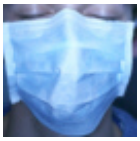

Count down to sepsis biomarkers

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By combining immunodepletion, three-dimensional liquid chromatography (LC) and a data analysis technique known as spectrum counting, US researchers have identified 10 low-abundant blood plasma proteins that could be biomarkers for sepsis.

Sepsis is a massive immune reaction that can occur in response to infection or injury, where the immune system essentially runs out of control. As such, it can be much more serious than the original bodily trauma, causing widespread inflammation, blood clotting and organ failure. After heart attack, it is the leading cause of death for intensive care patients.

If caught early enough, sepsis can readily be treated with antibiotics - to remove the sepsis-causing infection - fluid injections, medication to restore normal blood pressure, and specific treatments for failing organs. But the trick is catching it early, because sepsis can currently only be diagnosed from physical symptoms or by identifying the sepsis-causing infection, both of which take time.

Monitoring sepsis-specific biomarkers in blood plasma (the liquid part of the blood) offers a much speedier means of diagnosis, but definitive biomarkers have proved difficult to find. For a start, any biomarkers would need to achieve the difficult task of distinguishing between sepsis and normal immune reactions. Furthermore, as with other disease biomarkers, they would probably not be very

abundant in blood plasma, especially in comparison with the six most abundant proteins - albumin, immunoglobulin (Ig) G, IgA, haptoglobin, transferrin and antitrypsin - which account for up to 90% of the protein content.

So in order to find some candidate sepsis biomarkers, the team of US researchers, led by Gary Siuzdak from the Scripps Research Institute, California, had to design their study with great care. First off, they decided to compare the protein content of plasma from sepsis patients with that of plasma from patients experiencing a slightly milder immune reaction known as systemic inflammatory response syndrome (SIRS). Any proteins present at different levels in the samples should then be good candidates for specific sepsis biomarkers, rather than just reflecting normal immune system processes.

The researchers also decided to take advantage of Agilent Technologies' Multiple Affinity Removal System, which uses an antibody-filled column to remove the six most abundant proteins from blood plasma. They then used the enzyme trypsin to digest the remaining plasma proteins and separated the resultant peptides using three sequential LC columns, in order to deal with the huge complexity of blood plasma (which can contain over 10,000 different proteins).

This involved passing the plasma samples through a reverse phase (RP) column, which separated the peptides by hydrophobicity, and then through a strong cation exchange (SCX) column, which separated them by ion strength. The final separation was conducted on another RP column, with the separated peptides then analysed by tandem mass spectrometry (MS).

Finally, to determine the concentrations of the detected proteins the researchers used a data analysis technique called spectrum counting, which calculates protein concentrations based on the number of detected peaks rather than signal intensity. "We knew from our

experience that for low abundance proteins intensity didn't work well because the MS chromatograms are typically very noisy," explains team member Zhouxin Shen from Mass Consortium Corporation, an analytical technology company set up by Siuzdak. "On the other hand, although spectrum counting only gives semi-quantitative results, it allowed us to compare low abundant proteins."

In total, the researchers were able to detect around 3,000 proteins in the plasma samples, of which only 10 showed distinct concentration differences between the sepsis and SIRS samples (with seven proteins up-regulated in the sepsis samples and three proteins down-regulated). However, many of these 10 proteins were found to be involved in two major immune system pathways: the complement pathway, which helps rid the body of pathogens; and the coagulation pathway, which instigates blood clotting.

This gives confidence that these 10 proteins could well be specific biomarkers for sepsis, and Siuzdak and his team are now exploring them in more detail.

Related links:

- ***Journal of Proteome Research* 2006, 5, 3154**: "Sepsis Plasma Protein Profiling with Immunodepletion, Three-Dimensional Liquid Chromatography Tandem Mass Spectrometry, and Spectrum Counting"
- **Gary Siuzdak's research group**

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