Scientists develop inflammation test that may predict cardiovascular disease

October 14 2016, by Bruce Goldman

A blood test developed by Mark Davis and his colleagues may be able to predict the development of cardiovascular disease. Credit: Timothy Archibald

An assessment blending several measures of immune-cell responsiveness predicted cardiovascular problems in individuals who likely would have slipped under the radar.

A blood test devised by Stanford University School of Medicine scientists spits out a single number that strongly predicts the development of the world's most prevalent medical disorder: cardiovascular disease.

While more research remains to be done, there's good reason to suspect that this test could be used to predict many other diseases of old age, said Mark Davis, PhD, professor of microbiology and immunology.

A paper describing the test was published online Oct. 13 in *Cell Systems*. Davis is the senior author. Lead authorship is shared by former postdoctoral scholars Shai Shen-Orr, PhD, who is now an assistant professor at Technion-Israel Institute of Technology in Haifa, and David Furman, PhD, now a consulting associate professor at Stanford.

Mounting evidence suggests that many diseases that become more common with advancing age do so because the immune system begins to malfunction, said Davis, a Howard Hughes Medical Investigator and the director of Stanford's Institute for Immunity, Transplantation and Infection.

In addition to responding more sluggishly to dangers such as infectious pathogens or incipient tumors, the aging immune system tends to spend its downtime—periods when it has no imminent challenge to respond to—in a low-grade inflammatory state. Medical experts are increasingly convinced that this constantly thrumming, systemic, inflammatory activity threatens diverse tissues throughout the body.

A better test?

Cholesterol testing, a diagnostic mainstay, flags cardiovascular risk only about half of the time, said Davis, who is also the Burt and Marion Avery Family Professor. "For too many men experiencing a heart attack or stroke, the first observed hint of cardiovascular risk is their death," he said.

Testing for levels of CRP, a circulating protein linked to inflammation, has been shown to further enhance the prediction of cardiovascular risk, even among patients with normal cholesterol levels. A CRP reading is relatively simple to get, requiring only a blood draw and relatively straightforward lab tests.

The new test developed by the Stanford researchers is more complicated but appears to have superior diagnostic value to either the cholesterol or CRP test. Rather than testing circulating inflammatory proteins, it tests for the response of immune cells themselves to inflammation—a signal that appears to be more stable and hence a more robust diagnostic. In the study, it was able to detect early cardiovascular irregularities in otherwise asymptomatic individuals.

The researchers took advantage of data from the first three years of what was to become a nine-year longitudinal project carried out at Stanford under the direction of study co-author Cornelia Dekker, MD, a professor of pediatric infectious disease. In all, 90 adults, divided into two groups—people younger than 40 and people older than 60—were assessed annually at high resolution with a battery of tests as to the state of their immune system, how it varied from year to year within and between individuals and how this related to clinical markers of inflammation.

Isolating immune cells

For the Cell Systems study, the researchers isolated several types of immune cells from individual blood samples and measured these cells' responses to stimulation by circulating signaling proteins called cytokines. Many cytokines tend to shift the immune system into high gear.

The investigators recorded the extent to which several different types of immune cells mixed with cytokines in a lab dish increased the activation of intracellular substances called STAT proteins. In response to this stimulation, STAT proteins are known to undergo small chemical changes, causing them to head into a cell's nucleus and turn on batteries of genes that stir the erstwhile quiescent cell into a frenzy of immunological activity.

When immune cells from young people were stimulated with certain cytokines, the activation levels of STAT proteins skyrocketed. When the same thing was done to immune cells from old people, STAT-protein activity rose a lot less.

However, pre-stimulation levels of STAT-protein activation in immune cells from old people were substantially higher than in those from young people, suggesting that older people's immune systems are constantly somewhat revved up when they should be at rest.

Still, older people's pre-stimulation STAT-protein activation levels, and these proteins' activation in response to cytokine stimulation, varied widely between individuals. Some showed few signs of it, while in others it was pronounced.

Cytokine response score

The investigators blended 15 separate cytokine-responsiveness measurements to generate a single number called a Cytokine Response

Score. This measure, which varied considerably among different older adults, was quite stable from year to year for any given individual. A higher CRS is better, as it indicates a more-responsive immune system and lower background inflammation.

Intriguingly, individuals taking fish-oil supplements had higher scores. (Fish oil is known to have anti-inflammatory properties.) No other drug tested showed this correlation.

At the suggestion of study co-author Francois Haddad, MD, a clinical associate professor of cardiovascular medicine, the cytokine response score of each of 40 older subjects was then cross-referenced against cardiovascular-health assessments they underwent up to two years later. These assessments included a comprehensive clinical history and tests of atherosclerotic plaque, arterial stiffness and ventricular function.

Of the 40 subjects, 18 were in good cardiovascular health. Ten had already suffered cardiovascular events, and seven had sufficient atherosclerosis to be considered subclinical. Five others had lesser signs of cardiovascular problems and were considered borderline.

The researchers found that cytokine response scores were inversely correlated with clinical signs of atherosclerosis and with two measures associated with the heart's ability to relax between beats. Importantly, the borderline subjects also had low cytokine response scores. The scores' predictive value exceeded that of CRP tests, the current standard for measuring inflammation-based cardiovascular risk.

For now, the test to obtain a cytokine response score is not available in clinics; it's too complex and expensive. Davis said he and his colleagues want to try to simplify it and drive down the cost.

"The CRS may be a useful proxy for healthy aging," said Davis. "And its

predictive accuracy in cardiovascular disease further substantiates the inflammatory underpinnings of that prevalent, age-related condition."

The team's work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Provided by Stanford University Medical Center