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Abstract:
In modern pediatric emergency medicine, biomarker-based assays that enable quick bedside diagnostics and subsequent disease management can be valuable. There is a growing need for novel, disease-specific biomarkers that can improve the outcome of pediatric infectious diseases commonly encountered in the emergency department (ED). Viral respiratory infections, central nervous system infections, sepsis, and septic shock are acute disease states frequently encountered in the ED. In this review, we describe a host of novel biomarkers, including a diverse set of cytokines, chemokines, and nitric oxide–based metabolites. Based on disease pathophysiology, a rationale is provided for a molecular- or biomarker-based approach in the ED. Throughout this review, emphasis is placed on diagnostic rapidity because this relates directly to timeliness and quality of care in a busy ED. Once the biomarkers become more clinically available, in a rapid ED setting as bedside point-of-care assays, quality of care will be enhanced, not only by means of diagnostics but also in prognosticating severity of illness.

Keywords:
acute care; biomarkers; bronchiolitis; influenza-like infections; meningitis; rapid diagnostics; acute respiratory infections; sepsis; shock

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A Biomarker-Based Approach to Infectious Disease in the Pediatric Emergency Department

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A 4-month-old former full-term infant without any medical history presents to the emergency department (ED) on day 4 of her illness comprising of coughing, nasal congestion, and fever (T_{max}, 101°F). On physical examination, the child is alert and interactive. Vital signs are significant for tachypnea but with a normal oxygen saturation as measured by pulse oximetry. The respiratory examination reveals a child that is in moderate respiratory distress; there are subcostal and intercostal retractions, coarse breath sounds, and expiratory wheezing audible in all lung fields. A nasal wash is sent off for a rapid viral screen and comes back positive for respiratory syncytial virus (RSV). A bronchodilator trial is ineffective, as is a trial of racemic epinephrine. Although the child is in respiratory distress, she is able to maintain hydration and can possibly be managed at home. However, the ED physician is uncomfortable in discharging her home. Therefore, the physician decides to obtain a blood sample from the infant to analyze peripheral blood leukocyte global gene expression by means of an RNA microarray

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("RNA chip"). Based on the RNA expression profile, that is, the set of genes overexpressed vs those underexpressed, the prediction is that the patient will get sicker from the RSV bronchiolitis. Hence, the physician decides that the safest course would be to observe the infant for 23 hours.

The ED provides a unique clinical venue to develop, test, and implement novel biomarkers that can be cost-effective and rapid in their ability to manage acute as well as chronic disease processes with acute exacerbations. The loss of health insurance coverage that has followed the economic recession in the United States has, in turn, led to an increase in ED utilization.[1,2] Given the increasing numbers of pediatric patients being seen, there is an unmet need for viable and rapid biomarkers for various infectious disease states commonly encountered in the ED.

The use of biomarkers is crucial from both diagnostic and prognostic perspectives because early and accurate detection of infectious disease states in the ED setting can potentially impact morbidity and mortality, by way of improving disease management. Acute viral bronchiolitis, influenza-like illness (ILI) from seasonal or nonseasonal viruses, meningitis, encephalitis, sepsis, and septic shock are frequently encountered infectious disease states in children presenting to the ED. We use these diseases states as examples in this review. This review hopes to speak to the rationale for and the current state of biomarker-based analyses for the above-mentioned infectious disease states. We also make the case that if these biomarker-based tests are found to be useful for the pediatric ED setting, then developing them further as point-of-care assays would be a logical next step.

BIOMARKERS FOR ACUTE RESPIRATORY INFECTIONS

Acute bronchiolitis and ILI are 2 major respiratory infections commonly encountered in the pediatric ED. Respiratory syncytial virus is the most common cause of lower respiratory tract infections, particularly in children, including bronchiolitis and pneumonia.[3,4] There is a broad spectrum of disease severity for bronchiolitis, the leading cause of hospitalization for infants in the United States with associated hospitalization costs in excess of $500 million per year, and is also responsible for frequent ED visits.[5,6]

Pandemics like the 2009 novel swine-origin influenza A (H1N1) virus pose significant challenges: increased transmission relative to seasonal influenza, severe disease in patients with medical comorbidities, and difficulty in predicting which children with viral respiratory symptoms are at risk for influenza complications.[7] Given the immense disease burden of the above respiratory infections, development of rapid diagnostic and prognostic tools is a pressing need. In the evaluation of acutely ill children with respiratory infections in the ED, risk stratification by using biomarkers might augment clinical decision making and thus improve clinical outcomes.[8]

One of the proposed mechanisms for RSV pathophysiology relies on toll-like receptor functionality. Toll-like receptor 4 (TLR4), along with its coreceptor CD14, is activated by bacterial lipopolysaccharide. The RSV-specific F-protein elicits a similar TLR4 response, but the response is significantly weaker than the lipopolysaccharide response.[9] Another cofactor, the MD-2 polypeptide, is believed to play an important role in the TLR4 pathway described above, but this relationship is still under investigation. Lipopolysaccharide antagonist analogs that lower LPS mandated TLR4 activation also lower RSV F-protein–mandated TLR4 activation.[9] The measurement of RSV F-protein levels, TLR4 activation, and MD-2 levels offers a potentially novel biomarker system in the pediatric domain for prognostic purposes.[9] Serum vitamin D levels are another biomarker under research for acute bronchiolitis. Serum vitamin D levels have been shown to vary directly with immunoglobulin G and A levels and inversely with immunoglobulin E levels in the acute stages of bronchiolitis.[10] Data from a recent clinical trial showed that serum vitamin D levels were significantly lower in children with acute-phase bronchiolitis compared with healthy controls.[10]

Microarray-based whole-genome expression arrays have also been used in the ED to determine correlations with severity of RSV bronchiolitis.[11] We recently showed that there was systemic activation of interferon signaling networks during acute RSV infection in children presenting to the ED.[11] We also showed that this "biosignature" was effective in discriminating patients with acute RSV bronchiolitis from healthy age-matched controls.[11] These gene transcripts or their protein products could be promising biomarkers for acute RSV bronchiolitis

in future prospective studies. The case vignette at the beginning of this article describes one hypothetical situation in which microarray-based biomarkers might benefit clinical decision making. Before that, DNA- or RNA-based diagnostic and prognostic tests have to be cost-effective and rapid enough to be clinically effective in a busy ED setting. Irrespective of study, the invasive nature of blood collection for determining serum levels of any putative biomarker for acute bronchiolitis is a potential deterrent from use in an ED setting.

Serum interlukin-6 (IL-6) concentration has shown potential as a biomarker of disease severity in pandemic H1N1 influenza type A.[12] Interlukin-6 is a prominent cytokine in the immune response and plays a critical role in acute-phase response and fever. Interlukin-6 is also relevant to several pathogenic inflammatory states, with serum concentrations varying directly with sepsis severity, onset of H1N1 type A, and severe acute respiratory syndrome.[12] Serum IL-6 in pandemic H1N1 was significantly higher in patients who required critical care support compared with those who did not; however, this did not affect disease outcome.[12] This suggests that IL-6 shows potential as a biomarker of disease severity, but not as a therapeutic agent. As mentioned above for bronchiolitis biomarkers, serum levels of IL-6 would require a blood sample and thus are nonideal in the pediatric ED. During influenza virus infection, epithelial cells, macrophages, lymphocytes, and other cells release a complex series of cytokines and chemokines in an attempt to overcome viral replication and disease progression.[13–15] Nitric oxide (NO), a key mediator of airway inflammation, is also released. It is unclear if increased NO activity is a marker of clinical disease, a marker of the innate antiviral response, or associated significantly with a unique respiratory virus infection.[16] In the murine model of RSV for instance, NO production in the lungs appears to be associated with viral clearance and lung disease.[17] Induced NO synthase activity and production of NO were associated with enhanced clearance of RSV, yet they were also associated with airway inflammatory changes and airway dysfunction.[17] In murine studies of experimental influenza A–triggered pneumonia, excessive NO biosynthesis, as measured by its metabolites nitrate and nitrite (collectively referred to NOx), was demonstrated in the lungs.[18,19] This suggested that the metabolites may be markers of influenza-mediated disease in the respiratory tract.

In humans, NO is produced in the upper and lower respiratory tract. It can be detected in exhaled air of healthy individuals and at increased levels in subjects with pulmonary disease including asthma, bronchiectasis, and upper respiratory infections.[20–24] In aqueous solutions, NO is rapidly converted to distinct oxides of nitrogen, which may thus be potential biomarkers of disease severity in acutely ill patients presenting with ILI to the ED.[21,25]

During the 2009 novel H1N1 pandemic, we collected nasal wash specimens from children with ILI in the ED and then measured NOx concentration by high-performance liquid chromatography. Overall, our data suggested that concentration of NO-derived nitrate in nasal secretions in children in the ED may be related to type of viral pathogen causative for ILI: RSV subtype B–associated ILI had higher nasal wash nitrate compared with all other viruses combined (P = .002).[8] Ongoing studies will determine the predictive potential of this putative biomarker for important disease outcomes in sicker patients. Exhaled NO has also been studied as a putative biomarker for respiratory infections caused by influenza, rhinovirus, and other viral pathogens, but the data have been equivocal.[26,27] The conflicting data above may simply reflect technical issues in measuring exhaled NO. This methodology is cumbersome to achieve in the pediatric population, either for inpatients or in the ED. The newer NIOX-MINO (Aerocrine, Morrisville, NC) handheld NO detector has proven to be as reliable as the older, stationary chemiluminescence analyzer, the Ecomedics (ECO MEDICS, Duernten, Switzerland).[28] Its portability may make it easier to handle in the ED setting. Focusing on stable NO metabolites in nasal secretions from patients with ILI in the ED may still be the better approach because of ready accessibility. Future studies are needed that can establish correlations between nasal secretion NOx and exhaled NO for ILI-type disease processes in the ED. A major benefit of working with respiratory tract–based biomarkers, either in exhaled breath or via biochemical analyses of nasal secretions, is the independence from a blood collection in the ED.

BIOMARKERS FOR ACUTE CENTRAL NERVOUS SYSTEM INFECTIONS

Meningitis can be a life-threatening infection of the central nervous system (CNS). Bacterial meningitis, in particular, remains an important public health problem worldwide, with several studies confirming the devastating neurological sequelae that may result from it.[29] Cases of varying severity are likely to pass through the ED. Early cerebrospinal fluid (CSF)–based biomarkers are needed that may be able to predict severity.

Apolipoprotein E (ApoE) is a glycosylated protein with far ranging functionality. Apolipoprotein E has

traditionally been studied in lipid metabolism and vascular disease, but an emerging body of evidence suggests that it plays a key role in the immune response, including modulation of inflammation and oxidation.[30] Thus, ApoE is an intriguing candidate biomarker of CSF infections that cause inflammation, such as bacterial meningitis. Cerebrospinal fluid ApoE levels were significantly increased in patients with bacterial meningitis when compared with healthy controls (sensitivity of 85% and specificity of 100% for CSF values vs sensitivity of 80% and a specificity of 93% in serum).[31] Although the collection of both CSF and serum is invasive, if such high measures of validity can be replicated consistently, ApoE has potential as a prominent diagnostic biomarker for bacterial meningitis.

Nitric oxide and its metabolites in the CSF may represent another set of biomarkers. Studies have shown that patients admitted to the hospital with bacterial meningitis and influenza-associated encephalopathy had higher levels of CSF NOx as compared with controls.[32] In another study, patients with RSV infection and CNS symptoms (encephalitis, encephalopathy, or seizures) were found to have a higher concentration of CSF NOx compared with those with influenza-positive encephalopathy or those without meningitis.[33] Neither were the studies set up in the ED nor did they analyze CSF NOx in meningitis from different pathogens in the same setting. Using a rapid high-performance liquid chromatography–based assay for detection of these metabolites in CSF in the ED setting may add to the current armamentarium for helping in early diagnosis of different forms of meningitis and may be predictive of disease severity. Detecting CSF NOx in a rapid ED-based assay may be particularly helpful in cases that are difficult to interpret, such as in cases of CSF pleocytosis. The added information may help guide management, ultimately resulting in better disease outcomes.

BIOMARKERS FOR SEPSIS AND SEPTIC SHOCK

Sepsis is defined as the systemic response to infection, and about half of the patients whom encounter it progress to septic shock, which is characterized by refractory hypotension and multiple-organ failure.[34] Severe sepsis and septic shock are associated with mortality rates as high as 28% and have severe hemodynamic implications such as macrovascular and microvascular failure.[35] The central role of NO in maintaining microcirculation is highlighted in sepsis and septic shock, states in which the microcirculation has sustained an insult. Several studies have implicated the overproduction of NO as the cause for the decompensated state.[34] Sepsis, septic shock, and systemic inflammatory response syndrome in intensive care settings are associated with increased plasma levels of NOx.[36–38] Measuring both plasma and urinary NOx has revealed elevations in patients with sepsis and septic shock.[34] This may be caused by an elevated innate immune response to infection and inflammation, particularly in sepsis and shock. Such a response is associated with increased activity of induced NO synthase and thus more NO being released.[39,40] Urinary NOx, primarily nitrate, reflects both the production and use of NO.[41–43] Sepsis and shock can expose the kidney to ischemic injury, which can be reflected in low levels of urinary NOx excretion likely caused by low endogenous NO generation.[44,45] Conversely, because most (96%) of filtered nitrate and nitrite is reabsorbed by the renal tubules, high levels in urine secondary to reduced renal reabsorption may reflect acute renal failure concomitant with sepsis/septic shock.[44,46]

Although NO directly and indirectly, by conversion to peroxynitrite, can be bactericidal, excessive levels are counterproductive as evidenced by the refractory vasodilation, hypotension, and decreased tissue perfusion.[34] Irrespective of mechanism and level of metabolite, NO as a downstream effector of innate immune activation lends itself as a potential biomarker for sepsis. This may be particularly useful in the ED because the concentration of NOx in plasma or urine may predict important inpatient clinical outcomes, such as need for hospitalization, level of support (intensive care vs regular floor), and mortality.

Neonatal sepsis, as a subset of sepsis and septic shock, is a significant annual cause of morbidity and mortality, with infections accounting for a third of all neonatal deaths that occur globally.[47] Neonatal infections including sepsis, pneumonia, and meningitis account for an estimated 1.4 million neonatal deaths worldwide every year.[47,48] The clinical findings of neonatal sepsis are nonspecific, thus complicating diagnosis and resulting in overtreatment.[40] Early diagnostic/screening measures in blood such as complete blood count, C-reactive protein, tumor necrosis factor α, IL-6, granulocyte/macrophage–colony-stimulating factor, and procalcitonin (PCT), to name just a few, have been studied in newborns.[40,49] Of the above, PCT has shown limited promise in the biomarker realm but has consistently fallen short in its specificity. Procalcitonin levels are often increased in several noninfectious disease conditions, rendering it nonspecific to sepsis. Although PCT levels can be helpful in management of sepsis, the diagnostic link is unclear.[50] In addition,

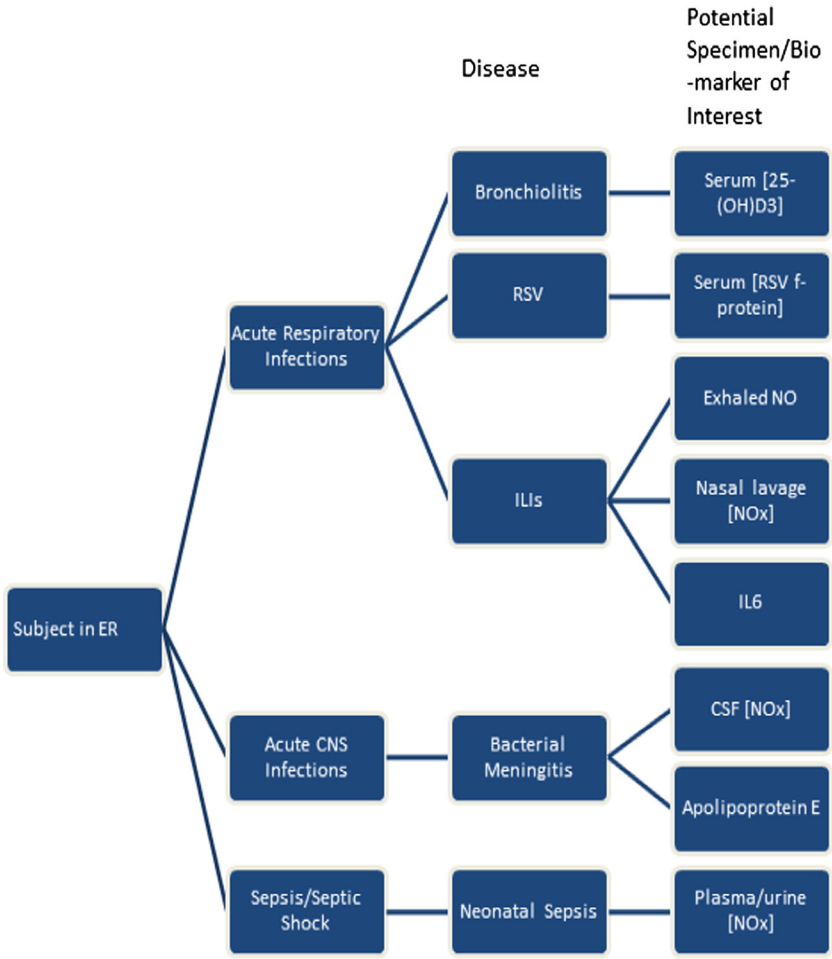

Figure 1. A hypothetical biomarker-based approach to commonly encountered infections in the pediatric ED. Putative biomarkers of interest for the respective pediatric infection or disease are shown in the last column. Different specimens (blood, nasal wash, exhaled air, urine, CSF) to be collected are described in detail in the text. Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; ER, emergency room; ILI, influenza-like illness; IL6, interlukin-6; NO, nitric oxide; NOx, nitric oxide metabolites; RSV, respiratory syncytial virus.

prohibitive costs and dependency on a blood draw for basing therapeutic decision making are major detractors in the ED setting.

No biomarker has been entirely satisfactory to sepsis and septic shock owing to lack of sensitivity, specificity, and diagnostic economy.[40,49] However, urinary biomarkers may provide a novel approach because obtaining a noninvasive (bagged) urinary specimen is much more practical and less cumbersome than obtaining blood.[40] The urinary analysis of NOx levels may be particularly useful in identifying neonates at high risk for serious bacterial infection and resultant sepsis or shock in a tertiary ED or in-patient setting, or in a nontertiary care community setting.

SUMMARY AND FUTURE DIRECTIONS

There is an obvious value of obtaining a noninvasive specimen in the ED for determining levels of biomarkers for disease entities. This will require the development, validation, and then clinical usage of relatively simple, rapid, and cost-effective techniques that may sample multiple body fluids (plasma, urine, nasal secretion, CSF).[51] This has been summarized in Figure 1. Regarding the hypothetical clinical vignette at the outset, instead of an invasive blood specimen, the nasal wash that was initially obtained for a rapid viral diagnosis can also be used for obtaining host cellular material. RNA expression profiling can be done on that cellular material. Assuming a relatively rapid turnaround, the gene expression signature might correlate with severity of respiratory illness, and the information thus obtained might be used for making a patient disposition.

Concentrations of biomarkers of interest might also be reliably obtained in a rapid manner in the ED by point-of-care tests on various biological samples. Adaptation to and validation of point-of-care tests

with the assistance of an industry partner will enable inexpensive screening capacity on a dipstick, point-of-care platform, to aid treatment decisions. This has certain global health implications that go far beyond the ED.[52,53] Analysis of NOx has shown promise in the dipstick domain, particularly with salivary NOx levels and their detection using the Griess reaction.[54] If such a dipstick could be developed for a rapid urinary analysis, there are high-impact implications in diverse settings ranging from a busy ED to rural community-type settings with little access to laboratory or clinical analytical systems.[55]

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