Immune activity shortly after surgery holds big clue to recovery rate, study finds

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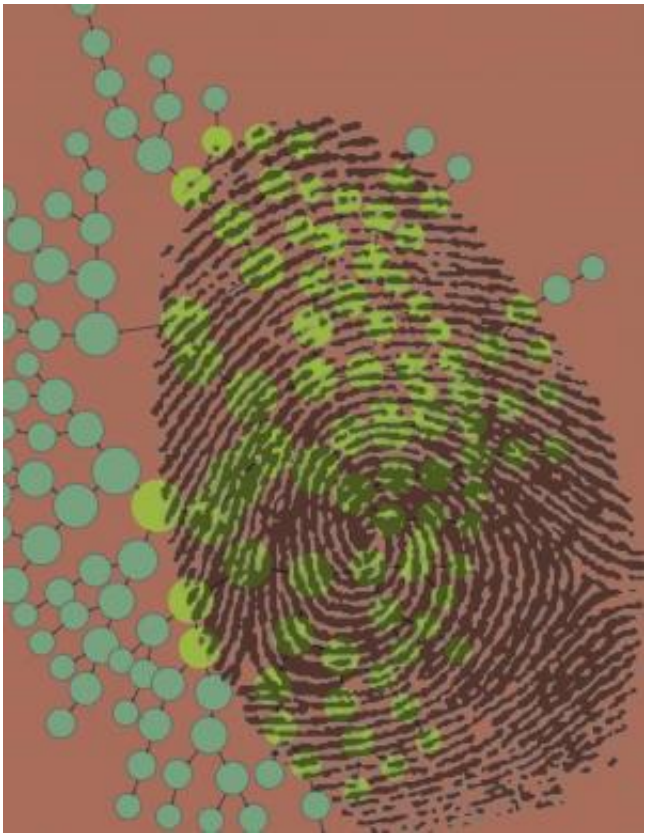

A thumbprint uniquely identifies an individual. Comprehensive mapping of the human immune system, using a technique called mass cytometry, may help reveal patient-specific immune signatures or "thumbprints" that predict the speed of recovery from surgery. Credit: Dr. Quentin Baca and Erika Saving / Stanford University School of Medicine

The millions of people who undergo major surgery each year have no

way of knowing how long it will take them to recover from the operation. Some will feel better within days. For others, it will take a month or more. Right now, doctors can't tell individual patients which category they'll fit into.

Now, researchers at the Stanford University School of Medicine have discovered that the activity level of a small set of immune cells during the first 24 hours after surgery provides strong clues to how quickly patients will bounce back from surgery-induced fatigue and pain, and be back on their feet again.

The finding, based on an in-depth analysis of blood samples drawn from middle-aged to older patients undergoing a hip-replacement procedure, is described in a study to be published Sept. 24 in *Science Translational Medicine*. The study may be the most comprehensive characterization to date of the immune system's response to trauma.

The Stanford researchers were able to make this discovery because they used a highly sensitive technology called single-cell mass cytometry. Developed in the laboratory of microbiology and immunology professor Garry Nolan, PhD, the method enables simultaneous monitoring of large numbers of biochemical features both on the surfaces of immune cells and within the cells, telling the scientists not only what kind of cells they are looking at but how active they are.

Strong Clues in First 24 Hours

"We learned that within the first 24 hours after surgery you can find strong clues in blood that reveal what shape a particular patient is going to be in two weeks later," said Martin Angst, MD, professor of anesthesiology, pain and perioperative medicine, who shared senior co-authorship of the study with Nolan.

The discovery could lead to the development of a personalized diagnostic blood test for predicting recovery after major surgery. (Stanford holds a provisional patent on the associated intellectual property.) Such a diagnostic could both help physicians make early decisions about which patients to put on enhanced recovery-optimizing regimens and help recovering patients know what to tell loved ones and bosses to expect. A full understanding of the molecular mechanisms identified in the study might even make it possible for clinicians to manipulate the immune system so as to foster faster recoveries.

Most of the more than 200 million surgical procedures performed annually worldwide are minor, Angst said. But in the United States alone, millions of those procedures—hip replacements, for example—are sufficiently traumatic to trigger profound inflammatory responses in patients.

Healing Versus Impeding

That initial blast of inflammation is essential to the healing process, said Angst. "You need to unleash the dragon," he said. "But you need to be able to ride it. Too much inflammation spells a protracted recovery." The immune system's components must continually rebalance their contributions in a dynamic that speeds rather than impedes healing.

A few years ago, Angst attended a talk by Nolan explaining mass cytometry, which permits simultaneous measurements of more than 50 different surface and internal features of single cells, while standard cell-sorting methods typically max out at 12-15 such measurements. This triggered a collaboration between Angst and Nolan, whose specialties were bridged by postdoctoral scholar Brice Gaudilliere, MD, PhD, now a clinical instructor in anesthesia. Gaudilliere shared lead authorship of the study with Gabriela Fragiadakis, a graduate student in Nolan's lab.

This ability to count so many features at once gives researchers a window to a cell's soul, said Nolan, who is the Rachford and Carlota Harris Professor. "We can observe not only an immune cell's identity but its state of mind," he said. Nolan holds an equity position in Fluidigm, a company that manufactures cell-cytometry instrumentation and reagents used in the study.

The study recruited 32 otherwise healthy patients, mostly between ages 50 and 80, who were undergoing first-time hip-replacement procedures carried out by Stanford orthopedic surgeons. Blood samples from these patients were drawn one hour before surgery, then at one, 24 and 72 hours post-surgery and again four to six weeks after surgery. The samples were quickly delivered to Nolan's lab, where cytometric analysis of 35 features in and on each sample's roughly half-million constituent cells yielded profiles of the cells' identities along with key activities underway inside them.

Every three days for a full six weeks after surgery, patients filled out questionnaires probing the degree of pain and fatigue they were experiencing and how well their refurbished hip was functioning.

Zeroing in on key predictor

The Stanford team observed what Angst called "a very well-orchestrated, cell-type- and time-specific pattern of immune response to surgery." The pattern consisted of a sequence of coordinated rises and falls in numbers of diverse immune-cell types, along with various changes in activity within each cell type.

"Amazingly, this post-surgical signature showed up in every single patient," Angst said. However, the magnitude of the various increases and decreases in cell numbers and activity varied from one patient to the next.

One particular factor—changes, at one hour versus 24 hours post-surgery, in the activation states of key interacting proteins inside a small set of "first-responder" immune cells—accounted for 40-60 percent of the variation in the timing of these patients' recovery. There was also a notable expansion in the numbers of these front-line cells soon after surgery, but the size of that increase didn't correlate with recovery on an individual basis nearly as strongly as did the changes in activity within the cells.

The cells in question account for only about 1-2 percent of all the white blood cells found in a typical sample of a healthy person's blood, so the changes within them could easily have been missed had a less-thorough detection technology been employed.

The strength of the observed correlation far exceeds the strengths reported in previous studies linking inflammatory responses to clinical recovery. Such studies have typically looked at secreted substances in blood or the trafficking of different kinds of cells at various time points after surgery. But those studies lacked the power to simultaneously observe, for example, which types of immune cells were secreting those blood-borne substances at any given time, or exactly what else those particular cell types were up to at the same time.

Possible Way to Predict Recovery

"If a correlation explains only 2 percent or even 10 percent of the variability in recovery rates, this may clinically not be all that relevant," said Angst. "Our robust correlation may lead to a clinically useful way to predict recovery from surgery."

The researchers believe, but have not yet proven, that the cells whose activity patterns correlated most strongly with patients' recovery trajectories are so-called "myeloid-derived suppressor cells," shown in

other studies to damp inflammation. Those cells have been linked to negative outcomes in cancer, where too much activity on their part appears to prevent the body's immune system from attacking tumors.

But in the post-surgery environment, their activity may be just what the doctor ordered.

The Stanford group is now looking to see if they can identify a pre-operation immune signature that predicts the rate of recovery. "If we could predict recovery time before surgery even took place," said Gaudilliere, "we might be able to see who'd benefit from boosting their immune strength beforehand, or from pre-surgery interventions such as physical therapy. It might even help us decide when or if a patient should have surgery."

More information: "Clinical recovery from surgery correlates with single-cell immune signatures," by B. Gaudilliere, et al. *Science Translational Medicine*, stm.sciencemag.org/lookup/doi/ … scitranslmed.3009701

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