G2 INTELLIGENCE

Diagnostic Testing & Technology Report

Competitive Intelligence & Analysis for an Expanding Global Market

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Study Finds 'Considerable Discordance' Between Estimated, Direct Measures of LDL-C

Standard cholesterol tests that rely on the Friedewald equation underestimate low-density lipoprotein cholesterol (LDL-C) levels, particularly in cases of high triglycerides, according to a large study published online March 21 in the *Journal of the American College of Cardiology*. The authors say this discordance may result in the undertreatment of some high-risk patients and they call for additional evaluation in these patients.

In this study, researchers examined lipid profiles in 1,310,440 patients (52 percent women) who underwent vertical density spin gradient ultracentrifugation lipid profiling between 2009 and 2011 at Artherotech Diagnostics Lab (Birmingham, Ala.), including 191,333 patients with Friedewald LDL-C less than 70 mg/dl. The researchers found that there were greater differences between Friedewald estimates and direct LDL-C measurements, particularly in patients with lower LDL-C and higher triglyceride levels. When triclycerides were 150 to 199 mg/dl, median direct measures of LDL-C were 9 mg/dl higher than Friedewald estimates, and when triglycerides were 200 to 399 mg/dl, direct measurement was 18.4 mg/dl higher than the estimated LDL-C.

"The Friedewald equation tends to underestimate LDL-C most when accuracy is most crucial . . . and therefore additional evaluation is warranted in high-risk patients," writes lead author Steven Jones, M.D., from Johns Hopkins (Baltimore).

For more on how diagnostics are being used to assess cardiovascular risk, please see the *Special Focus* section on page 8.

Rapid Growth Expected to Continue in Indian Diagnostics Market

The diagnostics and laboratory service industries are experiencing significant growth in India. With a number of strong drivers in place, this growth is expected to continue, presenting American laboratories and diagnostics manufacturers an opportunity to penetrate this nascent, fragmented market. Ironically regulation, the factor attributed with slowing commercialization efforts in the United States, poses the only threat to growth in India, although in India it is the lack of regulation and the resulting questionable quality of some tests that could derail confidence in the testing market.

Driving the growth in diagnostic testing is India's overall economic success in the past decade with double-digit growth in the gross domestic product.

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▲ **Rapid Growth Expected,** *from page 1*

Increasing consumer wealth combined with an increasingly connected global marketplace is raising expectations of Indian consumers.

"Twenty-five years ago a doctor would look at your ears, your eyes, your throat and give you a yellow liquid or a blue liquid," recalls Ravi Kiron, Ph.D., managing partner at Biopharma Strategy Advisors (San Jose, Calif.). "There was no system to collect or transport specimens to outside labs. Whatever the doctor could do in the office was what was done. Today the common man in India wants quality diagnostics."

Additionally, there has been a shift in disease profiles. Previously, communicable diseases were the biggest health threat. Now, with adoption of some of the negative influences of Western lifestyles, chronic diseases including diabetes, heart disease, and cancer are growing in prevalence—all of which require diagnostic testing.

India's reimbursement structure will also drive growth. Experts say that vast majority of patients pay for diagnostic testing out of pocket. The limited insurance that exists in India only covers inpatient hospital and surgical services. All outpatient testing is paid for by the individual.

"There are over a 100 million educated Indians who can afford anything. . . . There are also 200 million other Indians who can afford 'almost' anything," says Zoya Brar, co-founder of Core Diagnostics (Gurgaon, India). "With growing awareness . . . Indians are demanding the best that health care has to offer. This is what is at the heart of the growing utilization."

Brar tells *DTTR* that the Indian diagnostic and pathology labs test services market in India was valued at approximately $2.2 billion and is expected to more than double to $5.5 billion by 2020. The diagnostics market in India is described as having two informal segments—the organized market and the unorganized market. The unorganized market includes a staggering 30,000 to 40,000 laboratories, which are not necessarily owned by a pathologist or doctoral laboratory scientist. By contrast, the organized segment has much better oversight, but not necessarily by a government or regulatory agency. The organized segment is significantly smaller with centers numbered in the hundreds. Only a few of the laboratories in this sector are performing complex molecular diagnostics, including sequencing.

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—Zoya Brar

"There is also a severe lack of availability of high-end diagnostics," says Brar. "Most immunehistochemistry is just starting up and there is almost no FISH/molecular-based testing. There is also a lack of subspecialist pathologists, leading to deficiency in diagnosis. Some tests are sent to the United States or Europe, but in these cases, there are long turnaround times (two to three weeks). Essentially, there is a demand-supply imbalance."

Core Diagnostics, which has received venture capital funding from Artiman (Palo Alto, Calif., and Bangalore, India) is focused on offering advanced, high-value clinical diagnostics including in oncology and reproductive health in India.

In contrast to the over-regulation cited in the U.S. market, it is a lack of regulation in India that poses one of the few challenges to growth. With no single agency approving tests or determining their clinical applicability, nothing formally holds doctors back from ordering tests. Commercial laboratories just need to persuade a doctor, mak-

ing it relatively easy to launch a test. The challenge, experts say, is that with a lack of regulation there is no guarantee patients are receiving quality, medically necessary services. Several people familiar with the Indian laboratory market say labs commonly cut corners by buying cheaper products from China and Taiwan that have no regulatory approval and that unscrupulous practices, including kickbacks to prescribing doctors, could affect patient care.

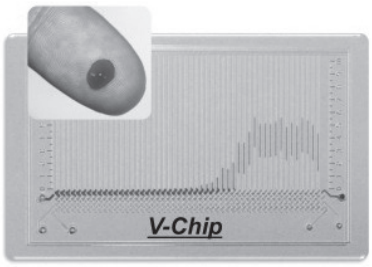
V-Chip POC Device Allows for Testing of up to 50 Analytes

With one drop of blood and a new business card-sized point-of-care (POC) device, clinicians may soon be able to test for up to 50 blood analytes including proteins, DNA, or infectious agents, all at the same time. In validation studies the V-chip was capable of quantitatively analyzing common markers like insulin as well as multiple gene expression profiles in breast cancer samples. The developers of the technology say the device is capable of simplifying analyses for personalized diagnostics.

"We have created a new volumetric POC platform that allows quantitative, multiplexed and instrument-free protein measurement," write the developers, led by Lidong Qin, Ph.D., from Methodist Hospital Research Institute (Houston), in a validation study published in *Nature Communications* in December.

The microfluidics-based chip incorporates enzyme-linked immunosorbent assay technology that generates a volumetric increase in oxygen pressure visualized as bar charts, hence its name—the volumetric bar-chart chip (V-chip). The device is made of two 2 inch-by-3 inch pieces of glass with wells for hydrogen peroxide; up to 50 different antibodies to specific proteins, DNA or RNA fragments, or lipids of interest, and the enzyme catalase; the blood or urine sample; and a dye. The wells are initially isolated but are brought into contact through a shift in the glass plates creating a contiguous, zig-zagged space from one end of the V-chip to the other. As the analyte of interest binds to antibodies bound to the glass slide, catalase is activated and splits the hydrogen peroxide into water and oxygen gas. The increased oxygen pressure pushes the dye up the column, with the distance the dye travels proportional to the amount of substrate present. The result is a visual bar chart displayed directly on the device—without the need for optical instruments (chemiluminescence or fluorescence), data processing, or plotting steps.

V-Chip



Source: *Lidong Qin, Ph.D., and Yujun Song, Ph.D., Methodist Hospital Research Institute*

The device's anticipated affordability and simple user-interface might make it suitable for disease monitoring in resource-limited areas or in home blood testing. The researchers are in the early stages of commercializing the product. A prototype has been made for approximately $10 per card, although they expect the eventual manufacturing costs to be less.

"We are in early-stage discussions with a number of companies who have interests in a wide variety of different applications for the chip. The strategy is to license the rights to commercialize the V-chip to a company or companies that can develop the chip into a final product," Joanne Mitchell, Ph.D., director of technology transfer at the Methodist Hospital Research Institute, tells *DTTR*. Ultimately, the licensing party would submit the device for regulatory approval.

Cell-Free DNA Best for Monitoring Metastatic Breast Cancer

Circulating cell-free DNA is better at detecting changes in tumor burden in women undergoing treatment for metastatic breast cancer than other blood-based markers. Circulating cell-free DNA (also called circulating tumor DNA) had higher sensitivity and a greater dynamic range than either circulating tumor cells (CTCs) and cancer antigen 15-3 (CA 15-3), according to a study published in the March 28 issue of the *New England Journal of Medicine*.

The researchers recruited women undergoing active treatment for metastatic breast cancer. Serial blood samples were collected at intervals of three or more weeks. Circulating cell-free DNA improved sensitivity compared to CA 15-3 by 26 percent (85 percent versus 59 percent, respectively). Similarly, a 27 percent improvement in sensitivity was seen using circulating tumor DNA compared to CTCs (90 percent versus 67 percent). CTCs were quantified using the U.S. Food and Drug Administration-approved CellSearch test by Veridex. There were 133 times the number of amplifiable copies of circulating tumor DNA as CTCs when comparing the medians. As might be expected, increasing levels of both circulating cell-free DNA and CTCs were associated with significantly inferior overall survival, but cell free-DNA provided the earliest measure of treatment response in 10 of 19 women (53 percent).

"This proof-of-concept analysis showed that circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer," write the authors, led by Sarah-Jane Dawson, Ph.D., from University of Cambridge in the United Kingdom.

In an accompanying editorial, Marc Lippman, M.D., from University of Miami, and C. Kent Osborne, M.D., from the Baylor College of Medicine in Houston, caution that unlike other cancers with more homogenous or common mutations, breast cancer (as witnessed by the fact that mutations or structural variants could only be identified in the blood of 30 of the initial 52 patients) would require "substantially deep sequencing" thus making a universal panel unlikely to work. Personalized panels would be costly and rate-limiting. Additionally, they say, more research is needed both in determining if analyzing circulating tumor DNA improves patient outcomes and if the strategy is cost-effective.

Cynvenio Announces LiquidBiopsy Lab Service

As a sign that rare cell capture technology is emerging on the clinical scene, Cynvenio Biosystems (Westlake Village, Calif.) is launching what it says is the first lab analysis service available to clinical oncologists for circulating tumor cells (CTC) and circulating tumor DNA. The company previously launched services for researchers in 2012. The company's proprietary automated CTC isolation and staining platform utilizes enhanced immunomagnetic capture technology within a patented microfluidic flow chip design.

The platform's high purity levels (recovery rates greater than 90 percent from whole blood and low non-target cell capture rates of less than 80 cells/mL blood) enable next-generation sequencing of the captured rare cell populations without the need for whole-genome amplification, thereby improving turnaround of actionable clinical information. The laboratory, which underwent its Clinical Laboratory Improvement Amendments inspection in mid-March, has an annual volume capacity of 16,000 samples based on one shift per day, five days per week.

The company tells *DTTR* that it offers three commercial LiquidBiopsy lab services: CTC Sequencing using a targeted 50 Oncogene panel and more than 2,100 mutations (turnaround time [TAT] from sample receipt is seven days; list price is $7,500 per sample), CTC PCR Analysis of 45 cancer mutation hot spots in EFGR, BRAF, or KRAS (TAT five days; $1,750 per sample), and CTC Recovery and Enumeration involving staining and quantification with optional biobanking or return of target cells for downstream analysis at customers' labs (TAT three days; list price $1,250 per sample). Currently the tests require self-payment.

G2 INTELLIGENCE
Inside the Diagnostics Industry

Ambry Genetics Leading the Way With Clinical Next-Generation Sequencing

Charles Dunlop, CEO, Ambry

Ardy Arianpour, vice president, Ambry

In early March, Ambry Genetics (Aliso Viejo, Calif.) reached the milestone of having sequenced 10,000 clinical diagnostic samples with next-generation sequencing (NGS) and processed 100,000 total NGS samples. Ambry has a proud history of being on the forefront of genetic testing, having been among the first purchasers of commercially available sequencing instruments back in 2007. The company says it was the first to offer an NGS-based diagnostic test in April 2010, with the introduction of its 81-gene panel for X-linked intellectual disability. Ambry continued with its list of firsts by launching the first clinically available whole-exome sequencing test at a CLIA-certified laboratory in 2011.

Evolving from its roots in cystic fibrosis (CF) testing, Ambry continues to develop and enhance its comprehensive testing menu with significant investments in automation. This has allowed the company to focus on reducing test turnaround time while retaining its focus on quality client service and steadfast attention to their guiding ethical principles. Recently, Ambry CEO Charles Dunlop and Ardy Arianpour, Ambry's vice president of business development, spoke to *DTTR* about the evolution of NGS in clinical care and Ambry's commitment to rapid, yet responsible, growth in the future.

Ambry Genetics has been a leader in clinically utilizing NGS technology. Tell us about how Ambry is using NGS and how it makes the decision of which tests to transition over to an NGS-platform?

Arianpour: We started NGS back in 2007 with one of the first Illumina Genome Analyzers. We took the technology and applied it in the clinical market to yield actionable data. We now have 12 dedicated clinical sequencers running 24 hours a day. Double shifts here are important because they allow our throughputs to be super high.

Dunlop: We are using NGS technology with a lot of things, but I still like to use Sanger technology with single genes because it is quick and reliable. But next-gen technologies are very good for a larger amount of genes to analyze—things like cancer, XLMR [X-linked mental retardation], exomes. We have 10 next-gen tests on our menu.

Arianpour: You really need to have volume to have it at a certain cost point, as well. Not every lab can launch NGS unless they have the volumes for those tests.

Dunlop: When you look at the data—enough good-quality data for making real clinical calls—you need to have a lot of samples on one run to take advantage of the economics and you also need to do a lot of replications per run to make it accurate enough. It's not the cost savings that people think it is and that is why not all of our tests get transferred over. For a lot of our neonatal tests it is better to keep them on Sanger. It is more cost-efficient and more cost-effective. . . . Whether things go to next-gen or not is basically economics. It is not as medical of a decision as you would think. Our cancer panel or XLMR are good examples of tests that

you couldn't do without next-gen because there are just so many genes. But CF is perfectly good on Sanger. If we were to move it over it would be an economic decision. It wouldn't be a technological decision at all.

Ambry has a wide test menu covering everything from carrier screening and prenatal testing to cancer and cardiology panels. Which segments are seeing the most growth at Ambry?

Dunlop: We are seeing growth all over the menu, which is at over 300-plus tests including a couple clinical versions of exome testing. There is no test that is not growing—even our CF business keeps growing. There is no really hot segment inside Ambry, which is a safer type of growth for us.

I would say the market likes the clinical exome tests. Clinical exome testing has so much application potential. We are really just scratching at the surface of that test, in terms of where that test will eventually wind up. But we are doing it in a responsible way and not pushing it into the market or trying to do it on autism kids, where we are not really ready for that kind of data analysis. We are sensitive in how we treat those markets. But for really sick infants we are doing really well with that test. With our biomarkers pipeline, we are seeing about a 15 percent higher detection rate in our exome than other people who are using commercially available bioinformatics software.

Bioinformatics is spoken of as one of the current rate-limiting steps to wider NGS adoption. How does Ambry address the bioinformatics needs associated with sequencing?

Dunlop: I look at bioinformatics not as a solution. Bioinformatics, for us, just gives geneticists tools, not necessarily creating these automatic reporting engines that you hear about, which I think are a little irresponsible and a little risky. We have developed our own bioinformatics in house and it is awesome.

> ***"Clinical exome testing has so much application potential. We are really just scratching at the surface of that test, in terms of where that test will eventually wind up."***
>
> ***—Charles Dunlop***

Arianpour: We call it the Ambry Variant Analyzer (AVA). The great thing about AVA is that it has been developed around patient samples and around our systems. We started developing it four years ago and it custom fits our system well. It is quite elegant. Using existing databases and our own curated databases it filters and comes up with data points. The pipelines we have developed are so good at detecting mutations—again we are having 10 percent to 15 percent higher detection rates than anyone else with the exome right now.

You mentioned that Ambry has genetic counselors on staff. How are they utilized and as an industry, how will their role evolve with the clinical adoption of more widespread molecular testing?

Dunlop: Everyone is all excited about NGS and they think that we might sequence everybody's genome. But that is very complicated and can be very touchy. We don't really know how it will work. Let's look at both extremes. If nothing changes then genetic counselors will basically be doing what they are doing and you can

use bioinformatics tools like AVA to help as more genetic material and analysis is going through each sample. If genome sequencing becomes a fad of our civilization and 5 million to 10 million are done a year, which are really absurd numbers, I don't know what their role could be. . . . Would counselors be doing data analysis? Counseling patients? If it comes to that point there aren't enough geneticists and counselors in the world to do all of that counseling.

Ambry has 30 or so genetic counselors and some have migrated to operations, some to R&D, a lot do reporting and double check results. We have a few counselors full-time, which is kind of unique, in accessioning. When it seems like there is a doctor that has ordered too much, or something that doesn't seem right for the indication, or maybe there is a paperwork error and they checked the wrong box, they'll go ahead and give them a call. We try to do it responsibly, not to call back and order 50 more tests. We do the opposite. We want to make sure we are doing what is appropriate for patient care. At Ambry the geneticists and counselors define what good patient care means—not the CEO, not the people in research. They live and breathe quality patient care, quality results, and quality ethics. They define in large part how and what we launch as well as what we report out and how we report it out.

"At Ambry the geneticists and counselors define what good patient care means—not the CEO, not the people in research. . . . They define in large part how and what we launch as well as what we report out and how we report it out."
—Charles Dunlop

Labs have been anxious about reimbursement with the recent transition to the new molecular pathology codes. How is Ambry handling this transition?

Dunlop: I'm cautiously optimistic. We live in a civilization that places a high premium on the value of human life, generally speaking, and I don't think we, as a culture, are going to block out the coolest technology that can frankly save lives just to save health care dollars. Exome comes with a price tag. At the same time, think about how much money the health care system is wasting on diagnostic odysseys to figure out a condition.

If payments are an issue in the lab, you are playing with products that don't have utility in the health care system. Tests have to make sense for insurance companies when they do their cost-benefit analysis. When I find out some of the things that are done out there in the somatic cell world and they are taking more money out of the overall health care system than chemotherapy is and the prognosis of the cancer patients with these new technologies isn't really any better, then why should we pay for that? . . . If a lab launches quality products that make sense for the system and they focus on the ethics of what they are launching—not what they can get away with—like we have and some of our competitors that I respect for have, then I don't think you need to be too worried about the reimbursements.

Ambry Genetics By-the-Numbers

- Year Founded: 1999
- Employees: 200-plus
- Capital Raised: $500,000
- Clinical Samples Sequenced with NGS: 10,000
- Test Menu: 300-plus

Interest Seen in Biomarkers for Cardiovascular Disease Risk, But Adoption, Evidence of Clinical Utility Lags

Despite advances in the management of heart disease, cardiovascular (CV) disease remains the leading cause of death. While a lot of effort has been dedicated to the treatment of diagnosed disease, there is increasing focus on screening biomarkers capable of differentiating at-risk individuals to better target preventive therapy. While discovery of novel CV biomarkers, including genetic markers, has garnered attention in recent years, to date proof of clinical utility lags.

Biomarker Discovery

CV biomarkers have been identified for a number of cardiac conditions including acute coronary syndrome, coronary artery disease (CAD), congestive heart failure (HF), and myocardial infarction (MI). Use of diagnostic biomarkers in patients with symptoms of disease is well-established clinically. But other than for cholesterol, screening biomarkers to assess future CV disease risk has not taken hold in routine practice.

"The primary prevention of cardiovascular disease relies on the ability to identify at-risk individuals long before the development of overt events," writes Thomas Wang, M.D., in a biomarker review piece published in the October 2012 issue of the *Journal of Internal Medicine*. "In the past decade, research into circulating, genetic, and imaging biomarkers to augment traditional methods of risk prediction has only achieved modest success."

In the 2010 American College of Cardiology Foundation and the American Heart Association guidelines on the use of existing markers, the group only recommended six circulating markers. While newer circulating biomarkers have been evaluated since that publication, leading candidates including C-reactive protein (CRP) and B-type natriuretic peptide (BNP) have only shown the ability to modestly improve discrimination and reclassification, Wang explained. He says that some have turned their hopes to multimarker combinations or scores, but there have only been a few large studies and they too have only had moderate success.

Traditional risk factors (cigarette smoking, diabetes, high cholesterol, and hypertension) do not identify all individuals who will develop CV disease, and the current prevention strategy of lifestyle changes, lipid panel testing, and lipid-lowering drug therapy constitute the predominant prevention strategy for managing CV disease risk. In a meta-analysis published in *Lancet* in 2005 that included more than 90,000 patients from 14 randomized trials of statins, results showed that lipid-lowering therapy only reduced CV events by 21 percent.

Some Promising Studies

There are signs though that continued biomarker discovery efforts may be paying off. At the recent American College of Cardiology (ACC) Annual Scientific Session & Expo (March 9-11; San Francisco) there were a number of studies presenting evidence that efforts to use novel risk markers to improve identification of at-risk individuals for targeted preventive treatment may be working.

Among the studies presented was the five-year STOP-HF study, which enrolled 1,374 asymptomatic patients (age 40 years and older) with risk factors for HF.

Participants were randomized into either the control group, which received standard care, or the intervention group, which was screened at least annually for CV risks and blood level BNP. BNP is a hormone that indicates how well the heart is functioning. The researchers found that significantly fewer patients in the intervention group had new-onset HF requiring hospitalization or had left ventricular dysfunction. Additionally, intervention patients also had significantly lower rates of emergency hospitalization for major CV events, compared to the control group.

"The results of this study indicated that use of BNP in the community may facilitate prevention strategies aimed at reducing heart failure, left ventricular dysfunction and cardiovascular events," said co-author Kenneth McDonald, M.D., director of the Heart Failure Unit at St. Vincent's University Hospital (Ireland), in a statement. "The STOP-HF project provides the first example of how a structured screening and intervention strategy can prevent heart failure."

Aside from identifying novel candidates, improvements in instrumentation technology may enable more sensitive exploration of established biomarkers. Such is the case with cardiac troponin I (cTnI).

In another study presented at the ACC meeting, data showed that using a high-precision digital assay enabled the measurement of cTnI below traditional test measurements. With a highly sensitive assay, researchers from Brigham and Women's Hospital (Boston) found that monitoring levels of cTnI over time can identify post-coronary syndrome patients who are at increased risk for future death from CV death or HF. Traditional tests can only measure cTnI levels above 30 pg/mL to 50 pg/mL. But using Singulex's (Alameda, Calif.) digital single molecule counting "smart" enzyme-linked immunosorbent assay reader, the analytical measurement range is extended to 0.1 pg/mL.

"The STOP-HF project provides the first example of how a structured screening and intervention strategy can prevent heart failure."
—Kenneth McDonald, M.D.

Serial measurements of cTnI levels taken in 2,664 patients with stable ischemic heart disease at 30 days and four months predicted likelihood of CV disease and death from heart failure at the two-year follow-up. Patients whose cTnI levels were elevated or newly rising above 9 pg/mL at four months experienced more than three times the rate of CV disease or death from heart failure within two years compared with those with low cTnI levels (below 9 pg/mL). The findings potentially expand the clinical applications of cTnI beyond diagnosing MI in acute-care settings due to improved sensitivity in testing. A previous study showed that patients with cTnI levels of 9 pg/mL or greater benefited from intensive statin therapy versus moderate dose therapy.

Genetic Markers

While hopes are high that incorporating genetic markers into CV disease risk profiling will ultimately improve identification of patients most likely to benefit from targeted preventive therapy, many clinicians don't feel routine genetic screening for CV disease is ready yet. Routine genetic screening is unlikely until management is improved by genetic testing. Currently, they say, risk variants are less potent predictors of common CV disease, like CAD, compared with biomarkers.

To date, 33 genetic variants of genomewide significance have been identified and replicated. However, these genetic variants have only been found to have modest to minimal risk effect, experts say. Based on an evidence report on the use of genomic profiling to assess risk for CV disease, the Evaluation of Genomic Applications in Practice and Prevention group (EGAPP; commissioned by the U.S. Centers for Disease Control and Prevention's Office of Public Health Genomics) made the recommendation in 2010 that there was insufficient evidence to recommend testing in the general population for 58 variants (including 9p21) in 29 genes encompassed in eight available genomic tests. The group said the magnitude of any health benefit from use of any of the tests, either alone or combined, is negligible.

"It is a tall order to develop a screening test accurate enough to identify those who will get disease in a low-risk population. The test performance characteristics have to be great."
—Thomas Wang, M.D.

"It is fair to say that the human genome turned out to be more complicated than people thought, and its clinical usefulness less than people thought," says Doug Campos-Outcalt M.D., a member of the EGAPP group and chair of family, community, and preventive medicine at the University of Arizona College of Medicine, Phoenix. "The real problem is these genomic-based cardiovascular risk panels are not very predictive beyond common clinical factors—age, gender, family history, blood pressure, weight."

Among EGAPP's unresolved questions related to the clinical utility of such tests are the biological mechanism underlying the most convincing marker's (9p21) association with CVD, the level of risk that changes intervention, whether long-term disease outcomes will improve, how individuals ordering direct-to-consumer tests will understand and respond to test results and interact with the health care system, and whether testing will actually stimulate behavior change.

"It is a tall order to develop a screening test accurate enough to identify those who will get disease in a low-risk population. The test performance characteristics have to be great," Wang, who is now chief of cardiovascular medicine at Vanderbilt University (Nashville), tells *DTTR*. "It is useful to remember we are early in discovery. I still think there is reason for optimism, but it will take time."

Among those who are optimistic about the prospects that a genetic-based score can predict CV risk are researchers from CardioDx (Palo Alto, Calif.). The company has developed a gene expression score (GES) based on an algorithm incorporating 23 genes, age, and sex that delivers a score on a 1 to 40 scale.

In a study published online Feb. 15 in *Circulation: Cardiovascular Genetics*, the gene expression score outperformed clinical factors and myocardial perfusion imaging in identifying CAD in symptomatic patients referred for nuclear imaging. In the multicenter study, of 431 patients, the researchers found that GES has high sensitivity and negative predictive value (NPV) for obstructive CAD, with an area under the curve of 0.79. The GES had a sensitivity, specificity, and NPV of 89 percent, 52 percent, and 96 percent respectively. Over six months of follow-up, 27 of 28 patients with adverse cardiovascular events or revascularization had GES scores higher than 15. The authors said the test is most suitable as a "rule-out" test, but 54 percent of patients had scores above 15.

Gregory Thomas, M.D., lead author of the study, who is a paid consultant for CardioDx, tells *DTTR* he believes such a gene expression test is complimentary to existing diagnostic methods, like nuclear stress tests, and the combination of the two "may optimize diagnostic performance and utilization of health care resources."

He acknowledges though that widespread clinical utilization of new screening tests of CV risk will be a challenge.

"Medicine changes very slowly. With guidelines it takes five years for predominate acceptance and 10 years for substantial acceptance," says Thomas, who is medical director of the MemorialCare Heart and Vascular Institute at Long Beach Memorial Medical Center in Long Beach, Calif. "The challenge is physician comfort. They are very comfortable with a stress test developed 60 years ago. Labs can put their toe in the water with testing, but they must show each doctor. It is a stretch to understand how a blood test can show blockages."

Funding for Cardio Dx companies

Despite some guarded skepticism about the prospects for rapid adoption of new CV diagnostics, several companies have announced closing successful rounds of private financing.

Critical Care Diagnostics (San Diego) announced in March that it raised $2.6 million to expand commercialization efforts of its Presage ST2 Assay. The company says the soluble ST2 protein is a predictor of adverse patient outcomes and can identify high-risk heart failure patients so that doctors can adjust care accordingly. The test is CE marked and U.S. Food and Drug Administration-approved and is commercially available as a kit. The company reportedly has other formats of the test in the pipeline, including a point-of-care test and an automated platform.

Luoxis Diagnosis (Greenwood Village, Colo.) was spun out of Ampio Pharmaceuticals in February and is raising $5 million in private financing to develop and commercialize diagnostics based on the Oxidation Reduction Potential (ORP) technology platform. From a single drop of blood the ORP point-of-care reader can assess the presence of oxidative stress and anti-oxidant reserves in a patient. The company says ORP is an important homeostatic measure of patient morbidity in a range of diseases and conditions, yet there is no currently available test. Multiple clinical trials with stored blood samples from several diseases are under way.

Aviir (Irvine, Calif.), which is developing multiplex protein-based diagnostics tests for CV conditions, announced in February that it had raised the second $10 million tranche of an expected total $30 million of financing. The round was triggered by achievement of revenue milestones and was led by the Merck Global Health Innovation Fund. Proceeds from the financing will be used for a broad U.S. commercial launch. International distribution will begin midyear. The MIRISK test measures the blood levels of seven proteins associated with the development of vulnerable plaque and employs an algorithm that includes both those results and other known risk factors to determine an individual's probability of experiencing a heart attack within the next five years.

CytoVas (Philadelphia) was the first funding recipient from the American Heart Association's Science and Technology Accelerator Program, which directs philanthropic contributions toward product development through investments and loans. CytoVas will use the funds for a proof-of-concept trial of its Vascular Health Profile blood test, which integrates multiple measures of endothelial stress and response. Blood vessel stress is indicated by the presence and concentration of microparticles that are formed from endothelial cells, platelets, and leukocytes in response to activation or cell death, while blood vessel reparative capacity is determined by circulating endothelial progenitor cells. The Vascular Health Profile test uses flow cytometry platforms to assess both the negative and positive indicators of blood vessel health in conjunction with a proprietary computational algorithms.

POC Syphilis Tests Comparable to Lab Testing

Rapid point-of-care tests (POC) for syphilis have sensitivities and specificities comparable to conventional laboratory tests, according to a review published online Feb. 26 in *PLOS One*. The authors say a major change is necessary in syphilis testing strategies and recommend replacing first-line laboratory tests with POC tests globally to expedite screening, particularly in resource-limited settings.

"This meta-analysis is important as it includes all global sub populations at risk, thus generating high quality evidence to inform global policy," write the authors, including Nitika Pant Pai, M.D., Ph.D., from MzcGill University in Canada.

The gold standard for syphilis testing is done using two laboratory tests—a non-Treponema pallidum (non-TP) and Treponema pallidum (TP)-specific tests. First-line screening is usually performed with non-TP tests that detect anti-cardiolipin antibodies. These tests are cheaper compared to TP tests, but they can give false positives with malaria, immune disorders, and pregnancy. For positive non-TP results, confirmation testing is performed with a TP-specific test, which is limited in its ability to distinguish past and active infections.

The researchers extracted data from 33 articles published from 1980 to 2012 to compare 18 globally used rapid and POC treponemal tests. Four tests accounted for the majority of tests studied in published papers. The Determine (Abbott Diagnostics, United Kingdom) had a sensitivity of 86.3 percent and specificity of 95.9 percent, SD Biolines (Standard, South Korea) had an accuracy of 84.5 percent and 98 percent, Syphicheck (Qualpro, India) had an accuracy of 74.5 percent and 99.6 percent, and VisiTect (Omega Diagnostics, United Kingdom) had an accuracy of 74.3 percent and 99.4 percent.

"Despite being less than 100 percent accurate . . . rapid and POC tests have the potential to facilitate rapid improved detection of syphilis, allowing for treatment initiation in the same visit, reducing missed opportunities for detection and timely intervention to prevent transmission to infants and to partners," conclude the authors.

Therapeutic Folate Benefit May Be Explained by Genes

Functional variants in genes that regulate folate absorption significantly influence treatment response in patients given folate plus vitamin B12 supplementation to improve negative symptoms of schizophrenia, according to a study published online March 6 in *JAMA Psychiatry*. These findings, the authors say, support a personalized medicine approach for the treatment of negative symptoms in schizophrenia, and possibly other folate interventions in additional clinical areas.

Negative symptoms in schizophrenia (apathy, social withdrawal, and loss of emotional expressiveness) are not improved with anti-psychotics and have previously been tied to reduced blood folate levels. Given mandatory folate fortification of grain products in the United States, the authors explain that the benefits of folate supplementation may be less readily detected and clinical response may depend on genotype.

In a multicenter clinical trial, 140 stable patients with schizophrenia who chronically displayed symptoms despite anti-psychotic treatment were randomized (2:1) to receive daily, oral supplementation with 2 mg of folic acid and 400 μg of vitamin B12 (n=94) or placebo (n=46). DNA extracted from whole blood samples in 120 consenting partici-

pants was genotyped for four variants previously associated with negative symptom severity (FOLH1 484C>T, MTHFR 677C>T, MTR 1298G>A, and COMT 675G>A).

The researchers found that in the study cohort, baseline red blood cell folate levels correlated significantly with FOLH1 T and MTHFR T allele loads, despite equivalent dietary folate intake in the genotype groups. During validation of these results in a separate cohort of 89 healthy individuals, there was only a significant inverse relationship between red blood cell folate concentration at baseline and FOLH1 C allele load. The researchers found that improvements in patients' negative symptoms with supplementation were only significant when considering genotype. Specifically, only patients homozygous for the 484T allele, the high-functioning variant of folate hydrolase 1 (FOLH1), showed significantly greater benefit with active treatment. While the treatment effects seen when considering genotype were "modest," the authors say they could still be clinically meaningful.

"The present results have direct treatment implications not only for schizophrenia but also for folate-related interventions in other areas of medicine," write the authors, led by Joshua L. Roffman, M.D., from Massachusetts General Hospital in Boston. "Well-replicated associations of reduced folate and elevated homocysteine concentrations as risk factors for stroke, cardiovascular disorders, and dementia have been tempered by large, prospective studies that have failed to find a benefit of folate supplementation on disease progression. The current results suggest that individual differences in folate metabolism related to the presence of common functional genetic variants may have a bearing on treatment outcomes in these other disorders, as well as negative symptoms of schizophrenia."

Roffman tells *DTTR* that the findings provide "general support for the idea of personalized genotype-based interventions" but that the evidence in not yet sufficient to recommend routine genetic testing.

Blood Test May Uncover Subconcussive Brain Damage

A test being developed by Cleveland Clinic researchers can detect increased serum levels of the protein S100B indicating blood-brain barrier disruption (BBBD) in football players who experience head hits below the diagnostic threshold for a concussion. Additionally, according to the study published online March 6 in *PLOS One*, there is evidence supporting a potential link between elevated S100B levels and future risk for cognitive changes.

Sixty-seven volunteers from three college football teams were studied. None of the players experienced a concussion in the games analyzed. A review of filmed game footage and post-game interviews documented head hits. Complete season data were available for 15 players including blood samples drawn before and after five games. S100B serum levels and S100B auto-antibodies were measured with direct and reverse immunoassays. Multiple magnetic resonance imaging (MRI)-diffusion tensor imaging (DTI) scans and cognitive assessments were also performed in a subset of players.

The researchers found that nine players experienced an above BBBD threshold post-game increase in serum S100B. The ceiling for changes indicative of BBBD on this test, the authors say, is 0.12 ng/ml. Transient, increases in serum S100B (post-game compared to baseline) were detected only in players experiencing the greatest number of subconcussive head hits. Players receiving a maximum score of 6 on the Head Hit Index (HHI) had a significantly higher level of S100B (post-game compared to baseline) than players with an HHI of 0. There was no correlation between serum S100B elevation and

the number of body contacts or hits. Similarly, players who remained on the sideline had significantly lower measures of S100B than players who experienced head hits.

Auto-antibodies to S100B (an immune response triggered by BBBD) were also only elevated after repeated subconcussive events. Serum levels of S100B auto-antibodies predicted persistence of MRI-DTI abnormalities, which in turn, the authors report, correlated with cognitive changes.

"The clinical significance of elevation of S100B, and therefore of BBBD, measured in players who are not diagnosed with a concussion remains unclear. Our findings show that elevations in S100B correlated with the occurrence of head hits and their intensity," write the authors, led by Nicola Marchi, Ph.D., from the Cleveland Clinic in Ohio. "This suggests that BBBD follows repeated non-concussive episodes during a game. Thus, S100B may be useful to objectively quantify risk for subsequent pathological sequelae."

BRCA Preventive Service Designation to Benefit Myriad

In a clarification released last month, the U.S. Department of Health and Human Services, along with the U.S. Department of Labor and the U.S. Department of the Treasury, have designated BRCA testing as a preventive service under the Affordable Care Act. This designation allows for coverage of BRCA testing with no cost sharing for asymptomatic women at high risk for breast and ovarian cancer due to a qualified family history covered under all nongrandfathered private insurance plans.

There had been some initial confusion if the Affordable Care Act listed just the genetic screening or both the genetic counseling and the BRCA testing as preventive services. As such, many (nongrandfathered) insurance plans had only waived cost sharing for patient screening for the associated genetic counseling but not for the BRCA test itself. With this ambiguity removed, women who are members of nongrandfathered insurance plans and who are determined to be high-risk for mutations in breast and ovarian cancer susceptibility genes BRCA1 or BRCA2 by their health care providers will have no out-of-pocket costs, including copays, deductibles, and coinsurance, when ordering BRCA testing. This also applies to the increasing number of patients enrolled in high-deductible plans.

This designation is expected to have a positive financial impact on Myriad Genetics (Salt Lake City), the only laboratory offering BRCA analysis testing services.

"Given that patient out-of-pocket requirements (particularly for high-deductible plans) are a barrier to adoption in the asymptomatic market, it should help Myriad to continue gaining traction in this underpenetrated market," writes Amanda Murphy, an analyst at William Blair & Co., in a research note. "While it is difficult to put specific numbers around the opportunity we estimate that each 1 percent increase in asymptomatic market penetration (assuming 52 percent of the patients are covered by non-grandfathered plans) yields incremental revenue of just under $40 million."

This designation is in alignment with a draft guidance the U.S. Preventive Services Task Force (USPSTF) released in early April reaffirming its recommendation of genetic counseling and, if indicated, BRCA testing in asymptomatic women at increased risk of BRCA genetic mutations due to family history of breast or ovarian cancer.

Congress Considering Reauthorization of Newborn Screening

Lawmakers have introduced legislation designed to help states with their newborn screening (NBS) programs. The Newborn Screening Saves Lives Reauthorization Act (H.R. 1281) was introduced in U.S. House of Representatives on March 20 by Lucille Roybal-Allard (D-Calif.) and Mike Simpson (R-Idaho) to reauthorize critical federal activities that assist states in improving and expanding their NBS programs and ensuring laboratory quality and surveillance.

Prior to passage of the original 2008 NBS legislation, there was great state-by-state variation in the number and quality of public health NBS. Only 10 states and the District of Columbia require infants to be screened for all recommended core conditions. These low numbers prompted the March of Dimes (MOD) to lead a nationwide campaign to urge all states to adopt a full NBS panel to identify infants who may have genetic, metabolic, or hearing disorders that may not be apparent at birth. As a result of those efforts, 44 states and the District of Columbia now require screening of at least 29 of the 31 treatable core conditions.

The reauthorization calls for funding of newborn screening initiatives at $25.8 million per year for five years. Specifically, the bill's key provisions:

- Extend the Health Resources and Services Administration (HRSA) grants to states to expand their screening programs, educate parents and health care providers, and improve follow-up care for infants with a condition detected through NBS.
- Reauthorize the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC), which is scheduled to expire in April 2013. SACHDNC provides states with a Recommended Uniform Screening Panel. "Allowing the committee to expire would eliminate a well-established and scientific process for evaluating NBS and providing guidance to states," the MOD said in a statement.
- Continue HRSA's Clearinghouse for Newborn Screening Information and the National Newborn Screening and Genetic Resource Center.
- Reauthorize the U.S. Centers for Disease Control and Prevention (CDC) Newborn Screening Quality Assurance Program.
- Authorize a CDC grant program to provide technical assistance to state newborn screening programs to track outcomes of infants identified through NBS.
- Fund the National Institutes of Health Hunter Kelly Newborn Screening program, which finances research aimed at identifying new treatments for conditions detected through NBS and developing new screening technologies.

G2 Insider

Can Metal Ion Testing Predict a Higher Risk of Metal Hip Failure? . . . Two recent U.K. studies have found that elevated blood metal ion levels are an indicator of metal hip implant failure. However, the two studies disagree as to whether metal ion testing is appropriate as a screening test in asymptomatic patients. Blood and serum cobalt (Co) and chromium (Cr) concentrations are considered reliable indicators of wear rates following metal-on-metal (MOM) hip arthroplasty. But the exact relationship between metal ion testing results and outcomes is still debated.

According to a study published March 12 in *BMJ Open*, blood metal ion tests can be used as a clinical indicator of increased risk of joint failure in asymptomatic patients. The researchers followed 278 patients with "no pain" or "slight/occasional" pain for a mean of 70 months. The researchers found that elevated blood Co levels were associated with an increased likelihood of early joint failure and Co concentrations greater than 20 µg/l were frequently associated with adverse local tissue response. Male patients with low Co concentrations had a very low incidence of adverse tissue reactions.

"Ion concentrations, if low, can be reassuring to both patients and surgeons and can also allow rationalization of resources," write the authors, led by David Langton, MRCS, who reports that 40 patients with blood Co greater than 20 µg/l have undergone revisions with macroscopic metal staining of the local tissues detected in all patients and bone loss in 35. "In light of these findings, at our unit patients with grossly elevated metal ion concentrations are now offered revision surgery in the absence of symptoms."

Another group of researchers led by Shiraz Sabah, M.D., similarly found that blood metal ions from 907 patients were significantly elevated in failed hips. However, Sabah's group concluded that "blood metal ions had poor sensitivities and negative predictive values making them an inadequate screening test" and "discourage surgeons from performing revision surgery based on raised blood metal ions alone" in a presentation at the 2013 American Academy of Orthopaedic Surgeons Annual Meeting (March 19-23; Chicago). G2

Company References

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American Heart Association 214-570-5978

Aviir 949-398-6300

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Critical Diagnostics 877-700-1250

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