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p 2885**Research Profile****Digging for disease markers of sepsis**

Using a novel combination of techniques, Gary Siuzdak and colleagues at Mass Consortium Corp.; Becton, Dickinson and Co. (BD); and the Scripps Research Institute identified 10 potential biomarkers that distinguished the serum of patients with sepsis from that of other very sick patients. They report their findings in this issue of *JPR* (pp [3154–3160](#)). This disease “turned out to be one of the more difficult problems we could have gone after, because sepsis can result from many different sources,” says Siuzdak. Sepsis, which kills >200,000 Americans every year, develops when the bloodstream is overwhelmed by toxin-producing microorganisms.

*Photodisc*

In the blood. To find biomarkers for sepsis, researchers immunodepleted high-abundance proteins from serum, a component of blood, and analyzed the remaining proteins by multidimensional LC and tandem MS.

The researchers' initial attempt to find sepsis markers simply showed that sick people differ from healthy people, and they detected no differences in easily identifiable serum proteins that might indicate early sepsis. Then, scientists at BD performed a longitudinal study of intensive care unit (ICU) patients who did and did not develop the disorder. As expected, the typical high-abundance serum proteins dominated the mass spectral data, making it impossible to obtain high-quality quantitative information on the less-abundant proteins.

At about this time (2003), the multiple affinity removal system (MARS) was unveiled. These immunodepletion columns, consisting of antibody-coated beads, cleanse serum of its six most abundant proteins: albumin, transferrin,

haptoglobin, antitrypsin, and immunoglobulins G and A. The Scripps researchers applied the BD samples to a MARS column. The serum came from 25 ICU patients with systemic inflammatory response syndrome (SIRS) and 25 with sepsis, in which the inflammatory response is associated with a documented infection. Sepsis is more deadly than SIRS and, therefore, requires more aggressive medical attention. “For a physician to be able to identify [sepsis] at an early stage would be very valuable,” Siuzdak says.

The immunodepletion step removed 85% of the protein from the samples. The remaining proteins were digested with trypsin, which resulted in ~1 million peptides. Instead of the typical 2D LC separation, a 3D approach was used to fractionate these peptides. First, the digests were passed through a reversed-phase column from which peptides were eluted with organic solvent and fractionated by hydrophobicity. The fractions were then run on a strong-cation exchange (SCX) column, which separates peptides by ionic strength. The third step was another reversed-phase column. “Doing the initial reversed-phase fractionation simplified each of the SCX runs, ultimately allowing us to have a manageable number of peptides for the final reversed-phase runs,” Siuzdak explains.

The resulting fractions were analyzed by MS/MS, which generated ~10 million spectra. Only 2% of the spectra represented the 6 high-abundance proteins, compared with 59% from those without immunodepletion.

Having successfully obtained so many spectra from low-abundance proteins, the researchers’ next challenge was data analysis. “The difference between our paper and previous papers is that we acquired a huge amount of data using the MS instrument that was available at that time,” says Mass Consortium Corp.’s Zhouxin Shen, who generated and analyzed the data. Using a proprietary proteomics analysis platform, Shen found 2810 proteins in the samples. Of those, 484 could be identified. Shen used the spectral count—the number of mass spectra produced for a particular protein—to assess the relative abundance of each one. He then compared the relative concentration of each protein in a SIRS sample to that of the same protein in a sepsis sample. This comparison showed that 103 proteins were either more concentrated or less concentrated in some sepsis samples than in some SIRS samples.

Using the database for annotation, visualization, and integrated discovery (known as DAVID), William Nussbaumer from BD grouped the 103 proteins into biologically meaningful subsets. The complement and coagulation pathways were the best-represented systems.

When all the sepsis samples were compared with all the SIRS samples, seven proteins were found to be consistently up-regulated and three consistently down-regulated. Acute-phase proteins, complement proteins, coagulation proteins, and lipid transporters were all represented. The up-regulated proteins were complement component C4 and the precursors to C-reactive protein, plasminogen, apolipoprotein A-II, plasma protease C1 inhibitor, transthyretin, and serum amyloid P-component. Among the proteins that were consistently less abundant in the sepsis samples than in the SIRS samples were the precursors to apolipoprotein A-I, antithrombin-III, and serum amyloid A-4. “There were clearly more proteins that we could have listed, but those were the most significant ones,” says Siuzdak.

MS is already widely used in neonatal screening to diagnose inborn errors of metabolism. “This work further demonstrates that [MS] can also be a powerful research tool for untargeted screening of disease-related proteins,” Siuzdak says.

Shen says their approach is likely to be even more powerful than demonstrated in the paper because mass spectrometers have improved dramatically in the 2 years since the study ended. “Our method is universal,” he says. “We picked the most difficult disease but still found potential biomarkers from small differences.”