New testing system predicts septic shock outcomes

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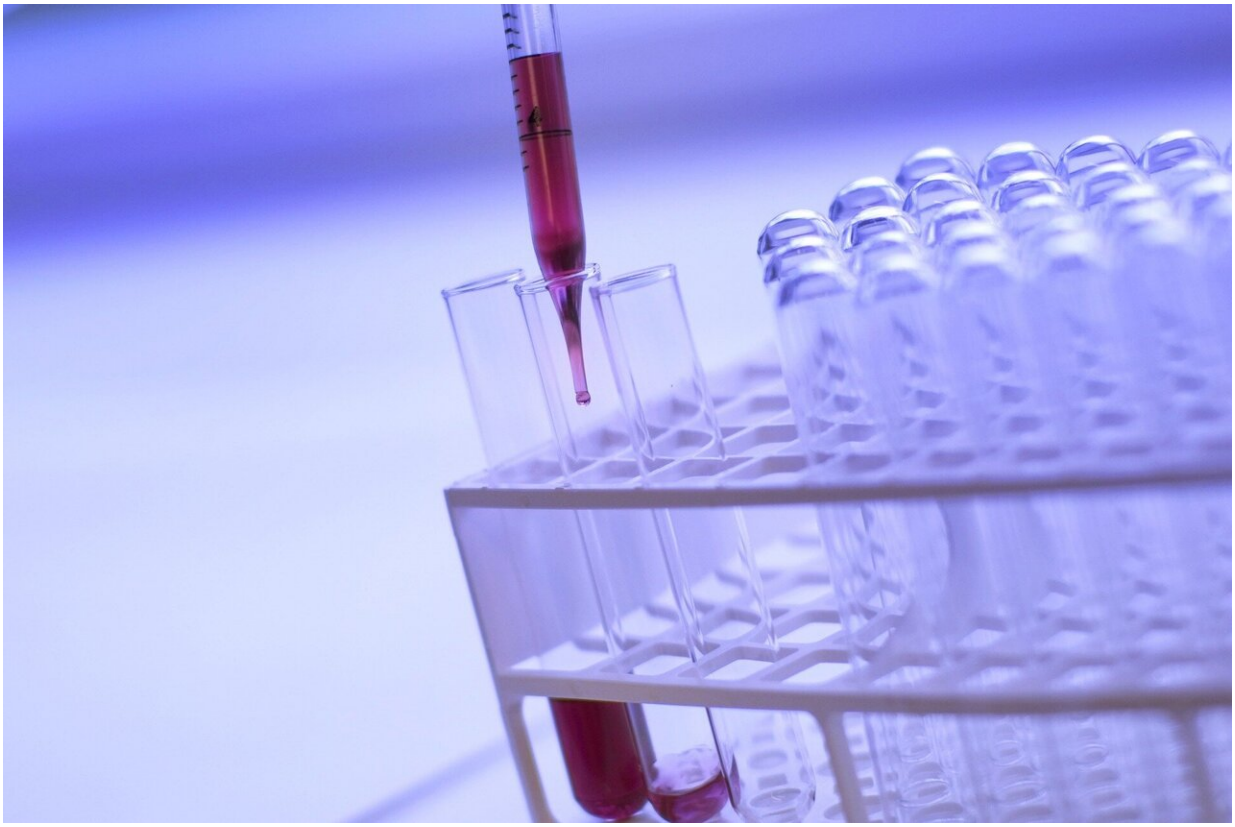

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More than 1.7 million Americans develop sepsis each year, and more than 270,000 die from it. The condition—which happens when the body has an extreme response to a bacterial or viral infection, causing a chain

reaction that can lead to organ failure and death—has few strategies for treatment.

That's what Savas Tay found a few years ago, when his mother died from sepsis. "I learned that there is very little they can do to really monitor and diagnose these patients," said Tay, associate professor of molecular engineering at the Pritzker School of Molecular Engineering (PME) at the University of Chicago. "A good percentage of them will ultimately die, which is unacceptable, considering the high-quality facilities, physicians, and therapies we have available. I was kind of enraged with the situation."

So Tay set out to do something about it. Now, he and his collaborators have developed a new, extremely sensitive method that can quantify bacteria, an antibiotic resistant gene, and immune molecule levels within sepsis patients, far more rapidly than current protocols.

By deploying these tests at intervals, the researchers also found that it wasn't the absolute levels of these markers that mattered—it was the change in the levels. Using machine learning, they accurately predicted which patients with sepsis would recover quickly, recover later, or ultimately succumb to the condition. That information could ultimately help physicians diagnose and treat patients in a more personalized way.

"Our findings provide a new approach to the diagnosis of sepsis with the potential to identify the causal pathogen early," said Gokhan Mutlu, professor of medicine and chief of pulmonary and critical care medicine at UChicago and co-author of the research. "This will allow us to use the appropriate antibiotics earlier before the culture results are available and minimize the use of antibiotics that are needed to treat the infection. By combining the pathogen-related and host response data, we are able to predict outcomes in patients with sepsis."

The results were published May 25 in the journal *Nature Communications*.

Understanding how to treat sepsis

Because sepsis is often caused by microbial infections, the condition is usually initially treated with antibiotics. Treatment must happen quickly—any delay in the administration of correct antibiotics increases the chances of the patient dying. But doctors often aren't sure which bacteria is causing the infection, and growing cultures to pinpoint the bacteria can take days.

Even if doctors can treat the infection directly, the condition can cause the body's immune response to become exaggerated. By attacking the pathogens, the immune system can release too many immune system proteins called cytokines, which can ultimately overwhelm the body and kill the patient. Anti-inflammatory drugs can help treat this, but often physicians do not know when this "cytokine storm" is taking place until it's too late.

"The immune system has a gas and a brake," Tay said. "You need the gas to kill the pathogens, but you need the brake so you don't overshoot inflammation and harm the patient. In all of this, timing is critical. We wanted to know if we could monitor bacterial load and cytokines at the same time, and monitor their changes, to provide better guidance about who should get certain treatments."

Creating an extremely sensitive test

Tay, an expert in single-cell analysis and microfluidics, and his team developed a digital polymerase chain reaction (PCR) test that uses digital proximity litigation assays to quantify the levels of certain genes and

proteins in the blood.

Specifically, the test uses a blood sample to test for gram-negative (GN) and gram-positive (GP) bacterial DNA, which is abundant in many septic patients. It also tests for levels of the IL-6 and TNF proteins, the cytokines that the immune system releases to attack pathogens. In addition, it tests for the blaTEM gene, which signifies antibiotic resistance.

The test is extremely sensitive—able to quantify very small changes in the concentrations of these molecules—and provides results within a few hours. Tay worked with pulmonologists at University of Chicago Medicine to try out the test on samples from septic patients.

The researchers took samples once a day for two days from 32 patients and tested their bacterial and protein levels. They found that the bacterial levels of the patients who lived decreased as time went on.

However, in almost every patient that died, IL-6 levels increased throughout their time at the hospital. Even patients who had low bacterial levels to begin with still died if their IL-6 levels increased, showing that the immune system potentially overshot and attacked their own body.

Though IL-6 has been considered a major biomarker in sepsis before, previous researchers did not realize that it was the change in the levels—not the levels themselves—that predicted this outcome.

In addition, the researchers found several patients with the gene that indicates antibiotic resistance, which would be helpful information for the physicians treating them.

"Sepsis manifests itself differently in each person, therefore having a

test like this to shed light on that variation could one day be used by providers to identify which patients may respond better to certain treatments or interventions," said Krysta Wolfe, a pulmonologist and assistant professor of medicine at UChicago, and co-author of the research.

Using machine learning algorithms, the researchers could ultimately use these biomarkers to predict who would recover early, recover late, or die, with nearly 100% accuracy.

"All of the sudden we have this method that allows us to really understand how these patients are going to fare," Tay said. "If there are patients that are going to do badly, then you can start treating these patients in different ways, perhaps with drugs that will help block the immune system from overshooting."

Extending the test to other diseases

Right now, the test happens in a lab, but Tay and his group are developing a machine that can quickly test samples on site at ICUs. They are proceeding with a clinical trial and hope to extend the test to include more groups of bacteria beyond just the GN and GP levels, to help physicians better understand which antibiotics are needed in order to help reduce antibiotic resistance.

This test could also be extended to other infections where cytokines can overtake the body, including viral infections like COVID-19.

"A rapid test like this is needed in many situations and could really change the game for treatment of sepsis," Tay said. "This is a disease that can kill everybody, regardless of your situation."

More information: M. Fatih Abasıyanık et al, Ultrasensitive digital

quantification of cytokines and bacteria predicts septic shock outcomes, *Nature Communications* (2020). DOI: 10.1038/s41467-020-16124-9