Diagnostics for Bioterrorism Agents on the Way

BY JEFF EVANS
Senior Writer

WASHINGTON — Several rapid diagnostic tools for detecting the unique profiles of both common pathogens and bioterrorism pathogens in exhaled breath and body fluids may soon be tested on patients in emergency departments, speakers said at a biodefense research meeting sponsored by the American Society for Microbiology.

In a real bioterrorism (BT) event, physicians in multiple disciplines will likely collaborate, but in particular, it will be emergency department, hospital, and research laboratory personnel who will use such diagnostic equipment in real clinical settings, said Dr. Richard E. Rothman, of the emergency medicine department at Johns Hopkins University, Baltimore.

"The current diagnostic tools that we use in clinical practice are really limited" in terms of a prolonged wait time for results and sampling problems such as multiple blood draws and tedious steps, he said.

From the perspective of an emergency physician, he said, the key diagnostic questions that physicians need to ask routinely when patients come to the ED include, "Is there an infection? What is the organism? Is it uncommon? Could it be a BT agent?"

Dr. Rothman and his associates have developed several large-scale institutional review board protocols to sample breath condensates initially in animals, then in volunteers, and ultimately in ED patients. Other protocols have been developed to collect excess sera, cerebrospinal fluid, or nasopharyngeal aspirates from large numbers of ED patients and compare the results of standard microbiologic assays with those of new assays under development.

The rationale for analyzing breath condensates is based on evidence that pathogens in the respiratory system may elicit a host response that can be detected in the breath of an infected patient. Analyses of the patient's immune response could potentially discriminate between bacterial and viral infections and possibly determine what microbe is causing the symptoms.

In vitro studies have shown that specific cytokines and other inflammatory mediators from murine macrophages are activated in a time-dependent manner in the presence of bacterial lipopolysaccharides.

Researchers collaborating with Dr. Rothman have used a mask device called the ECoScreen to hold condensate for analysis by a mass spectrometer. The investigators also have developed their own mask to collect exhaled breath condensate.

In one study, collections of breath condensates from a group of 60 piglets that were exposed to bacteria showed a peak in interleukin-2 as early as 1 hour after exposure to *Staphylococcus aureus* enterotoxin B—long before the onset of symptoms, Dr. Rothman said.

The work has already shown that animals have unique cytokine profiles to different pathogens, and "now we're moving on to try to define baseline mass spectrometry profiles in healthy humans" and choose an optimal device for sampling, he said. From there, Dr. Rothman and his colleagues plan on sampling ED patients who are healthy or have acute respiratory symptoms.

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An ideal diagnostic assay for detecting microorganisms would be sensitive and specific, applicable to multiple types of specimens, able to detect common organisms and BT agents, and useful as a triage tool, Dr. Rothman said.

Several years ago, Dr. Rothman conducted a pilot study with Charlotte A. Gaydos, Dr.P.H., to determine if a group of 51 febrile intravenous drug users were bacteremic when they arrived at the ED. At the time, most of these patients were hospitalized while diagnostic tests were run. The study findings suggested that it might be possible to detect bacteremia by running polymerase chain reaction (PCR) assays for 16S ribosomal RNA, which is highly conserved among bacteria. Different species of bacteria have differences in the 16S nucleotide sequence.

The PCR assay had 87% sensitivity and 87% specificity for bacteremia, compared with blood culture; all eight patients who had culture-positive infective endocarditis also were determined to be positive by PCR (J. Infect. Dis. 2002;186:1677-81).

Real-time PCR assays that detect single category A BT agents already exist. But in real BT events the particular agent isn't known. It is better to be able to detect multiple pathogens in a single reaction, Dr. Rothman said.

Dr. Rothman and Dr. Gaydos have developed universal primers and probes to use in their quantitative, real-time PCR assay for 16S ribosomal RNA. They also designed species-specific primers and probes to detect agents commonly seen in ED and infectious disease settings or in BT events.

The assay was able to detect 25 common bacterial pathogens (such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Staphylococcus aureus*) and inactivated BT agents (such as *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, and *Brucella* species) in about 3 hours and 45 minutes, compared with 24 or more hours for detection by culture, said Dr. Gaydos, of the school of public health at Johns Hopkins.

In all cases, the probes for common bacterial pathogens picked up the intended target bacteria and did not cross-react with contaminants or other pathogens. The BT-specific probes detected all of the agents they were intended to find and did not cross-react with other BT pathogens, except in the cases of the *B. anthracis* probe picking up *B. cereus* and the *Y. pestis* probe picking up *F. philomiragia* and *Y. pseudotuberculosis*. In each of those cases, a confirmatory test is required to distinguish the species from each other, she said. "This method could be used for spinal fluid or blood or any other sterile fluid or tissue. This shows a lot of promise for being used in emergency departments." ■

Procalcitonin-Guided Protocol Can Cut Duration of Antibiotics for Pneumonia

BY KERRI WACHTER
Senior Writer

WASHINGTON — A procalcitonin-guided protocol can cut the duration of antibiotic use in patients with community-acquired pneumonia by roughly 50%, according to data presented at the annual Interscience Conference on Antimicrobial Agents and Chemotherapy.

"Procalcitonin seems to be a more reliable parameter for the individual tailoring and discontinuation of antibiotics as compared with commonly and routinely used clinical and laboratory parameters," said Dr. Mirjam Christ-Crain, an endocrinologist at the University Hospital Basel (Switzerland).

She and her colleagues proposed using procalcitonin as a biomarker to guide antibiotic treatment because the propeptide of calcitonin is increased with increasing severity of bacterial infection.

For this study, patients with community-acquired pneumonia (CAP) were randomized to receive therapy of standard duration (151 patients) or therapy whose duration was guided by procalcitonin (151 patients). Patients in both groups averaged age 70 years. Only 20% had antibiotic pretreatment. Most patients in both groups had comorbidities. More than two-thirds of patients had severe or very severe pneumonia.

Among those in the procalcitonin group, patients with levels greater than 0.25 mcg/L were started on antibiotic therapy. Those with levels of 0.25 mcg/L or less were not given antibiotics.

Procalcitonin measurements were performed on all patients on days 0, 2, 4, 6, and 8, though the results were available only for those in the procalcitonin group. The decision to continue or discontinue antibiotic therapy in the procalcitonin group was based on the cutoff levels described above. Follow-up, including a chest x-ray, was performed at 4-6 weeks. In patients with clinical uncertainty, there was follow-up remeasurement of procalcitonin in 6 hours.

Patients in the standard therapy group received antibiotics initially, and almost all of them were on antibiotics for more than 8 days. In comparison, only 85% of those in the procalcitonin group initially received antibiotics. In this group, "only about 50% had antibiotics for more than 4 days and about 30% for more than 6 days," Dr. Christ-Crain said.

Patients in the procalcitonin group received antibiotics for an average of 6 days, compared with 13 days for the standard therapy group. "This is a highly significant reduction of antibiotic use and antibiotic duration," said Dr. Christ-Crain, at the meeting sponsored by the American Society for Microbiology.

Clinical outcomes—as assessed by a visual analog scale and clinical parameters such as temperature, oxygen saturation, and pulse rate—were similar in both groups. Laboratory outcomes—C-reactive protein and procalcitonin levels in the normal reference ranges—assessed at 4-6 weeks were also similar.

Most experts recommend a 10-14-day course of antibiotic therapy to treat CAP, but the optimal duration is unknown. "In our opinion, the correct duration of antibiotics varies from patient to patient," Dr. Christ-Crain said.

New tests for the determination of procalcitonin levels have improved sensitivity, enabling physicians to distinguish clinically relevant bacterial infections from other infections. ■

Low BMI Predicted Increased Mortality Risk in Septic Shock

SAN FRANCISCO — For at least one medical condition, it's low and not high BMI that predicts mortality, a study has shown.

Patients admitted to the ICU for septic shock had a significantly greater risk of death if they had a lower-than-normal BMI, according to a poster presented by Dr. Almothana Shanaah at the annual congress of the Society of Critical Care Medicine. Patients with BMIs in the overweight or obese ranges had no significantly increased risk of dying.

Dr. Shanaah of Cooper University Hospital, Camden, N.J., and colleagues used a multicenter database to extract data on patients admitted with septic shock. A total of 1,745 patients were included. The researchers considered patients with BMIs of 18.5-24.9 kg/m^2 to be of normal weight, those with BMI of less than 18.5 to be underweight, those with BMIs of 25-29.9 to be overweight, and those with BMIs of at least 30 to be obese.

The groups did not differ significantly in age or APACHE II (Acute Physiology and Chronic Health Evaluation) severity of disease score. Ventilator dependency and chronic renal failure also were associated with mortality.

—Robert Finn