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New Trends, Applications, and IVD Industry Analysis

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Lead Testing Under-Used; Geographic Variability in Elevated Levels Can Target Testing

While the water crisis in Flint, Mich. dominated headlines at the beginning of the year, that city is not alone in concerns over lead harming young children. Over the past four decades, blood lead concentrations among U.S. children have declined due to the elimination of lead from gasoline, paints, and other consumer products. Yet, there are worrisome population pockets of young children with elevated blood levels of lead, particularly among those living in poverty in older homes.

New data shows a significant lead problem is potentially harming millions of U.S. children and that blood lead screening is under-used. As was the case in Flint, lead can leach into drinking water from pipes—a problem similarly discovered recently in Mississippi, Ohio, New Jersey, and Oregon schools. Lead can also be ingested through old houses' remaining lead paint, as well as from contaminated soil and dust. A blood lead test is a cheap and reliable way to identify a lead-poisoned child.

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Genotyping Reveals Relevant, Incidental Pharmacogenomic Findings

Clinically relevant pharmacogenomic findings are seen in single nucleotide polymorphism (SNP) genotyping and exome sequencing data, according to a study published in *Genetics in Medicine*. These findings suggest the need to refine strategies for reporting pharmacogenomics incidental findings as a means to improve patient care and to further personalize treatment.

The American College of Medical Genetics and Genomics (ACMG) has focused on disease-associated genes, but pharmacogenetic incidental findings can be clinically actionable and hold potentially significant medical benefit given the possibility of life-threatening adverse drug reactions or therapeutic inefficacy.

Pharmacogenetic analysis was performed on a research basis for individuals participating in the National Institutes of Health (NIH) Undiagnosed Diseases Program (UDP) between 2009 and 2014. SNP chip analysis was performed on 1,101 individuals (from 308 families, including 355 affected

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Preferred Sample Guidance

The U.S. Centers for Disease Control and Prevention (CDC) say there is no safe blood lead level in children, and research has identified a blood lead level of 5 µg/dL or more as a threshold to trigger the need for clinical and public health interventions. Children are most at-risk for lead-related complications, including lower IQ, in early childhood.

In 1991, the CDC recommended universal blood lead testing for children. Because of wide variation in lead exposure, in 2005 the American Academy of Pediatrics (AAP) recommended that states and cities develop their own lead screening strategy based on local data. The AAP, like the CDC, recommended universal, blood-based, lead screening of children living in communities with more than 27 percent of housing built before 1950 or a prevalence of blood lead concentrations 10 µg/dL or higher in children 12 to 36 months old of 12 percent or greater.

"In the primary care office, primary prevention begins with education and counseling."
—Council on Environmental Health, AAP

AAP's Environmental Health Council published a policy statement on the prevention of childhood lead toxicity in the June issue of *Pediatrics*. The paper, written by lead author Bruce Perrin Lanphear, M.D. makes several new recommendations regarding testing asymptomatic children for elevated blood lead concentrations.

AAP calls on the Centers for Medicare & Medicaid Services to "expeditiously revise" current regulations for allowable laboratory error permitted in blood lead proficiency testing programs from ±4 µg/dL to ±2 µg/dL for blood lead concentrations of 20 µg/dL or less. AAP says that this range of error can result in children's blood lead levels being misclassified.

Clinically, AAP says pediatricians are in a unique position to work with public health officials to improve testing adherence and refer for environmental assessments of older housing. Some specific clinical testing recommendations include:

- Testing asymptomatic children for elevated blood lead concentrations according to federal, local, and state requirements and testing immigrant, refugee, and internationally adopted children when they arrive in the United States because of their increased risk.
- Working with public health officials to conduct surveys of blood lead concentrations among a randomly selected, representative sample of children in their states or communities at regular intervals to identify trends in blood lead concentrations.
- Monitoring children who have blood lead concentrations 5 µg/dL or higher until environmental investigations and remediation are complete and blood lead concentrations decline.
- Screening children for iron deficiency and insufficient dietary calcium intake.

"In the primary care office, primary prevention begins with education and counseling," writes the AAP's Council on Environmental Health. "Blood lead surveillance data can be used to identify cities, communities, or housing units at higher than typical risk for lead poisoning. Technologies using geographic information system-based

analyses and surveillance from electronic medical records are important tools to identify at-risk children who should have their blood lead concentration measured."

"These alarming findings show that while our nation has made progress in addressing lead exposure, our public health successes are neither complete nor demographically consistent."

— Harvey Kaufman, senior medical director, Quest Diagnostics

Evidence of Under-Testing

In addition to the CDC's call for universal testing, blood lead tests are mandated for all children in 11 U.S. states (Alabama, Connecticut, Delaware, Iowa, Louisiana, Maryland, Massachusetts, New Jersey, New York, Rhode Island, Vermont) and Washington, D.C. Additionally, Medicaid requires that enrolled children be tested for lead toxicity at ages one and two years as part of its Early and Periodic Screening, Diagnostic and Treatment) benefit. Yet, new evidence shows that testing is inconsistently performed.

On June 9, Reuters published the results of their investigation showing that millions of children are falling through the lead testing safety net, leaving them vulnerable to poisoning, beyond the extent seen in Flint. The news agency reviewed data from state health departments, the Centers for Medicare and Medicaid Services, and the CDC. The Reuters investigation found that:

- Only 41 percent of Medicaid-enrolled one- and two-year-olds had been tested, as required.
- In some states requiring tests, more than half the children were not tested.
- Medicaid claims data showed wide variability in screening. In California, Medicaid paid for enough lead tests to cover just one in three enrollees in 2014, whereas Massachusetts screens around 80 percent of children.
- Utah, Kansas, and Alaska report not recognizing or following a federal requirement to test Medicaid children.

Some of the reasons cited for under-testing include; Some doctors don't order the tests or are unaware of the rules, parents don't follow up on test referrals, and Medicaid and health departments do little to enforce testing requirements. Additionally, data is often incomplete and surveillance funding and data collection have been cut over the years, so the true scope of the lead exposure problem may not be adequately captured.

Scope of the Lead Problem

Quest Diagnostics (Madison, N.J.) published findings from its Health Trends study online June 10 in the *Journal of Pediatrics*, in what is believed to be the largest analysis of lead blood level test results in children in the United States.

The study found that based on more than 5 million venous blood lead level results (May 2009 to April 2015), approximately 3.0 percent of children nationally have high blood lead levels (at or above 5 µg/dL). There were significant differences in high blood lead levels based on sex, pre-1950s housing construction quintiles, and poverty income ratio. Health and Human Services regions, states, and 3-digit ZIP code areas also showed "drastically" different frequencies of high blood lead levels and very high blood lead levels (above 10 µg/dL).

Reuters' analysis similarly found wide geographic variances. For example, across Pennsylvania, 9.4 percent of children tested in 2014 had levels above the 5 µg/dL threshold, while in Cleveland, 13.7 percent of children tested had lead levels above

the threshold. For comparison, following the Flint water contamination, 4.9 percent of children exceeded the threshold.

"These alarming findings show that while our nation has made progress in addressing lead exposure, our public health successes are neither complete nor demographically consistent," said Harvey Kaufman, senior medical director, Quest Diagnostics and a study author. "We have a long way to go, both in terms of contaminated water and residual lead-based paint, to reduce disparities that put some of our children at disproportionate risk of exposure to lead."

Takeaway: Low levels of lead toxicity screening compliance may be masking a large public health problem of lead exposure in U.S. children.

Criteria-Based Ordering Improves Vitamin D Testing

Implementing a criteria-based restriction on laboratory requisitions can sharply cut vitamin D testing, according to a study published online May 23 in *JAMA Internal Medicine*. The authors say that this strategy improves the clinical utility of testing without missing clinically relevant conditions.

"Criteria-based approaches to the implementation of the Choosing Wisely recommendations have been examined in practice for other tests, including the commonly ordered tests for rheumatoid factor antibody and anti-nuclear antibodies, as well as advanced imaging."

— Robert Ferrari, M.D.

Laboratories in Alberta, Canada noted excessive test volume for vitamin D assays, so in 2013 laboratory services decision-makers used feedback from endocrinology specialists in the province and Choosing Wisely guidelines to identify when a 25-hydroxy vitamin D assay was likely to be most clinically useful. This resulted in the development of a new laboratory services requisition form that required physicians to identify the medically necessary indication for testing vitamin D levels (metabolic bone disease, abnormal blood calcium level, malabsorption syndrome [including celiac disease, small-intestinal surgery, anticonvulsant agents], chronic renal disease, and liver disease). Other indications did not qualify for testing.

The researchers found that in the first nine months of use of the new requisition form (from April 1 to Dec. 31, 2015), 20,609 vitamin D tests were ordered. Historical data would have predicted that 256,027 tests would have been ordered during this time frame. Thus, the intervention led to a 92.0 percent reduction in the number of vitamin D tests ordered, translating to a savings of $3 million for the study period.

"Criteria-based approaches to the implementation of the Choosing Wisely recommendations have been examined in practice for other tests, including the commonly ordered tests for rheumatoid factor antibody and anti-nuclear antibodies, as well as advanced imaging," writes co-author Robert Ferrari, M.D., from University of Alberta, Edmonton. "Using a criteria-based approach to test ordering not only reduces the number of tests that would be ordered but it does so without missing clinically relevant conditions."

Takeaway: Criteria-based ordering can help improve the medical necessity of Vitamin D test orders, but is an applicable strategy for other over-ordered tests as well.

Inside The Diagnostics Industry

Rheonix Sees Automation as Key to Expanding Molecular Testing

Gregory Galvin, Ph.D., CEO, Rheonix

Richard Montagna, Ph.D., SVP, scientific and clinical affairs, Rheonix

The molecular firm Rheonix (Ithaca, N.Y.) developed a microfluidic platform capable of fully automating multiplex molecular testing—from raw sample to result—at a lower cost. One novel feature of the platform is the Rheonix CARD (Chemistry And Reagent Device) that fully automates sample preparation for molecular testing for a wide array of diagnostic applications including next-generation sequencing (NGS).

Rheonix believes the platform's small size, affordability, and flexibility will enable the expansion of molecular testing applications both in the clinical realm, as well as in the food and beverage industry. *DTET* recently spoke to Gregory Galvin, Ph.D., Rheonix's CEO, as well as Richard Montagna, Ph.D., senior vice president of scientific and clinical affairs, regarding the company's technology and its commercial launch.

How can Rheonix's platform expand the applications and settings for molecular diagnostics (MDx)?

Galvin: Historically the issue with MDx assays is that they are complicated and require highly skilled labor and a room full of different instruments. That complexity and high labor requirement have impeded the rollout of molecular assays for a really broad spectrum of applications, for which they are ideally suited. Rheonix is focusing on creating a completely automated platform that allows, from the user's perspective, to take the complexity out. The complexity is there in what actually transpires, but users are not involved in it, so you can disperse MDx into a much broader arena, more economically, than has been possible.

At the 40,000-foot level, Rheonix is not primarily in the business of creating biochemistry or genetics or science. We provide the platform that allows a very wide variety of genetic-based science to be delivered in an economic manner.

In what areas is Rheonix planning on applying its technology?

Montagna: We think there is potential in a lot of different areas. Obviously there is the clinical diagnostics market. In addition, there are food and beverage applications. If you look at the food and beverage market, up until recently, and even now, most testing is relying on 200-year-old methods. Sub-cultures take a long time and do not deliver the most sensitive results. Now with molecular opportunities you can get answers much more quickly and with much greater accuracy.

A third arena would be NGS, which yields a tremendous amount of data, but the issue is again, complexity. The actual sequencing is very automated, but on either side of it you still have complexity—the upfront sample preparation, to prepare the DNA to analyze it and the backend, bioinformatics analysis of the data. Rheonix is not tackling the backend, but upfront we know we can use the automated capabilities of our system to prepare the DNA libraries for NGS.

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Automated Sample
The Encompass Optimum workstation can take up to 24 samples of blood, saliva, urine, or formalin-fixed, paraffin-embedded tissue and automatically lyse, extract, and purify DNA and RNA for analysis. Total sample preparation and load in requires less than five minutes.

We are in the middle of a project right now with New York Center of Excellence at University of Buffalo. Their library preparation methods typically take, using multiple pieces of equipment, about a day and a half. Once they get the raw sample it takes a day and a half to get everything ready before they can begin the sequencing. We have been able to reduce that day and a half to 146 minutes. It is truly automated. You put the sample in and hit a button. The sophistication is still there, but it is handled by an instrument. The user can basically go get a cup of coffee while this is happening. That allows laboratories to decrease the cost of the labor. Highly skilled people are not needed because the instrument is capable of taking complex steps and automating it without intervention on the part of the user.

With personalized medicine there are a number of gene sequences known to predict whether an individual will respond to a particular therapy. Rheonix plays a role by providing the opportunity to reduce the cost and the labor to generate the sample.

Rheonix is simultaneously pursuing the clinical MDx market, as well as the food and beverage market. Can you explain the strategy?

Galvin: My experience with technology companies is that companies want to pursue as many different things as the technology allows them. For the business to be successful, you have to contain that spread because you cannot do everything. The Rheonix platform is extremely flexible. So one of our business challenges is to focus on those areas we know we can rapidly deploy a solution, since we can't do them all simultaneously. We evolved to the three-pronged product/market strategy.

First is the clinical in vitro diagnostics (IVD), which ultimately is the largest value and largest dollar market, but it is also the longest to market. Second, is the laboratory developed test (LDT) market, which is still a clinical market, but somewhat of a lower barrier to entry. Third is the applied markets, the non-clinical markets. We were surprised by the inbound interest for customers in this space. There is a lot of interest in the food and beverage world in molecular testing because of the sensitivity and specificity of the tests. But, the industry historically could not afford the labor, nor did they have access to the highly skilled staff and complex laboratories needed for molecular testing. For us this is an opportunistic, underserved market that allows us to get product into nonregulated market very quickly. Our solution is extremely simple to use and addresses the key pain points in this market. So, our strategy is partially technology driven and partially financially driven—to generate revenue sooner rather than later.

The LDT market is one of the core areas the company is pursuing. Can you talk about how Rheonix is preparing for the possibility of the U.S. Food and Drug Administration's (FDA's) expanded regulation of LDTs?

Montagna: Our philosophy is to be very open with the FDA. We have purposely engaged FDA early and have shown the agency our intended approach. If the guidance documents are finalized in their current format, the FDA does not want to see companies

Inside The Diagnostics Industry

working with CLIA laboratories to help them develop tests, which due to our DNA capture probe technology we would have to do. If you want to target ABCD, you get a specific probe and you would have to contract with us to put that probe on the array. We have come up with a novel approach that we call the universal CARD that allows the end-user to modify and functionalize the array for their own purposes. We have proven we can do this in a lot of different arenas and showed that to the FDA. As part of a presubmission meeting, we essentially reached an agreement with them for how we could proceed to get the platform approved for our own products and for laboratories that develop their own LDTs, which we call user-defined assays, to stay away from the hot button of LDT.

Galvin: Because the platform is so simple to use the food and beverage sector also can develop their own assay. It is very easy to functionalize the CARD automatically. The beauty of the system is that if you put a raw sample in and think about what has to happen, the cells have to be lysed, the DNA or RNA extracted, purified, amplified, and detected. While that is happening, simultaneously the CARD is automatically functionalized. The user doesn't lose any time. They can amplify the targets of interest and when they want to detect them, by the time the amplicons make their way to the array the probes will already be there.

Can you speak of the current development status along your product lines?

Galvin: In food and beverage the first assay to be commercially released is a beer spoilage organism assay that will be introduced this August. The LDT or user defined assay is presently in beta testing at a couple of different sites, so that will be the next to roll out. And in the traditional clinical IVD segment, products in sexually transmitted infections are entering clinical trials and will need to be submitted for 510k approval. So, that is furthest away, but they are all moving forward in parallel.

In addition to STI, you are also working on HIV and Zika testing. How does the company evaluate which assays to pursue?

Galvin: It is very much a subject of internal discussion with some guidance from outside consultants. It really is the intersection of the business opportunity and the technology. Some of the questions we ask are can we do it? Will it be near or far to enter the market? Is the market sufficient? Is it a fit with the volume and throughput of our platform? Is reimbursement appropriate? Who is competing in the space?

As an industry, how will molecular testing evolve in the next several years?

Galvin: We are excited about where molecular testing is going. Fundamentally, from a scientific basis, the idea that you can look at a gene sequence and know very accurately the diagnosis of a pathogen or the susceptibility to a disease or medical condition or which medicines will be effective is all good for patient care. We are just at the tip of the iceberg of the science and are bringing the business and the economics of it in line with the technical opportunities. The changes in economics will drive new markets.

Rheonix By-the-Numbers

- 7 publications in scientific journals
- 24 samples processed simultaneous with the automated Encompass workstations and CARD technology
- 51 U.S. issued patents, plus 16 pending U.S. patent applications
- 70 employees
- 2008 year founded

Genotyping Reveals Relevant, Incidental Pharmacogenomic Findings, *Continued from bottom of p.1*

individuals), while a subset of these participants underwent exome sequencing (645 individuals, including 182 affected, from 158 families).

The Pharmacogenomics Knowledgebase (PharmGKB; 868 pharmacogenetic loci) was used to identify incidental findings based on its listing of variant–drug associations. Combined, the SNP chips and exome sequencing provided coverage of 65 percent of the SNPs in the PharmGKB database and 81 percent of the PharmGKB 1A and 1B SNPs (top two levels of variants based on evidence).

The researchers found that SNP chip sequence data identified 395 sequence variants, including 19 PharmGKB 1A and 1B variants, while exome sequencing data uncovered 388 variants, including 21 PharmGKB 1A and 1B variants. No participants had been prescribed a medication associated with a PharmGKB 1A or 1B category variant they carried. However, five pharmacogenomically relevant incidental variants were identified in nine individuals, although these associations ranked lower in PharmGKB—category 2 or category 3 associations. Yet, these variants altered efficacy of a prescribed medication. Three of the variants were detectable with both the SNP chip and exome sequencing technology and two were detectable only by exome sequencing.

"Despite the small size of the NIH UDP patient cohort, we identified pharmacogenetic incidental findings potentially useful for guiding therapy," writes co-author Murat Sincan, M.D., from the NIH's UDP (Bethesda, Md.). "Consequently, groups conducting clinical genomic studies might consider reporting of pharmacogenetic incidental findings."

In the present study, the choice to report an incidental variant to the participant was made by the clinical team, which elected not to return PharmGKB category 2 or 3 variants. Several reasons were cited.

"The study consent reflected routine practice from the early days of the application of genome-scale sequencing to medical diagnostics—only DNA variants that might contribute to the test indication were to be returned." "Second," the authors write, "current ACMG guidelines do not include such variants among those recommended to be returned. The UDP recognizes that these are areas of intense debate in the literature and elsewhere; the program is prepared to adjust its practice as the standard of care evolves."

Takeaway: Despite a current focus to date on disease-associated, genetic incidental findings, pharmacogenomic incidental findings may be detectable using SNP chip or exome sequencing technology and may be clinically relevant.

Matched Tumor, Germline Sequencing Yields Actionable Pharmacogenetic Variants

Matched tumor-germline sequencing can provide additional information relevant to the patient's treatment, including identification of actionable pharmacogenomics variants, according to an abstract presented at the 2016 American Society of Clinical Oncology annual meeting (Chicago; June 2-6).

Tumor and germline exome sequencing occurred in 125 pediatric patients with refractory tumors enrolled in the Michigan Pediatric Oncology Sequencing study. Nonsynonymous germline variants (TPMT, DPYD, CYP2C19, CYP2C9, G6PD, and CYP3A5) were assessed for discovery of clinically actionable variants based on Clinical Pharmacogenetics Implementation Consortium guidelines.

The University of Michigan (Ann Arbor) researchers identified clinically actionable variants in 17 of 125 patients (14%). The majority was in TPMT, which informs 6-mercaptopurine (6-MP) dosing, while one DPYD variant that increases the risk of severe myelotoxicity to fluoruracil (5-FU) treatment was discovered.

"Future work will assess whether patients carrying these variants received the relevant drug, and if so, whether they experienced toxicity as predicted, and estimate the potential cost-benefit of integrating pharmacogenetic analysis into tumor genetic profiling programs," writes lead author Daniel Louis Hertz, Pharm.D., Ph.D.

Evidence Emerging for More Use Cases for High-Sensitivity Troponin

In recent years there has been mounting interest in the clinical use of circulating biomarkers for prediction of risk and diagnosis of cardiovascular diseases. High-sensitivity cardiac troponin (hs-cTn) is one of these biomarkers. To date, the U.S. Food and Drug Administration (FDA) has not approved an hs-cTn assay for use in the United States, but experts predict these tests (currently used in Europe) could be approved in the United States within the next year.

"Use of hsTn for rapid triage of patients presenting to the emergency department with chest pain is an application likely to be embraced by practitioners."

— David Morrow, M.D.

Two new studies published in *JAMA Cardiology* show how new, high-sensitive assays can speed the time to rule in or rule out diagnosis of an acute myocardial infarction (AMI) in patients presenting with chest pain and how the utility of this marker can be extended to evaluate risk of coronary heart disease (CHD) or heart failure (HF).

For Diagnosis of Myocardial Infarction

Patients with a possible AMI can be triaged within one hour after admission with no additional risk compared to the standard three-hour approach used in Europe, according to a study published online June 1 in *JAMA Cardiology*. The authors say that using a "low and sensitive" cutoff for hs-cTnI enables an accelerated diagnostic protocol resulting in either a safe discharge or more rapid treatment initiation.

"Use of hsTn for rapid triage of patients presenting to the emergency department with chest pain is an application likely to be embraced by practitioners," writes David Morrow, M.D., from Brigham and Women's Hospital (Boston) in an accompanying editorial. "Despite this low pretest probability, physicians are obligated to exclude myocardial ischemia with a high degree of probability and engage in time-consuming and costly testing strategies to do so. To manage costs and the adverse effects of overcrowding in the emergency department, it is a high priority to rapidly and safely identify patients with a sufficiently low probability for acute coronary syndrome (less than 0.5 to 1.0 percent) so that they can be discharged efficiently and avoid unnecessary testing."

The German-based researchers tested a one-hour algorithm to diagnose AMI using an hs-cTnI assay (ARCHITECT i2000SR; Abbott Diagnostics). The Biomarkers in Acute Cardiac Care study prospectively applied the assay for the diagnosis of AMI in 1,040 patients (median age, 65 years; 64.7 percent male) presenting to the emergency department at the University Medical Center Hamburg-Eppendorf with acute chest pain from July 19, 2013 to Dec. 31, 2014. A one-hour diagnostic algorithm was compared to the standard three-hour algorithm (currently included in the European Society of Cardiology guidelines) for diagnostic accuracy of the lower cutoff versus the recommended 99th percentile. Follow-up mortality was also evaluated (median follow-up time, 313 days). The one-hour diagnostic algorithm was validated in two additional independent cohorts totaling 4,009 patients.

The researchers found that the best performing cutoff value for the hs-cTnI assay was 6 ng/L. This cutoff was lower than the routinely used 99th percentile. With the application of the lower troponin I cutoff value of 6 ng/L, the rule-out algorithm showed a high negative predictive value of 99.8 percent (95% CI, 98.6%-100.0%) after 1 hour for non–ST-segment elevation MI type 1. The one-hour approach was

comparable to a three-hour approach. Similarly, a rule-in algorithm based on troponin I levels provided a high positive predictive value with 82.8 percent. The lower cutoff also resulted in lower follow-up mortality (1.0 percent) versus the use of the 99th percentile (3.7 percent) for this assay.

"Currently, it seems irrefutable that biomarkers can predict risk in population-based cohorts."

— James Januzzi Jr., M.D.

Long-Term Changes in hs-cTnT Predict Poor Outcomes

Increases in hs-cTnT levels over a six-year timeframe are tied to incident CHD, death, and, HF, according to a study published online June 8 in *JAMA Cardiology*. Serial measurement of hs-cTnT adds clinically relevant information to baseline testing and may be useful in targeting prevention strategies to high-risk individuals, especially among persons with stage A or B HF, the authors say.

Previous studies established that single measurements of hs-cTnT are independently tied to adverse cardiovascular outcomes in those with disease, but in the present study the researchers assessed changes in hs-cTnT in asymptomatic, middle-aged adults participating in the multi-site Atherosclerosis Risk in Communities Study. Hs-cTnT levels were measured twice (six years apart) in 8,838 participants (mean age, 56 years; 59.0 percent female; 21.4 percent black), who were initially free of CHD and HF between Jan. 1, 1990 to Dec. 31, 2011.

The researchers found that incident, detectable hs-cTnT (baseline, less than 0.005 ng/mL; follow-up, 0.005 ng/mL or greater) was independently associated with subsequent CHD, HF, and death, compared to an hs-cTnT level less than 0.005 ng/mL at both visits. Among individuals with the most marked hs-cTnT increases (baseline, less than 0.005 ng/mL; follow-up, 0.014 ng/mL or greater) hazard ratios were as high as 4 for CHD and death and 8 for HF. On the other hand, risk for subsequent outcomes was lower among those with relative hs-cTnT reductions greater than 50 percent from baseline.

"Currently, it seems irrefutable that biomarkers can predict risk in population-based cohorts," writes James Januzzi Jr., M.D., from Massachusetts General Hospital (Boston) in an accompanying editorial. "What is needed now are efforts toward developing strategies to upwardly bend the survival curves of those with a biomarker signature of risk, leveraging the knowledge gained."

Takeaway: As evidence mounts for the ability of hs-cTn to more rapidly diagnose AMI and better predict risk of cardiovascular disease and death, experts expect hs-cTn assays to soon be approved in the United States.

Novel HCV Antigen Test Allows 1-Step Screening, Diagnosis

A novel hepatitis C virus antigens enzyme immunoassay (HCV-Ags EIA) can reliably serve as a one-step test to screen and diagnose active or viremic HCV (V-HCV) infection, according to a study published online June 6 in *Hepatology*. The highly specific and sensitive test relies on simultaneous detection of four HCV proteins and non-denaturation of serum samples to achieve results comparable to HCV RNA reverse transcription-polymerase chain reaction (RT-PCR) results in differentiating V-HCV infection from resolved HCV (R-HCV) infection.

While the latest generation of anti-HCV tests are highly sensitive and specific, they are relegated to screening tests as they remain incapable of diagnosing acute HCV infection and differentiating between V-HCV and R-HCV infection. The gold standard for HCV diagnosis remains HCV RNA RT-PCR, which is costly and requires specialized equipment not available in low-resource settings. The availability of new treatment options are increasing the need for HCV screening and diagnosis to link these individuals to appropriate care. HCV core antigen (HCVcAg) has been considered a serologic marker of viral replication and holds promise for one-step diagnosis of HCV infection, but HCVcAg-based tests have been plagued by low sensitivity and specificity.

"For the first time, we demonstrated that serum sample denaturation is the main reason for the low specificity of HCV-Ags related tests, including current HCVcAg assays, and results in failure to differentiate V-HCV infection with R-HCV infection."

— Ke-Qin Hu, M.D.

To develop the HCV-Ags EIA the researchers first assessed the expression and detectability of the HCV non-structural proteins, other than HCVcAg in HCV-infected serum specimens (HCV NS3, NS4b and NS5a). These HCV proteins are expressed in all six HCV genotypes. For comparison, anti-HCV test was performed using the Architect Anti-HCV Assay, a chemiluminescent microparticle immunoassay (Abbott Laboratories) and serum HCV RNA was quantitated by real-time RT-PCR using the Roche COBAS AmpliPrep/COBAS TaqMan HCV assay.

The University of California Irvine researchers found their novel HCV-Ags EIA has high specificity and sensitivity for detection of V-HCV infection. Furthermore, sample non-denaturation is needed to guarantee specificity high enough to diagnose V-HCV. In 189 cases with V-HCV infection by all six different genotypes, three with acute HCV infection, and 186 with chronic V-HCV infection, there was 100 percent positivity concordance between the results of HCV-Ags EIA and HCV RNA RT-PCR. Additionally, the HCV-Ags EIA could diagnose acute HCV infection 59 days to 65 days before anti-HCV appearance.

"For the first time, we demonstrated that serum sample denaturation is the main reason for the low specificity of HCV-Ags related tests, including current HCVcAg assays, and results in failure to differentiate V-HCV infection with R-HCV infection. As most reported HCVcAg assays employ pre-test serum sample denaturation to increase the test sensitivity, these current HCVcAg tests cannot be used for one-step diagnosis of V-HCV infection," writes co-author Ke-Qin Hu, M.D. "Our data support further clinical development of this novel HCV-Ags EIA assay as a cost-effective and convenient one-step alternative clinical assay for HCV screening and diagnosis to replace the current two-step approach."

Hu tells *DTET* that the technology has been exclusively licensed to DiligenMed (Irvine, Calif.). He expects that following an additional clinical trial, and once the test subsequently becomes available, it can replace the current two-step approach to hepatitis C screening and diagnosis—promoting universal screening and diagnosis of HCV infection.

Takeaway: Simultaneous detection of four HCV-Ags in a novel EIA that uses non-denaturation of serum samples may eliminate the need for two-step testing for HCV.

G2 INSIDER

C. Diff Screening at Admission Can Cut Hospital-Acquired Infection

Detecting and isolating Clostridium difficile (C. diff) carriers upon admission to the hospital can a significantly decrease the incidence of health care-associated C. diff infection (HA-CDI), according to a study published in the June issue of *JAMA Internal Medicine*. The authors say C. diff screening upon admission is an easily implemented and effective strategy for preventing HA-CDI. It is estimated that there are half a million cases of C. diff in the United States annually, causing 29,000 deaths. But, current control measures do not target asymptomatic carriers, despite evidence that they can contaminate the hospital environment and cause health care-associated infection.

The Québec Heart and Lung Institute had endemic C. diff infection, despite "significant" efforts to control it. So, in 2013 the facility adopted a policy to detect and isolate asymptomatic carriers. The present study reports the effects of implementation. The intervention of screening asymptomatic patients admitted through the emergency department occurred Nov. 19, 2013, through March 7, 2015. Identified carriers were placed under infection control measures similar to those for C. diff infection, but tailored to minimize the effect on bed management and work flow. Admission screening was conducted to test for the tcdB gene using polymerase chain reaction on a rectal swab (BD GeneOhm Cdiff Assay; BD Diagnostics). A pre-intervention control period was defined as an epidemic period (Aug. 22, 2004 to July 21, 2007) and a post-epidemic period (July 22, 2007 to Nov. 18, 2013).

The researchers found that 92.5 percent of 8,218 eligible patients were screened. Of those screened, 4.8 percent (n=368) were identified as C. diff carriers. During the intervention period significantly fewer patients developed an HA-CDI—38 patients during the intervention (3.0 per 10.000 patient-days) versus 416 patients (6.9 per 10.000 patient-days) during the post-epidemic, pre-intervention period. The change in the level of HA-CDIs significantly decreased overall, through a gradual progressive decrease over time (7 percent per 4-week period). The researchers estimated that the intervention prevented 63 of the 101 expected HA-CDI cases (62.4 percent). In total, 121 patients needed to be screened and six asymptomatic carriers needed to be isolated to prevent one HA-CDI. Preliminary data shows this strategy may be cost-effective. The intervention cost $130,000 over 17 periods and prevented approximately 63 cases. Each HA-CDI case costs between $3,427 and $9,960, so the intervention's presumable savings ($216,000 to $627,000) were greater than the costs of the intervention.

"Prevalence of carriage on admission was similar to that in other investigations, suggesting that our screening strategy was adequate to identify carriers," writes lead author Yves Longtin, M.D., from Jewish General Hospital in Canada. "To date, no commercial test is approved in Canada or the United States to detect asymptomatic carriers, and the optimal detection method is unknown."

Company References

Abbott Diagnostics
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American Academy of Pediatrics
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American College of Medical Genetics and Genomics
301-718-9603

BD Diagnostics
410-316-4000

Centers for Disease Control and Prevention
404-639-3311

DiligenMed
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