

REVIEW

Diagnostic markers of infection in neonates

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Diagnostic markers of infection are useful indicators of neonatal sepsis. Serial measurements of infection markers can improve diagnostic sensitivity, and the use of multiple markers can enhance diagnostic accuracy. Current evidence suggests that promising markers may be useful for early termination of antimicrobial treatment, but none of the current diagnostic tests are sensitive and specific enough to influence the clinical decision for withholding antibiotic treatment at the onset of suspected infection.

clinical aspects of these infection markers and focuses on five main areas: (a) the "ideal" diagnostic marker of infection; (b) infection markers in clinical practice; (c) infection markers with prognostic significance; (d) limitations of the diagnostic tests in clinical applications; (e) future development.

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.^{9 10} 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.¹⁰ 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.^{9 10} Table 1 summarises other important clinical and laboratory characteristics of an ideal infection marker.

Advances in neonatal management have led to considerable improvement in survival of newborn infants, in particular, the very low birthweight group (< 1500 g).^{1–3} Early (< 72 hours of birth) and late (> 72 hours) onset systemic bacterial infection, however, remains a devastating complication and an important cause of morbidity and mortality in these infants.^{4–7} Recent surveys suggest that very low birthweight infants who develop neonatal infection have a significantly increased risk of prolonged oxygen supplementation,⁸ a longer hospital stay,^{4 5} and higher mortality⁹ than patients who are not infected. In both term and preterm infants, early warning signs and symptoms are often minimal, subtle, non-specific, and can easily be misinterpreted as being due to non-infective causes such as transient tachypnoea of the newborn, environmentally induced fluctuation of body temperature, apnoea of prematurity, and acute exacerbations of bronchopulmonary dysplasia.^{9 10} Although the onset of illness is often inconspicuous, the clinical course may be alarmingly fulminant, leading to septicaemic shock, disseminated intravascular coagulation, and death within hours of the onset of clinical manifestations.⁹ Infected infants must therefore be promptly identified and differentiated from non-infected patients, and antibiotics started without delay. However, as microbiological culture results and antimicrobial susceptibility data are not usually available until at least 48 hours after the specimen reaches the laboratory, early identification of genuine sepsis is a major diagnostic problem. In addition, antimicrobial treatment based solely on risk factors and clinical grounds is likely to result in overtreatment. Continuation of antibiotics for presumptive bacterial infection often leads to unnecessary and prolonged treatment. A wide variety of haematological and biochemical markers have been investigated for the evaluation of clinical sepsis. As the literature on this topic has grown substantially, this article examines the important

INFECTION MARKERS IN CLINICAL PRACTICE

Many infection markers have been evaluated in the intensive care and accident and emergency settings in neonatal, paediatric, and adult

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Abbreviations: CRP, C reactive protein; IL, interleukin; PCT, procalcitonin; SIRS, systemic inflammatory response syndrome; TNF, tumour necrosis factor

Table 1 Characteristics of an ideal infection marker**Clinical characteristics**

1. A well defined optimal cut off that is comparable between different NICUs
2. Favourable 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

NICU, Neonatal intensive care unit.

patients. Table 2 summarises the markers that have been studied in preterm or term infants. The clinical importance and usefulness of these tests are described below.

Haematological tests

In the early and mid 1980s, neonatal clinicians relied mainly on haematological indices as adjunct indicators for early diagnosis of neonatal sepsis. Total leucocyte count, total neutrophil count, immature neutrophil count, immature to total neutrophil (I/T) ratio, immature to mature neutrophil ratio, morphological or degenerative changes in neutrophil such as vacuolisation, Döhle bodies, intracellular bacteria, toxic granulation, and platelet count have been studied either singly or in combination.¹¹⁻¹⁷ Results of white cell counts and ratios varied widely across studies, with sensitivity and specificity ranging from 17% to 90% and 31% to 100%.¹⁴ In general, the abnormal leucocyte ratios, including the I/T ratio ≥ 0.2 , tend to have high sensitivity, whereas abnormal leucocyte counts, such as leucopenia and neutropenia, tend to have high specificity.¹²⁻¹⁵ The use of an elaborated haematological scoring system involving seven of the above variables (one point allocated to each abnormal variable) suggested that the higher the score, the greater the certainty that the suspected septic episode was genuine.¹⁵ Using a cut off of ≥ 3 , the score had a high sensitivity of 96%, but a disappointingly low positive predictive value of 31%.¹⁵ This scoring system was not widely adopted because of its unfavourable diagnostic values, complexity of the scoring method, and the fact that some of the tests were labour intensive and required a highly trained technician to produce an accurate result. Low platelet counts and morphological changes in neutrophils were often severe and late signs of infection.^{12 15 18} Moreover, thrombocytopenia without disseminated intravascular coagulation has been recently shown to be associated with increased pulmonary platelet consumption secondary to architectural lung damage in a rodent model.¹⁹ This phenomenon has also been observed in preterm infants with severe respiratory distress syndrome and is independent of any infective cause (unpublished data).

More recently, granulocyte colony stimulating factor, a mediator produced by the bone marrow for facilitating the proliferation and differentiation of neutrophils, has been proposed to be a reliable infection marker for early diagnosis

Table 2 Diagnostic markers of infection for preterm and newborn infants**Haematological 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

- α 1 Antitrypsin
- C Reactive protein (CRP)
- Fibronectin
- Haptoglobin
- Lactoferrin
- Neopterin
- Orosomucoid
- Procalcitonin (PCT)

Components of the complement system

- C3 α -desArg
- C3bBbP
- sC5b-9

Chemokines, cytokines and adhesion molecules

- Interleukin (IL)1 β , IL1 α , IL2, sIL2R, IL4, IL5, IL6, IL8, IL10
- Tumour necrosis factor α (TNF α), 11sTNFR-p55, 12sTNFR-p75
- Interferon γ (IFN γ)
- E-selectin
- L-selectin
- Soluble intracellular adhesion molecule-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	
Others		
Lactate		
Micro-erythrocyte sedimentation		
Superoxide anion (respiratory burst)		

of neonatal sepsis.^{20 21} Based on a cut off of 200 pg/ml, it has a high sensitivity (95%) and negative predictive value (99%) for predicting early neonatal bacterial and fungal infections.²⁰ Further, septic neonates were also prone to develop haemorrhagic and thrombotic complications. Activation of the clotting and fibrinolytic systems has been shown both in adult patients and preterm neonates with severe infection.^{8 22-24} Circulating thrombin-antithrombin III complex, plasminogen activator inhibitor-1, plasminogen tissue activator, fibrinogen, and D-dimer concentrations are significantly raised in infected infants compared with non-infected patients.^{22 24} However, sick preterm newborns with respiratory distress syndrome also have deranged coagulation and fibrinolysis. Whether granulocyte colony stimulating factor and coagulation products can in future be used as clinical indicators of sepsis, or whether the suppression of thrombin and plasmin production may improve mortality and clinical outcomes, will require further investigation and direct comparison with other infection markers.

Acute phase proteins and other proteins

Acute phase proteins are produced principally by the liver as part of an immediate inflammatory response to infection or

tissue injury. This group of endogenous peptides was widely studied in the late 1980s and early 1990s, as it was recognised that haematological indices alone could not be confidently used as decision criteria for the diagnosis of infection or for guiding antimicrobial treatment. The most extensively used and investigated acute phase reactant is C reactive protein (CRP).^{9-14 25-32} CRP is synthesised within six to eight hours of exposure to an infective process or tissue damage. It has a half life of 19 hours and may increase more than 1000-fold during an acute phase response.³³ CRP as a diagnostic marker in neonates has higher sensitivity and specificity than total neutrophil count and I/T ratio.¹⁴ Previous studies suggest that CRP is particularly useful in managing late onset nosocomial bacterial or fungal systemic infection and necrotising enterocolitis.^{9 12 13 27} However, as the concentrations of CRP increase rather slowly in the initial phase, the sensitivity at the time of sepsis evaluation is only 60%.⁹ Serial measurements at 24 and 48 hours after the onset of illness considerably improve the sensitivity (82% and 84% respectively).⁹ The specificity and positive predictive value of CRP range from 93% to 100% throughout the study period.⁹ Thus, CRP can be considered as a "specific" but "late" marker of neonatal infection.^{9 13} Further, the change in pattern of CRP and normalisation of raised concentrations are considered to be useful in monitoring the progress of treatment and for guiding antibiotic treatment.^{9 13 25 26 29 30} Despite the promising characteristics of the test, we have previously reported cases of life threatening central nervous system candidiasis that were not identified by CRP and other sophisticated markers of infection.³² The localised and chronic low grade nature of these infections are probably the main reasons for the persistent normal plasma values observed.³² Conversely, increased CRP concentrations have been observed in non-infected clinical conditions such as meconium aspiration, tissue necrosis, recent vaccination, and surgery.^{9 31}

Another acute phase marker that has attracted much attention recently is procalcitonin (PCT). The increase in circulating PCT concentration is independent of calcitonin, and PCT has been shown to be associated with neurotransmission, immunomodulation, and vascular control during infection and in the systemic inflammatory response syndrome (SIRS). Although the exact sites of production of PCT in sepsis have not been identified, monocytes and hepatic cells are believed to be potential sources. Serum concentrations of PCT begin to rise four hours after exposure to bacterial endotoxin, peak at six to eight hours, and remain raised for at least 24 hours.³⁴ The half life is estimated to be about 25–30 hours, and the serum concentrations do not appear to be affected by the gestational age.^{24 35} However, serum PCT concentrations vary widely in the first few days of life.^{24 35 36} The kinetics of PCT in non-infected newborns in the immediate postnatal period suggests that concentrations are relatively low soon after birth (< 0.08 mg/ml), rise to a peak at 21–24 hours (0.6 ng/ml), and return to the baseline by 48 hours of life.³⁵ The reason for this "physiological" phenomenon is not known, but has been postulated to be the result of rapid colonisation of bacteria in the gastrointestinal tract, followed by translocation of endotoxin through the bowel wall. Several studies have shown that serum PCT concentrations increase appreciably in systemic bacterial infection and necrotising enterocolitis during early and late onset neonatal infection.^{24 35 37} Its diagnostic profile has been claimed to be superior to other acute phase proteins, including CRP, with sensitivity and specificity ranging from 87% to 100%.^{24 35} In contrast, infants with viral infection, bacterial colonisation, and non-infected inflammatory stress, such as birth trauma, aspiration syndrome, and hypoxaemia, have normal or only slightly raised concentrations.³⁷ Further, definitively raised PCT concentrations in bacterial infection

can be easily differentiated from the relatively small "physiological" increase in the immediate postnatal period.³⁵ Unlike haematological indices, PCT has been reported to be useful in indicating the severity of infection, following progress of treatment, and predicting outcomes.^{35 38} However, false negative cases have also been found,^{36 39} and very high serum concentrations have been detected in patients with respiratory distress syndrome, acute lung and inhalation injuries, haemodynamic failure, and severe trauma, without bacterial infection.

Many other acute phase proteins, including α_1 antitrypsin,¹¹ fibronectin,¹⁶ haptoglobin,¹¹ lactoferrin,⁴⁰ neopterin,²⁷ and orosomucoid, have been evaluated in relation to neonatal sepsis. Although most markers reveal significant increases in concentrations in infected infants, none of these peptides have been routinely used clinically, either because of their limited diagnostic accuracy or because they were superseded by better and more sophisticated tests.

Chemokines, cytokines, adhesion molecules, and components of the immune pathway

This group of infection markers were extensively studied in the mid and late 1990s. Although it is widely believed that preterm as well as term newborns have immature inflammatory responses, a recent study has shown that these infants display a higher percentage of interleukin (IL) 6 and IL8 positive cells than do adults.⁴¹ The rationale for investigating this diverse group of intercellular messengers is that leucocyte indices and CRP are "late" markers and are not sensitive enough for early diagnosis of neonatal sepsis.⁹ Of the many mediators studied (table 2), much attention has been focused on IL6, IL8, and tumour necrosis factor (TNF) α .

IL6 is an important cytokine of the early host response to infection. Its concentration increases sharply after exposure to bacterial products and precedes the increase in CRP. Umbilical cord blood IL6 has been consistently shown to be a sensitive marker for diagnosing neonatal infection within 72 hours of birth, the sensitivities and negative predictive values being 87–100% and 93–100%.⁴²⁻⁴⁴ Less promising results from some studies^{45 46} are probably due to the use of less sensitive assay methods.⁴⁷ IL6 is equally effective as a diagnostic marker for late onset nosocomial infection in preterm infants.^{9 48} At the onset of infection, IL6 has the highest sensitivity (89%) and negative predictive value (91%) compared with other biochemical markers, including CRP, IL1 β , TNF α , and E-selectin.⁹ However, it has a very short half life, and the concentrations fall precipitously with treatment and become undetectable in most infected patients within 24 hours.^{9 49} The sensitivity is therefore reduced to a much lower concentration at 24 and 48 hours (67% and 58% respectively).⁹ IL6 can be considered as an early and sensitive marker of neonatal infection.⁹ Clinically, the measurement of IL6 (early and sensitive) in combination with CRP (late and specific) in the first 48 hours of presumed septic episodes have been shown to yield a better sensitivity than either marker alone.^{9 42 50-52} In addition, one study has shown that the use of IL6 and IL1 receptor antagonist (IL1ra) together can predict neonatal sepsis two days before clinical manifestations and can result in earlier initiation of antimicrobial treatment with correspondingly better clinical outcome.⁴⁸

In many aspects, the characteristics and kinetic properties of IL8 and TNF α are very similar to those of IL6. Both are proinflammatory chemokines or cytokines produced predominantly by activated phagocytes in response to systemic infection and inflammation. Such proinflammatory responses do not seem to be affected by the gestational or postnatal age of infants. In both early and late onset sepsis,

IL8 and IL8 mRNA concentrations are substantially higher in infected than non-infected newborns.^{29 30 53–55} IL8 is considered to be a highly accurate marker with sensitivities ranging from 80% to 91% and specificities from 76% to 100%.^{29 30 54 55} The diagnostic accuracy is further enhanced by simultaneous measurement of either CRP^{29 30} or neutrophil cell surface marker CD11b.⁵⁵ The combination of IL8 and CRP has also been suggested to be useful in restricting unnecessary antibiotic use.^{29 30} Although TNF α has been shown to be an important mediator in the pathophysiology of septic shock and SIRS, its usefulness as a diagnostic marker has not been found to be as good as either IL6 or IL8.^{9 42 43 52 53 56 57}

Similarly, other cytokines (IL1 β , sIL2R, interferon γ),^{18 27 54 56 57} adhesion molecules (ICAM-1, VCAM-1, E-selectin, L-selectin),^{9 52 58} and complement activation products (C3a-desArg, C3bBbP, sC5b-9)⁵⁹ have been found to be significantly increased during sepsis, but none has fulfilled the stringent criteria (table 1) to be considered suitable for clinical application in newborns.

Cell surface markers

In the late 1990s and early 2000s, advances in flow cytometric technology paved the way for easy detection of cell surface antigens on blood cells. Specific leucocyte surface antigens are known to be expressed in substantial quantities after inflammatory cells are activated by bacteria or their cellular products.^{60 61} To date, published reports on leucocyte cell surface antigens in newborns are limited. Flow cytometric analysis has the advantage over conventional immunological assay methods of being able to localise the activated markers to a specific cell type. Such tests can be readily performed on an ad hoc basis, and each test requires only a minimal volume of blood (0.05 ml whole blood).¹⁰ Further, as the circulating concentrations of cytokines may not necessarily reflect their biological activities, assessing the cellular response to cytokines can be a better way of identifying an early immunological response to bacterial invasion.

In newborns, cell surface antigens have been studied in connection with congenital, early and late onset sepsis. Neutrophil CD11b and CD64 have been found to be promising markers for diagnosis of early and late infections respectively. CD11b is an α subunit of the β_2 integrin adhesion molecule. It is normally expressed at a very low concentration on the surface of non-activated neutrophils.^{62 63} Its expression, however, increases considerably within a few minutes after the inflammatory cells come into contact with bacteria and endotoxins.^{60 61} These unique characteristics enable CD11b to be used as a potential early warning marker for prediction of bacterial infection. The sensitivity and specificity of CD11b for diagnosing early onset neonatal sepsis are very high, being 96–100% and 100% in two studies.^{55 63} However, its accuracy in diagnosing late onset nosocomial infection in preterm infants is more variable.^{10 64} The discrepancy of results between studies may be related to different infant populations being evaluated, the methodology of performing the test, and at which phase of infection blood is obtained for determination of CD11b.¹⁰ In contrast, CD64 is a highly effective marker for the diagnosis of late onset infection.^{10 62} CD64 is normally expressed in very low concentrations by unstimulated neutrophils. It is considerably upregulated on the trigger of bacterial invasion,^{10 62} and has been shown to be involved in the process of phagocytosis and intracellular killing of pathogens.^{65 66} More importantly, neutrophils from preterm infants express CD64 during bacterial infections to the same degree as those from term infants, children, and adults.⁶² In our recent study using two neutrophil (CD11b, CD64) and two lymphocyte surface markers (CD25, CD45RO), CD64 had the highest sensitivity (97%), specificity (90%), and negative predictive value

(99%).¹⁰ Diagnostic accuracy at the onset of infection and 24 hours later were equally impressive. Combining the use of CD64 with IL6 or CRP further enhances the ability to diagnose localised infections, and improves the sensitivity and negative predictive value to 100%.¹⁰ The other three cell surface antigens, however, were not considered to be useful markers for nosocomial infection. To date, most reports on cell surface antigen expression use a semiquantitative method of measurement.^{63 64} Our latest study provides an additional advantage of being able to quantify the number of antigens expressed on the cell surface, and therefore able to provide a numerical cut off value for the test.¹⁰ Despite the encouraging results in neonates, neutrophil surface antigens do not appear to be useful for febrile neutropenic oncology patients, as too few neutrophils are available for cytometric measurement (unpublished data).

CD45RO, a memory antigen for T lymphocytes, is probably more useful in detecting congenital viral infections⁶⁷ than acute bacterial infections.^{10 68} Prolonged CD45RO expression for several weeks or months after exposure to bacterial and viral infections⁶⁹ renders it unsuitable for use in the neonatal intensive care unit. Other cell surface markers have been investigated in preterm and term infants. Weinschenk and coworkers⁶⁴ studied a panel of lymphocyte (CD3, CD19, CD25, CD26, CD71 and CD69) and neutrophil (CD11b, CD11c, CD13, CD15, CD33 and CD66b) antigens, and found that leucocytes of preterm newborns responded to infection with an increased expression of CD19, CD33, and CD66b. However, the diagnostic utilities of these markers are not known.⁶⁴ HLA-DR expression on monocytes was found to be significantly lower in adult septic patients after trauma⁷⁰ or surgery⁷¹ than in similar patients without sepsis. An interim analysis of the current study by my group suggests a decrease of HLA-DR expression in term infants with early onset sepsis, although the diagnostic accuracy may not be as good as the conventional acute phase reactants, cytokines, or other cell surface markers (unpublished data).

Undoubtedly, the demand of flow cytometry for cell surface antigen detection and other haematological analyses will continue to grow. This technology is likely to become an essential and integral part of routine laboratory investigations in future.

INFECTION MARKERS WITH PROGNOSTIC SIGNIFICANCE

As chemokines and cytokines are endogenous mediators that orchestrate the inflammatory cascade of the human body,⁷² the interaction between proinflammatory (IL6, TNF α , IFN γ) and anti-inflammatory mediators (IL4, IL10) may therefore play an important role in the clinical manifestations and outcomes of SIRS.^{18 73 74} Proinflammatory cytokines are primarily responsible for initiating an effective defence against exogenous pathogens. However, overproduction of these mediators can be harmful and may result in tissue destruction. Conversely, anti-inflammatory cytokines are crucial for downregulating the exacerbated inflammatory process and maintaining body homeostasis, but excessive anti-inflammatory response may also result in the suppression of immune function. It has been suggested that persistently high concentrations of IL10 or a high IL10/TNF α ratio could indicate poor prognosis and increased mortality in adult febrile patients.⁷⁵ Others have found that high IL6/IL10 ratios may be associated with poor outcomes in patients with SIRS.⁷⁴ Such evidence supports the hypothesis that exaggeration of proinflammatory activity coupled with inadequate anti-inflammatory compensation may result in shock, multi-organ dysfunction, and death. Recent data from my research team on very low birthweight infants also suggest that both the proinflammatory and anti-inflammatory

counterregulatory mechanisms are probably operational very early in gestation, and septic infants with disseminated intravascular coagulation show disproportional increases in plasma IL6, IL10, and TNF α concentrations or IL10/TNF α and IL6/IL10 ratios.¹⁸ However, transient increases in these concentrations and ratios do not necessarily indicate a poor outcome, as these indices fall precipitously after successful treatment. I speculate that key mediators such as IL6, TNF α , and IL10 play a crucial role in balancing the risks and benefits of the inflammatory cascade during systemic infection. Recent advances in flow cytometric technology allow quantitative measurement of a large panel of inflammatory mediators with a minimal volume of blood and would therefore be very suitable for assessing the pathophysiological process of infection in newborn infants.

LIMITATIONS OF INFECTION MARKERS IN CLINICAL APPLICATIONS

Despite the favourable claims by many studies, most diagnostic markers fail to meet the stringent demands required for clinical practice. Cost, availability of specimens at the appropriate time, complexity of the assay methods, laboratory turnover time, reliability of the tests, and attitude of attending clinicians are all important factors in determining the suitability of a diagnostic marker for clinical application.

Assays of chemokines and cytokines are expensive tests. Most research laboratories tend to perform the analyses in batches, and this defeats the purpose of using them as "early" warning markers. Unless the assay methods become automated,^{29 30} ad hoc measurement of these blood samples will not be cost effective. In contrast, expression of cell surface antigen is usually measured on an individual basis, but these tests are also very expensive. Specimens are required to be transported to the laboratory for immediate processing, and the experiment has to be performed under strictly specified conditions.^{10 63} Thus a highly trained technician needs to be available at all times to satisfy the rapid turnaround time required clinically. This arrangement is not practicable in most institutions and would incur a substantial cost. The collection of specimens for CD64 can probably be delayed until the next working morning, as antigen expression is sustained for at least 24 hours after the onset of illness.¹⁰

More importantly, the relatively small sample size in most studies, the lack of a reference cut off for most diagnostic markers, and different cut offs of the same marker given in the literature render the results difficult to interpret and the diagnostic tool wearisome to apply clinically. For an infection marker to be useful as a routine clinical tool, high comparability and reproducibility across different laboratories is required. In addition, the heterogeneous methods of laboratory measurement and the wide variations in data analysis and in reporting results have virtually excluded the possibility of performing a meaningful meta-analysis.⁴⁷ Thus it is often difficult to formulate a definitive opinion on the clinical usefulness of infection markers from the published reports.

In most of these tests, there are no problems in obtaining specimens from the patients, as blood or other sterile body fluids are routinely collected for haematological, biochemical, and microbiological investigations. Iatrogenic anaemia is not a major concern, as most modern tests require only a minute volume of blood for measurement.^{9 10} The flow cytometric technique, in particular, needs only 0.05 ml whole blood for each surface antigen measurement¹⁰ or 0.05 ml plasma per panel (six cytokines) of cytokine markers.¹⁸

Despite the very high sensitivity and negative predictive value suggested for some diagnostic markers,^{9 10 29 30 35 37 48 55 63}

most neonatal clinicians are reluctant to withhold antibiotic treatment for a deteriorating infant at the onset of a suspected septic episode. This is because none of the current infection markers can consistently diagnose 100% of infected cases, and the consequence of misdiagnosing a potentially treatable but life threatening condition would be devastating and ethically unacceptable. Serious localised infections such as pneumonia, deep tissue abscess, and cerebral ventriculitis have been associated with normal concentrations of circulating markers.^{9 10 32} Current evidence suggests that the use of promising diagnostic markers such as CD11b, CD64, IL6, IL8, PCT, and CRP, either alone or in combination, can allow neonatal clinicians to confidently discontinue antibiotics at an early phase (24–48 hours of onset) of the disease process, and without waiting for the definitive microbiological results, provided that the infant remains clinically well. There is, for the time being, not enough evidence to justify daily "prophylactic" blood sampling for predicting nosocomial sepsis 48 hours in advance,⁴⁸ and larger studies are required to further delineate the diagnostic utility of measuring these cytokines for such an application. No ideal diagnostic marker has been identified so far because the mechanisms that activate the inflammatory cascades are probably similar whatever the initial trigger (infective *v* non-infective).

FUTURE DEVELOPMENT

The search for an "ideal" diagnostic marker or a set of markers for diagnosis of neonatal sepsis will definitely continue. Many new chemokines, antimicrobial peptides, acute phase reactants, and cell surface antigens such as epithelial neutrophil activating peptide-8 (ENA-78), growth related oncogene α (GRO- α), human β defensin 1 and 2, cathelicidin LL-37/hCAP-18, calprotectin, and soluble CD14^{75–78} are being investigated. The advances in quantitative flow cytometric technology, which simultaneously measures a wide variety of inflammatory mediators or cell surface antigens and requires only a minimal volume of blood, will be useful for identifying the appropriate cytokines or cell surface markers most suitable for this clinical purpose. Other molecular diagnostic techniques, primarily based on the polymerase chain reaction, have also been used to detect the genetic materials of pathogens in blood, sterile body fluids, and tissue samples.⁷⁹ The latter technique is particularly useful in identifying the exact type of pathogen responsible for the infection.

CONCLUSIONS

Diagnostic markers are useful indicators of neonatal sepsis. PCT, IL6, IL8, CD11b, and CD64 are "early sensitive" markers of infection,^{9 10 48 63} whereas CRP is a "late specific" diagnostic test.⁹ CD64 is probably one of the most useful infection markers for diagnosis of late onset nosocomial sepsis.¹⁰ Serial measurement of infection markers will certainly improve the diagnostic sensitivity of these tests, because in most circumstances it is not certain at which stage of the infection the specimen should be taken for analysis. In addition, the use of multiple markers, in particular, combining an early sensitive marker with a late specific test will further enhance the diagnostic accuracy of these mediators in identifying infected cases.^{9 10} Thus promising markers may be used for early termination of antibiotic treatment in non-infected infants. 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.

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