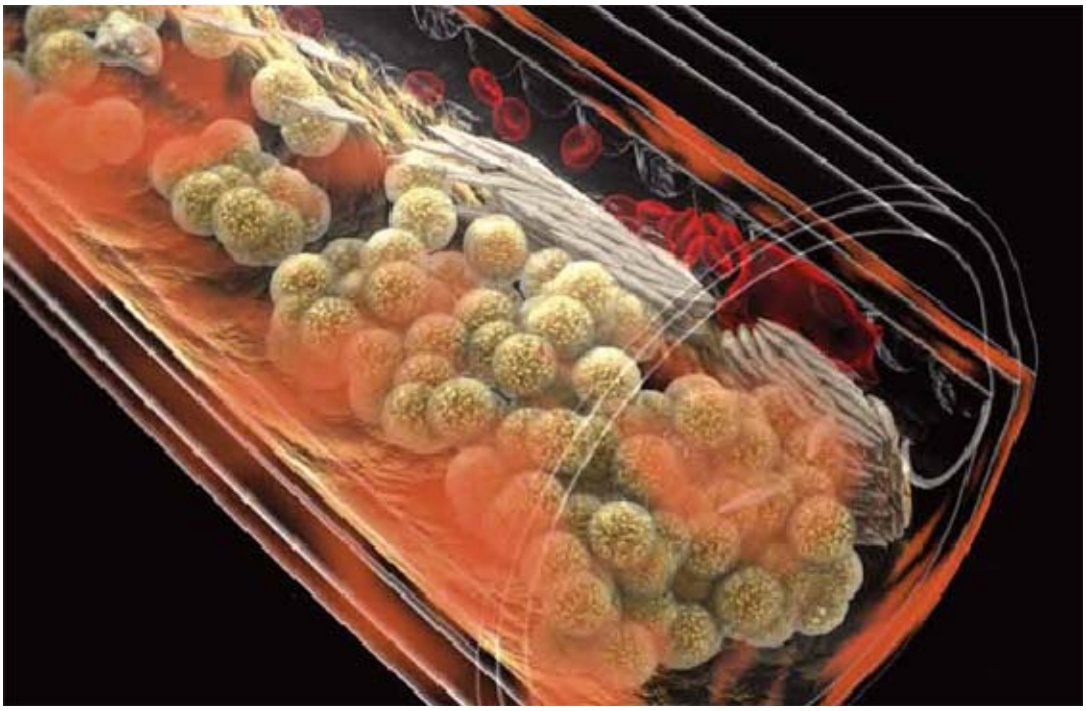
The Prophetic Protein

Who is at risk for heart attack?

by Tamar Nordenberg



Scientists believe most heart attacks are caused by plaques that burst suddenly, causing a blood clot that blocks the artery and impedes blood flow. Credit: Bryan Christie

Tense hours in the emergency room while tests confirm a heart attack may be rolled back to mere minutes, thanks to a telltale protein marker identified by Case Western Reserve University School of Medicine researchers. Better yet, a routine blood test for the nefarious protein could serve as an early warning to people at high risk: Take steps now, and you may dodge the dangerous attack altogether.

When the proverbial elephant takes a seat on one's chest, it is a decided hint: That person might be having a heart attack, or myocardial infarction (MI). Every 25 seconds, someone in the United States has one, according to the American Heart Association, but the oft-reported sensation of chest tightening or pain is just that-a clue. Even in the hospital, it can take eight to 12 hours for current tests to conclusively rule a heart attack in or out. Common alternative culprits in chest pain are intense heartburn or a gallstone attack.

Led by top physician-researcher Daniel I. Simon, MD, investigators at the School of Medicine, however, have discovered a marker of heart attack that promises to cuthours off the time for

definitive MI diagnosis-to the tune of confirmation within 10 to 15 minutes of arriving at the emergency room. What's more, a simple blood test for the novel myeloid-related protein-8/14 (MRP-8/14) marker could give long-used cholesterol screening a run for its money as a signal of MI in the making, years ahead of the cardiac attack.

"Though we gain great insight into patients' potential risk for cardiovascular disease using conventional biomarkers, we are limited in identifying some people at risk," says Douglas Vaughan, MD, professor of cardiology at Northwestern University's Feinberg School of Medicine and chair of its Department of Medicine. "An additional marker measured in people's blood could valuably refine our ability to take care of patients with coronary artery disease."

To hone in on the up-and-coming predictive protein MRP- 8/14, researchers applied an unprecedented scientific approach that scoured entire human genomes for cardiac warning signs. "We were on the hunt. We wanted to know what genes turn on or off in heart attack patients," explains Dr. Simon, the Herman K. Hellerstein Professor of Cardiovascular Research at the School of Medicine and director of University Hospitals Harrington-McLaughlin Heart & Vascular Institute. Dr. Simon and his team of researchers identified MRP-8/14 as their best-bet marker for heart attack for use in emergency settings and as a potential companion to routine cholesterol screening in the doctor's office.

Dr. Simon's account is a tale of finding a little molecule with big potential-a project born in a lab in New England that has grown on a campus in Cleveland.

Nature's Time Capsules

Dr. Simon, then a researcher at Harvard Medical School's Brigham and Women's Hospital, and his colleagues, canvassed the human genome for differences in heart attack patients versus those with stable coronary disease. "The field has been struggling to understand this: What are the final triggers that transition stable blockages into life-threatening plaque rupture and thrombosis?" Dr. Simon explains. Scientists believe that most heart attacks are caused by plaques that burst suddenly, causing a thrombus, or blood clot, that blocks the artery and impedes blood flow. Atherosclerosis, often referred to as "hardening of the arteries," is the process responsible for depositing plaques made of cholesterol and other fatty materials, which thicken the artery wall. But atherosclerosis exists in stable blockages, too. So what, then, causes the enduring problem to take its life-threatening turn?

To answer this question, Dr. Simon and his team looked at the entire genome using a process called transcriptional profiling. While treating a patient for heart attack in the hospital, researchers drew their blood and compared it with samples from patients with stable angina. They examined mRNA , which contains the blueprint for making proteins, for all of a person's 30,000 or so genes.

Researchers were challenged to analyze the blood in a way that could discern which differences they found preceded the heart attack and did not result from it. "It's a chicken-and egg problem. Which came first?" Dr. Simon explains. The team chose to examine platelets-fragments of bone marrow cells called megakaryocytes that break off and enter the bloodstream and, significantly, have no DNA -housing nucleus. The signs of gene expression are frozen in time during the 7- to

10-day lifespan of the platelets, earning them Dr. Simon's designation as "nature's time capsules."

Theirs is the first published report of platelet profiling in human health or disease; since then, the method has been applied in sickle cell disease research. The scientists dubbed their platelet-based approach the "Lois Lane-Superman phenomenon" because the pre-heart attack biological state is reflected in post-heart attack blood. In Dr. Simon's words, "By sampling the platelets, we can turn back time-just like Superman before Lois Lane dies in the crash-and look at gene expression in the days and hours before the heart attack."

By collaborating on the transcriptional profiling with Millennium Pharmaceuticals, Dr. Simon and his academic colleagues from Harvard were able to leverage the company's personalized medicine program; in particular, the research benefited from a high-fidelity amplification method used to make crucial health observations, such as distinguishing high-risk from low-risk tumors. The approach-a method "as mindbogglingly complex and expensive as it is powerful," as Dr. Simon describes it-magnified the number of molecules so that a small amount of blood taken from each patient sufficed for analysis that would normally require 1,000 times that amount.

Mukesh Jain, MD, came from Harvard to become the Ellery Sedgwick Jr. Chair and Distinguished Scientist and director of the Case Cardiovascular Research Institute at the School of Medicine. He describes himself as "consultant and sounding board" on the MRP-8/14 research, and says, "The partnership is an extraordinary example of how bright people from industry and academia can collaborate to play up their respective research strengths."

Heart Attack Ahead?

As detailed in the May 16, 2006, issue of the journal Circulation, the transcriptional profiling project bore encouraging fruit in the form of 59 differences spotted between the platelets of those with heart attack and those with stable coronary disease. It identifies the disparity with the greatest promise for further scientific study as the protein MRP-14-which is bonded with its sister molecule MRP-8 and so is studied in its MRP-8/14 combination state.

MRP-8/14 was the most abundant protein found inside infection-fighting cells called neutrophils. Neutrophils play a starring role in acute inflammation-a process that scientists today understand as a considerable contributor to atherosclerosis and heart attack-and are the cells associated with plaque ruptures seen in autopsies after death from heart attack.

Dr. Simon and his team knew from examining platelets that MRP-8/14 was present in patients' blood in relatively high levels before their heart attack. This suggested the protein might serve as a prompt indicator of which people sensing an elephant on their chest were having an actual heart attack. What they still did not know, however, was how far in advance of the coronary calamity that elevated levels of MRP-8/14 appeared. Does the protein lurk inside apparently healthy people, even years before a heart attack occurs? In that case, tracking MRP-8/14 levels-which can be measured in the plasma with a simple blood test-could prove to be an enormously powerful tool in heart attack prediction for the general population.

For a prospective validation study that would elucidate the timeline, the researchers were granted access to scientifically precious blood from the Women's Health Study-a renowned cardiovascular disease prevention study during which blood from more than 28,000 apparently healthy women was collected and frozen. Over the years following the blood collection, some of the women had a heart attack or stroke, while others did not.

In those women who went on to have heart attacks, Dr. Simon's group found the expected elevated cholesterol levels, high blood pressure and other accepted risk indicators. Notably, women who went on to have MIs also had much higher levels of the study's protein of interest, MRP-8/14, in their blood when they were seemingly healthy. This served as the hoped-for validation of the compound's predictive potential. Abnormally elevated levels of MRP-8/14 were associated with a more than doubled heart attack risk, a more significant association than even LDL cholesterol.

Cholesterol testing is known to be a meritorious, but by no means foolproof, method to appraise cardiovascular risk. "We know that many people with modest cholesterol levels are at relatively high risk, while those with high levels can be at pretty low risk," says Carl Orringer, MD, the Harrington-McLaughlin Chair in Preventive Cardiovascular Medicine at the School of Medicine and director of preventive cardiovascular medicine at University Hospitals Case Medical Center. MRP-8/14 has been compared with the "gold standard" biomarker C-reactive protein (CRP) as a possible partner with cholesterol and other measures to help predict cardiovascular events, including heart attack.

Studies in Mice and Men

The research team was not content to stop at their breakthrough finding of MRP-8/14's association with heart disease. Dr. Simon went back to the laboratory-this time as a faculty member of the School of Medicine-and created a pertinent mouse model to study whether MRP-8/14 played a fundamental role in the vascular inflammation involved in atherosclerosis. "As physician-scientists, these researchers committed to bridging the clinical and scientific observations: 'Let's go back to the bench to understand how this works,'" Dr. Jain says.

The first step in the research, published in the August 4, 2009, issue of Circulation: Create a mouse with raging atherosclerosis by feeding it a high-fat diet. Step two: Delete a gene involved in cholesterol metabolism. Finally: Cross this mouse with one engineered without its MRP-8/14 gene.

This way, the scientists were poised to examine the gene's role in vascular inflammation. Finding significantly less inflammation in the custom-designed mouse-the one missing MRP-8/14-the researchers were able to conclude that the protein plays a fundamental role in vessel wall inflammation. Says Kevin Croce, MD, PhD, an instructor of medicine at Harvard Medical School, adjunct assistant professor at Case Western Reserve, and first author on the study, "It's very powerful to go back-and-forth from test tubes, to mice, to patients. Both Dan [Simon] and I have the opportunity to work with patients every day, and it's incredibly exciting to find new targets in the lab that have real clinical promise."

Dr. Simon and his colleagues have turned their attention now to a crucial question of causation in thrombosis. "We know a person is prone to a heart attack if MRP-8/14 is present at a high level," he says. "We're trying to figure out now whether this is only a sign that a person is at risk for heart attack, or if it actually is the culprit." An upcoming paper will address whether MRP-8/14 influences the clotting that sets up a heart attack.

The research uses models with induced clots; if the time to clot is longer in a specimen whose MRP-8/14 gene has been removed than in a normal model, researchers will have their answer that MRP-8/14 plays a causational role in thrombosis.

The researchers also want to confirm whether MRP-8/14 predicts a second event in those who have established coronary disease. It seems predictive of a recurrence, based on a study published in the January 2008 American Heart Journal, and now the question must be studied in a larger number of patients. David A. Morrow, MD, MPH, a Harvard Medical School clinical cardiologist and lead author of the important recurrence study says of the ongoing collaboration among researchers from Harvard, Case Western Reserve and other distinguished research institutions, "I think this reflects the best of what academic centers have to offer in moving science forward from bench-top research to potential bedside application."

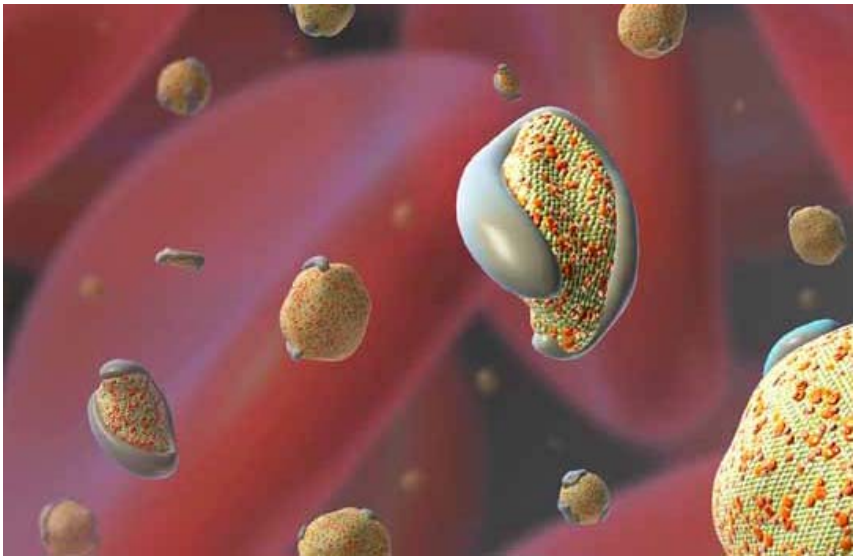
Another project is being designed by the remaining team of Brigham and Women's researchers together with Dr. Simon's School of Medicine group to compare MRP-8/14 head-to-head with other heart attack markers to better gauge what it might add to the existing field.

To be determined, ultimately, is whether medications can be developed that thwart any destructive power of MRP-8/14. Dr. Simon hopes this kind of drug will be available in 7 to 10 years. "We still have a lot to learn," acknowledges the physician-scientist, "but we view our glass as half-full. We didn't even know before that this protein might be involved in vascular inflammation and thrombosis, and in three short years we have all this dramatic information to build on."

In the near term, clinical trials are ongoing to gain approval from the Food and Drug Administration for quick-working MRP-8/14-based tests. European researchers have found MRP-8/14 to be the most sensitive and rapid predictor of heart attack for patients in the emergency room-substantiating an MI in a matter of minutes, not hours like a traditional test that tracks the substance troponin slowly leaking into the bloodstream. Dr. Simon estimates that MRP-8/14 tests could be widely available within two years, not only for use in the emergency room to confirm a heart attack, but also for routine testing in the physician's office to help avoid one.

(Sidebar)

Much-needed complement to cholesterol testing



Various high-density and low-density lipoproteins (HDL and LDL) and chylomicrons comprise the “good” and “bad” cholesterol of circulating blood.

For patients outside the highest and lowest traditional risk factor categories, based on factors like high cholesterol, smoking, diabetes, hypertension and family history of heart disease, MRP-8/14 could become a prominent diagnostic tool. “We are attempting to determine whether the use of MRP-8/14 should sway us toward more aggressive preventive therapies,” says Carl Orringer, MD, the HarringtonMcLaughlin Chair in Preventive Cardiovascular Medicine at the School of Medicine.

Currently, a “high-sensitivity C-reactive protein” (hs-CRP) assay is sometimes used in conjunction with cholesterol tests to assess heart disease risk. Like hs-CRP, MRP-8/14 represents a different biological process than cholesterol and is likely to serve as a complement to, not a substitute for, cholesterol screening. Of cholesterol testing’s shortcomings, Dr. Orringer says, “Relying on cholesterol alone is ignoring the inflammation that lights the fuse that sets off the explosion that is the heart ttack. Dr. Simon’s approach is a very good way to look at the inflammation part of the heart attack equation, which in many cases is hiding, so the clinician does not know about it.”

Dr. Orringer, who developed an innovative heart attack risk assessment program that uses CT scans to see whether a person has hardening of the arteries, believes that MRP-8/14 may come to be incorporated to aid in risk estimation.

“A person’s heart attack risk is related to how much calcium is in the arteries—the more calcium, the greater the risk,” Dr. Orringer explains. “Those with calcium in their arteries indicating atherosclerosis might be really good candidates for MRP-8/14 evaluation to see who is at the highest risk.”

(Sidebar)

Do “bad” genes make a heart attack inevitable?

With the discovery of genes like MRP-8/14 that predispose a person to a heart attack—coupled with long-known risk factors like cholesterol—it may seem that people whose genes spell high risk for heart attack are bound to have one. But even among participants in the Women’s Health Study whose blood contained MRP-8/14 levels in the highest quartile, most did not go on to have a heart attack or stroke.

No doubt, genetics play an “incredibly important role in a person’s risk for heart attack,” Dr. Orringer says, emphasizing that lifestyle, too, plays a major role. “So far, however, we have no clear understanding of which is more important.”

Using assessment tools, such as cholesterol screening and maybe soon MRP-8/14, to identify those whose genetics put them at particular risk is key to reducing that risk over time, Dr. Orringer says. “Even if the genetics are bad, there are many things we can do to reduce people’s risk.”

Dr. Orringer’s recommendations depend on the particular patient, but his short list of options includes a heart-healthy diet with fish oils; regular exercise; and medicines such as lipid-reducing statins, blood pressure medications and aspirin.