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NGS Poised to Reshape IVF With Preimplantation Genetic Screening

Next-generation sequencing (NGS) may improve the feasibility of preimplantation genetic screening (PGS) of embryos during the in vitro fertilization (IVF) process, according to several studies presented at annual American Society for Reproductive Medicine (ASRM) meeting (Honolulu; Oct. 18-22). Employing NGS for screening embryos before implantation, experts say, increases the chances of successful implantation, cuts miscarriage rates, and could ultimately cut the number of multiple births associated with IVF.

To date, PGS has not been widely adopted, in large part because of the high cost associated with traditional genetic approaches. Preimplantation genetic screening falls into three main categories: specific, single-gene disorders (like cystic fibrosis); structural chromosomal rearrangements and translocations; and aneuploidies. Microarrays have been employed for detection of targeted mutations and aneuploidies with some success. Quantitative polymerase chain reaction has proven faster but still requires a search for a specific erroneous

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Lawrence Livermore Supersizes the Microarray

Developers assess many considerations when making the determination of the size of a multiplex panel: comprehensiveness versus actionability, efficiency versus sensitivity. While targeted, moderately sized solutions are likely ideal for most common conditions, researchers at Lawrence Livermore National Laboratory (California) are developing a super assay for global disease surveillance and for instances when there is not a suspected pathogen. The Lawrence Livermore Microbial Detection Array (LLMDA) is capable of detecting virtually any microbe that has been sequenced, with results in 24 hours.

The researchers see configuration of the assay as possible for a wide range of clinical applications. It has demonstrated success, for instance, in detecting bacterial pathogens in the wounds of U.S. soldiers. The LLMDA was able to detect at least one bacterial pathogen in roughly one-third of wound samples in which no bacteria were detected using the standard culture method, according to a study published in the July issue of the *Journal of Clinical Microbiology*. One of the study's key findings, the researchers say, is that the assay detected bacteria,

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▲ **Lawrence Livermore Supersizes the Microarray,** *from page 1*

commonly seen with hospital-related infections, which were associated with wounds that did not heal successfully.

The technology combines bioinformatics with a microarray that in its current iteration has probes for about 8,100 microorganisms, including 3,855 bacteria and 3,856 viruses (as well as fungal targets). The researchers say several microarray configurations are possible on a single slide, including a single square array containing all 360,000 probes, four arrays of 72,000 probes each, or for human clinical use, 12 arrays on one slide, each with 135,000 probes.

Compared to polymerase chain reaction and next-generation sequencing, LLMDA is midrange in cost, processing time, and sensitivity, the Livermore researchers say. Crystal Jaing, Ph.D., a group leader in applied genomics and part of the LLMDA team, acknowledges that while the group believes the array could be applied clinically in cases of burns with large surface areas, people injured by trauma, or people with diabetic ulcers, there are reimbursement and regulatory hurdles given the lack of a precedent for such a comprehensive test.

Jaing tells *DTET* that right now the turnaround takes 24 hours, which is still "reasonable" for clinical use, but can be shortened by adding automation to the test. Future iterations of the assay could also incorporate databases containing virulence and resistance profiles of pathogens, which would provide "very rich information."

Takeaway: The LLMDA is a supersized microarray capable of detecting virtually any microbe that has been sequenced. Its developers are finding it to have clinical utility but acknowledge potential regulatory and reimbursement hurdles in the near term.

CDC Develops Multiplex Hepatitis Assay

The U.S. Centers for Disease Control and Prevention (CDC) has developed a multiplex polymerase chain reaction (PCR) assay capable of simultaneously detecting all five known hepatitis viruses, according to an abstract presented at the annual meeting of the American Association for the Study of Liver Diseases (Nov. 7-11, Boston).

While the five viral hepatitides are clinically indistinguishable, various testing algorithms are employed to determine their etiology. Nucleic acid testing (NAT) remains the gold standard for diagnosis of active and viremic stages of infection, the CDC says. The CDC researchers designed standardized TaqMan (Life Technologies) assays for simultaneous detection of RNA for hepatitis A, C, D, and E viruses (HAV, HCV, HDV, and HEV) and the DNA for hepatitis B virus (HBV). After individual PCR assays for each virus were optimized to run under identical experimental conditions, the assays' performances were evaluated on TaqMan Array Cards (TAC) to detect the five viral genomes simultaneously.

"The hepatitis TAC assay has great potential for simplifying laboratory testing of viral hepatitides," Maja Kodani, Ph.D., an associate service fellow in CDC's Division of Viral Hepatitis, tells *DTET*. "It was not developed to replace current hepatitis testing algorithms; instead, it was developed to address a need for rapid testing in situations where traditional testing may be challenging and expensive."

Based on 329 clinical specimens, the TAC assay was able to identify all positive samples for HCV, HDV, and HEV, yielding 100 percent sensitivity for those strains. The TAC assay positively identified 43 of 46 HAV-NAT positive samples (sensitivity, 94 percent) and 36 of 39 HBV-NAT positive samples (sensitivity 92 percent). No false positives were detected for HBV, HCV, HDV, or HEV in NAT-negative samples (specificity 100 percent), while for HAV-NAT positive samples, the TAC assay' specificity was 93 percent.

While the group says they are working to further optimize and validate the assay before it is transferred to public health laboratories for routine use, they believe there are additional opportunities to expand the utility of the panel beyond global health and disease surveillance. Kodani says, for example, future work will add more pathogens such as HIV and other sexually transmitted diseases to make the panel useful for donor blood and organ testing, as well as for prenatal testing.

Takeaway: A multiplex PCR-based assay can simultaneously detect all five known hepatitis viruses. With the addition of other sexually transmitted diseases, future iterations of the panel may expand its utility beyond public health disease surveillance and into the realm of donor blood and organ testing and prenatal testing. G2

▲ **NGS Poised to Reshape IVF,** *from page 1*

sequence. With NGS, reproductive experts say simultaneous testing for gene mutations and aneuploidies is possible.

"NGS allows for higher throughput sequencing and while you can look for known sequence errors, you can run bigger regions. It's like a high-def television—more detail," says Thomas Molinaro, M.D., a reproductive endocrinologist at Reproductive Medicine Associates of New Jersey (RMANJ) (Eatontown, N.J.).

Molinaro says that with the plummeting price of NGS, the technology can foster better precision, more information, and the combined assessment of chromosome screening and targeted single-gene analysis at a cheaper price tag. Additionally, NGS can evaluate more embryos at a time than microarrays.

Unlike some IVF centers that send out PGS, RMANJ is developing its NGS-based PGS in-house, with full-time Ph.D.-scientists working on applying emerging technologies rapidly into the clinical practice. RMANJ has validated a chip that can screen 96 embryos at a time with identifying barcode tags inserted during the DNA amplification process. They plan to roll out the NGS-based assay for clinical use next year following completion of validation studies, Molinaro says.

The biggest benefit NGS can offer in the IVF setting, Molinaro says, is to aid in the push for fewer multiples during IVF pregnancies. Multiples, which make up to one-third of IVF pregnancies, add significant health care costs to the system. Molinaro says to ensure patients' IVF success, genetic technologies are needed to do a "better job picking" chromosomally sound embryos, making single embryo transfers more appealing, which could potentially save the health care system $1 billion over time.

"Although we believe PGS will improve IVF, insurance companies do not cover the costs of genetic testing, as it is not the standard of care," Molinaro tells *DTET*. "If they

wake up and see the cost [difference] from a singlton versus a $300,000 twin preterm pregnancy, then they'd realize the tremendous cost savings from the $3,000 spent for chromosome sequencing."

Multiple abstracts presented at ASRM echo RMANJ's findings and validated the application of NGS to embryonic aneuploidy screening and detection of structural chromosomes in embryos. In multiple abstracts reviewed by *DTET*, researchers from several institutions (Johns Hopkins, Reprogenetics, Gene Diagnostix, and Good Start Genetics) all found that NGS results are 100 percent concordant to comparative genomic hybridization microarrays, while often providing superior sensitivity and specificity, leading researchers to conclude that NGS is an "attractive," "valid," and possibly a "superior" platform alternative to current screening techniques.

"If [insurance companies] wake up and see the cost [difference] from a singlton versus a $300,000 twin preterm pregnancy, then they'd realize the tremendous cost savings from the $3,000 spent for chromosome sequencing."

—Thomas Molinaro, M.D., RMANJ

Another study presented at ASRM found that there is strong public support for PGS, for select conditions. In an online survey of more than 1,000 individuals, researchers from University of California, San Francisco, uncovered strong public support (72.9 percent) for PGS for diseases "fatal early in life" and those "causing lifelong disability (66.7 percent). By contrast, only 48 percent support PGS for "diseases that manifest late in life."

NGS Compelling for Carrier Screening Too

NGS provides a more comprehensive assessment of carrier status and yields higher detection rates of uncommon carrier mutations in couples treated at assisted reproductive technology centers, according to an abstract presented at ASRM by researchers from Good Start Genetics (Cambridge, Mass.). Good Start offers GoodStart Select, a menu of NGS-based genetic carrier screening tests.

Traditional carrier screening assays are designed to detect only the most common mutations in a gene, due in part to both cost and technological limitations. These traditional assays, the authors say, are suboptimal for ethnicities outside of specific high-yield screening populations.

In the Good Start study, carrier status for 14 disorders was evaluated in 22,296 IVF patients. Assessment was conducted using a proprietary methodology that included multiplex gene capture, NGS, and computational analysis.

The researchers found that NGS was able to detect less common mutations, mutations that would not be detected by traditional screening assays routinely used in IVF centers. Positive carrier status was identified in 6.6 percent of patients. The majority of the 235 distinct pathogenic mutations identified (63.4 percent) were uncommon or never-before reported. The researchers estimate that 12.3 percent to 17.9 percent of carriers would have been missed by other major laboratories' traditional carrier tests.

Takeaway: NGS is poised to reshape IVF treatments by making PGS part of routine care. Aside from yielding a potentially higher pregnancy success rate, the the additional information yielded through PGS may allow for single embryo transfers, which could cut the number of multiples and associated costs. G2

One Drop of Blood Going a Long Way to Diagnose Disease

It was just over a year ago that Theranos (Palo Alto, Calif.) set the laboratory industry abuzz by unveiling its microsample testing services at Walgreens locations. While Theranos's rapid results and transparent, low-cost pricing is still potentially transformative for the entire health care system, interest in other microsample testing scenarios continues to gain momentum.

Among the purported benefits of microsample testing is the ability to conduct more frequent monitoring, especially if drop-sized samples can be self-collected. This scenario not only eliminates the inconvenience of traveling to blood draw sites for the sick, the elderly, or those in underserved areas, but it also eliminates the barrier to testing imposed by the fear of needles. All of the companies *DTET* spoke to involved in microsample testing herald the benefits of more frequent monitoring and improved patient compliance.

"It is hard to get a good sample in the hospital, let alone in the wilds of our kitchens, so the HemaSpot device tries to make it foolproof."

—Jeanette Hill, Ph.D., Spot On Sciences

Spot On Sciences (Austin, Texas) believes it can transform testing through innovation in the collection and storage of biological fluid samples. What if patients didn't need to worry about coming in for blood draws? What if with a finger stick they could mail in their own self-collected, two-drop sample? Spot On Sciences' CEO Jeanette Hill, Ph.D., believes this is possible through some enhancements to the collection of dried blood spot (DBS).

"The idea came from talking to my mother, who lives in a rural area," Hill tells *DTET*. "It is really hard for her to travel and get to a site to get a blood draw."

While DBS technology is not new (it has been used for newborn screening for 50 years), it is not widely used outside of newborn screening due to challenges with collecting quality samples. Traditional DBS requires a multistep process, and samples could be degraded from moisture, contamination, and sample loss.

"It is hard to get a good sample in the hospital, let alone in the wilds of our kitchens, so the HemaSpot device tries to make it foolproof," Hill says.

The company's patented HemaSpot device improves sample quality, simplifies collection, and allows for stable sample storage at room temperature. It collects and dries two drops of blood within a protective, credit card-sized cartridge that can be immediately shipped. The moisture-tight cartridge has a tamper-resistant latch and can be labeled with a unique bar code for sample identification that can be read by standard readers such as smart phones or laboratory scanners. Smart phone-based bar code recognition applications can be used to alert a lab that the sample has been shipped along with patient information and a time and date-stamp for the sample.

The company was founded in 2010, and Hill was awarded DARPA Small Business Innovation Research (SBIR) Program Phase I funding to improve access to medical testing for elderly, homebound, rural, and economically disadvantaged patients by allowing easy remote sample collection and shipment. The award al-

lowed the company to prove collection and analysis of time-relevant blood samples for medical research in chronic disease and population studies was feasible. This summer, Spot On received a $750,000 DARPA SBIR Phase II Option Award to scale-up manufacturing and quantification methods, and produce field-use data.

Hill says the need to better understand the effects of circadian rhythms on biomarkers is behind the recent DARPA award. A Spanish study has shown that the survival of at-risk patients is increased up to fivefold by simply taking blood pressure medications at bedtime instead of morning. The cause of this is likely due to circadian rhythms, but such studies have been difficult, given the need for phlebotomist collections in the early morning and late evening. Ramon Hermida, the chronobiology expert behind the Spanish study, will utilize HemaSpot to initiate large-scale, low-cost patient studies in blood pressure and hypertension next year.

"With advances in mass spectronomy and polymerase chain reaction, you can do so much with two drops of blood."

—Jeanette Hill, Ph.D., Spot On Sciences

The HemaSpot device is designed to absorb a target volume of 80µL of blood. The fan-shaped, dried sample can be removed in portions (each blade is approximately 11.5 percent of the total fan, meaning that each blade can carry approximately 9µL to 10µL of blood per blade), enabling multiple tests to be run from a single self-collected sample. Markers that can be measured from the DBS include proteins, nucleic acids, and small molecules.

"You can do almost all tests from HemaSpot samples," Hill says. "With advances in mass spectronomy and polymerase chain reaction, you can do so much with two drops of blood. It works really well with these newer technologies."

The company sells directly to laboratories. Initial interest has been strong in the areas of HIV and infectious disease testing as well as with chronic conditions and wellness testing. The company is awaiting CE-mark for Europe and is working with the U.S. Food and Drug Administration. Development is also currently under way on devices for collection of additional fluids (urine and saliva) and tissue.

ELISA-Based Nucleosome Testing for Cancer

Another company reinvigorating an old-school technology is VolitionRx (Belgium). The company was founded in 2010 with the goal of making cancer diagnosis as simple as a blood test for cholesterol by bringing together the long-established enzyme-linked immunosorbent assay (ELISA) diagnostic technology with cutting-edge nucleosome detection and analysis techniques.

The company believes this is possible using its Nucelosomics technology, which measures signatures of nucleosomes in circulating blood. Like a "beads-on-a-string" necklace, each bead or nucleosome is DNA wrapped around a core of histone proteins. As cells die, the string is naturally broken down with individual nucleosomes released into the blood. With the rapid cell turnover associated with cancer, nucleosome levels rise in cancer patients' blood.

"The basic idea is that remnants of chromosomes are circulating in blood and that the chromosomes of cancer patients are different than healthy patients'," Jake Micallef, Ph.D., VolitionRx's chief science officer, tells *DTET*. "We are extending that to look at histone variants as well."

The company's NuQ assays can be run on multiple ELISA platforms, including manual plates, automated machines, or point-of-care configurations, which the company will pursue in "relatively short order." NuQ assays have been shown to both distinguish caner patients from healthy ones or those with related, but noncancerous, conditions as well as distinguish different types of cancer.

"The reason ELISA is very good for what we do is that it is 'old hat' and that every hospital has a machine that they can do one more application on," Micallef says.

The company is developing assays able to detect methylation in the DNA, methylation in the histones, as well as variants in histones, and nucleosome-protein complexes. The company's first commercially available clinical application of the Nucelosomics technology will be in colorectal cancer (CRC) testing. CRC screening is plagued by compliance issues. Yet CRC screening remains a large market, 150 million people in Europe and 87 million in the United States, the company says.

"We chose CRC in the first instance because it has the best gold standard. Yes, there is the greatest competition with Exact Sciences and EpiGenomics; nonetheless, because there is competition shows there is a market and there is a gold standard to compare the assay to," Micallef says. By comparison, with lung cancer, he says, if the assay is positive in an apparently healthy person, it could be 10 years to 15 years before symptoms develop, and there is no other diagnostic for comparison.

"With small samples, you move to pin pricks, which can enable tests to be performed more often. You can almost take patient compliance out of the equation."

—Jake Micallef, Ph.D., VolitionRx

The company is currently conducting analysis of its 4,800-subject CRC trial. However, they recently reported encouraging top-line results from an initial group of 938 subjects, which shows the NuQ test accurately detected colorectal cancer in 84 percent of subjects (78 percent specificity) and detected 60 percent of adenomas (polyps). The assay performed with similar accuracy for the detection of both early (I or II) and late-stage (III or IV) disease and was able to differentiate CRC from other gastrointestinal conditions. The company will seek CE mark in Europe next year and will start a pivotal trial in the United States next year.

"With small samples, you move to pin pricks, which can enable tests to be performed more often. You can almost take patient compliance out of the equation," Micallef says. "At the moment [in Europe] only 50 percent do fecal screenings. With small blood samples you move from actively opting into a nasty test to a situation where it can almost be done automatically, like cholesterol tests, liver enzymes, and cancer screening."

The company also recently released pilot study results of the NuQ platform's ability to detect lung cancer in both blood and sputum. Both sample sources could detect early- and late-stage lung cancer with high sensitivity and specificity and could differentiate lung cancer from chronic obstructive pulmonary disease, independent of smoking status and age. This was the first trial of nonblood samples on the NuQ platform, "greatly extending [Nucleosomics technology's] potential applications," the company says. The company has other ongoing trials in colorectal cancer, lung cancer, prostate cancer, and endometriosis.

An Immunosignature

A single drop of blood also holds an immunosignature that can provide clues about the body's disease states. This approach avoids the need to measure the pathogen directly and capitalizes on the body's own immune-related amplification processes to ensure sensitive results, while using miniscule sample sizes.

HealthTell's (San Ramon, Calif.) Immunosignature technology is applicable across over 30 diseases, including neurological, autoimmune, oncologic, metabolic, and infectious diseases. It was developed as "an alternative to the typical reductionist biomarker paradigm" and leverages the response of antibodies to disease-related changes, as well as the inherent signal amplification associated with antigen-stimulated B-cell proliferation.

"It is simultaneous detection and identification of multiple diseases with a single assay that underlies the true potential of this approach as a disruptive force in healthcare," writes Stephen Albert Johnston, Ph.D., from Arizona State University (Tempe), whose work provided the foundation for HealthTell, in a July 14 *Proceedings of the National Academy of Sciences* paper. "This, combined with the fact that serum antibodies are robust to handling such that a drop of blood can be sent dried on filter paper through the mail, should enable frequent, inexpensive monitoring for many different diseases."

"It is simultaneous detection and identification of multiple diseases with a single assay that underlies the true potential of this approach as a disruptive force in healthcare."

—Stephen Albert Johnston, Ph.D., Arizona State University

The company says it can provide a real-time measurement of an individual's health. The platform is based on a high-density peptide array. The antibodies in diluted blood are incubated with a microarray of thousands of random sequence peptides. The pattern of binding to these peptides is the immunosignature.

"An important aspect of this approach is that it senses essentially all antibodies raised to the disease and detects each of the antibodies as separable binding patterns composed of unique molecular recognition elements," Johnston says. "This differs from, for example, an ELISA, which might sum the contributions of many different antibodies using a single protein, cell, or virus capsid."

Johnston also says that by highly diluting the blood it improves sensitivity and specificity, prevents other blood proteins from significantly binding to the arrays, and ensures the assay is sample-sparing.

The fabrication of these arrays leverages equipment and processes similar to those used in the manufacturing of silicon-based electronics, making it both cost-effective and scalable, unlike printed microarrays. The company has currently demonstrated success using 10 million peptides per slide (330,000 peptides per assay) on silicon wafers. This October, the company announced $13.5 million of funding to help expand the development and commercialization.

Takeaway: Advances in microsample testing are enabling companies to conduct a whole host of diagnostic testing on a single drop of blood. Advocates say that by minimizing the pain and inconvenience of phlebotomy, more frequent monitoring can provide a better overall picture of patients' health.

Provista Assay Improves Precision Of Breast Cancer Imaging Diagnosis

Women with a Breast Imaging Reporting and Data System (BI-RADS) mammogram score of 3 or 4 often pose a diagnostic and management conundrum for radiologists. These women fall into a gray diagnostic zone where the subjective nature of image analysis can prevent a definitive diagnosis, and patients often wait for reimaging in six months, during which time some may have progression of invasive breast cancer and some with benign lesions face a nervous waiting game. Provista Diagnostics (New York City) is developing a comprehensive proteomic panel that can better differentiate benign lesions from invasive breast cancer, in conjunction with standard imaging.

"In lay terms, BI-RADS 3 is 'most likely benign' and BI-RADS 4 is 'possibly malignant,' and most BI-RADS 3s are sent away to re-examine in six months. But, 1 percent to 2 percent sent away will actually have invasive breast cancer," David Reese, Ph.D., Provista's CEO tells *DTET*. "This is problematic for radiologists, with 40 percent of medical malpractice suits for breast cancer radiologists resulting from the BI-RADS 3-4 juncture. We hope to give radiologists a way to go home and know that their nos are actually nos."

The company recently presented results of a randomized trial of 351 women under the age of 50 with suspicious mammograms (scored as either BI-RADS 3 or 4) at the San Antonio Breast Cancer Symposium (Texas; Dec. 9-13). In a multisite trial the Videssa Breast panel of 20-plus biomarkers (equal numbers of serum protein biomarkers and tumor-associated autoantibody markers) was used in conjunction with standard imaging and improved differentiation of benign from cancerous lesions.

"We believe our proteomics approach, which combines the sensitivity of autoantibodies with the specificity of known serum protein biomarkers along with standard-of-care imaging, may reduce the number of missed actionable breast cancers, while avoiding the stress and added diagnostic costs of false negative reports," says Reese. "For BI-RADS 4 cases, one in eight biopsies will find a tumor. If we can modestly drop that to two or three in eight, or even better, we think there is terrific health economic argument for the utility of the test."

Unlike progress made in other areas of cancer, definitive biomarkers for breast cancer diagnosis are lacking. But Reese believes in the future "integrated" models of breast cancer diagnosis and management will incorporate protein and genetic markers, as well as imaging modalities.

The company will make the Videssa Breast commercially available in the first half of 2015, initially as a laboratory-developed test in its Scottsdale, Ariz., College of American Pathologists-accredited CLIA-compliant laboratory, while it works toward U.S. Food and Drug Administration clearance of the assay. Reese says that while the company is not yet ready to disclose final pricing, it will be in the "hundreds, not thousands" of dollars. Still, the potential market is large, with millions of women diagnosed with a BI-RADS 3 or 4 score each year.

Takeaway: Provista aims to improve breast cancer diagnosis for patients in whom conventional imaging is nondefinitive by incorporating a comprehensive proteomic panel into clinical decisionmaking and patient management.

Cardiac Biomarker Testing Overused in ER

Cardiac biomarker testing in the emergency department (ED) is common even among those without symptoms suggestive of acute coronary syndrome (ACS), which should drive such testing, according to a study published online Nov. 17 in *JAMA Internal Medicine*. The researchers say that extrapolating their findings to all ED visits nationally shows that over a two-year period, there could have been 8.5 million instances of inappropriate testing, a pattern they define as "concerning."

Cardiac biomarker testing is not routinely indicated in the emergency department for all chest pain patients because of low utility and potential downstream consequences from false positive results. However, with the emergence of increasingly sensitive assays, testing for cardiac biomarkers is seen as a powerful tool to rapidly detect myocardial necrosis, a hallmark of ACS.

The researchers analyzed retrospective data from the 2009 and 2010 National Hospital Ambulatory Medical Care Survey, a probability sample of ED visits in the United States. Of the 44,448 visits analyzed, cardiac biomarkers were tested in 16.9 percent of visits, representing 28.6 million visits nationally over a two-year period. In patients lacking ACS-related symptoms, biomarker testing occurred in 8.2 percent of visits, almost one-third of all visits with biomarker testing. The researchers estimate that up to 18.3 million visits involved testing among individuals "in the absence of any clinical suspicion of ACS." Even among individuals subsequently hospitalized for any cause, including noncardiac diagnoses, cardiac biomarkers were tested in 47 percent of all visits, with more than one-third (35.4 percent) of these patients lacking ACS-related symptoms.

The researchers note that independent of ACS symptoms, the strongest predictor of cardiac biomarker testing among all ED visits was the number of other tests or services performed. The higher the total number of tests performed, the higher the chances cardiac biomarker testing was also performed.

"The high rates of testing in a population without suspicion of ACS are particularly concerning in the context of the impending adoption of highly sensitive cardiac biomarker assays in the United States, which yield more false-positive test results," write the co-authors Anil Makam, M.D., and Oanh K. Nguyen, M.D., both from UT Southwestern Medical Center, Dallas. Makam and Nguyen argue that if there was a 2 percent ACS prevalence ("likely an overestimate," they say) among the 8.5 million visits tested with no ACS symptoms and assuming that biomarker testing characteristics are equivalent to the highly sensitive troponin T assay (95 percent sensitivity and 80 percent specificity), "1.7 million individuals would have a false-positive biomarker test result (e.g., an elevated biomarker test result in the absence of confirmed ACS). In other words, even this highly sensitive biomarker test would have only an 8.8 percent positive predictive value in this low-risk population."

Takeaway: Researchers are in the early stages of quantifying potentially costly and harmful downstream effects of inappropriate cardiac biomarker testing among patients not presenting to the emergency department with clinical symptoms of ACS. Experts are concerned this pattern may be exacerbated by increasing usage of highly sensitive cardiac biomarker tests that come with higher rates of false positive tests.

Physicians Split on Genetic Cancer Screening

A poll conducted by the *New England Journal of Medicine* (*NEJM*) finds that physicians are split on whether or not they would recommend genetic screening for an asymptomatic patient expressing an interest in testing.

The *NEJM* presented a fictional asymptomatic, 45-year-old man to readers. This patient was concerned about his cancer risk and asked his internist about genetic testing. In addition to the scenario, views from two experts were shared. The more than 900 *NEJM* readers who participated in the poll were split in their opinion, 40 percent against any testing and 60 percent saying they would recommend testing. Of those favoring testing, 12 percent of all voters say they would screen with whole-genome sequencing, while 47 percent would chose targeted sequencing.

Respondents raised concerns about the management of the patient following test results as well as the financial consideration of genetic testing (both the cost of the test as well as potential downstream costs if screening showed increased risk).

"The mixed results of the polls reflect, in part, concerns about genetic testing and about genome sequencing in asymptomatic persons who do not have a strong family history of diseases for which there are known genetic risk variants and available treatments," writes Joann Schulte, D.O., in a *Clinical Decisions* piece published on Nov. 13 regarding the poll results. Schulte and co-authors conclude that at a time when "the cost of DNA sequencing is declining as our knowledge about genetic risk is increasing, conclude that many patients, like [the fictional 45-year-old man], are curious and will actively seek knowledge about their genetic risk."

Split Seen on Returning Kids' Secondary Findings

One of the challenges to clinical implementation of whole-genome or whole-exome sequencing is the issue of secondary findings. In a separate survey, researchers found that the majority of American College of Medical Genetics and Genomics (ACMG) members feel that patient preferences should dictate the return of secondary findings (formerly called incidental findings) uncovered during sequencing.

The study, published Nov. 13 in *Genetics in Medicine,* found that most respondents agreed that returning secondary findings is best practice and is consistent with medical standards, has sufficient evidence, and, for adults, the benefits generally outweighing potential harms. Despite ACMG's recent update to its policy calling for routine analysis of "pathogenic variants associated with severe but preventable disease" in children, the survey showed a lack of agreement among members regarding benefits versus harms for reporting in children. However, consistent with ACMG policy, the majority agreed that patient preferences should be considered, including the ability to opt out, and that informed consent was both feasible and critical. ACMG added in its policy statement that given the "ever-changing list" of disorders, opt-out should be for the entire set of ACMG's actionable genes and due to "practical concerns," patients should not be offered the option of choosing a subset of medically actionable genes for analysis.

Takeaway: The medical community is aware of rising patient-initiated interest in genetic screening for cancer. Yet physicians are divided in their reported views for recommending such testing in asymptomatic patients, in part over patient management and cost concerns.

G2 Insider

Hepatitis B Screening, Follow-Up Testing 'Suboptimal'

Among veterans testing positive for hepatitis B surface antigen (HBsAg), the initial screening test for hepatitis B virus (HBV), the rates of recommended follow-up serologic testing for treatment stratification is low, according to an abstract presented at the annual meeting of the American Association for the Study of Liver Diseases (AASLD; Boston; Nov. 7-11). While the chronic HBV infection seems to be substantially higher in veterans, compared to the general U.S. population, improved adherence to testing guidelines is likely needed by care providers both in and out of the Department of Veterans Affairs (VA), the authors say.

Data from the VA Corporate Data Warehouse was used to identify adult patients with any positive HBsAg result from 2002 to 2014 and to determine if additional serologic and biochemical testing recommended by the AASLD (HBeAg, HBeAb, HBcIgM, HDVAb, HDV RNA, HCVAb, HIVAb) was performed at any time following the positive HBsAg result. The researchers also determined if alanine aminotransferase (ALT; liver function testing) and HBV DNA testing was performed within 180 days from the first HBsAg-positive result.

The researchers found that HBsAg screening was performed on 2,643,089 veterans, with 1.9 percent (n=50,109) testing positive. The vast majority of these positive results were new diagnoses (95.2 percent). Just over three-quarters (77.6 percent) of those testing positive had an ALT liver function test within six months of the initial positive result, while only 14.2 percent had HBV DNA polymerase chain reaction testing. Among HBsAg-positive individuals, 17.2 percent received testing for HBcIgM, 23.7 percent for HBeAg, 20.7 percent for HBeAb, 78.2 percent for HCV Ab, 33.2 percent for HIV Ab, and 4.1 percent for HDVAb or HDV RNA.

These "suboptimal" rates of HBV serologic testing are likely to directly impact patient outcomes, the authors suggest, as appropriate testing is necessary to determine a patients' qualification for treatment. As for initial screening, which the authors also determined was low, the U.S. Centers for Disease Control and Prevention calls for screening all persons born in countries where hepatitis B is endemic (more than 2 percent, like Asia and Africa), health care workers, HIV-positive patients, men who have sex with men, and pregnant women.

"While other chronic viral infections such as hepatitis C and HIV have received tremendous educational efforts hepatitis B has received far less attention," the study's principal investigator, David Kaplan, M.D., from the Philadelphia VA Medical Center, said in a statement. "We suspect that in the baby boomer population there is a significant population of injection drug use-related chronic HBV that is undiagnosed and will not be captured by current U.S. [Preventive] Screening Task Force screening guidelines."

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