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Diagnostic Testing & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

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Value of Multigene Panels Emerging

Multiple-gene sequencing panels may provide benefit beyond BRCA testing alone, according to a study published online April 14 in the *Journal of Clinical Oncology*. This study begins to build an evidence base demonstrating that multigene panels hold enhanced clinical value, guiding interventions that can prevent incident cancer.

Forty-two genes associated with cancer risk were sequenced from frozen samples from 198 patients referred for clinical BRCA 1/2 testing (from 2002 to 2012) using a customized germline-DNA sequencing panel. Fifty-seven of the women carried germline BRCA1/2 mutations, which was fully concordant with prior BRCA testing. An additional 16 pathogenic variants (five novel ones) were identified in other genes among the 141 women without BRCA mutations. Fifteen of these pathogenic variants were actionable and warranted a change in care (i.e., targeted screening). An average of 2.1 variants of unknown significance were seen across all genes.

"This is a significant yield of potentially actionable results, comparable to the 5 percent to 10 percent probability threshold endorsed by guidelines and payers for BRCA1/2 and Lynch syndrome testing," write the authors, led by Allison Kurian, from Stanford University in California. "Although further research is required to guide practice, these findings provide an early signal for the clinical relevance of multiple-gene sequencing in cancer-risk assessment."

Several authors report financial ties to genetic testing company InVitae (San Francisco), which partially funded the study. For more information on trends affecting BRCA testing, please see *Inside the Diagnostics Industry* on page 5.

Drug Monitoring Can Impact Utilization In Patients on Opioid Therapy

Quantitative drug testing and monitoring can reduce utilization of high-risk medications in injured workers on chronic opioid therapy, according to a study presented at the Academy of Managed Care Pharmacy 26th Annual Meeting and Expo (April 1-4; Tampa, Fla.). Laboratories can play an important role in addressing the national opioid abuse epidemic, as increased utilization of drug monitoring is proving it can improve outcomes both for patients and payers.

Existing guidelines do call for periodic monitoring for prescription compliance, but noncompliance remains a substantial burden with fraud, addiction, and cost implications for both patients and payers. The current study evaluated clinical

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benefits for 100 injured workers enrolled in workers' compensation service provider Progressive Medical/PMSI's Drug Testing and Monitoring (DTM; March to April 2013) program. Enrollment was due to urine drug test results inconsistent with the drug regimen reported by the prescribing provider. Prescription transaction history and urine samples were evaluated during the 90-day period prior to enrollment in the DTM service, 90-days after enrollment, and six months post enrollment.

Lab testing was performed by Millennium Laboratories (San Diego), which specializes in medication monitoring and pharmacogenomics testing. Liquid chromatography tandem mass-spectrometry, which is becoming the gold standard for drug screening given its increased specificity over immunoassays and lower levels of detection, was used in the study to test for a broad array of drugs.

> ***"It is a win-win demonstrating both cost savings and enhanced safety."***
> ***—Steve Passik, Ph.D., Millennium Laboratories***

With monitoring, a decrease in all measures of drug utilization, including high-risk medication, was seen. This decline was driven primarily by opioids (a 32 percent decrease) and benzodiazepines (a 51 percent decrease), as well as a 26 percent reduction in total utilization of all medications, regardless of drug class. There was a reduction in the average morphine equivalent dose (MED), from 123.3 mg to 83.3 mg, after six months. The total percentage of claimants with a daily MED less than 120 mg increased from 65 percent to 77.7 percent.

"It is a win-win demonstrating both cost savings and enhanced safety," says Steve Passik, Ph.D., director of clinical addiction research and education at Millennium.

Passik tells *DTET* that generally about 60 percent of urine test results are consistent with the doctor's prescribed regimen. Roughly one-third of missing results can be explained by "more innocent explanations" such as clerical errors, including a prescription being inadvertently left off the requisition. However, a substantial number of patients are not taking their medications or are "diverting" them by only taking part of the pill and sharing or selling the other part. However, in some cases where the patient is reporting proper drug usage, yet the metabolite is not consistently showing up, pharmacogenomic testing may be ordered.

With the benefits of monitoring becoming apparent, Brooke Mueller, Pharm.D., a senior clinical pharmacist at Progressive Medical/PMSI (Tampa, Fla.)., says she expects to see a trend toward higher volumes of patients referred as continued monitoring becomes the standard of care. Yet, she tells *DTET* that open questions remain regarding just how broad monitoring should be. Not only are other often-prescribed medications potentially high-risk with opioids (i.e., antidepressants, anti-convulsants, muscle relaxants), but there are perpetually new drugs of choice emerging for abuse. Both Millennium and Progressive Medical/PMSI have ongoing studies to further demonstrate the economic impact of systematic drug monitoring, as well as which patients and at what point they should be referred for pharmacogenomic testing. Additionally, the necessary frequency of monitoring needs to be settled.

"To realize the benefit, the frequency has to be frequent enough to be systematic," says Passik. "Once a year is not enough and three times a week is too much. The truth will lie in the middle somewhere."

Passik says that in the future, throughout medicine, he believes there will be increased testing for medication adherence, even outside of opioids. "Labs will be in a better position to help with adherence—to get information to doctors and to package it in a way that they can understand," says Passik. "With more testing, overall patients will be more adherent, and pain patients, in particular, will be safer, if taking their medications as prescribed and avoiding illicit drugs."

Takeaway: Continuing drug monitoring in those prescribed chronic opioid therapy will likely increase in the coming years as part of a trend toward greater emphasis on adherence in clinical care. Data obtained from this systematic monitoring both saves payers money and enhances medication safety for patients. G2

New Guidelines, High-Cost Treatments Driving Surge in HCV Testing

Laboratories can expect to see a surge in hepatitis C virus (HCV)-related testing given adoption of expanded guidelines calling for one-time screening of all baby boomers, who are now more likely to undergo screening given the availability of a new generation of effective HCV treatments. However, this surge in HCV testing is not likely to last long term after the initial wave of screening and associated treatment passes.

"In light of the policy implications of greater screening coverage and the emerging therapeutic trends in the clinic, we believe clinical labs will play an increasingly important and visible role to the investment community as the hepatitis market gains steam over the next 12 to 24 months," writes Darren Lehrich, an analyst with Deutsche Bank, in a research note from March.

National estimates say that there are likely more than 3 million people chronically infected with HCV in the United States, but more than half of them are unaware that they are infected. The availability of new, high-cure-rate, direct-acting anti-viral drugs is causing great enthusiasm for screening and treatment, but their high cost is giving insurers and many providers pause, possibly dampening the enthusiasm for screening.

"The cost of treating chronic HCV infection is set to increase by untold billions of dollars a year," writes co-author Robert Steinbrook, M.D., in an editor's note published May 5 in *JAMA Internal Medicine*. "At current projections, the cost of directly acting antiviral agents for HCV is likely to quickly overwhelm the budgets of any state Medicaid program or any private insurer."

In the United States, the costs of 12 weeks of therapy for drugs alone can range from $66,360 for simeprevir to $84,000 for sofosbuvir, or roughly $1,000 per tablet. Reports show that sofosbuvir (marketed as Sovaldi by Gilead) sales were $2.3 billion just in the first three months of 2014. According to Reuters, UnitedHealth Group Inc., the largest U.S. health insurer, spent more than $100 million to cover Sovaldi treatment in its initial months on the market, an amount that was "multiple" times what UnitedHealth had expected.

This, experts say, is partially explained by the pent-up demand for new, effective treatments but also due to company-sponsored, direct-to-consumer advertising to encourage HCV testing in asymptomatic persons.

"The most significant brake on enthusiasm for the new direct-acting antiviral drugs is their potential addition of many billions of dollars of costs to an already burdened health care system," writes Daniel A. Ollendorf, from the Institute for Clinical and Economic Review (Boston), in another paper published May 5 in *JAMA Internal Medicine*. "Unless the prices of the new drugs decrease, public and private insurers face an untenable spike in short-term costs and will be forced to develop stringent patient eligibility criteria as the only way to manage the tension between access and affordability."

These efforts to increase access can be seen in a final Decision Memo for Screening for Hepatitis C Virus published by the Centers for Medicare and Medicaid Services (CMS) June 2. CMS will cover a screening test for adults at high risk for hepatitis C virus infection, defined as a current or past history of illicit injection drug use, having received a blood transfusion prior to 1992, and all other adults born from 1945 through 1965.

"We believe the CMS action could further accelerate the HCV testing market beyond what was originally set forth by U.S. Centers for Disease Control and Prevention and the U.S. Prevent[ive] Services Task Force," writes Lehrich. "Our model sizes the HCV testing market at ~$300 million per annum for the foreseeable future, and assumes the following: (1) ~10 percent of the 80M baby-boomer cohort gets tested each year; (2) ~3 percent of the screened population cohort tests positive for HCV (2012 CDC report cites 3.25% prevalence in baby-boomer cohort) with a one-time test (86803—Clinical Laboratory Fee Schedule [CLFS]~$19/test); (3) patients placed on therapy receive a one-time genotype test (87902—2014 CLFS ~$351/test) and then once on therapy are tested four times with follow-up RNA quantitative tests (87522—2014 CFLS ~$58/test) as well as a battery of routine tests per guidelines (80053/84443/G0306—2014 CLFS ~$48 total for tests)."

Lehrich adds that the reimbursement rates in his model are assumed to be 15 percent below current CLFS rates, reflecting a mix of discounted patients and potential for future CLFS reductions.

"We will start to see primary care physicians screen the birth cohort in agreement with guidelines when they feel they can improve quality of patient care," Nancy Reau, M.D., a member of the American Liver Foundation Medical Advisory Board, tells *DTET*. "With the simplicity of the newer therapies, I strongly think we will see primary care doctors screening and treating their own patients. Like, with H. pylori, they will just send nonresponders [to specialists]."

Reau says it is important that laboratories are prepared for this influx of testing by getting rid of archaic options and focusing on sensitive assays with low thresholds for detection for monitoring.

Takeaway: Laboratories can expect to see a surge in HCV-related screening and testing in light of recent expanded guidelines and greater availability of new, effective treatments. This wave will be passing as higher cure rates are achieved and unknowingly infected baby boomers receive one-time screenings.

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Inside the Diagnostics Industry

BRCA Testing Growing; Market Increases Seen With New Players, Intended Populations

The 2013 revelation of Angelia Jolie's BRCA status and the U.S. Supreme Court's ruling invalidating the patentability of the BRCA gene the following month thrust BRCA testing into the limelight. Already recognized in clinical circles as perhaps the most prominent example of how knowledge of genetic variants can be used to reduce disease risk, the high-profile status the BRCA gene garnered in lay media increased public awareness of genetic testing and its potential implications. Given the anniversary of both of these events, *DTET* examined how BRCA testing has changed over the course of the past year and how the rapidly expanding market will evolve in the coming years.

The most immediate implication of the Supreme Court's unanimous decision against gene patenting in June 2013 was competition for Myraid Genetics (Salt Lake City), which had previously held exclusive testing rights for all BRCA testing in the nation. Not only did other laboratories, such as Ambry Genetics and GeneDx, immediately begin offering a BRCA test, but additional entrants have entered the market throughout the year, including national laboratories LabCorp and Quest Diagnostics.

Competition

"BRCA testing now has quite a lot of competition," Amanda Murphy, an analyst with William Blair & Co., tells *DTET*. While intellectual property litigation is ongoing, Myriad has no IP protection and if they do, the other companies believe they can work around it. You now have a free-for-all."

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>
> ***—Amanda Murphy, William Blair & Co.***

Competition is entering the marketplace in many forms. For some laboratories, BRCA testing is their first next-generation sequencing (NGS)-based test, while others have NGS experience but now have the freedom to offer the test or to add the mutation to panels.

CLIA-certified Counsyl (San Francisco) became one of the newest entrants in the BRCA market with its May announcement of the launch of its Inherited Cancer Screen. The company, however, was not new to NGS-based testing as it had adopted the upgraded platform for its Family Prep Screen test.

"We had already booted NGS as a platform doing our Family Prep Screen, a recessive carrier screening test. So we were comfortable with it to move into inherited diseases, and BRCA was fairly natural, given our patient experience," Eric Evans, a co-founder and chief scientist at Counsyl, tells *DTET*. "We began development before the Supreme Court announcement because it was seeming obvious the direction it was headed."

Evans characterized the uptake of the test as "fairly substantial." The company also announced it raised $28 million in Series D financing that will further expand commercialization of the inherited cancer test, which the company offers for $999 (cash price), substantially lower than the $3,000-plus price tag for Myriad's BRACAnalysis test. Declines in BRCA test pricing have been heralded by patient advocates as one of the advantages of an increased number of players in the market.

Medicare will reimburse the test at a rate of $2,184 in 2015, substantially lower than Myriad's historic price.

Private payers are also noticing cost and emerging evidence shows that they will direct testing volumes. In late May it was reported that two insurers (Horizon Blue Cross Blue Shield of New Jersey and Amerigroup) independently notified health care providers in their networks to discontinue using Myriad for BRCA testing and to rely instead on in-network laboratories for BRCA testing.

In addition to a broad financial and competitive impact, Murphy says that the Supreme Court decision has caused diagnostics industries to question their intellectual property (IP) positions.

"Composition of matter claims had traditionally been stronger than method claims, but it didn't hold up," explains Murphy. "Generally, the strength of IP to protect a market position has gone down and companies must differentiate in other areas, like with harder to replicate clinical data. Generally publication of validity and clinical utility becomes the mode, rather than IP."

BRCA Market Variability

- Cost $999 to $2,870
- Turnaround time 14 days to 29 days

Source: William Quirk, Piper Jaffray.

Murphy stresses that the technical component is not the biggest challenge in launching BRCA testing. The more difficult aspect is understanding the clinical significance of variants. This is where, despite efforts to construct a public database, some believe Myriad maintains an advantage with its vast proprietary database. However, with the trend toward panels, rather than single-gene tests, Myriad's advantage dwindles.

Increasing Use of Panels

The Supreme Court's decision permits the inclusion of BRCA analysis in broader, inherited cancer panels that are permeating the commercial market. This trend toward higher complexity is one that industry experts expect to continue, especially as the cost of sequencing continues to decline. The outstanding question is how many clinically relevant variants should be included. In the midterm it is believed targeted panels will be preferred over whole-genome and whole-exome sequencing due to the ability to limit analysis to actionable genes.

Even Myriad is migrating its BRCA testing away from the BRACAnalysis test to its myRisk panel. The NGS panel analyzes 25 genes associated with eight hereditary cancers, including breast, colon, ovarian, endometrial, pancreatic, prostate, and gastric cancers, and melanoma.

Payers are showing early commitments to migrating to these panels. Early in May, Myriad signed a three-year deal with UnitedHealthcare for coverage of Myriad's myRisk panel for beneficiaries who meet certain eligibility criteria for hereditary cancer risk. Murphy says it is encouraging that a large payer sees benefits from testing additional genes. She says the agreement even allows for incremental gene testing in previously tested patients. Myriad called the agreement "transformational" and said it was the first major coverage decision by a payer for a multigene panel.

Future Testing

Without a doubt the "Jolie dilemma" boosted BRCA testing volumes, and its effect may continue to drive a shift in testing volumes in the coming years.

"The benefit of competition and a broadening market is greater BRCA awareness," says Murphy. "It is not a zero-sum game and [every competitor] is not taking away from Myriad. The market has grown."

Continued growth will emerge in part from changes to clinical guidelines, which are both expanding which patient population the test is relevant for as well as for additional BRCA components. But the larger growth opportunity is expected through greater penetration of the asymptomatic market.

"The oncology market—those diagnosed with breast or ovarian cancer—is relatively penetrated. The real growth will be in asymptomatic people—those with a family history," explains Murphy. "It is underpenetrated. Less than 10 percent of them get the test, so there is a lot of growth there."

Also, there are drugs that are poly ADP-ribose polymerase inhibitors that will only work with BRCA mutations, so if these drugs are approved in 2014 or 2015 it will further expand the market for BRCA testing in all patients as a companion diagnostic.

Despite optimism over the future growth of BRCA-related testing, the fierce competition among the new market entrants has raised some concerns among genetic counselors. In response to what they call "aggressive and manipulative tactics" in which genetic counseling centers have been pressured by clinicians with financial ties to specific laboratories and reports of laboratories threatening to "siphon-off referring clinicians if their laboratory isn't used," counselors from the Yale Cancer Center Genetic Counseling Program posted a "Genetic Testing Lab Position Statement." They call on providers to utilize "patient-centric criteria, including open access to data, to make decisions about genetic testing laboratories."

> ***"The benefit of competition and a broadening market is greater BRCA awareness. . . . The market has grown."***
> ***—Amanda Murphy***

While acknowledging some caveats in which laboratory choices "must sometimes be based on insurance plan regulations, test availability, or the lab's previous experience with a rare familial mutation," the counselors say that decisions on laboratory selection should be based on quality, turnaround time, cost, and open access to data generated from testing.

"Whenever possible we will choose laboratories that have pledged to make all of their past, present, and future gene data publicly available in order to allow this important information to be freely accessible to all clinicians and researchers, to further the advancement of medical knowledge and to best serve patient care," write the counselors in a supporting blogpost.

"The benefits of more market entrants is that it raises the profile to make the test more routine, and of course there has been a cost reduction," says Evans. "But it has also made testing more open with data-sharing, which is a positive trend."

Takeaway: BRCA testing volumes are likely to grow in the short term as more asymptomatic patients with a family history of breast and ovarian cancer seek testing due to increased public awareness. A growing number of market entrants will service this volume with a decided shift toward multigene panels for hereditary cancers.

DTC Testing May Pose Actual Clinical Risk

Misinterpretation of direct-to-consumer (DTC) genetic testing results could pose a clinical risk to individuals, according to a case study published in *Clinical Pharmacology & Therapeutics*. In the reported case, rare genotypes for the gene encoding thiopurine methyltransferase (TPMT) were misinterpreted by a DTC company and could have affected dosing treatment decisions. The authors say that professional interpretation and test validation, comparable to a CLIA-certified lab, are necessary to ensure that DTC test results are not misapplied.

"We are not advocating the end of DTC testing, nor are we universally denouncing DTC genetic testing companies," the authors write. "However, we feel that the interpretations must be accurate and reasonable, with adequate and freely available interpretation support for consumers and physicians."

A male patient received a DTC genetic test (company unnamed) based on the standard HumanHap550 panel (Illumina) supplemented with a custom set of 25,000 additional single nucleotide polymorphisms selected by the company. His results report that for TPMT he has "one *3B mutation and one *3C mutation, a homozygous nonfunctional alleles pattern resulting in significant enzyme deficiency. A person with these mutations has an increased risk of toxicity when treated with thiopurine drugs at standard doses."

The report did contain the disclaimer that "the information contained in this report should not be used to independently establish a thiopurine regimen, or abolish or adjust an existing course of treatment" and that "only a medical professional can determine whether a thiopurine drug is right for a particular patient."

"In our opinion, these results need to be held to the same standards as clinical institutions'," co-author Catherine Brownstein, from Boston Children's Hospital, tells *DTET*. "Pharmacogenomics serves no other purpose than clinical. It is not entertainment."

Members of the Boston Children's Hospital Clinical Pharmacogenomics Service (CPS; which provides nonpediatric outpatient consults) questioned some general technical aspects of the finding and further questioned the probability of the results, given this was the third incidence of this specific *3B/*3C call in three, unrelated, ethnically diverse test recipients. CPS decided to genotype the patient's family (parents, wife, and children) using Sanger sequencing in a CLIA-certified laboratory (Claritas Genomics; Cambridge, Mass.), which confirmed his *1/*3A status, demonstrating the DTC summary is "misleading," the authors say.

"The most reasonable interpretation of the genotype was not presented clearly, and patients may not realize that they need interpretative support," write the authors. "These tests should be held to the same standards as clinical institutions[']because of the burden or risk for the prescriber. Repeating testing may not be possible with time [critical] dosing decisions."

Shannon Manzi, a study co-author, tells *DTET* that retesting is needed in half of cases they see, particularly if the patient-reported test results are not able to be tracked down at another institution, if they were performed as part of a research protocol or not in a CLIA-certified laboratory, or sometimes if it is necessary in order for the patient to accept a different interpretation.

So what harm was done? While acknowledging that cases like this are likely not "widespread," Brownstein tells *DTET* misapplication of test results is "possible." If the patient

required 6-mercaptopurine treatment for his Crohn's disease and the treating physician had based treatment on the *3B/*3C status in the report, "it would not be catastrophic," but he would be prescribed a dose 10 percent of standard versus a dose reduced 30 percent to 70 percent with the *1/*3A genotype, the patient actually has, leading to potential disease progression, worsening of symptoms, or unnecessary discomfort.

However, they write, had the patient had leukemia, which is standardly treated with a thiopurine, and the DTC result was used to inform treatment (a scenario the authors call not likely, but possible) there could have been "serious consequences including progression of disease" from the "dramatic" dose reduction called for with the reported genotype.

"This data needs to be supported with adequate disclaimers, so that consumers can understand there are risks. Like any other test, DTC genetic testing is not immune from risks," Brownstein says.

Takeaway: This case study provides the first evidence of potential harm resulting from misinterpretation of DTC genetic test results. Enhanced validation requirements may make DTC pharmacogenomic results more clinically relevant. G2

Direct-to-Patient Reporting Genetic Results Through ePHRs

Web-based disclosure of genetic screening results through electronic patient health records (ePHRs) is both feasible and acceptable to cancer patients, according to a study published in *Genetics in Medicine*. ePHRs are being studied for their effectiveness in meeting new requirements for direct return of results to patients, as well as expanded meaningful use criteria, but haven't been widely used for returning genetic test results.

Per institutional standards, immunohistochemistry (IHC) testing was performed for mismatch repair (MMR, MLH1, MSH2, MSH6, and PMS2 expression) in colorectal and endometrial cancer specimens for identification of Lynch Syndrome carrier status. For study participants, MMR results were returned via institutional ePHRs. An automated e-mail message alerted participants that the result was available with a reminder e-mail for those not viewing results within three days. Participants completed a distress measure by telephone within 36 hours of viewing the result.

The researchers found that more than half of the approached patients (65 of 121) were ineligible for study participation due to lack of Internet access or use of the Internet or e-mail less than once per week. Nonwhite patients and those 65 years of age and older were significantly more likely to be ineligible. Of those eligible, 74 percent consented to participate, exceeding the first feasibility benchmark of at least 50 percent participation.

Forty-nine participants had MMR results successfully posted to their ePHR, with 89 percent viewing the results. Participant-rated acceptability of the study was high, with 97 percent having a mean score of 4 or higher on a 7-point scale. There were no differences seen between anxiety levels, regardless of result. The majority viewed their result within 24 hours after posting, although some took up to 18 days. The "More Information" hot link was used by over three-quarters of participants, including four of the five participants with abnormal results. An equal percentage of participants, regardless of result, reported discussing their result with their doctor.

The authors, led by Michael J. Hall, from Fox Chase Cancer Center (Philadelphia), say they deliberately chose IHC testing so that the pilot study would be applicable to community settings without on-site molecular testing capabilities, but they believe centers with more comprehensive universal MMR testing protocols could develop ePHR-based notification modules that report results of all elements in a summary format. The researchers will conduct a randomized trial to assess if a Web-based educational intervention coupled with the MMR screening result can improve downstream outcomes of MMR screening, including uptake of genetic testing among family members at risk for Lynch Syndrome.

Takeaway: As laboratories and providers explore efficient means for direct return of results to patients, ePHRs seem to be acceptable for return of genetic test results. G2

Circulating Cell-Free RNA Provides 'Dynamic' Diagnostic Picture

Measuring circulating cell-free RNA can provide a noninvasive, tissue-specific way to monitor fetal development and changes in disease status, according to a study published May 5 in the *Proceedings of the National Academy of Sciences*.

The authors say that RNA, a measure of gene expression, provides a more dynamic picture of health and development than the snapshot provided by DNA in applications like noninvasive prenatal testing, molecular tissue-typing, and tumor characterization.

"We've moved beyond just detecting gene sequences to really analyzing and understanding patterns of gene activity," said senior author Stephen Quake, Ph.D., Stanford University (Palo Alto, Calif.), in a statement. "Analyzing the RNA enables a much broader perspective of what's going on in the body at any particular time."

Using a combination of microarray and next-generation sequencing technologies, the researchers were able to characterize the sequences and relative levels of RNA in the blood of pregnant women, healthy volunteers, and Alzheimer's patients. By focusing on RNA messages, encoding proteins produced only in certain tissues, the researchers were able to track the relative proportions of specific RNA circulating and could assess the development or health of particular organs over time.

By analyzing combined cell-free RNA transcriptomes (100 genes whose RNA transcripts contained paternal single nucleotide polymorphisms that were distinct from the maternal inheritance) in 11 pregnant women across all three trimesters, the researchers were able to track longitudinal phenotypic changes and trace the development of specific tissues, including the fetal brain, liver, and placenta. The weighted average fetal fraction of cell-free RNA increased from 0.4 percent in the first trimester to 3.4 percent in the second trimester and 15.4 percent in the third trimester. The increase in the number of genes detected across the different trimesters suggests that these unique transcripts are expressed specifically during particular time intervals in the developing fetus.

Additionally, by comparing neuron-specific transcripts from blood samples of both healthy adults and those with Alzheimer's disease, disease-specific neural transcripts were identified at increased levels in affected participants.

"We think of this technique as a kind of 'molecular stethoscope,' and it's broadly useful for any tissue you care to analyze," said Quake. In the paper he adds, "We anticipate

these results are a stepping stone toward translating the temporal dynamics of plasma mRNA for clinical diagnosis of pregnancy-associated complications and developmental diseases, especially those that are temporal in nature and involve cellular apoptosis."

Takeaway: RNA may prove to be a broadly applicable diagnostic marker, as it provides a dynamic picture of disease state and development, yet can be tissue-specific.

New Multimarker Panel May ID Pancreatic Cancer Earlier

A panel of four blood biomarkers, comprised of the previously identified pancreatic cancer biomarker CA 19-9 plus three new protein biomarkers, can successfully identify individuals with pancreatic cancer from those who have other pancreatic conditions, according to a study presented at the American Association for Cancer Research's special conference on pancreatic cancer (May 18-21; New Orleans).

Blood-based biomarkers could markedly improve the ability to identify early-stage pancreatic cancer.

Most of the time, pancreatic cancer is identified too late for good outcomes, with only 10 percent of pancreatic patients presenting with localized disease. The previously identified CA 19-9 marker for pancreatic cancer has been of limited utility and is not detectable in 5 percent to 10 percent of subjects with fucosyltransferase deficiency. So researchers have undertaken further validation of a multimarker panel intended to complement CA 19-9.

A training set of plasma samples from 138 pancreatic cancer patients and 81 controls (52 healthy subjects and 29 subjects with chronic pancreatitis) yielded a combination rule which was then tested in plasma samples from an independent cohort of 42 early-stage pancreatic cancer patients, 50 healthy controls, 29 subjects with chronic pancreatitis, and 14 subjects with benign pancreatic cysts.

In these analyses, CA 19-9 alone correctly identified as negative for pancreatic cancer blood samples from 76 percent to 78 percent of the time in the cohorts, compared with 90 percent to 94 percent for the four-biomarker panel. Negative predictive values (NPVs) at 98 percent sensitivity were substantially improved compared to CA 19-9 alone when identifying early-stage pancreatic cancer versus healthy controls and subjects with chronic pancreatitis or benign cysts (NPVs of the panel = 0.96, 0.95, and 0.88, respectively versus NPVs of CA 19-9 alone = 0.83, 0.83, and 0.88).

"Our biomarker panel was much better at distinguishing patients with pancreatic cancer from those who were healthy, had chronic pancreatitis, or had pancreatic cysts compared with CA 19-9 alone," said lead study author Ayumu Taguchi, Ph.D., M.D., from MD Anderson Cancer Center (Houston). "This means that our panel has the potential to substantially reduce the number of patients who would have to undergo extremely invasive screening procedures."

Taguchi tells *DTET* that the panel still requires further validation, including in prediagnostic samples. He plans to license the intellectual property behind the test.

Takeaway: While not ready for clinical use, the use of protein biomarkers along with CA 19-9 offers hope that a noninvasive test will be able to diagnose pancreatic cancer at an earlier stage, when improved outcomes are possible.

G2 Insider

Automated Urinalysis Underreports Signs of Kidney Damage . . . Manual microscopy outperforms automated urine analysis, according to a small study presented at the National Kidney Foundation's 2014 Spring Clinical Meetings (Las Vegas; April 23-26). Automated urinalysis underreported the value of granular casts in a cohort of acute kidney injury (AKI) patients, a key determinant of the underlying cause of kidney damage.

The researchers compared results in the reported ranges of granular and muddy brown casts using manual microscopy and an automated urine analyzer (IRIS 200 system) for evaluation of 10 samples from patients with acute kidney injury. A single observer analyzed the same urine sample with both technologies, spending an average of 10 minutes per patient specimen. An attending physician validated the first count. Manual counts were divided by 144 to approximate the automated system's high-power focus (HPF). Results were characterized by number of casts as none, few (0 to 5 casts/HPF) and many (21 to 50 casts/HPF).

The researchers found that there were significant differences between the results produced by each modality. Manual microscopy coded no patient as having "none," while the automated system coded 70 percent as "none." Manual analysis coded 100 percent of specimens as "few." The automated system coded two samples as "many," which manual microscopy characterized as "few."

"Nowadays most hospitals use some form of an automated urinalysis system, and how often the data is actually supplemented with manual microscopy is hard to quantify," said lead author Natasha Sharda, M.D., of the University of Arizona, in a statement, acknowledging that the automated systems can offer a cost, labor, and efficiency benefit. Manual microscopy can take up to six minutes per sample compared to less than one minute for automated systems. "The automated system still has utility as a screening test, but manual microscopy should be done in all cases of abnormal kidney function, as accurate quantification of casts could have some prognostic benefit to patients," adds Sharda.

Joseph Vassalotti, M.D., the chief medical officer of the National Kidney Foundation, tells *DTET* that AKI complicates at least 1 percent of hospital admissions in the United States, including especially critically ill patients and those undergoing cardiac surgery. Vassalotti, says that while initial reaction would be to assume that detecting more casts in manual examination is better, he urges further studies to determine if this discrepancy in results has prognostic implications with regard to renal recovery, dialysis dependency, or mortality.

Company References

American Liver Foundation 212-668-1000

Boston Children's Hospital 617-355-6000

Centers for Medicare and Medicaid Services 800-633-4227

Counsyl 888-268-6795

Fox Chase Cancer Center 215-728-6900

Millenium Laboratories 858-217-3800

Myriad Genetics 801-584-3600

Progressive Medical/ PMSI 800-777-3574

Yale Cancer Center Genetic Counseling Program 203-764-8400

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