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The Future Possibilities of Diagnostic Testing for the Evaluation of Febrile Infants

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Fever is one of the most common reasons for childhood visits to emergency departments and primary care practitioners worldwide. Most febrile infants younger than 3 months have nonbacterial causes of fever, and these children are frequently clinically indistinguishable from those with serious bacterial infections (SBIs) (including bacterial meningitis, bacteremia, and urinary tract infections).

The evaluation of well-appearing febrile infants continues to be challenging and controversial for clinicians. This is particularly true for infants younger than 3 months because although the risks of bacteremia and bacterial meningitis are low in those with normal routine screening laboratory test results (eg, complete blood cell counts and urinalyses), the outcomes of bacteremia and meningitis could be devastating.¹ The need for performing routine lumbar punctures in well-appearing febrile infants younger than 3 months is also part of the controversy.¹ The essence of the controversy is that no definitive clinical criteria or laboratory screening tests reliably exclude SBI, and blood cultures have a not-inconsequential rate of false-positive and false-negative results.² Clinically apparent viral syndromes such as bronchiolitis reduce but do not exclude the possibility of SBI,³ and although clinical prediction rules can help identify those infants with bacterial meningitis, they are not perfect, particularly in the youngest infants.⁴

As a consequence, clinicians depend on a variety of guidelines and complex algorithms initially developed in the early 1980s for evaluation and management of these infants.¹ Most of these algorithms require comprehensive and invasive procedures with examination of

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blood, urine, and cerebrospinal fluid and frequently lead to hospitalization and empirical antibiotic therapy until bacterial cultures are reliably negative (typically 48 hours). Although these strategies are conservative and aim to ensure identification and treatment of all young febrile infants with SBIs, the costs of and risks for iatrogenic complications of these approaches may be unacceptably high. Clinician and parental risk tolerance and preferences also vary, complicating the ability to set one standard for evaluation. The controversy over the evaluation of the young febrile infant is documented with more than 300 publications to date and continues to remain highly relevant for clinicians worldwide. In 2004, *JAMA* published the results of a collaborative study of 3066 infants younger than 3 months who were evaluated for fever in 573 practices participating in the Pediatric Research in Office Settings network.⁵ The results of that study highlighted the different approaches clinicians use to evaluate young febrile infants depending on the clinical setting.⁵ In this article, we describe a potentially new laboratory paradigm for evaluating and managing febrile infants, which may fundamentally alter our diagnostic and treatment approaches in the near future.

The current laboratory approach for the evaluation of young febrile infants has substantial limitations. Screening tests for SBI, including complete blood cell counts, absolute neutrophil counts, band cell counts, band to neutrophil ratios, and inflammatory markers such as the erythrocyte sedimentation rate, C-reactive protein, and, more recently, procalcitonin, lack the test characteristics to make them sufficiently discriminative to distinguish between febrile infants with bacterial infections vs those with nonbacterial infections. Perhaps most problematic is that the standard criterion for diagnosis of SBI, cultures of relevant body fluids (and more specifically the blood culture), is a suboptimal reference standard.² Despite advances in microbiological techniques including continuous-monitoring blood culture systems using fully automated instruments, the ability of culture techniques to identify true pathogens depends on various factors including time between sample collection and incubation, volume of blood collected, duration for which inoculated blood culture bottles are left at room temperature, the presence of fastidious pathogens that grow slowly or require complex culture media, and prior antimicrobial therapy. The time to growth of pathogens frequently leads to hospitalization or long-acting antibiotics until lack of growth can be confirmed. In addition, blood cultures may also be falsely negative if bacteremia is transient or intermittent. Finally, contaminant growth of bacteria is known to increase both the duration and cost of care.¹ Dependence on cultures to discriminate between bacterial and nonbacterial infections is therefore inaccurate, costly, inconvenient, and impractical. Thus, there is a clear need to develop new, more precise, efficient, and rapid laboratory diagnostic strategies that would allow a less invasive and more accurate, cost-effective evaluation of young febrile infants.

Fortunately, in the current era there are novel methods that circumvent the limitations of culturing for pathogens, and they are approaching bedside clinical use. Some of these methods involve higher fidelity in pathogen identification.² Others examine the host response to infection as a means of identifying which patients are infected with pathogenic bacteria.^{2,6}

The application of molecular assays is transforming the diagnostic approach to infectious diseases. Identification of pathogens based on nucleic acid detection, such as polymerase

chain reaction, has become the new standard for identification of viral infections, including those that frequently affect young infants such as enteroviruses, herpes simplex viruses, and respiratory viruses. These assays have limitations, however, as the detection of a viral pathogen does not completely exclude the presence of a concomitant bacterial infection.³ In addition, the polymerase chain reaction–based assays for detection of bacterial pathogens, including the promising “universal polymerase chain reaction” assay based on detection of the bacterial 16S ribosomal RNA gene, have not yet demonstrated sufficient sensitivity for detection of bacteria in the blood, as required for diagnosis of SBI in febrile infants.²

Alternatively, it is now possible to detect the presence of infection by assessing the specific host responses. Different pathogens induce distinct transcriptional “biosignatures” in the RNA of blood leukocytes that can be reliably measured by microarray analysis using small blood samples. Studies conducted in hospitalized children indicate that this method allows discrimination between infections caused by different types of pathogens with high fidelity.^{2,6} This fundamentally paradigm-shifting strategy allows for a non–culture-dependent approach for detecting infections and should be prospectively examined in the evaluation of young febrile infants. Preliminary data suggest great potential in pursuing this strategy.⁷ Indeed, there are several advantages of pursuing the host RNA signature: (1) host response patterns can differentiate between classes of pathogens, eg, bacteria vs viruses, as well as gram-positive vs gram-negative bacteria; (2) it allows for assessment of disease severity for patient stratification; and (3) it can identify concomitant viral and bacterial coinfections.⁸ The Figure shows an example of a microarray analysis using a “heat map” representing the differences in gene expression patterns that allow discrimination of patients with bacterial and viral infections.⁶

Important issues that need to be resolved prior to widespread application of host–gene expression analysis, however, include validation of host signatures across different platforms, at different times in disease, at different severities of illness with the same pathogen, and in patients with bacterial and viral coinfections. As the technology for conducting molecular analyses advances and costs decrease, it will soon be possible to apply these techniques in the clinical setting and potentially replace the need for cultures of body fluids as the reference standard in the evaluation of young febrile infants. It is imperative that these technologies are rigorously evaluated before clinical implementation, however, which will require the establishment of a clinical genomics infrastructure. The potential benefit is substantial, though: these novel approaches may ultimately obviate many of the lumbar punctures performed, reduce unnecessary hospitalizations, and decrease the use of unnecessary empirical antibiotics, leading to safer, more timely, and more efficient evaluation and management of young febrile infants.

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Figure. Gene Expression Patterns Discriminate Viral vs Bacterial Infections

A, Set of 35 genes that discriminates patients with viral infections (influenza A; green) and bacterial infections (*Escherichia coli* and *Streptococcus pneumoniae*; red). The discriminative pattern is shown by the gene expression patterns in the heat map (red indicates overexpressed genes; blue, underexpressed genes). B, The diagnostic signature was tested in an independent set of patients that confirmed its accuracy. K-NN indicates nearest neighbor algorithm.