G2 INTELLIGENCE

Diagnostic Testing & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

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Digital Microfluidics Increases Complexity of Paper Diagnostics

Complex multiplex and multistep assays have traditionally posed a challenge for paper-based microfluidic diagnostic devices. However new research shows that paper-based digital microfluidics (DMF) devices have comparable performance to photolithographically patterned chromium-on-glass DMF devices at a fraction of the cost, according to a study published in *Advanced Materials*.

DMF involves manipulating liquid drops on an array of electrodes using electrostatic forces. A challenge in their application is a lack of scalable, cost-effective device fabrication. University of Toronto researchers showed that using an inkjet printer and silver nanoparticle-based ink they could create a paper DMF device that is capable of performing a homogeneous chemiluminescence assay that requires 22 discrete steps, which the authors say would be "difficult or perhaps impossible" to perform on a capillary-driven paper device.

"DMF is emerging as a useful tool for implementing fully automated, low-volume magnetic particle-based immunoassays, and we propose that paper-based DMF devices could make this diagnostic method feasible for resource-poor settings," write the authors, led by Ryan Fobel.

For more information on the re-emergence of interest in paper-based point-of-care diagnostics, please see *Inside the Diagnostics Industry* on page 5.

Facing Limited Early-Stage Funding Opportunities, Diagnostics Companies Turn to Crowdfunding

Facing decreasing access to capital, life science companies and researchers are turning to popular crowdfunding sites to reach out to lay audiences to promote their ideas in hopes of generating financial support. Experts say that crowdfunding for biotech projects, including diagnostics, remains in the early stages, and while some projects' successes at raising funds are encouraging, it remains too soon to measure the ultimate viability and success of the platform for furthering the diagnostics industry.

There are several varieties of crowdfunding. Some, especially for mainstream technology ideas, include product giveaways in which backers are rewarded with early versions of the product. Life science projects are usually backed by philanthropic supporters. In this case there is no tangible product reward, just knowing that they contributed to research they care about. Finally, there are

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emerging equity-backed platforms, which are awaiting final rules from the U.S. Securities and Exchange Commission (SEC).

"The spirit of crowdfunding is to break down barriers and open the curtains—to let the public take some ownership [of funding] previously reserved for the elite few," says Fazila Seker, Ph.D., director, technology and venture development, MaRS Innovation (Toronto), who herself was involved with an ultrasound-based breast cancer monitoring technology that raised funds on Indiegogo.

Life science researchers and companies have faced enormous challenges in recent years obtaining funding. These challenges arise both from cuts from granting agencies, like the National Institutes of Health, as well as a pullback among venture capitalists, who are reserving limited capital for deployment in later-stage companies that are closer to commercialization, thereby shrinking their risk and building the likelihood of creating a positive return for investors.

"There is a huge constriction of early-stage capital broadly in the life sciences, especially for seed stage or Series A rounds," says Paul Grand, managing director of RCT Ventures, which actually specializes in this early-stage funding. "Crowdfunding is definitely going to grow. These platforms are filling a gap."

Seker adds that for life sciences and medtech companies it has been really tough to get funding in that stage beyond initial proof of concept. She says that the innovation cycle has four phases of development: discovery, feasibility, optimization, and commercialization.

"Discovery and feasibility stages don't cost quite as much and have [access to] smaller pots of grant funding. But the valley of death phenomenon, when they really fall off the cliff, is between feasibility and optimization where there is a need for huge amounts of money for clinical trials to show the regulatory bodies that the technology is safe for the general population," Seker explains. "This takes tens of millions of dollars and there isn't readily available grant funding or that much immediate interest from the investment perspective. There can be great ideas but it is tough to cross that chasm."

"The big question is how to measure success given it is such a new platform," says Seker. "The community is in flux. It is a global experiment. While crowdfunding has been picking up for the past few years, last year was a turning point with data coming out to start analyzing."

Diagnostics Companies, Researchers Actively Crowdfunding

Below is a sampling of diagnostics companies and researchers seeking support on crowdfunding sites. These examples demonstrate the variety of projects seeking support both in terms of research area and stage of development.

Indiegogo is a general crowdfunding platform. A recent search uncovered two prostate cancer diagnostics: a test spun-off from an Italian public research agency that discovers a patient's proneness to develop prostate cancer and to provide a further measure to quantify its advancement, and Cellanyx Diagnostics (Boston), which uses advanced microfluidics to analyze molecular, biophysical, and phenotypic biomarkers to guide therapy for patients in all stages of treatment. Additionally, a London-based group is raising funds to perform proof of concept for a rapid celiac disease diagnostic device, and a woman is seeking funds to open her own clinical laboratory.

Experiment (formerly Microryza), initially launched in April 2012, uses a donation-based crowdfunding platform for research scientists to fund their projects from interested lay people. Experiment evaluates projects using three criteria: if the projects answer a scientific question (is it actually research), if the goals are within the capabilities of the researcher, and verification of the researcher's identity.

No clinical diagnostics were currently listed on the platform, but according to *Forbes*, during beta testing Experiment funded over 80 projects in a wide variety of research topics ranging from cancer research to marine biology, raising over $600,000 from over 5,000 individuals.

AngelList is a hybrid platform (between lay crowdfunding and traditional venture capital) for startups to meet qualified individual investors. It launched in January 2010. Some diagnostics-based companies seeking support include Prime Genomics (breast cancer screening through salivary samples) raising $4 million, Station X (improving genomics understanding) raising $2 million, Lazarus Bioscience (cancer molecular diagnostics) raising $750,000, and EvaGen (library preparation for next-generation sequencing) raising $100,000.

Ethan Perlstein, Ph.D., a crowdfunding scientist conducting research on precision orphan drug discovery, conducted analysis of 115 science projects ("fitting the academic profile") that together raised $5,082,028 from 47,958 donors. He found that the median project goal is $3,029, and the median number of project donors is 39. The $100 donation per project was consistent across projects, regardless of goal.

"There is a ceiling between $25,000 and $35,000 above which Microryza [Experiment] and RocketHub science projects don't go, but above which Kickstarter and Indiegogo science projects do boldly go. What's causing this separation?" asks Perlstein in a blog post. "Briefly put: tangible rewards shift the average donation size higher than people might spontaneously donate."

Critics are concerned that while crowdfunding allows the public to become more involved in science, not all of these potential funders have a strong understanding of science, and some scientifically questionable research may be funded because of a compelling YouTube pitch.

"Once the metrics are better established the bar is going to be raised," believes Seker. "Once success is defined, certain standards will be established. Life science companies will have a better sense of how to do this and it will become a more standard part of the funding toolkit to supplement grants."

But Grand says the ultimate measure of crowdfunding success will be in the longer term whether or not the funded projects can use the funds toward the goal of commercialization and generating returns for investors in equity-based platforms.

"Ultimately you need exits to define success in terms of returns. With any new funding platform you have to look at returns," says Grand.

Takeaway: Life science researchers and early-stage companies are turning to popular crowdfunding platforms to fill funding gaps. However, the platform remains too young to have demonstrated long-term success in terms of the commercial viability of funded companies.

Bucking the Trend, Several Diagnostics Companies Diversify Offerings

While headlines tell of large divestitures, with diagnostic companies selling off noncore or underperforming business lines, some companies are boldly expanding their assay portfolios into clinical diagnostics. *DTET* has studied the business cases of two diagnostics start-ups that have plans to adapt their platform outside of their core business and move into clinical diagnostics and health care.

Invisible Sentinel (Philadelphia), established itself in 2006 by enhancing food safety testing with its rapid molecular diagnostics. As of late March, the Association of Analytical Communities, the global standardization organization for the food industry, certified Sentinel's Veriflow assays for salmonella, listeria, and campylobacter. The Veriflow platform uses vertical flow technology to enable testing in food products and various surfaces in contact with food during preparation and packaging.

The food industry, a $1 billion testing market, provided an "interesting opportunity to bring the power of molecular diagnostics—its sensitivity and its accuracy—but to make it more accessible," says Benjamin Pascal, co-founder and chief business officer of Invisible Sentinel. "Veriflow makes molecular diagnostics accessible with easy-to-

read results, and it eliminates the need for capital equipment and maintenance so that it empowers the bulk of the food industry."

The pressing need for improved testing to ensure food safety, combined with a simpler path to regulatory approval, made the food and beverage industry Sentinel's first choice. However, clinical molecular diagnostics was always a consideration given the founders' experiences in the health care industry. The amount of capital needed for U.S. Food and Drug Administration regulatory approval was initially daunting, though. Now with steady revenue from food and beverage products, the company is looking to expand its Veriflow product portfolio across additional industries through partnerships established in a custom-solution program.

The company says that its patented vertical flow technology allows for the sensitivity of real-time polymerase chain reaction tests but with the ease of use of lateral flow assays. The system minimizes sample preparation (by eliminating the need for gel electrophoresis or fluorophore-based detection of target amplification), speeds time to results, and provides easy-to-interpret data for the end user. New, validated prototypes can be turned around in 12 weeks, Pascal tells *DTET*.

Another company that is rapidly validating clinical assays is Menon Biosensors (San Diego). Its Molecular Mirroring (M^2) platform is based on nuclear magnetic resonance technology but was originally developed as a classified government project for the detection of agents that could be used in a bioterrorism attack, such as anthrax and the plague. Over a six-year period, more than 3,000 samples studied with the Department of Homeland Security's Detect to Protect bio detection project, the platform demonstrated better than 99 percent accuracy.

Now the company has expanded the applicability of its portfolio of assays for pathogen detection, including Clostridium difficile (C. diff) and the mycobacterium tuberculosis complex and plans to add HIV and hepatitis C, as well as applications in food safety and livestock screening. Development of clinical assays for the health care industry began in mid-2013, and the company believes they have already achieved "a multiple level of improvement over current industry standards," said Suresh Menon, Ph.D., the president of Menon Biosensors, in a statement.

Based on validation studies that are currently under way at Scripps Memorial Hospital laboratories, the company says the M^2 technology has shown a "wide dynamic range" for pathogen detection (1 colony-forming unit [CFU] to 100 million CFU per sample). M^2 was able to detect tuberculosis and C. diff in concentrations of 1 CFU per sample in water and 50 CFU/mL to 100 CFU/mL in sputum and stool. Sample prep took between five minutes and 15 minutes, with results within one hour. The company says they will further optimize assays for sputum and stool samples and will publish clinical validation data in 2014.

The platform may have applications in low-resource areas as well, as the platform requires less than 10 watts of electricity or a battery and the system weighs less than 10 pounds. Additionally, the reagents are lyophilized, allowing for storage at room temperature for one year. Results are displayed on a tablet.

Takeaway: Despite the trend toward divestitures of noncore business lines, some diagnostics companies are investing in expanding their platforms to address unmet needs in clinical diagnostics. G2

G2 INTELLIGENCE
Inside the Diagnostics Industry

Technology Advancements Bringing Resurgence of Interest in Commercialization of Paper-Based POC Diagnostics

Paper-based diagnostics are not that new. Dipstick assays and lateral flow tests, like home pregnancy tests, date back to the 1960s and 1970s. Yet there was never a broad proliferation of the technology to encompass a multitude of clinical applications. While there has always been hope that paper-based products' ease of use, rapid time to results, and low cost would make them prime targets to increase the accessibility of medical care, particularly in resource-limited settings, the reality is that diagnostics manufacturers have not pursued the strategy.

"The principles today are the same, but what's changed is that we've refined more ways to detect more things," says Andrew Warren, a Massachusetts Institute of Technology (MIT) graduate student and lead author of a recent paper on the development of a paper-based diagnostic for cancer. "Today we have expanded our thoughts as to what else it is useful for and moved beyond a binary yes/no. With nanotechnology we have a good sense of how to do this in a robust manner."

There has been a resurgence of interest in paper-based diagnostics resulting from advances in the technology led by renowned chemist George Whitesides' group at Harvard University. Patterning paper can be fabricated in 2-D or 3-D to transport the fluidics and patterned channels and wells now allow for filtering and multistep reactions. Recently, paper-based microfluidics has emerged as a point-of-care platform that is capable of bringing sophisticated multiplex assays to resource-limited settings without the need for any large equipment.

Use Cases

One of the leaders in the paper-based diagnostics space is Cambridge, Mass.-based Diagnostics For All (DFA), a 6-year old spinoff based on technology from Whitesides' lab. DFA was established as a nonprofit and is funded through philanthropic grants. It holds the exclusive worldwide license for Whitesides' patterned paper technology.

The Process of Making Paper-Based Microfluidic

Below outlines the steps in DFA's making of its liver function paper-based microfluidic assay.

- Paper is printed on a sheet with wax defining areas for 55 tests. Assembled tests include a sandwich of two such sheets.
- The sheets are baked for 30 seconds at 130 degrees Celsius to allow the wax to melt completely through the paper's 0.2 millimeter thickness.
- The test wells are wax-free circles 2 millimeters across.
- Specified amounts of the reactant chemicals are deposited on each sheet. The first sheet's reagents react with enzymes, while the second sheet receives dyes that change color if exposed to products released by the first reactions.
- The two sheets are fused together with adhesives in a press.
- A protective laminate is affixed to the top of the package.
- Completed tests are cut into individual squares.

(Adapted from *MIT Technology Review*)

Describing the technology as "elegantly simple," DFA says that patterned paper-based technology is not only inexpensive but also requires minimal training to use, practically no sample preparation, and no electricity or additional equipment to process a sample.

In addition to the liver function assay (see box on page 6) that DFA says will be the first approved paper-based microfluidics test, they are working on other paper-based diagnostics. Developed through a grant from the Bill & Melinda Gates Foundation, the second product in the DFA pipeline is a low-cost, rapid diagnostic test for

immune markers to determine successful vaccination against tetanus and measles. In a U.S. Defense Department, Defense Threat Reduction Agency-funded effort in conjunction with Harvard University, DFA is developing nucleic acid amplification- and immunoassay-based paper-microfluidic devices for Brucella abortus.

Commercialization

Whitesides has been quoted as saying that ''if the science of something is still interesting, the 'something' is probably not ready to be a product."

The technology behind paper-based diagnostics, particularly microfluidics, has come a long way in just the last few years. Currently, though, researchers are in the midst of finding the best use cases for the technology, including which clinical conditions are most in need of these increasingly sophisticated assays and in which populations. But historically, commercialization and adoption of these tests has been plagued, despite good intentions, by a lack of a business case supporting their use.

"There has been a lot of promise in the field, but not a lot of delivery," writes Ali Kemal Yetisen from the University of Cambridge (United Kingdom) in a critical review published February 2013 in *Lab on a Chip*.

"Diagnostics for resource-limited settings does not always receive attention from large companies while limited research activity in smaller biotechnology companies and academia struggle to make an impact in creating real world products, scaling up and achieving market penetration. . . . This aspect parallels the disinterest of industrial partners in these applications due to the limited market and low profit margins."

These low margins are also accompanied by low manufacturing and development costs. Yetisen and colleagues say in the review that low-cost lateral flow tests can be manufactured for 10 cents to $3 per test, and development costs for lateral-flow immunoassays range from $30,000 to $100,000 per test if the target analyte and necessary antibodies are available. Paper-patterning costs are estimated to be less than 1 cent to 3 cents, including the price of the paper, although a large number of tests are needed to achieve economies of scale for production.

"It will be very challenging, yet not impossible, for a biotechnology or a diagnostic company, research group or investors to capture value from paper-based microfluidics," writes Yetisen. "Different strategies need to be considered based on the

Paper-Based Microfluidic Liver Assay

Routine monitoring of liver function for patients on anti-retrovirals (ATV) and therapy for tuberculosis is the standard of care. However, this monitoring for liver toxicity is "severely limited" in the developing world, because of expense and lack of access to modern laboratory instrumentation. While access to ATVs has expanded in underserved parts of the world, there has not been similar improvement in access to monitoring tests.

Diagnostics For All (Cambridge, Mass.) is validating and looking for a partner to commercialize a liver function assay, in what will likely be the first approved paper-based microfluidic diagnostic, the company says. The assay monitors for drug-induced liver injury through serial measurements of serum transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]). The 3-D multilayered design allows a single 30 ml to 35 ml sample of whole blood to be split into five separate "streams" of plasma, which are tested with five independently optimized assays in parallel. The test requires no preanalytical sample prep, no additional agents, and no reader and returns results in 15 minutes, at ambient temperature. The researchers say that when the test is mass produced, the cost could be less than 10 cents per test.

According to a study published in *Science Translational Medicine* (September 2012), an earlier version of the liver assay was used to test 223 blood samples obtained by venipuncture and 10 finger-stick samples from healthy volunteers. The test allowed visual measurements of AST and ALT, in both whole blood and serum with more than 90 percent accuracy.

"Our test performed well compared to automated methods, even in specimens that were obtained from critically ill patients with multiple derangements in other analytes and that were up to 5 hours old at the time of testing," write the authors, led by Nira R. Pollock, M.D., Ph.D., associate medical director, Infectious Diseases Diagnostic Laboratory at Beth Israel Deaconess Medical Center in Boston. "The experiments presented here have firmly established proof of concept and clinical relevance and will allow us to move into clinical field studies."

geographical placement (developed or the developing world) of the product and also on the therapeutic area (established or new). If the market is mature for a particular therapeutic area, then expecting a shift from current diagnostic methods to paper-based microfluidics is unrealistic."

But Yetisen and colleagues do offer optimism that recent advances in capillary-based microfluidics might improve the translation of hope into products. While the DFA's liver function assay was specifically designed to be used in underserved areas, there has been quite a bit of interest in the developed world.

"We are seeing a dual-market use case," says Patrick Beattie, director of operations at DFA. "Pharmaceutical companies are interested in the assay as a companion diagnostic and for trials, although no official partnerships have yet been established."

Beattie says that regulatory submission will depend on the ultimate market selected and could range from U.S. Food and Drug Administration approval to CE-mark, World Health Organization prequalification, or local regulatory approval.

The interest and cooperation from what Beattie describes as "traditional" diagnostics manufacturers is welcome, given that some in the laboratory and diagnostics industry see sophisticated, highly sensitive, point-of-care technology as a threat.

"What we are developing is disruptive and will be viewed by some as negative, but we have had great discussions with traditional diagnostics manufacturers. They see us an opportunity," Beattie tells *DTET*. "Being a nonprofit, for us it is important to focus on technology development and not manufacturing and distributions, which could get in the way of our own success. It is not simple to build a diagnostic company. We want to take advantage of the market-based focus of a potential partner. "

Looking Ahead

While all involved in paper-based diagnostics are on a mission to make the tests commercially available to underserved markets as rapidly as possible, they believe they are able to push the technology to new levels of complexity.

"We are taking clinical chemistry tests that have been performed on a machine and cartridge-based system and moving them to solid, disposable platforms," says Beattie. "The approaches we are using is to look at what internists do in the developed world and see what isn't available in the developing world. An obvious next step is renal function testing and normal blood chemistry."

Paper-Based Tests for Cancer, Blood Clots

Among the unmet global health needs are noninvasive methods to diagnose noncommunicable diseases, which are becoming an increasing burden, in low-resource areas. MIT researchers have developed exogenous agents that can serve as synthetic biomarkers, measurable in urine and quantifiable by a companion paper test, for noncommunicable diseases including cancer and blood clots.

According to a mouse-model study published Feb. 24 in the *Proceedings of the National Academy of Sciences*, the test relies on injected nanoparticles (synthetic biomarkers) that interact with tumor proteins called proteases or thrombin in clots. At the diseased tissue, local up-regulated proteases (matrix metalloproteinases or thrombin, respectively) cleave their surface coat of peptides, releasing hundreds of reporters that are easily detectable on a paper strip from a sample of the patient's urine. From injection to results is about one hour in mice, and the researchers expect similar time to results in humans. The researchers are also working on a nanoparticle formulation that could be implanted under the skin for longer-term monitoring.

While testing in humans is necessary, senior author Sangeeta Bhatia, M.D., Ph.D., said in a statement that the technology would likely first be applied to high-risk populations, such as those with a personal or family history with cancer, although eventually, she would like to see it used for early detection in developing nations. She said that applications in the United States and other developed countries could be "transformative," enabling image-free cancer detection in a home or pharmacy clinic.

Clinical Benefit of NGS Panel-Based Colorectal Cancer Testing Over Sequential Testing Remains Uncertain

While there may be cost and time savings associated with the use of next-generation sequencing (NGS), panel-based testing for inherited colorectal cancer (CRC), questions remain as to whether the identification of moderate-penetrance genes currently enhances clinical care and management, according to a study published online March 20 in *Clinical Genetics*. This study is the largest to date to describe results found among clinical patients undergoing panel-based CRC testing and critically assesses the benefits and challenges associated with this testing method.

While many tout the potential time and cost savings associated with NGS panel-based testing, these tests increase the complexity of interpreting results in part because of the higher rate of inconclusive results, which may result in lengthier counseling and unnecessary screening and follow-up. However, the hope is that by including moderate-risk genes along with continued analysis of variants of unknown significance (VUS), understanding of cancer risk and case management will improve over time. It is estimated that between 10 percent and 30 percent of patients with CRC have an inherited predisposition, making them prime targets for enhanced risk-based management.

"[The] reality remains that syndrome-based testing would have been sufficient to identify the majority of patients with deleterious mutations."
—Deborah Cragun and colleagues

Patients who underwent hereditary CRC panel-based testing (ColoNext; Ambry Genetics [Mission Viejo, Calif.]) from March 2012 to March 2013 were identified from a comprehensive data repository. ColoNext includes analysis of 14 genes, a mix of mostly highly penetrant, actionable genes and some moderately penetrant genes with lower established clinical utility. The researchers estimated mutation and VUS rates and determined whether patients with a mutation met national genetic testing criteria (2013 National Cancer Center Network [NCCN] guidelines) for the respective cancer syndromes identified.

Based on demographic data stored in Ambry's comprehensive repository, those tested tended to have a personal history of CRC (53 percent) and a positive family history of CRC or other cancers (54 percent). The researchers found that just over 10 percent of the 586 patients tested had a pathogenic mutation. When excluding the patients with CHEK2 mutations (n=8; clinical relevance remains uncertain) and patients with only one MUTYH mutation (n=11; MUTYH-associated polyposis are autosomal recessive, but monoallelic carriers may have a moderately increased CRC risk), the number of patients with actionable mutations decreased to 7.2 percent, primarily in Lynch syndrome genes. Just over 20 percent of all patients had a VUS. Nearly 12 percent of those with a VUS also had a pathogenic mutation.

The majority of the 42 patients with an actionable mutation (71 percent) met NCCN syndrome-based testing, screening, or diagnostic criteria. Twelve patients with actionable mutations did not clearly meet the testing criteria, including six in whom panel-based testing may have identified mutations otherwise missed because of limited medical or family history or an atypical presentation.

"On the basis of our findings, although there are scenarios where panel-based testing may have been more cost-efficient, [the] reality remains that syndrome-based testing

would have been sufficient to identify the majority of patients with deleterious mutations. Consequently, the optimal and most cost-effective use of panel-based testing as a first-tier test vs a second-tier test (i.e. after syndrome-based testing is negative), remains to be determined," write the authors, led by Deborah Cragun, from the H. Lee Moffitt Cancer Center (Tampa, Fla.). "Finally, it remains uncertain whether identification of moderate penetrance genes truly helps guide cancer screening decisions over and above what would be recommended based on comprehensive collection of family history without conducting testing."

Several authors report financial ties to Ambry Genetics, which performs ColoNext testing.

Takeaway: While the expanded information profile and cost efficiencies made possible with panel-based CRC testing are appealing, it remains to be seen whether this can be translated into improved management of potentially higher-risk patients. G2

Longitudinal Variance in Gene Expression Score Predicts Transplant Rejection

Variability in gene expression profiling test scores in post-transplant heart patients over time may provide prognostic utility for risk for organ rejection, according to a study published March 27 in *Transplantation*. The importance of variability in predicting clinical stability is independent of a single ordinal test score.

The noninvasive, blood-based gene expression profiling test (AlloMap; XDx [Brisbane, Calif.]) incorporates expression levels of the 11 genes and was developed to minimize serial endomyocardial biopsies needed to identify heart transplant recipients at risk of rejection. The U.S. Food and Drug Administration-approved test to date has been used to rule out acute cellular rejection.

The researchers analyzed data from patients participating in the Monitoring Attenuation by Gene Expression Profiling (IMAGE) study, with rejection surveillance gene expression profiling tests performed at one- to six-month intervals. For most patients (86 percent) surveillance began 12 months post-transplantation. The standard deviation of an individual's cumulative test scores was used to define variability in gene expression profiling scores. Over a median follow-up of 19 months, 297 patients were monitored with gene expression profiling (mean, 4.4 tests) and 305 patients were monitored with biopsies. Rates of adverse events were similar between the groups.

Being nonwhite, younger at time of transplantation, and having an earlier time of study entry post-transplantation were significantly associated with future events. Twenty percent of patients had variability less than 0.5, 43 percent had variability over 1.0, 18 percent had variability greater than 1.5, and 7 percent had variability over 2.0. Gene expression score variability was significantly associated with future clinical events, even when controlling for gene expression ordinal score.

"This new prognostic information complements and validates our clinical intuition during routine patient encounters and demonstrates the value of longitudinal genomic testing. This longitudinal genomic testing is part of a very exciting transition toward

the paradigm of personalized medicine," said lead author Mario Deng, M.D., from University of California, Los Angeles, in a statement.

Such information, the authors say, can lead to reducing immunosuppressive maintenance regimens in low-risk patients or evaluating higher-risk patients for overlooked infections or medication noncompliance. Ongoing studies are evaluating whether longer follow-up and the time interval for repeat testing confirm the current findings of the significance of variability.

Several authors report financial ties to XDx, which funded the study.

Takeaway: Changes in gene expression over time may predict the risk of negative transplantation outcomes better than a single measurement.

New Blood-Based Markers May Be Capable of Guiding Post-Concussion Return-to-Play Decisions

Several recent studies indicate progress in the search to find blood-based biomarkers associated with concussions. Forty-nine states have passed so called return-to-play (RTP) laws requiring better management of head injuries in student athletes, including requiring clearance by a medical professional before resumption of regular activity. Yet a definitive biomarker demonstrating safe RTP remains elusive, and these decisions are made based on subjective clinical evaluations and the absence of self-reported symptoms by the athlete.

"Establishing a serum marker of injury and recovery would assist bedside clinicians . . . bringing this highly prevalent diagnosis in line with other common conditions, such as abdominal pain and fever, in which laboratory studies are routinely combined with clinical predictors to allow clinicians to stratify patients by risk," writes lead author Rebekah Mannix, M.D., from Boston Children's Hospital in Massachusetts, in a study published online ahead of print on Feb. 4 in the *Journal of Neurotrauma*.

CSF Protein Measurable in Blood

For the first time, an ultrasensitive diagnostic platform has been able to detect total tau (T-tau), a highly specific cerebrospinal fluid protein in blood, and correlate these blood measurements to a concussive diagnosis and readiness for RTP, according to another study published March 13 in *JAMA Neurology*.

The Simoa platform (Quanterix; Lexington, Mass.), a single molecule array technology, was used by researchers in Sweden to study sports-related head injuries. Neuronal proteins, like T-tau, have concentrations in peripheral circulation below the detection limit of conventional tests, the company says, but the Simoa platform offers a 3,000-fold improvement in sensitivity compared to available tests. The ability to measure low-abundance biomarkers of brain function in a simple blood test may provide new insights for diagnosis, monitoring, and treatment of other neurological and neurodegenerative conditions.

From Sept. 13, 2012, to Jan. 31, 2013, 35 Swedish Hockey League players suffered a concussion, of whom 28 had repeated blood sampling (four times in the 144 hours after injury and at RTP). Post-concussion levels of three markers (T-tau, S-100 calcium-binding protein B, and neuron-specific enolase) were compared to baseline measurements taken in the preseason.

The study found that T-tau is significantly elevated following mild to severe concussion and remains elevated, compared to preseason levels, for at least six days post-concussion. Concussed players had significantly elevated levels of the axonal injury biomarker T-tau (difference in median levels, 5.5 pg/mL) following injury compared to baseline measurements. In the first hour following the injury, concentrations were highest, but the level of T-tau one hour after injury was not able to significantly differentiate between concussion severity categories. However, there were trends toward higher concentrations in players who had symptoms lasting for more than 10 days or had loss of consciousness (both indicators of more severe injury). T-tau concentrations immediately after injury did predict the number of days it took for the concussion symptoms to resolve and the players to have safe RTP. Additionally, high T-tau levels 144 hours after concussion correlated with persistence of post-concussive symptoms.

Glial Fibrillary Acidic Protein

Another serum biomarker—glial fibrillary acidic protein (GFAP)—shows promise in aiding in the diagnosis and prognosis in pediatric concussion cases, according to a small study published in the *Journal of Neurotrauma*. GFAP has previously been shown to correlate with markers of injury severity in adult patients.

In the present study, the researchers examined 13 children and young adults (11 years to 21 years of age) presenting to the emergency department (ED) within 24 hours of concussion. Initial serum samples were obtained in the ED, and follow-up samples were collected within 24 hours to 72 hours of injury. Samples were tested using the GFAP assay (Banyan Biomarkers; Alachua, Fla.), a sandwich electro chemiluminescent immunoassay. For the assay, the lower limit of detection was 0.008 ng/ml. Laboratory personnel performing the tests were blinded to clinical data.

The researchers found that the mean initial GFAP level was 0.12 ng/ml. Initial GFAP levels were significantly associated with the burden of symptoms both initially and at follow-up, up to one month after injury. However, GFAP levels at follow-up did not correlate with symptom burden.

While hopeful that GFAP could offer an objective measure of injury and recovery after pediatric concussion, the authors caution that it is "likely that no single biomarker, but rather a combination of biomarkers and other clinical variables, will offer the best [concussive] diagnostic and prognostic utility."

Takeaway: This research furthers a trend to improve diagnosis of brain injury through objective measures in a practice area notoriously based on subjective measures. While further validation is necessary, clinicians are eager for a biomarker that can guide RTP decisions.

New Study Suggests Molecular Products in Urine Reflect Brain Injury

Development of diagnostics based on brain injury urinary signatures using either combinatorial quantitative models or pattern-recognition methods may serve a role in addressing the need for incorporating a great number of markers representing the multifactorial nature of brain injury, according to a study published March 27 in the *Journal of Neurotrauma*.

Researchers from Virginia Commonwealth University (Richmond, Va.) analyzed urine specimens from head trauma subjects admitted for acute brain injury rehabilitation, as well as nontraumatized matched controls. They utilized an innovative data-independent mass spectrometry approach for molecular quantification of small metabolic byproducts across osmolarity-normalized samples. They found that human urine contains 10,929 reproducible traumatic brain injury discriminant measures of pathobiological relevance and diagnostic potential, representing a diverse class of molecular products.

"These results support further development of pattern-based urinary metabolite diagnostics and theragnostics to assess rehabilitation readiness and efficacy of intervention applicable broadly to brain injuries from traumatic, ischemic, and hemorrhagic insults," conclude the authors.

G2 Insider

Study Finds Hurdles Persist for Clinical Use of Whole-Genome Sequencing. . . While clinical whole-genome sequencing (WGS) is being employed by early clinical adopters, it may still not be ready for prime time, especially for prediction of disease risk, according to a small study published March 12 in the *Journal of the American Medical Association*. Challenges include both technical issues and the "considerable" human resources needed for clinical interpretation that remains rather subjective.

Stanford University researchers conducted WGS in 12 adult patients (seven women; five white and seven East Asian). Coverage and consistency of clinically relevant genetic variation were evaluated between sequencing systems (San Diego-based Illumina and Mountain View, Calif.-based Complete Genomics). Agreement of interpretation was evaluated both for potentially reportable genetic findings and proposed clinical follow-up.

The researchers found that reportable genes associated with inheritable conditions were commonly not covered at adequate standards (10 percent for Illumina and 19 percent for Complete Genomics) and would thus require supplementation with other genetic assays. Genotype concordance was high (99 percent) for previously described single nucleotide genetic variants, but low for small insertion or deletion variants that can be clinically meaningful (53 percent to 59 percent). For each participant, 90 to 127 genetic variants of potential personal risk and carrier status were identified and required a median of 54 minutes of investigation per variant, making the median cost for sequencing and variant interpretation $14,815 (not including costs of computing infrastructure and data storage).

Two to six personal disease-risk findings were reported in each participant, including one frameshift deletion in the BRCA1 breast cancer gene in a woman with no known family history. Physician review of sequencing findings prompted consideration of a median of one to three initial diagnostic tests and referrals per participant, ranging in cost from $351 to $776.

"We need to be very honest about what we can and cannot do at this point in time," Euan Ashley, M.D., a senior study co-author, said in a statement. "It's clear that if we sequence enough cases, we can change someone's life . . . Our hope is that the identification of specific hurdles will allow researchers in this field to focus their efforts on overcoming them to make this technique clinically useful."

For more information on the clinical adoption of next-generation sequencing (NGS), don't miss G2's conference, MDx NEXT, which will be held June 11-13 in Baltimore (*www.mdxconference.com*). Sherri Bale, Ph.D., FACMG, managing director of GeneDx and senior vice president of Bio-Reference Laboratories, will be discussing challenges ahead for NGS. Kevin Davies, founding editor of *Nature Genetics* and Bio-IT World and author of *The $1,000 Genome*, will address the $1,000 genome and beyond.

Company References

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