# Sepsis Biomarkers In Early Onset Neonatal Infections: A Review

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**Abstract**

The septic response is an extremely complex chain of events involving inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities (1, 2). The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature of the signs and symptoms of sepsis (3). However, the early diagnosis and stratification of the severity of sepsis is very important, increasing the possibility of starting timely and specific treatment (4, 5). Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis (6, 7), and can differentiate bacterial from viral and fungal infection, and systemic sepsis from local infection. With this background in mind, we reviewed the literature on sepsis biomarkers that have been used in clinical or experimental studies to help better evaluate their utility.

## INTRODUCTION

The septic response is an extremely complex chain of events involving inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities (1, 2). The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature of the signs and symptoms of sepsis (3). However, the early diagnosis and stratification of the severity of sepsis is very important, increasing the possibility of starting timely and specific treatment (4, 5). Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis (6, 7), and can differentiate bacterial from viral and fungal infection, and systemic sepsis from local infection. Other potential uses of biomarkers include roles in prognostication, guiding antibiotic therapy, evaluating the response to therapy and recovery from sepsis, differentiating Gram-positive from Gram negative microorganisms as the cause of sepsis, predicting sepsis complications and the development of organ dysfunction (heart, kidneys, liver or multiple organ dysfunction). However, the exact role of biomarkers in the management of septic patients remains undefined (8) C-reactive protein (CRP) has been used for many years (9, 10) but its specificity has been challenged (11). Procalcitonin (PCT) has been proposed as a more specific (12) and better prognostic(13) marker than CRP, although its value has also been challenged(14). It remains difficult to differentiate sepsis from other noninfectious causes of systemic inflammatory response syndrome(15), and there is a continuous search for better biomarkers of sepsis. With this background in mind, we reviewed the literature on sepsis biomarkers that have been used in clinical or experimental studies to help better evaluate their utility.

## THE IDEAL DIAGNOSTIC MARKER OF INFECTION

As most infection markers are essential mediators of the inflammatory cascade, their concentrations are likely to be influenced by infective as well as non-infective inflammatory stimuli such as toxic and tissue damaging processes. Establishing a statistically significant correlation between the concentration of a circulating marker and the severity of infection, or showing a significant increase or decrease in the marker's concentration in an infected infant, is not sufficient to qualify the diagnostic test as being competent or suitable for clinical use. Considering the high mortality and serious morbidity associated with neonatal sepsis, a diagnostic marker with a very high sensitivity (infected infants have a positive test) and negative predictive value (a negative test confidently rules out infection) approaching 100% is desirable because all septic infants with life threatening infection that is totally curable when diagnosed early should be identified and treated.(16, 17) Withholding or delaying antibiotics in false negative cases could have a fatal outcome. Conversely, the lack of reliable

clinical signs often results in anticipatory antimicrobial treatment. Thus a competent diagnostic marker also needs to have a reasonably high specificity (the test is negative if infection is absent) and a good positive predictive value (infection is present when the test is positive), preferably better than 85%, in order to minimise unnecessary use of antibiotics in false positive cases.(17) In addition, an optimal cut off should be defined in a specified patient population using the receiver operating characteristics curve for each marker, so as to allow comparison of results between different neonatal centres.(16, 17) Table 1 summarises other important clinical and laboratory characteristics of an ideal infection marker. Table 2 summarises the markers that have been studied in preterm or term infants.

**Figure 1**

Table 1 Characteristics of an ideal infection marker

| |
|---|
| **Clinical characteristics** |
| 1. A well defined optimal cut off that is comparable between different NICUs |
| 2. Favorable diagnostic utilities: |
| Sensitivity (approaching 100%) |
| Specificity (.85%) |
| Positive predictive value (.85%) |
| Negative predictive value (approaching 100%) |
| 3. Detects infection at an early stage |
| 4. Differentiates between different types of pathogen (viral v bacterial) |
| 5. Guides antibiotic use (type and duration) |
| 6. Monitors progress of treatment |
| 7. Prognostication |
| **Laboratory characteristics** |
| 1. Stable compound |
| 2. Adequate time window for specimen sampling (sustained increase or decrease in level for at least 48 h after the onset of clinical manifestations) |
| 3. Quantitative measurement |
| 4. Small volume of specimen |
| 5. Easy method of measurement |
| 6. Quick laboratory turnover time |
| 7. Results comparable between laboratories |
| 8. Low cost |

**Figure 2**

Table 2 Diagnostic markers of infection for preterm and newborn infants

| | | |
|---|---|---|
| **Hematological tests** | | |
| Total white blood cell count | | |
| Total neutrophil count | | |
| Immature neutrophil count | | |
| Immature/total neutrophil ratio | | |
| Neutrophil morphology: vacuolisation, toxic granulations, DÖhle bodies, intracellular bacteria | | |
| Platelet count | | |
| Granulocyte colony-stimulating factor (G-CSF) | | |
| D-dimer | | |
| Fibrinogen | | |
| Thrombin-antithrombin III complex (TAT) | | |
| Plasminogen activator inhibitor-1 (PAI-1) | | |
| Plasminogen tissue activator (tPA) | | |
| **Acute phase proteins and other proteins** | | |
| α1 Antitrypsin | | |
| C Reactive protein (CRP) | | |
| Fibronectin | | |
| Haptoglobin | | |
| Lactoferrin | | |
| Neopterin | | |
| Orosomucoid | | |
| Procalcitonin (PCT) | | |
| **Components of the complement system** | | |
| C3a-desArg | | |
| C3bBbP | | |
| sC5b-9 | | |
| **Chemokines, cytokines and adhesion molecules** | | |
| Interleukin (IL)1b, IL1ra, IL2, sIL2R, IL4, IL5, IL6, IL8, IL10 | | |
| Tumour necrosis factor a (TNFα), 11sTNFR-p55, 12sTNFR-p75 | | |
| Interferon c (IFNc) | | |
| E-selectin | | |
| L-selectin | | |
| Soluble intracellular adhesion moleucule-1 (sICAM-1) | | |
| Vascular cell | | |
| adhesion molecule-1 (VCAM-1) | | |
| **Cell surface markers** | | |
| Neutrophil | Lymphocyte | Monocyte |
| CD11b | CD3 | HLA-DR |
| CD11c | CD19 | |
| CD13 | CD25 | |
| CD15 | CD26 | |
| CD33 | CD45RO | |
| CD64 | CD69 | |
| CD66b | CD71 | |

## ANTENATAL DIAGNOSIS

Intra-amniotic infection is an important and potentially preventable cause of preterm births,

early onset neonatal sepsis, periventricular leucomalacia/ cerebral palsy, and maternal febrile morbidity. Overt or subclinical intra-amniotic infection is present in at least 50% of extremely preterm births; an inverse relation has been shown between gestational age at birth and both the frequency of micro-organisms recovered from the chorioamnion and histological chorioamnionitis.(18, 19) Potential pathogens largely arise from the ascending route and from the maternal endogenous vaginal flora, causing chorioamnionitis. The release of endotoxins and/or exotoxins from micro-organisms results in stimulation and production of inflammatory cytokines, prostaglandins, metalloproteinases resulting in maternal sepsis (chorioamnionitis, septicaemia, postpartum endometritis), fetal loss (extreme prematurity), and preterm delivery (infant prematurity and its consequences, including increased

susceptibility to cerebral palsy),

in addition to severe neonatal sepsis(18) To determine whether a patient in preterm labour has intra-amniotic inflammation, and thus should be delivered rather than treated with tocolytic agents, is a critical decision with clinical implications for both mother and fetus.(20) Early diagnosis of intra-amniotic infection is problematic, however, because clinical signs and symptoms (including preterm labour) tend to be late manifestations of this condition. Furthermore, the available non-invasive diagnostic tests have limited predictive value, or, as in the case of measurement of interleukin (IL) 6, polymerase chain reaction (PCR) tests, or microbial cultures, the results are often delayed and amniocentesis is required. Therefore improved diagnostic methods are needed to identify women and fetuses who may benefit from specific interventions, such as antibiotics or anti-inflammatory agents.(19) Amniotic fluid tumour necrosis factor a (TNF) is a marker for the prediction of early onset neonatal sepsis in patients with preterm labour and intact membranes, and a better independent predictor of early onset neonatal sepsis than placental histology or amniotic fluid Gram stain and/or culture. Amniotic fluid TNF concentration >41 pg/ml had a sensitivity of 82% and specificity of 79% in the prediction of early onset neonatal sepsis.(21) Although a strong association has been found between maternal serum C reactive protein (CRP) concentrations and cytokine concentrations in the amniotic fluid, in general, maternal measurements of CRP alone do not have a high sensitivity in predicting underlying asymptomatic intra-amniotic sepsis and are not recommended.(22) IL1b was the best predictor of vascular extension of chorioamnionitis, and TNF was the best predictor of the development of severe early onset neonatal infection(23).Although TNF is an important mediator in the pathophysiology of septic shock and systemic inflammatory response syndrome, its utility has not been found to be as good as either IL6 or IL8.(24, 25)

POSTNATAL DIAGNOSIS

Multiple studies have examined total leucocyte count, immature to total neutrophil ratio, platelet count, and CRP, and shown that these routine investigations either have low sensitivity and specificity or varying delayed responses early in the course of infection.(26) Leucocyte indices and CRP are considered to be ‘‘late’‘ markers and are not sensitive enough for early diagnosis of neonatal sepsis.(16) However, abnormalities in these markers soon after a birth complicated by clinical signs and obstetric risk factors of sepsis are highly

suggestive of early onset neonatal sepsis. Recent investigations have focused on various groups of chemokines, cytokines, adhesion molecules, and components of the immune pathway that could be used as earlier markers to diagnose infection in neonates.

## ACUTE PHASE REACTANTS

These groups of endogenous peptides are produced by the liver as part of an immediate response to infection or tissue injury. CRP has been extensively investigated,(26) but there has been more recent interest in procalcitonin. Many other acute phase proteins, including 1 antitrypsin, fibronectin, haptoglobin, lactoferrin, neopterin, and orosomucoid, have been evaluated in relation to neonatal sepsis. Although most markers show significant increases in infected infants, none have been routinely used clinically, either because of their limited diagnostic accuracy or because they have been superseded by better and more sophisticated tests. CRP is synthesised within six to eight hours of exposure to an infective process or tissue damage, with a half life of 19 hours, and may increase more than 1000-fold during an acute phase response.(27) The ranges of sensitivity and specificity for diagnosis of early onset sepsis ranges are 43–90% and 70–78% respectively.(26)The specificity and positive predictive value of CRP ranges from 93% to 100% in late onset sepsis. Thus CRP is a ‘‘specific’‘ but ‘‘late’‘ marker of neonatal infection.(16) CRP as a diagnostic marker in neonates has higher sensitivity and specificity than total neutrophil count and immature to total neutrophil ratio.(28) We have previously reported that the combination of CRP (.10 mg/l) with full blood examination (abnormal film and/or immature to total neutrophil ratio >0.2) and/or gastric aspirate (>5 polymorphs/ high power field or potential pathogen on Gram stained smear and/or culture of potential pathogen) has a sensitivity of 97%, specificity of 61%, negative predictive value of 98%, and likelihood ratio of 49 for early onset neonatal sepsis.(29) Procalcitonin is another important acute phase reactant produced by monocytes and hepatocytes which begins to rise four hours after exposure to bacterial endotoxin, peaking at six to eight hours, and remaining raised for at least 24 hours(30) with a half life of 25–30 hours. Several studies have shown that serum procalcitonin concentrations increase appreciably in systemic bacterial infection, necrotising enterocolitis, and during both early and late onset neonatal sepsis.(31) It may be superior to other acute phase proteins, with sensitivity and specificity ranging from 87% to 100%. It may be useful in

assessing the severity of infection, following the progress of treatment, and predicting outcomes(31, 32). However, it is not a readily available diagnostic assay in most institutions.

## CELL SURFACE MARKERS

Advances in flow cytometric technology have opened up ways of detecting cell surface antigens on blood cells. This technology appears to be superior to conventional immunological assay methods for localising the activated markers to a specific cell type. Further, as circulating concentrations of cytokines may not necessarily reflect their biological activities, assessing the cellular response to cytokines may be a better way of identifying early immunological response to bacterial invasion.(33, 34) Neutrophil CD11b and CD64 appear to be promising markers for diagnosis of early and late onset infections.(35, 36) CD11b is a subunit of the b2 integrin adhesion molecule, normally expressed at a very low concentration on the surface of non-activated neutrophils. There is a 2–4-fold increase in neutrophil CD11b expression in infants with blood culture positive sepsis,1 (36, 37) similar to that seen in adults with blood culture positive sepsis. The sensitivity and specificity of CD11b for diagnosing early onset neonatal sepsis are 86.3–100% and 100% respectively. A recent study assessing two neutrophil (CD11b, CD64) and two lymphocyte surface markers (CD25, CD45RO) showed that CD64 had the highest sensitivity (97%), specificity (90%), and negative predictive value (99%) as a diagnostic marker for early onset neonatal infection both at the onset of infection and 24 hours later.(38) Combining CD64 with IL6 or CRP further enhances the ability to diagnose localised infections and improves the sensitivity and negative predictive value to 100%.(38) There is an increase in the number of lymphocyte (CD3, CD19, CD25, CD26, CD71 and CD69) and neutrophil (CD11b,CD11c, CD13, CD15, CD33 and CD66b) antigens in preterm newborns in response to infection, with increased expression of CD19, CD33, and CD66b. However, the diagnostic utilities of these markers are yet to be evaluated.(39) Furthermore, none of these white cell surface markers are readily available diagnostically.

## GRANULOCYTE COLONY STIMULATING FACTOR

Granulocyte colony stimulating factor, a mediator produced by bone marrow, facilitates proliferation and differentiation of neutrophils, and has been proposed to be a reliable infection marker for early diagnosis of neonatal sepsis. A concentration >200 pg/ml has a high sensitivity (95%) and negative predictive value (99%) for predicting early onset neonatal bacterial and fungal infections.(40, 41)

## CYTOKINES

Cytokines play an essential role in maturation of progenitors in the bone marrow, in innate immunity, and in the maturation of antigen specific adaptive immunity. As antigen specific immunity develops later—for example, at 2 years of age in the case of encapsulated bacteria—neonates initially depend on natural (innate) immunity. This includes phagocytosis (by monocytes, tissue macrophages, and neutrophils), natural killer cells, and humoral mediators (CRP, complement, and transplacentally acquired maternal antibodies). In response to antigens such as bacterial endotoxins,(42) activated tissue macrophages produce TNF and IL1. These proinflammatory cytokines stimulate endothelial cells to express receptors for intercellular adhesion molecule on white blood cells. This initiates the cytokine cascade towards increased production of IL6, IL8, and chemokines.(43) Some bacteria activate epithelial cells directly to produce inflammatory cytokines. Newborn infants display a higher percentage of IL6 and IL8 positive cells than do adults.(42) There is sharp rise in IL6 concentration on exposure to bacterial products, which precedes the increase in CRP. Umbilical cord blood IL6 has been consistently shown to be a sensitive marker for diagnosing early onset neonatal sepsis, with sensitivities of 87–100% and negative predictive values of 93–100%.(40, 44) IL6 has the highest sensitivity (89%) and negative predictive value (91%) at the onset of infection compared with other biochemical markers, including CRP, IL1b, TNF, and Eselectin, but sensitivity is reduced at 24 and 48 hours (67%and 58% respectively) because IL6 concentrations fall rapidly and become undetectable after 24 hours.(40, 45) The combined measurement of IL6 (early and sensitive) with CRP (late and specific) in the first 48 hours of presumed septic episodes improves the sensitivity compared with either marker alone.(40) IL8 is a proinflammatory cytokine that is predominantly produced by monocytes, macrophages, and endothelial cells,(46) with similar kinetics to IL6.(47) It is produced in response to various stimuli such as polysaccharide and TNF.(46) IL8 is considered to be a highly accurate marker with sensitivities ranging from 80% to 91% and specificities from 76% to 100%. IL8 and IL8 mRNA concentrations are substantially higher in infected than non-infected newborns.(40) The simultaneous measurement of either CRP(48, 49) or neutrophil cell surface marker CD11b with IL8 further enhances the diagnostic value in the diagnosis of neonatal sepsis.(50) A recent multicentred randomised controlled trial of 1291

clinically stable infants with clinical signs or obstetric risk factors suggesting early onset neonatal sepsis reported that the combination of IL8 .70 pg/ml and/or CRP .10 mg/l significantly reduced antibiotic therapy from 49.6% to 36.1% (p,0.0001) without missing infections; sensitivity was 80%, specificity 87%, positive predictive value 68%, and negative predictive value 93%.(51) Furthermore there was no significant difference in missed infections in the group not treated with antibiotics with the control group. Another group of proinflammatory cytokines often linked with sepsis is the IL1 family, including IL1a, IL1b, and IL1 receptor antagonist (IL1ra), the last of which exists in substantial excess over IL1b during sepsis. The diagnostic usefulness of IL1b is minimal given conflicting reports of both increasing(43) and decreasing(52) concentrations associated with sepsis. In contrast, concentrations of IL1ra have been shown to be consistently increased in septic patients with concentrations of 6–30 mg/l(53, 54) compared with concentrations in uninfected neonates of 2–3 mg/l. Furthermore, one study found that IL1ra had increased by 3 and 15 times the concentration in healthy neonates at 4 and 2 days of age respectively before the clinical diagnosis of neonatal sepsis.(54) TNF is a proinflammatory cytokine that stimulates IL6 production and has a broad spectrum of biological actions on several types of target cell, both immune and non-immune. Newborns developing early onset infection are born with higher TNF concentrations than non-infected infants.(55) Other markers studied over the last few years include adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-selectin, L-selectin)(40) and complement activation products (C3a-desArg, C3bBbP, sC5b-9),(56) which have been found to be significantly increased during sepsis, but require further evaluation for clinical application in the diagnosis of newborn infection.

## MOLECULAR GENETICS

During the past decade an increasing number of reports on the use of nucleic acid amplification techniques such as PCR in the detection of bacterial genomes in blood cultures have appeared. In particular, broad range PCR analysis, which relies on the fact that the bacteria specific 16S rRNA gene is highly conserved in all bacterial genomes, is a useful method for identification of bacteria in clinical samples.(57) Amplification targeting of this 16S rRNA gene is a potentially valuable clinical tool in samples with low copy numbers of bacterial DNA, as this gene is present at 1 to more than 10 copies in all bacterial genomes. The gene also has a number of divergent regions nested within it, so PCR can be targeted for species specific detection of bacteria in clinical samples. For example, using the adaptation of real time PCR, with the LightCycler system, Golden et al(58) detected a group Bstreptococcus (GBS) specific cfb gene target DNA sequence in blood specimens, reporting a 100% sensitivity and 100% specificity when tested against 26 non-GBS culture detected bacteraemic episodes. The test was capable of detecting 100 copies or 10 pg GBS genomic DNA. This technology has also been reported to be a very sensitive (100%) and rapid method for detecting potential pathogens in amniotic fluid commonly involved in the pathogenesis of preterm labour and adverse neonatal outcome.(59) However, the performance of broad range PCR analysis at a level of high analytical sensitivity is complex and remains one of the most challenging PCR applications in the diagnostic laboratory. For example, as 16S rRNA gene amplification targets all bacterial species, small amounts of inherent residual DNA present in reagents may be co-amplified, resulting in false positivity. Methods for the removal of potential background contamination include long wave UV light gamma irradiation DNAse, restriction endonuclease digestion, ultrafiltration, and low DNA polymerases.However, many of these methods result in a reduced sensitivity in detecting target DNA, with a detection limit range of 103–104 copies/ml, which is not ideal for diagnosing sepsis in clinical settings. We have found that a combination of pre-PCR culture with the use of AmpliTaq Low DNA achieves an acceptable level of sensitivity (5–50 copies/ml in a turnaround time of eight hours) for the real time amplification of bacteria in blood samples, without the need to remove any inherent DNA contamination (unpublished work). Consequently it is critical to ensure that high standards and appropriate evaluations of analytical as well as clinical sensitivity are met, if such methods are used in diagnostic laboratories. It is also advisable that all positive broad range PCR products are identified, preferably by a sequenced based method.(57) Detection by PCR does not result in the antimicrobial sensitivity pattern of the pathogen. However, amplification of known resistance genes allows quick identification of bacteria that are resistant to specific drugs—for example, methicillin resistance for staphylococcal species. With the application of real time PCR, DNA isolation can be accomplished in as little as 20 minutes.(60) Early exclusion of bacterial infection could help to reduce overuse of antibiotics. It is predicted that eventually real time PCR combined with DNA MicroArray technology will allow not only identification of the organism but also the antimicrobial

sensitivity pattern, which is so critical to clinical care.

## SUMMARY

Table 3 provides a summary of the sensitivities, specificities, and positive and negative predictive values for various markers of early onset sepsis. It was not possible to calculate all values from the data provided in the individual papers.

### Figure 3

Table3 Accuracy of diagnostic tests or combinations of tests for early onset neonatal sepsis

| Diagnostic test | Sensitivity | specificity | PPV | NPV |
|---|---|---|---|---|
| **Antenatal** | | | | |
| AmnioticTNFα>41 pg/ml[21] | 82 | 79 | 47 | 95 |
| PCR for genomic DNA in amniotic fluid[59] | 100 | 100 | 100 | 100 |
| **Postnatal** | | | | |
| CRP[16] | 60-82 | 93-96 | 95-100 | 75-87 |
| CRP, FBE, gastric aspirate[29] | 97 | 61 | 53 | 98 |
| Procalcitonin[31, 32] | 82-100 | 87-100 | 86-98 | 93-100 |
| CD11b[36] | 96-100 | 81-100 | 22-100 | 100 |
| CD64[35] | 64-97 | 72-96 | 64-88 | 84-98 |
| CD64, IL6 or CRP[35] | 81-97 | 71-87 | 63-74 | 86-98 |
| GCSF >200 pg/ml[40, 41] | 95 | 73 | 40 | 99 |
| Umbilical cord IL64[44] | 87-90 | 93 | 93 | 93-100 |
| IL64[45] | 67–89 | 89–96 | 84–95 | 77–91 |
| IL6 and/or CRP[40, 45] | 93 | 88–96 | 86–95 | 95 |
| IL84[51] | 80–91 | 76–100 | 70–74 | 91–95 |
| IL8 and/or CRP[51] | 80 | 87 | 68 | 93 |
| PCR for genomic DNA in blood culture[58] | 100 | 100 | 100 | 100 |

PPV, Positive predictive value; NPV, negative predictive value; TNF, tumour necrosis factor ; CRP, C reactive protein; FBE, full blood examination; GCSF, granulocyte colony stimulating factor; IL6, interleukin 6; IL8, interleukin 8; PCR, polymerase chain reaction

## CONCLUSIONS

The cost associated with preterm birth is substantial in economic, social, and emotional terms. Upper genital tract infection is important in the mechanism of preterm birth, although it is usually asymptomatic. Furthermore, the pathogens associated with intra-amniotic sepsis are those usually involved in early onset neonatal infections. To ensure accurate diagnosis and appropriate antimicrobial management, highly sensitive markers predictive of neonatal sepsis and with a rapid turnaround time are required. Although many putative markers (acute phase reactants, cell surface markers, cytokines) are reported in various clinical research settings, most are not available to the routine diagnostic laboratory. Furthermore, the greatest predictability usually results from a combination of assays. However, none of the current diagnostic markers are sensitive and specific enough to influence the judgment to withhold antimicrobial treatment independent of the clinical findings. Until further evidence from larger studies are available, daily routine screening for prediction of neonatal infection is not warranted.

## References

1. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003 Jan 9;348(2):138-50.
2. Gullo A, Bianco N, Berlot G. Management of severe sepsis and septic shock: challenges and recommendations. Crit Care Clin 2006 Jul;22(3):489-501, ix.
3. Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. BMJ 2007 Oct 27;335(7625):879-83.
4. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006 Jun;34(6):1589-96.
5. Zambon M, Ceola M, Almeida-de-Castro R, Gullo A, Vincent JL. Implementation of the Surviving Sepsis Campaign guidelines for severe sepsis and septic shock: we could go faster. J Crit Care 2008 Dec;23(4):455-60 Epub 2007 Dec 11.
6. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001 Mar;69(3):89-95.
7. Marshall JC, Reinhart K. Biomarkers of sepsis. Crit Care Med 2009 Jul;37(7):2290-8.
8. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008 Jan;36(1):296-327.
9. Povoa P, Coelho L, Almeida E, Fernandes A, Mealha R, Moreira P, et al. C-reactive protein as a marker of infection in critically ill patients. Clin Microbiol Infect 2005 Feb;11(2):101-8.
10. Schmit X, Vincent JL. The time course of blood C-reactive protein concentrations in relation to the response to initial antimicrobial therapy in patients with sepsis. Infection 2008 Jun;36(3):213-9 Epub 2008 May 10.
11. Clyne B, Olshaker JS. The C-reactive protein. J Emerg Med 1999 Nov-Dec;17(6):1019-25.
12. Nakamura A, Wada H, Ikejiri M, Hatada T, Sakurai H, Matsushima Y, et al. Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock 2009 Jun;31(6):586-91.
13. Luzzani A, Polati E, Dorizzi R, Rungatscher A, Pavan R, Merlini A. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003 Jun;31(6):1737-41.
14. Tang BM, Eslick GD, Craig JC, McLean AS. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007 Mar;7(3):210-7.
15. Giamarellos-Bourboulis EJ, Giannopoulou P, Grecka P, Voros D, Mandragos K, Giamarellou H. Should procalcitonin be introduced in the diagnostic criteria for the systemic inflammatory response syndrome and sepsis? J Crit Care 2004 Sep;19(3):152-7.

16. Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, Wong W, et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 1997 Nov;77(3):F221-7.
17. Ng PC, Li K, Wong RP, Chui KM, Wong E, Fok TF. Neutrophil CD64 expression: a sensitive diagnostic marker for late-onset nosocomial infection in very low birthweight infants. Pediatr Res 2002 Mar;51(3):296-303.
18. Garland SM, Ni Chuileannain F, Satzke C, Robins-Browne R. Mechanisms, organisms and markers of infection in pregnancy. J Reprod Immunol 2002 Oct-Nov;57(1-2):169-83.
19. Gravett MG, Novy MJ, Rosenfeld RG, Reddy AP, Jacob T, Turner M, et al. Diagnosis of intra-amniotic infection by proteomic profiling and identification of novel biomarkers. JAMA 2004 Jul 28;292(4):462-9.
20. Buhimschi IA, Christner R, Buhimschi CS. Proteomic biomarker analysis of amniotic fluid for identification of intra-amniotic inflammation. BJOG 2005 Feb;112(2):173-81.
21. Park KH, Yoon BH, Shim SS, Jun JK, Syn HC. Amniotic fluid tumor necrosis factor-alpha is a marker for the prediction of early-onset neonatal sepsis in preterm labor. Gynecol Obstet Invest 2004;58(2):84-90 Epub 2004 May 12.
22. Giles M, Garland S, Oats JJ. Management of preterm prelabour rupture of membranes: an audit. How do the results compare with clinical practice guidelines? Aust N Z J Obstet Gynaecol 2005 Jun;45(3):201-6.
23. Baud O, Emilie D, Pelletier E, Lacaze-Masmonteil T, Zupan V, Fernandez H, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestation: histological associations and neonatal outcome. Br J Obstet Gynaecol 1999 Jan;106(1):72-7.
24. Berner R, Tuxen B, Clad A, Forster J, Brandis M. Elevated gene expression of interleukin-8 in cord blood is a sensitive marker for neonatal infection. Eur J Pediatr 2000 Mar;159(3):205-10.
25. Dollner H, Vatten L, Austgulen R. Early diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6, soluble tumour necrosis factor receptors and soluble adhesion molecules. J Clin Epidemiol 2001 Dec;54(12):1251-7.
26. Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days--a systematic review. Arch Dis Child Fetal Neonatal Ed 1998 Mar;78(2):F92-8.
27. Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest 1993 Apr;91(4):1351-7.
28. Da Silva O, Ohlsson A, Kenyon C. Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J 1995 May;14(5):362-6.
29. Garland SM, Bowman ED. Reappraisal of C-reactive protein as a screening tool for neonatal sepsis. Pathology 2003 Jun;35(3):240-3.
30. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994 Dec;79(6):1605-8.
31. Guibourdenche J, Bedu A, Petzold L, Marchand M, Mariani-Kurdjian P, Hurtaud-Roux MF, et al. Biochemical markers of neonatal sepsis: value of procalcitonin in the emergency setting. Ann Clin Biochem 2002 Mar;39(Pt 2):130-5.
32. 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 Jan;49(1):60-8.
33. Simms HH, D'Amico R. Lipopolysaccharide induces intracytoplasmic migration of the polymorphonuclear leukocyte CD11b/CD18 receptor. Shock 1995 Mar;3(3):196-203.
34. Lehr HA, Krombach F, Munzing S, Bodlaj R, Glaubitt SI, Seiffge D, et al. In vitro effects of oxidized low density lipoprotein on CD11b/CD18 and L-selectin presentation on neutrophils and monocytes with relevance for the in vivo situation. Am J Pathol 1995 Jan;146(1):218-27.
35. Fjaertoft G, Hakansson L, Ewald U, Foucard T, Venge P. Neutrophils from term and preterm newborn infants express the high affinity Fcgamma-receptor I (CD64) during bacterial infections. Pediatr Res 1999 Jun;45(6):871-6.
36. Weirich E, Rabin RL, Maldonado Y, Benitz W, Modler S, Herzenberg LA. Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal infection. J Pediatr 1998 Mar;132(3 Pt 1):445-51.
37. Takala A, Jousela I, Jansson SE, Olkkola KT, Takkunen O, Orpana A, et al. Markers of systemic inflammation predicting organ failure in community-acquired septic shock. Clin Sci (Lond) 1999 Nov;97(5):529-38.
38. Ng PC, Li G, Chui KM, Chu WC, Li K, Wong RP, et al. Neutrophil CD64 is a sensitive diagnostic marker for early-onset neonatal infection. Pediatr Res 2004 Nov;56(5):796-803 Epub 2004 Sep 15.
39. Weinschenk NP, Farina A, Bianchi DW. Premature infants respond to early-onset and late-onset sepsis with leukocyte activation. J Pediatr 2000 Sep;137(3):345-50.
40. Mehr S, Doyle LW. Cytokines as markers of bacterial sepsis in newborn infants: a review. Pediatr Infect Dis J 2000 Sep;19(9):879-87.
41. Kennon C, Overturf G, Bessman S, Sierra E, Smith KJ, Brann B. Granulocyte colony-stimulating factor as a marker for bacterial infection in neonates. J Pediatr 1996 Jun;128(6):765-9.
42. Schultz C, Rott C, Temming P, Schlenke P, Moller JC, Bucsky P. Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. Pediatr Res 2002 Mar;51(3):317-22.
43. Berner R, Niemeyer CM, Leititis JU, Funke A, Schwab C, Rau U, et al. Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor-alpha, interleukin (IL)-1beta, IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. Pediatr Res 1998 Oct;44(4):469-77.
44. 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 May-Jun;8(3):88-94.
45. Buck C, Bundschu J, Gallati H, Bartmann P, Pohlandt F. Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. Pediatrics 1994 Jan;93(1):54-8.
46. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989 Oct;84(4):1045-9.
47. Martich GD, Danner RL, Ceska M, Suffredini AF. Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. J Exp Med 1991 Apr 1;173(4):1021-4.
48. Franz AR, Steinbach G, Kron M, Pohlandt F. Reduction

of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. Pediatrics 1999 Sep;104(3 Pt 1):447-53.
49. Franz AR, Steinbach G, Kron M, Pohlandt F. Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr 2001 Sep;90(9):1025-32.
50. Nupponen I, Andersson S, Jarvenpaa AL, Kautiainen H, Repo H. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics 2001 Jul;108(1):E12.
51. Franz AR, Bauer K, Schalk A, Garland SM, Bowman ED, Rex K, et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. Pediatrics 2004 Jul;114(1):1-8.
52. Atici A, Satar M, Alparslan N. Serum interleukin-1 beta in neonatal sepsis. Acta Paediatr 1996 Mar;85(3):371-4.
53. Dollner 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 Jul;80(1):41-7.
54. Kuster H, Weiss M, Willeitner AE, Detlefsen S, Jeremias I, Zbojan J, et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet 1998 Oct 17;352(9136):1271-7.
55. Kowalik K, Czeszynska MB, Celewicz Z. [Evaluation of diagnostic usefulness of the cord blood TNF-alpha levels as a marker of early onset neonatal infection]. Ginekol Pol 2003 Jun;74(6):439-45.
56. Zilow EP, Hauck W, Linderkamp O, Zilow G. Alternative pathway activation of the complement system in preterm infants with early onset infection. Pediatr Res 1997 Mar;41(3):334-9.
57. Maiwald M. Broad-range PCR for the detection and identification of bacteria, Molecular microbiology:diagnostic principles and practice. 2nd ed ed. Washington DC: American Society of Microbiology; 2004; 379–90.
58. Golden SM, Stamilio DM, Faux BM, dela Cruz WP, Shoemaker CT, Blackmon CL, et al. Evaluation of a real-time fluorescent PCR assay for rapid detection of Group B Streptococci in neonatal blood. Diagn Microbiol Infect Dis 2004 Sep;50(1):7-13.
59. Straka M, Dela Cruz W, Blackmon C, Johnson O, Stassen S, Streitman D, et al. Rapid detection of group B streptococcus and Escherichia coli in amniotic fluid using real-time fluorescent PCR. Infect Dis Obstet Gynecol 2004 Sep-Dec;12(3-4):109-14.
60. Jaffe RI, Lane JD, Albury SV, Niemeyer DM. Rapid extraction from and direct identification in clinical samples of methicillin-resistant staphylococci using the PCR. J Clin Microbiol 2000 Sep;38(9):3407-12.

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