Specialized test detects bacterial infections in youngest infants with fever

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Physicians from Children's Hospital of Michigan, Wayne State University, UC Davis Medical Center and Nationwide Children's Hospital, in collaboration with 19 other pediatric emergency

departments around the country, have established a "proof of principle" for measuring patterns of ribonucleic acid (RNA) expression in the bloodstream that can enable clinicians to distinguish bacterial infections from other causes of fever in infants up to two months old.

The diagnostic test—a high through-put RNA analysis that yields specific markers known as RNA biosignatures—means that emergency department physicians could someday avoid ordering painful, invasive exams for many of the more than 500,000 febrile infants who arrive at hospitals each year and must be evaluated to determine whether a bacterial infection is the cause of their fevers. Results that indicate no bacterial cause would also help reduce unnecessary hospitalizations and antibiotic treatments.

The findings of a study conducted in the Pediatric Emergency Care Applied Research Network (PECARN) and led by principal investigators Prashant Mahajan, Nathan Kuppermann and Octavio Ramilo are published in the current issue of the *Journal of the American Medical Association* in an article titled "Association of RNA biosignatures with bacterial infections in febrile infants 60 days of age or younger."

Typically, fewer than 10 percent of infants evaluated for fever in emergency departments have serious and potentially life-threatening bacterial infections, including bacteremia (bacteria in the blood), bacterial meningitis or a urinary tract infection. But because of their age and current treatment guidelines, many febrile infants undergo invasive testing and are hospitalized and given antibiotic treatment until a bacterial infection can be ruled out.

"Finding an accurate but less invasive method to determine if babies with fevers have bacterial infections is a 'holy grail' for emergency department physicians," said Kuppermann, professor and chair of the

Department of Emergency Medicine at UC Davis School of Medicine. "This proof-of-concept study demonstrates that the evaluation of RNA biosignatures could one day be that tool."

Current guidelines for evaluating young febrile infants call for culturing bacteria from blood, urine and cerebrospinal fluid samples. Cultures typically take 24 to 48 hours to determine if bacteria are present. The tests, while effective, are invasive and can be painful for young patients as well as stressful for parents. Testing also is costly, involves some degree of clinical risk and may require hospitalization, all of which pose important questions for pediatricians and emergency department physicians who do not want to miss a serious infection.

Although the RNA biosignatures approach has been shown to be valuable in detecting certain infections in older children and adults, the current study is the first to show that the test could also be used in very young febrile infants. Some physicians and researchers had concerns that RNA biosignatures may not work in this patient population because the immune cells in the blood of these youngest patients were too immature to mount a detectable response to bacterial infection.

"Despite the young age of the babies in this study, they did carry robust RNA biosignatures," said Ramilo, chief of infectious diseases at Nationwide Children's Hospital and professor of pediatrics at The Ohio State University. "Regardless of whether they had a viral or bacterial infection, their immune systems were already programmed to respond with specific patterns."

Although cultures are the current standard for diagnosing bacterial infections, they can deliver false results. If not enough blood is drawn—a common problem with the youngest babies—bacteria may not grow in a culture medium even if present, causing the diagnosis to be missed. In addition, bacteria can be picked up from the skin during a blood draw,

contaminating the culture and leading to a false-positive result.

In contrast to blood cultures, an RNA biosignature assay requires only a small amount of blood to detect immune system responses to pathogens. DNA within white blood cells are prompted to produce different RNAs according to environmental cues, the first step in making proteins that are essential for keeping the cell functioning and able to cope with changes in the surroundings. For example, RNAs associated with inflammation are produced in response to bacterial infection, and RNAs associated with interferons (a group of signaling proteins) are expressed in response to certain viruses. By analyzing the patterns of RNAs produced—the RNA biosignatures—it can be determined with a high degree of certainty whether an individual has a bacterial infection or not.

The prospective study was conducted with infants 60 days or younger with fever (defined as having a rectal temperature of at least 38°C/100.4 °F). RNA biosignatures were measured on a selected group of 279 infants, of whom 89 were determined to have bacterial infections. Nineteen healthy infants with no fever served as controls.

The research team found that RNA biosignature testing was highly sensitive and specific for categorizing patients with and without bacterial infections when compared with the current standard using bacterial cultures. In infants who had bacteremia (bacteria in the blood) the test had 94-percent sensitivity; meaning it aligned with the bacterial culture in 94 percent of the cases. For febrile infants whose fever was not caused by bacteremia, the RNA biosignature test agreed with the negative culture 95 percent of the time (95-percent specificity). For all cases of serious bacterial infections, which included bacteremia, meningitis and urinary tract infections, the RNA biosignatures test had an 87-percent sensitivity for detecting bacterial infection and an 88-percent specificity for infants without bacterial infection when

compared to the standard culture results. It may be that in the cases of disagreement between RNA biosignatures and standard cultures, the RNA biosignatures are more reflective of the true type of infection.

"The implications of these findings are potentially paradigm-changing," said Prashant Mahajan, professor of pediatrics and emergency medicine at Wayne State University and chief of pediatric emergency medicine at Children's Hospital of Michigan. "For 100 years, doctors have looked directly for bacteria in body fluids to make a diagnosis. We have now shown that genomic analysis to detect the response of the human immune system is also very accurate and potentially can be more rapid in determining if a young baby has a bacterial infection."

The RNA biosignatures testing was much more accurate than the Yale Observation Scale, which currently is used as a screening test in emergency departments to help determine if young infants with fever are likely to have a bacterial infection. The scale is based on behaviors such as the quality of a baby's cry, and reaction to parents and social response.

With a renewed five-year grant from the National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development, the research team will be validating the study findings on a larger patient population and evaluating whether the RNA biosignature is stable at two different time-points. They also will be testing a new RNA biosignatures polymerase chain reaction platform—currently available only for research purposes—which they anticipate will produce faster results and be more applicable in clinical laboratories. The new study will also determine if RNA biosignatures testing can detect the presence of a simultaneous bacterial and viral infection in a single patient.

This study was conducted through PECARN, a network of pediatric

emergency departments throughout the country that has established new, evidence-based standards for managing common and important problems in pediatric emergencies. PECARN, which annually evaluates more than 4,000 febrile infants ages 60 days or younger, offers an ideal setting to evaluate the application of RNA expression analysis for diagnosing and managing young infants with fevers in a prospective manner.

In addition to the three principal investigators, 36 other PECARN researchers and physicians from the network co-authored the study.

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