

Feature Article

Hearts and Minds: Biomarker Research Branches Out

- **Genomic and proteomic tools are increasingly deployed in the hunt for biomarkers to diagnose or characterize particular diseases. Much of this work centers on cancer, but researchers are starting to find promising markers for some of the world's other leading killers.**
- By Alan Dove, PhD

A man walks into a doctor's office with a duck on his head. "Can I help you?" asks the doctor, puzzled. "Yeah," says the duck, "can you get this guy off my rear end?"

The joke is old, but it hints at an even older problem: even patients who present with seemingly obvious symptoms can be strangely difficult to diagnose. Often, the problem seems to make no more sense than a talking duck. By one estimate, one out of every three presenting symptoms that show up in a primary care office will remain unexplained.

That two-thirds of symptoms can be explained, though, is a minor miracle, considering the complexity of human physiology and disease. While medicine still relies heavily on the knowledge and intuition of individual doctors, gradual advances in disease biomarker discovery over the past few decades have boosted their diagnostic abilities considerably.

Until recently, the search for new biomarkers was excruciatingly slow. Often, it took years to associate some measurable parameter of physiology, such as blood cholesterol, with a risk, such as heart disease. Understanding why a particular marker is associated with a particular disease could take decades longer, as clinicians waited for basic science to catch up with medical observations.

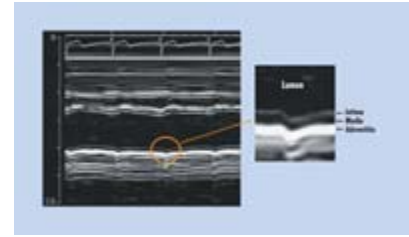
Using new genomic and proteomic tools, researchers are now engaged in an unprecedented large-scale hunt for new biomarkers. Because modern molecular biology and cell biology are so closely linked to cancer research, that family of diseases has received the lion's share of attention, but a few scientists are now developing molecular biomarkers for other prevalent conditions, like heart disease and brain injury. While borrowing some techniques from cancer research, these investigators are also developing some of their own approaches, and discovering some of their own obstacles.

Priming the pump

According to the Centers for Disease Control and Prevention, Atlanta, cardiovascular disease kills about 950,000 people a year in the United States alone, or one person every 33 seconds. Heart disease and stroke, the two main categories of cardiovascular disease, are the first and third leading causes of death nationally, with cancer sandwiched in between them at number two. Statistics for other developed countries are similar.

Besides its enormous prevalence, cardiovascular disease is an attractive target for biomarker researchers because it is so treatable. "One of the big things about cardiovascular disease is that there are so many things people can do to decrease risk compared to cancer," says Richard Lee, professor of medicine at Harvard Medical School, Cambridge, Mass. "The more things you can do to prevent it, the more things biomarkers make sense for."

By allowing physicians to diagnose cardiovascular disease early and accurately, new biomarkers could save numerous lives. But the search has been slow. "All the news this year is on C-reactive protein," says Lee. "That's a biomarker that has been around for 30 or 40 years; it's just assigned its role more specifically now."



click the image to enlarge

The thickness of the space between the intimal (thin top line in inset) and medial (dark area under the thin line) layers of the carotid artery was once considered a potential biomarker for cardiovascular disease, but the difficulty of interpreting the ambiguous images made it unworkable. (Source: Richard Lee, Harvard University)



Computerized tomography scan shows bleeding from hemorrhagic stroke (white area). Patients often suffer small strokes that go undiagnosed because of a lack of reliable biomarkers. (Source: Victor Wu, MD, Lakeland Radiologists, Effingham, Ill.)

To pick up the pace, Lee and his colleagues have turned to a combination of genomics and computational biology. The researchers first identify areas where new cardiovascular disease biomarkers are needed, then identify assays that might be able to fill those gaps. "For example, following people with heart failure to see who might benefit from more aggressive therapy. That's an area where we have big holes . . . as compared to diagnosis of myocardial infarction, where the [existing] tests are extremely accurate," says Lee.

Once they identify a need, the investigators turn to databases of clinical trials that have already been done and look for correlations between diseases and their experimental biomarkers. By using data that are already available, the team has shortened the search dramatically. In some trials, according to Lee, "You already have 10-year follow up, so you can essentially do a 10-year prospective study in a month. It's looking

for what is available and thinking about how that database fits with your ultimate goal."

Use of existing databases also helps the team get around some of the difficulties of sample collection (see "HIPAA Has Chilling Effect on Sample Access" on page 27). The strategy is already showing promise. A gene called ST2 emerged from genomic studies as a potential marker for heart disease, and Lee and his colleagues are now developing the assay for clinical use. Other disease markers will likely follow soon.

Besides better markers for heart disease, clinicians' wish lists also include more accurate diagnostic tests for stroke, and better markers for myocardial ischemia, which is the loss of blood flow that precedes the death of heart muscle tissue.

Why a duck?

Whatever they diagnose, there is a good chance that the cardiovascular biomarkers of tomorrow will be proteins and genes whose exact functions are not known. There are a few historical precedents for this blind approach. Cholesterol, for example, served as a marker of heart health for decades before its physiology was worked out. But genomic and proteomic technologies are now poised to produce a windfall of such poorly understood markers.

Experts in the field argue that these blind searches are the only way to proceed.

"Certainly the traditional approach has been to look at markers that have a known role in the pathophysiology of the disease," says Alan Wu, professor and director of the clinical chemistry laboratory at the University of California, San Francisco. "That has taken us so far to probably the limit of where we can go. [Now] we need to take the more blind approach of massive screening, and looking for proteins or genes for which we don't know why they are there."

While it may be less satisfying intellectually, a blind biomarker has pragmatic appeal for clinicians. "A marker's a marker," says Wu. "We're less concerned as to what it is and more concerned as to what it can do for us." To a clinician, any answer is potentially informative, whether it comes from the patient or the enigmatic duck on his head.

Even if their functions are unknown, though, researchers must be rigorous about new biomarkers' validity, particularly in cardiovascular disease. "There have been some terribly misleading and disastrous surrogates in cardiovascular development," says Lee. At one time, researchers thought that improving how hard the heart squeezes, and suppressing extra beats, would be good markers for the activity of cardiovascular drugs. In fact, neither measure correlates with drug efficacy.

"Many [cardiovascular disease] surrogates have turned out not to be trusted. That, unfortunately, has been discouraging for some companies," says Lee. Fortunately, the failures have not frightened all of the corporate support from the field. Biosite Inc., San Diego, for example, recently filed an application with the US Food and Drug Administration (FDA) for a new diagnosis system that uses a panel of biomarkers, in combination with traditional diagnostic techniques, to determine whether a patient has suffered a stroke.

Blunt instruments

Similar obstacles, and similar corporate skittishness, have confronted the search for biomarkers for traumatic brain injury (TBI). Though it is not quite as prevalent as cardiovascular disease or cancer, each year about 1.4 million Americans get hit on the head hard enough to cause TBI, and about 50,000 of them die as a result. Compared to the magnitude of the problem, tools for fixing it are scarce.

"There have been more than 200 negative clinical trials for TBI [treatments]. This has discouraged people," says Ronald Hayes, PhD, professor of neuroscience, University of Florida, Gainesville.

Anything from a fist to a floor can cause TBI, and the direction, force, and location of the impact all affect the injury, as does the patient's own brain chemistry and history. Patient management also makes an enormous difference. In the past 20 years, improvements in patient management alone have reduced severe TBI-related mortality from more than 50% to less than 20%.

The complexity of the problem creates a chicken-and-egg conundrum for clinical researchers. Because there are few reliable biomarkers to track the progression of TBI, it is extremely difficult to show that a new therapy works. Finding new biomarkers, meanwhile, requires comparing patient outcomes to differences in markers, but the shortage of treatments and diagnostic tools makes outcomes hard to compare.

Where others have thrown up their hands in frustration, though, Hayes sees opportunity. Even mild TBI can have far-reaching impacts on patients who are not

properly diagnosed. "More than half of [US military] casualties in Iraq have attendant concussive injuries from blast-related insults," says Hayes. "It's one thing to take a concussed football player and put him back on the field; it's quite another thing to put a concussed soldier back in harm's way."

At the University of Florida's McKnight Brain Institute, as well as at Banyan Biomarkers Inc., Alachua, Fla., a company Hayes founded, researchers are using an approach they call "smart proteomics" to find new biomarkers for TBI. Instead of screening all of the proteins in a sample, the investigators focus on a subset of post-translational modifications that have been associated with traumatic injury.

Once they find potential markers, Hayes says, "Our discovery platform is closely integrated with a preclinical validation platform that allows us to confirm or disconfirm the utility of these markers in *in vitro* and *in vivo* systems." Biomarkers that look good in a rodent model will then move into clinical trials in patients with TBI. Hayes estimates that some of the company's markers could be usable in the clinic within 18 months.

Indeed, the potential for a quick turnaround time from testing to approval is part of the appeal of biomarker discovery. In many ways, diagnostic tests face much lower regulatory barriers than drugs. "Basically in the FDA approval process, you help dictate what you think the utility of the biomarkers will be. You can posit fairly modest goals for specificity and sensitivity, and as long as you meet those goals . . . you can get FDA approval," says Hayes.

The sum of their parts

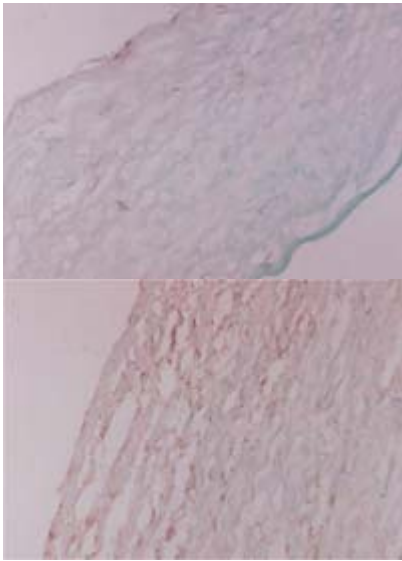
Though regulators may be happy with modest specificity and sensitivity, doctors prefer tests that give definitive answers. As the history of biomarkers has shown, human physiology can be evasive, a problem researchers are hoping to address with new tests that use multimarker panels. Each individual biomarker in a panel may yield vague results, but their individual weaknesses will cancel out, producing a robust assay. At least, that's the theory.

Cancer researchers have pioneered the multimarker approach, using gene chips to identify expression patterns associated with tumor growth. Some cancer tests now in development track hundreds of genes simultaneously, but scientists studying cardiovascular disease and TBI are still operating on a more modest scale.

"Smaller panels is the current direction that it's going," says Wu. "In the case of ischemia, we may see that it will require more [markers], in which case the clinical laboratory field will need to find new techniques to conveniently and inexpensively do a multimarker panel." Multimarker systems now being developed for cancer diagnosis and staging may pave the way for other panel-based tests, by encouraging clinical laboratories to invest in the necessary chip readers and other equipment.

In the longer term, translating the findings of basic biomarker research into practical

diagnostic tools may require more fundamental changes. Francesco Marincola, MD, senior investigator at the Clinical Center of the National Institutes of Health, Bethesda, Md., and founding editor of the *Journal of Translational Medicine*, says that many of the problems now confronting drug development efforts are also likely to slow biomarker development.



click the image to enlarge

On the top, tissue from a normal carotid artery, and on the bottom, dark staining showing the overexpression of eotaxin protein in a diseased carotid artery. Polymorphisms in eotaxin may be useful biomarkers for cardiovascular disease, but they only work for a small percentage of the population. (Source: Richard Lee, Harvard University)

As an example, Marincola cites a biomarker study [M. C. Panelli *et al.*, *Genome Biol.*, vol. 3, pp. research0035.1 - 0035.17 (2002)], in which a panel of 30 gene expression markers strongly predicted clinical responses in 25 patients with metastatic melanoma. Despite the assay's promise, though, Marincola says he has "never convinced anyone to do it on a larger group" of patients. Biomarker fields that are just getting started, like cardiovascular disease and TBI, will likely encounter similar disappointments soon.

Although some researchers have had luck starting their own companies, Marincola advocates a more radical change in the way biomedical research is done. "We should start considering having departments in a translational medicine-type of setting, where the department is dedicated to a specific problem rather than to a discipline, for example, a department dedicated to [research on] melanoma," he says. Such a department would hire faculty for different parts of the research pipeline, from basic discovery through clinical trials, to shepherd their findings all the way into application.

However researchers address the translational bottleneck, the availability of new biomarkers for some of medicine's biggest diagnostic challenges, such as cardiovascular disease and TBI, could eventually save millions of lives. And that's no joke.

Alan Dove is a contributing writer based in New York.