Friedman H, Taylor GH, Schluchter M, Fanaroff AA. Neurodevelopment and predictors of outcomes of children with birth weights of less than 1000 g: 1992–1995. Arch Pediatr Adolesc Med 2000;154:725–31.
[117] Volpe JJ. Postnatal sepsis, necrotizing enterocolitis, and the critical role of systemic inflammation in white matter injury in premature infants. J Pediatr 2008;153:160–3.
[118] Dammann O, Leviton A. Inflammatory brain damage in preterm newborns-dry numbers, wet lab, and causal inferences. Early Hum Dev 2004;79:1–15.
[119] Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birth weight infants. Developmental Epidemiology Network Investigators. Pediatr Res 1999;46:566–75.
[120] Dammann O, Phillips TM, Allred EN, O'Shea TM, Paneth N, Van Marter LJ, et al. Mediators of fetal inflammation in extremely low gestational age newborns. Cytokine 2001;13:234–9.
[121] Yoon BH, Park CW, Chaiworapongsa T. Intrauterine infection and the development of cerebral palsy. BJOG 2003;110(Suppl 20):S124–7.
[122] Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. Am J Obstet Gynecol 2000;183:1124–9.
[123] Martinez E, Figueroa R, Garry D, Visintainer P, Patel K, Verma U, et al. Elevated amniotic fluid interleukin-6 as a predictor of neonatal periventricular leukomalacia and intraventricular hemorrhage. J Matern Fetal Investig 1998;8:101–7.
[124] Kadhim H, Tabarki B, Verellen G, De Prez C, Rona AM, Sébire GH. Inflammatory cytokines in the pathogenesis of periventricular leukomalacia.. Neurology 2001;56:1278–84.
[125] Haynes RL, Baud O, Li J, Kinney HC, Volpe JJ, Folkerth DR. Oxidative and nitrative injury in periventricular leukomalacia: a review. Brain Pathol 2005;15:225–33.
[126] Glass HC, Bonifacio SL, Chau V, Glidden D, Poskitt K, Barkovich AJ, et al. Recurrent postnatal infections are associated with progressive white matter injury in premature infants. Pediatrics 2008;122:299–305.
[127] Shah DK, Doyle LW, Anderson PJ, Bear M, Daley AJ, Hunt RW, et al. Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. J Pediatr 2008;153:170–5.
[128] Chau V, Brant R, Poskitt KJ, Tam EW, Synnes A, Miller SP. Postnatal infection is associated with widespread abnormalities of brain development in premature newborns. Pediatr Res 2012;71:274–9.
[129] Chau V, Poskitt KJ, McFadden DE. Effect of chorioamnionitis on brain development and injury in premature newborns. Ann Neurol 2009;66:155–64.
[130] Adams E, Chau V, Poskitt KJ, Grunau RE, Synnes A. Tractography-based quantitation of corticospinal tract development in premature newborns. J Pediatr 2010;156:882–8.
[131] Procianoy RS, Silveira RC. Association between high cytokine levels with white matter injury in preterm infants with sepsis. Pediatr Crit Care Med 2012;13:183–7.

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