LEADING ARTICLE

Early Markers of Infection and Sepsis in Newborns and Children

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Biochemical markers may one day prove to be more objective and reliable than clinical signs and symptoms or physiological parameters in determining the onset of sepsis and quantifying the response to therapy. However, no single or combination of biomarkers has yet been shown to be sufficiently robust to fulfill these roles. Nonetheless, a rapid and reliable clinical or biological marker for the early diagnosis of infection or sepsis would be clinically invaluable. This article aims to review currently available markers for sepsis and infection in children, and to speculate on the possible future utility of novel markers currently under investigation. *Adv Sepsis* 2006;**5**(4):118–25.

At the International Pediatric Sepsis Consensus Conference in 2001, definitions for systemic inflammatory response syndrome (SIRS), infection, sepsis, severe sepsis, septic shock, and organ dysfunction for children were established for research purposes [1]. One of the key points was that biochemical markers of inflammation might one day prove to be more objective and reliable than physiological parameters in determining the onset of various stages of the sepsis syndrome and quantitatively measuring the response to therapy. However, it was pointed out that no marker has yet been confirmed as being sufficiently robust to be added to the general definition.

From a clinical perspective, a rapid and reliable clinical or biological marker for early diagnosis of infection or sepsis would be invaluable. The importance of early treatment with antibiotics in newborns and infants with sepsis has been demonstrated by the sepsis home-treatment study performed by Bang and colleagues in rural India [2,3]. Healthcare workers were trained and sent to villages to teach parents to recognize sepsis in babies with apnea, tachypnea, poor feeding, temperature instability, or diarrhea. Infants diagnosed with sepsis were randomized to receive supportive care or a 5-day course of intramuscular gentamycin and oral co-trimoxazole at home. None were referred to hospital or treated with intravenous fluids. The mortality rate decreased from 16% to 3% in the cohort that received antibiotic therapy.

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Antepartum antibiotic treatment of mothers with chorioamnionitis has been associated with a 97% reduction in newborn early onset Group B streptococcal sepsis and a five-fold reduction in the development of cerebral palsy [4]. This salutary effect on neurological morbidity is thought to occur because early antibiotic therapy prevents cytokine- and glutamate-induced apoptosis of white matter in the central nervous system. Likewise, it is accepted that early antibiotic treatment of infection in infants and children with an identified focus of infection such as pharyngitis, pneumonia, cellulitis, osteomyelitis, or subacute bacterial endocarditis also improves outcomes.

The purpose of this article is to review the currently available clinical and biological markers for sepsis and infection in children, and to speculate on the possible utility of novel biomarkers under investigation.

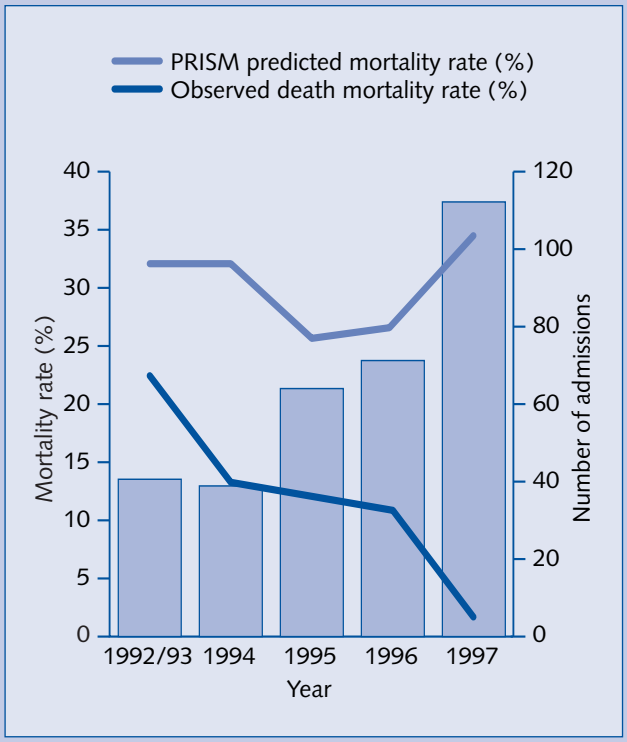
Early clinical signs

Fever and infection

The paramount importance of early clinical recognition of infection and sepsis is demonstrated by:

- The progressive increase in mortality rates as patients develop infection, sepsis, and septic shock [5,6].
- A >10-fold reduction in mortality rate and a five-fold reduction in neurological morbidity rate by early use of antibiotics to prevent and treat sepsis in newborns [2,3,7–15].
- A near 100% survival rate in children with early dengue shock who receive early fluid resuscitation [16,17].

Figure 1. Early recognition and treatment reduces mortality rate from meningococcal septic shock. In this figure, the bars show the annual admissions of children with meningococcal disease to a pediatric intensive care unit. The lines show the predicted (using PRISM score) and observed case fatality rates, indicating a falling mortality rate with improving care.



PRISM: Pediatric Risk of Mortality.
Redrawn with permission from [18].

- A 10-fold reduction in the mortality rate of infants and children with meningococcal septic shock who receive early fluid resuscitation and inotropic therapy (Fig. 1) [18–20].

The earliest clinical signs of infection are age-dependent changes in body temperature (Table 1). In immunocompetent children, the earliest sign is fever. In immunocompromised children and premature infants, the earliest sign may be hypothermia or, occasionally, fever. Fever is also an important sign of chorioamnionitis in mothers with premature rupture of membranes.

Not all infants and children require antibiotic therapy for fever, as many have benign viral infections rather than serious bacterial, viral, or fungal infections. The likelihood of a benign viral infection is greatest in a child who is happy, active, smiling, and playful. However, if the child is not smiling and playing, it is more likely that a serious bacterial infection is present. In fact, most serious bacterial infections occur subsequent to a viral infection, with influenza being the most notorious facilitator [21,22]. Hence, a child who has a fever and will not smile and play, particularly after the fever has been controlled with anti-pyretic therapy, is more likely to have a serious infection and would benefit from antibacterial, antiviral, or antifungal therapy [21–24]. The development of abnormal systemic signs in newborns and infants presages the onset of sepsis.

Tachycardia, tachypnea, and sepsis

Sepsis is diagnosed in infants and children who have a suspected or proven infection and signs of a systemic response, including tachycardia and/or tachypnea. Tachycardia is a useful sign of sepsis in the term newborn (Table 1). Graves and Rhodes evaluated 4530 consecutive newborns, 82 of whom underwent a sepsis work-up (Fig. 2) [25]. Tachycardia was present in only 21 patients (0.46%). Of these, 12 (92%) of the 13 babies with culture-positive sepsis had tachycardia, compared with six (9%) of the 69 with culture-negative sepsis, and only three of the 4268 who did not receive a sepsis work-up. Sepsis is also diagnosed in premature and term newborns by suspicion of infection and other clinical signs including decreased tone (extension rather than flexion, with hypotonia), activity (little spontaneous movement of extremities), color (pale or grey), and poor feeding or suck [26].

Fever can partly account for tachycardia, as each 1°C increase in body temperature can result in a 10% increase in heart rate. However, heart and respiratory rates should return to normal-for-age values once the fever is controlled with anti-pyretic therapy. Heart rates >150 beats/min in children and >160 beat/min in infants, and respiratory rates >50 breaths/min in children and >60 breaths/min in infants are associated with an increased mortality risk and commonly presage the development of septic shock [1].

Current biological markers of infection and sepsis

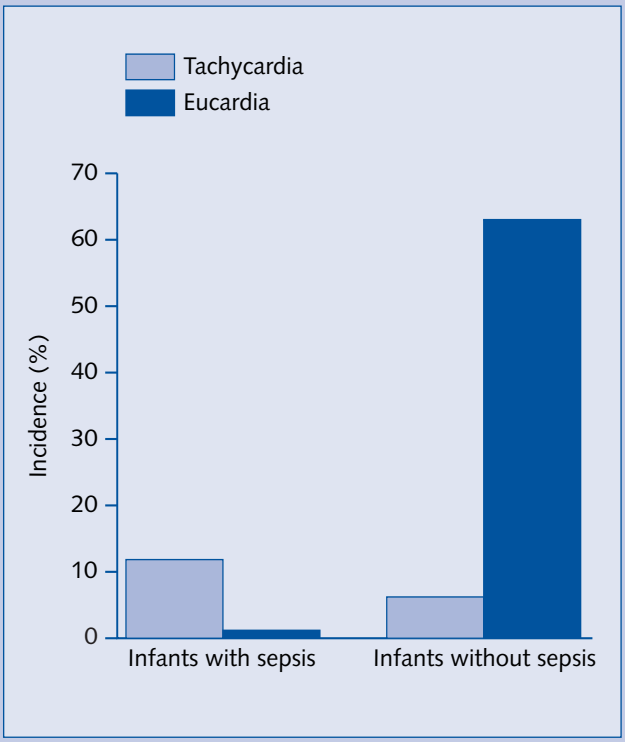
Marshall et al. published a thorough report from the Fifth Toronto Sepsis Roundtable on measures, markers, and mediators of clinical sepsis [27]. The meeting defined markers as measures that identify a biological state or that predict the presence or severity of a pathological process or disease. Specific types of markers include:

- Diagnostic markers, which are used to establish a diagnosis and therefore can identify patients who might respond to a particular therapy.
- Severity markers, which quantify the severity of the disease and therefore can indicate the likely outcome.
- Therapeutic response markers, which measure the response to therapy.

Table 1. Clinical signs of infection and sepsis in newborns, immunocompetent infants, and children.

Disease Progression	Clinical Signs
Infection	Newborn: fever or hypothermia Immunocompetent infant or child: fever
Sepsis (responds to antibiotics alone)	Newborn: apnea or tachypnea, bradycardia or tachycardia, poor feeding, decreased tone, poor color Immunocompetent infant or child: tachycardia, tachypnea, not smiling or playing

Figure 2. Tachycardia as a predictor of sepsis. In a study of 4530 live births, only 21 infants were tachycardic. Of 82 infants evaluated for sepsis, 13 had the condition and 69 did not. Twelve of the 13 infants with sepsis were tachycardic compared with six of the infants without sepsis ($p<0.001$), indicating that tachycardia is an important sign of neonatal sepsis.



Redrawn with permission from [25].

As Marshall et al. pointed out, while many circulating or cell-associated molecules have been proposed as useful markers of the presence, severity, or response to therapy of sepsis, none has been demonstrated to be 100% sensitive and specific, to have a clear utility in directing therapeutic decision-making, or to have proven reliability in defining optimal populations for clinical trials [27]. Nonetheless, it is useful to review those markers currently available, as a related test or a combination of them may prove to be more successful in the near future. Table 2 lists putative biological markers for sepsis. Table 3 lists diagnostic biological markers examined in pediatric studies.

This review will discuss several biomarkers of infection and sepsis. Only markers described in reports published after 2000 and from studies that included a readily-identifiable pediatric population have been included.

Peripheral white blood cell count

A peripheral white blood cell (WBC) count is almost universally performed as a screening test for infection. However, it is an inaccurate screen for bacteremia in febrile young infants and has proven insensitive and non-specific for invasive *Neisseria meningitidis* infections in children [28,29].

Procalcitonin

Procalcitonin (PCT) is a protein of 116 amino acids and a molecular weight of 13 kDa. It is a prohormone of calcitonin that is produced by the parafollicular cells of the thyroid gland, and is intracellularly cleaved by proteolytic enzymes to form the active hormone. In 1993, a study identified elevated level of PCT in patients with bacterial infection [30]. Since then, PCT has become the most widely studied and reported putative biomarker for sepsis in children [31–33].

While circulating levels of PCT in healthy subjects are below the limit of detection, production of PCT during inflammation correlates with both the presence of bacterial endotoxin and inflammatory cytokines [30]. PCT has been reported to:

- Differentiate between SIRS and sepsis [34].
- Serve as a marker for sepsis in neonates [35,36].
- Identify children at high risk of death from sepsis after bone marrow transplant [37].
- Have a better correlation with sepsis than C-reactive protein (CRP) or WBC count in patients admitted to a pediatric intensive care unit [38].
- Correlate with poor outcome in pediatric sepsis [39].
- Differentiate between fever of viral and bacterial etiology with more specificity than and similar sensitivity to CRP [40].

However, other studies have reported that PCT is non-specific and/or insensitive in the diagnosis of invasive fungal

Table 2. Changes in physiological signs, microbial products, and host proteins that may prove useful in the diagnosis or monitoring of sepsis.

Classification	Increased	Decreased
Microbial products	Endotoxin Enterobacterial common antigen *Candida* antigen Bacterial DNA	
Physiological parameters	Temperature Heart rate Cardiac Index Respiratory rate	Temperature Heart rate Blood pressure Systemic vascular resistance Urine output Level of consciousness
Hematopoietic cells	Neutrophils Monocytes	Neutrophils Monocytes Platelets
Cell surface markers	Polymorphonuclear neutrophil Monocyte CD11b Monocyte CD40 Monocyte CD63 Monocyte CD64 E-selectin	Monocyte human leukocyte antigen-DR Monocyte TNF receptor
Soluble receptors	sCD25 (IL-2R) sE-selectin sELAM-1 sTNF-R1 sTNF-RII sCD14 Soluble intercellular adhesion molecule-1	
Cytokines	IL-1 IL-1 receptor antagonist IL-6 IL-8 IL-10 IL-18 TNF Transforming growth factor Macrophage inflammatory protein-1 High-mobility group box-1 protein Hepatocyte growth factor Leptin Melanocyte-stimulating hormone	
Acute phase reactants	C-reactive protein Lipopolysaccharide-binding protein Fibrinogen α1 anti-trypsin	Albumin Prealbumin
Mediators of coagulation	Fibrin degradation products von Willebrand factor Fibrinopeptide A Plasminogen activator inhibitor Tissue plasminogen activator Prothrombin fragment 1+2 Thrombin–antithrombin complexes D-dimers Thrombomodulin Platelet thrombospondin Procoagulant activity	Antithrombin III Protein C Tissue plasminogen activator
Cellular processes	Lymphocyte apoptosis	Neutrophil apoptosis Whole blood synthesis of TNF

ELAM: endothelial leukocyte adhesion molecule; IL: interleukin; s: soluble; TNF: tumor necrosis factor.
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Table 3. Sensitivity and specificity of commonly reported single and combination diagnostic markers of infection in children.

Marker	Diagnosis	Sensitivity (cutoff value)	Specificity (cutoff value)	Positive predictive value (cutoff value)	Negative predictive value (cutoff value)	Reference
WBC	SBI	52% (≥15×10⁹/L)	74% (≥15×10⁹/L)	78% (≥15×10⁹/L)	45% (≥15×10⁹/L)	[89]
	MCD	69% (<4 or >15×10⁹/L)	67% (<4 or >15×10⁹/L)	77% (<4 or >15×10⁹/L)	56% (<4 or >15×10⁹/L)	[90]
	S pneumoniae vs. *M pneumoniae*	65% (15×10⁹/L)	79% (15×10⁹/L)	82% (15×10⁹/L)	61% (15×10⁹/L)	[91]
CRP	Acute pyelonephritis	94% (≥20 mg/L) 74% (≥50 mg/L)	32% (≥20 mg/L) 77% (≥50 mg/L)	61% (≥20 mg/L) 78% (≥50 mg/L)	83% (≥20 mg/L) 72% (≥50 mg/L)	[92]
	SIRS/sepsis	70% (23 mg/L)	89% (23 mg/L)	53% (23 mg/L)	94% (23 mg/L)	[35]
	SBI	79% (40 mg/L)	79% (40 mg/L)	61% (40 mg/L)	90% (40 mg/L)	[89]
	Sepsis/pneumonia	77% (≥20 mg/L)	75% (≥20 mg/L)	86% (≥20 mg/L)	73% (≥20 mg/L)	[48]
	MCD	81% (>30 mg/L)	89% (>30 mg/L)	91% (>30 mg/L)	76% (>30 mg/L)	[28]
	S pneumoniae vs. *M pneumoniae*	88% (>20 mg/L) 70% (>60 mg/L)	40% (>20 mg/L) 52% (>60 mg/L)	72% (>20 mg/L) 81% (>60 mg/L)	67% (>20 mg/L) 58% (>60 mg/L)	[91]
IL-6	SIRS/sepsis	55% (43.2 ng/L)	78% (43.2 ng/L)	30% (43.2 ng/L)	91% (43.2 ng/L)	[35]
	SBI	36% (100 pg/L)	80% (100 pg/L)	38% (100 pg/L)	77% (100 pg/L)	[89]
	Sepsis/pneumonia	68% (≥20 ng/L)	88% (≥20 ng/L)	71% (≥20 ng/L)	58% (≥20 ng/L)	[48]
	S pneumoniae vs. *M pneumoniae*	66% (>100 pg/L)	83% (>100 pg/L)	86% (>100 pg/L)	56% (>100 pg/L)	[91]
PCT	Acute pyelonephritis	90.7% (≥0.5 µg/L) 83.3% (≥0.8 µg/L) 81.4% (≥1.0 µg/L)	70.2% (≥0.5 µg/L) 93.6% (≥0.8 µg/L) 93.6% (≥1.0 µg/L)	77.7% (≥0.5 µg/L) 93.7% (≥0.8 µg/L) 93.6% (≥1.0 µg/L)	86.8% (≥0.5 µg/L) 83.0% (≥0.8 µg/L) 81.4% (≥1.0 µg/L)	[92]
	SIRS/sepsis	55% (7.7 µg/L)	80% (7.7 µg/L)	33% (7.7 µg/L)	91% (7.7 µg/L)	[35]
	SBI	93% (0.5 µg/L)	74% (0.5 µg/L)	60% (0.5 µg/L)	96% (0.5 µg/L)	[89]
	MCD	94% (>2 µg/L)	93% (>2 µg/L)	95% (>2 µg/L)	91% (> 2 µg/L)	[28]
	S pneumoniae vs. *M pneumoniae*	95% (>0.5 µg/L) 86% (>1 µg/L) 63% (>2 µg/L)	60% (>0.5 µg/L) 88% (>1 µg/L) 96% (>2 µg/L)	80% (>0.5 µg/L) 90% (>1 µg/L) 96% (>2 µg/L)	88% (>0.5 µg/L) 80% (>1 µg/L) 60% (>2 µg/L)	[90]
IL-6 and CRP	Sepsis/pneumonia	94% (≥20 ng/L and ≥10 mg/L)	63% (≥20 ng/L and ≥10 mg/L)	79% (≥20 ng/L and ≥10 mg/L)	87% (≥20 ng/L and ≥10 mg/L)	[48]
PCT and CRP	MCD	80% (>2 µg/L and >30 mg/L)	95% (>2 µg/L and >30 mg/L)	96% (>2 µg/L and >30 mg/L)	76% (>2 µg/L and >30 mg/L)	[28]
PCT and IL-8	Fever and neutropenia	94% (≥500 ng/L and ≥20 ng/L) 100% (≥150 ng/L and ≥5 ng/L)	90% (≥500 ng/L and ≥20 ng/L) 50% (≥150 ng/L and ≥5 ng/L)	79% (≥500 ng/L and ≥20 ng/L) 44% (≥150 ng/L and ≥5 ng/L)	92% (≥500 ng/L and ≥20 ng/L) 100% (≥150 ng/L and ≥5 ng/L)	[50]

CRP: C-reactive protein; IL: interleukin; MCD: meningococcal disease; *M pneumoniae*: *Mycoplasma pneumoniae*; PCT: procalcitonin; SBI: serious bacterial infection; SIRS: systemic inflammatory response syndrome; *S pneumoniae*: *Streptococcus pneumoniae*; UTI: urinary tract infection; WBC: white blood cell.

infections [41], sepsis in burns patients [42], meningococcemia [43], and neonatal sepsis [44].

CRP

CRP is an acute-phase protein. CRP concentrations in febrile young children who are at risk for occult bacteremia have been found to have a better predictive value than WBC count or absolute neutrophil count [45]. Their predictive value is similar to that of PCT in critically ill children for unexplained fever [46], but they are less reliable than PCT as a diagnostic marker of sepsis in critically ill children [47].

Lipopolysaccharide-binding protein

Lipopolysaccharide-binding protein (LBP) is an acute-phase protein involved in the endotoxin-mediated immune response [33]. An initial high LBP level may predict Gram-negative bacteremia in cancer patients with febrile neutropenia [48].

Interleukins 6 and 8

Interleukin-6 (IL-6) levels correlate with the severity of the inflammatory response, although they are not specific for bacterial infection [33]. The concentration of IL-6 increases in children with sepsis [49], and a persistent IL-6 concentration >500 pg/mL may be useful in identifying pediatric patients with intra-abdominal sepsis who are likely to have a prolonged length of stay and increased morbidity [50,51].

IL-8 is a chemokine responsible for migration of neutrophils and macrophages to the site of inflammation and is not normally present in high quantities in healthy children [52]. It has been shown to serve as a diagnostic marker for bacterial sepsis in febrile, neutropenic children.

Protein C

Acquired deficiencies in protein C during sepsis have led to a number of studies that have evaluated the safety and efficacy of replacement therapy with drotrecogin alfa (activated) (recombinant human Activated Protein C) [52–64]. Protein C levels at diagnosis correlate with severity of illness and outcome. In children and adults, acquired deficiencies in protein C are found in most patients with severe sepsis and are associated with an increased risk of mortality [65]. Both the levels and activation of protein C are considerably diminished during severe sepsis [52,54]. Approximately 87% of patients who go on to develop sepsis exhibit abnormal protein C levels upon presentation [52,66].

Endocan

Endocan (endothelial cell-specific molecule-1) is a 50-kDa dermatan sulphate proteoglycan that is expressed by endothelial cells in the lungs and kidneys, and is present at detectable levels in human blood [67]. Inflammatory cytokines (IL-1β and tumor necrosis factor-α [TNF-α]) stimulate the upregulation of endocan messenger RNA and a sustained release of the protein into the serum. In patients with sepsis, endocan blood levels are reported to be related to severity of illness and outcome, and are likely to be associated with endothelial injury [67].

Future biological markers of infection and sepsis

There are many biological markers that are currently being studied. They may prove to be significantly more accurate than clinical signs and currently available markers for screening, diagnosis, and determining the response to therapy of infection and sepsis in children.

Streptococcus pneumoniae antigen assay

A *Streptococcus pneumoniae* antigen-detection assay has been shown to have a high sensitivity for proven (bacteremic) and suspected (focal pneumonia) invasive pneumococcal infections [68]. The rate of false-positive results among febrile children without identified pneumococcal infection is approximately 15%, which is comparable to the WBC or absolute neutrophil counts commonly used to screen for clinically unsuspected pneumococcal infections.

PCR assays

PCR-based tests currently take too long to be useful at the bedside or in the classification of subjects for clinical trials. However, future technological advances may shorten the time required. PCR has proved useful in the identification of enteroviral [69,70], adenoviral [71,72], *S mutans* serotype k [73], *S agalactiae* (Group B *Streptococcus*) [74], and fungal infections [75].

Genomics

While not a direct biomarker for diagnostic or therapeutic purposes, host genetic information could be used to predict a patient's response to infection and sepsis and to determine therapy [76,77]. The known susceptibility of certain individuals with hereditary complement deficiencies to invasive meningococcal disease has led to numerous studies of the role of host genetic makeup in determining susceptibility, severity, and outcome. Several common genetic polymorphisms may influence susceptibility to invasive meningococcal disease or account for a higher mortality rate in some patients [78–83].

Combination biomarkers

The use of a combination of markers often produces a screening or diagnostic test with better sensitivity and

specificity than a test based on a single marker (Table 3). For example, IL-6 in combination with CRP appears to be a valuable parameter in the early diagnosis of pediatric infections [49]. Similarly, PCT and IL-8, but not IL-6, have been shown to be produced in response to bacterial infections [51], while increases in the pro-inflammatory cytokine TNF-α and the cytokine inhibitors soluble TNF-α receptor and IL-1 receptor antagonist were found in serum of newborns with sepsis at the time of diagnosis [84]. Finally, temporal patterns of changes in the serum concentrations of cytokines and cytokine inhibitors, along with the time course of acute-phase proteins such as PCT and CRP, have been demonstrated to allow evaluation of SIRS [85].

Proteomics

The development of traditional diagnostic tests has been hindered, in part, by an inefficient discovery process. Traditionally, a single marker (e.g. PCT) is described and its efficacy for the diagnosis in question is then undertaken. Proteomics represents a new approach in the discovery of disease biomarkers. It utilizes surface-enhanced laser deionization and mass spectrometry to identify, potentially, all peptides within a biological sample. Compared with traditional discovery methods, it is an extremely efficient technique for the identification of peptides that are differentially expressed in disease states within a short time period. Furthermore, as all differentially expressed peptides can be identified, fewer disease cases and controls are necessary to screen for potentially useful biomarkers. Novel peptides identified by proteomics may then form the basis for the development of assays that are extremely sensitive and specific. For example, proteomics has recently been utilized to identify novel serum and amniotic fluid markers for intra-amniotic infection [86–88].

Conclusion

There is a clear need for more accurate biochemical markers of infection and sepsis in children. To date, no biochemical markers are either robust or accurate enough to serve clinical requirements, particularly not for clinical trials or for determining therapeutic response. Perhaps the best hope for the future of biomarkers for infection and sepsis lies in combining different classifications of current biomarkers or in the development of rapid antigen, PCR, or proteomic-profile tests.

Disclosures

Dr Carcillo has no relevant financial interests to disclose. Dr Planquois is an employee and stockholder of Eli Lilly and Company. Dr Goldstein has acted as a paid consultant for Eli Lilly and Company and Xoma.

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