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Lab Institute
October 14-16, 2015
Hyatt Regency Washington DC on Capitol Hill
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Nanopore Sequencing Improvements Generate Enthusiasm

In February 2014, Oxford Nanopore Technologies (United Kingdom) rolled out the MinION nanopore-based sequencer. Initial user descriptions indicated that the device failed to live up to the company's promises—it was slower than expected and made "a worrying number" of errors. But Oxford Nanopore has made improvements and pilot users are excitedly publishing results and testing the use cases for the palm-sized sequencer.

Recent publications by pilot users have shown that a MinION can reliably sequence small genomes, such as bacteria. It can discriminate between closely related bacteria and viruses, read complex portions of the human genome, and differentiate between the genetic variants. Field trials have included Ebola sequencing in West Africa, biodiversity sequencing in forests and rain forests, and reportedly even upcoming trials in space. Researchers are hopeful that if the MinION can be modified to run on a smartphone instead of a computer, there could be endless field applications. Early users say the MinION's benefits are its real-time, portable nature and its "super-long" reads. The palm-sized device plugs into a laptop's USB port and data is displayed on the screen as generated, rather than at the end of a run.

Oxford Nanopore recently held its first user conference dubbed London Calling (London; May 14-15). Among the highlights of the early users were:

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Test Offers Hope for Catching Ovarian Cancer Earlier

The risk of ovarian cancer algorithm (ROCA), based upon serial measurements of cancer antigen 125 (CA-125), can double the number of screen-detected ovarian cancers, compared with a single measurement of the marker. The test, developed by Abcodia (United Kingdom) is set to be released both in the United States and the United Kingdom later this year.

"The test represents a unique opportunity to address a significant clinical unmet need for any post-menopausal woman or pre-menopausal women with a clear family history of ovarian cancer," said Julie Barnes, Ph.D., cofounder and CEO of Abcodia, in a statement. Recent clinical trial results "highlight the sophistication of ROCA in being able to accurately distinguish between asymptomatic women with and without ovarian cancer, despite in many cases, their serum CA125 level appearing within the normal range."

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Nanopore Sequencing Improvements Generate Enthusiasm, *Continued from top of p.1*

- Nick Loman, Ph.D. (University of Birmingham, United Kingdom) presented his experience using the MinION to identify Salmonella as the source of food poisoning in just 100 minutes of sequencing (30 minutes for species identification; 50 minutes for serotype; 100 minutes for full characterization).
- Jared Simpson, Ph.D. (Ontario Institute for Cancer Research, Canada) produced a complete Escherichia coli genome assembly with 98.4 percent nucleotide identity, compared to the reference genome.

One of the most talked-about presentations at London Calling was Oxford Nanopore's Chief Technology Officer Clive Brown's company update. It outlined improvements and specs for the company's next round of sequencers. In response, industry watchers called the company's announcements "game-changing," "revolutionary" and possibly heralding the arrival of field or at-home sequencing.

- **Sample Prep** - Brown announced the company's goal is to get sample prep down to 10 minutes. The company unveiled Voltrax, a fully integrated, nanoliter volume, automated sample prep solution, that will be available later this year. It is portable, programmable, and disposable, with between six and 12 sample input ports. It automates the entire sample prep process and loads directly onto the MinION and PromethION. It was reported that this significant improvement in sample preparation should allow direct sequencing from blood, with a time to results (for targeted sequencing or species identification) of about 30 minutes from sample collection.
- **High Performance Circuits** - The company announced upgrades to the ASIC (Application Specific Integrated Circuit), the core of the PromethION and MinION sequencers, which controls and measures the ionic current flow. The current ASIC chip has 512 channels, but the new chip will have 3,000. Oxford Nanopore announced that they are separating the nanopore sensing membrane from the ASIC, which will permit better manufacturing control of the two and allow for reuse of the ASIC, which will lower consumable costs.
- **Throughput** - "Fast mode" will improve pore speed. "Normal mode" reads at 30 bases per second, but the new "fast mode," which will be released within a few months, will read at 500 bases per second, with no decrease in base-calling quality. Experts say that "fast mode" makes the MinION comparable in throughput and cost to HiSeq.
- **PromethION** - A prototype of the PromethION was unveiled at the conference. The device will be launched in a restricted, early-access program later this year. The PromethION is essentially 48 MkII flow cell units, providing 144,000 sequencing channels that can work in unison on the same sample, individually, or multiplexed. The laptop-sized device can accommodate one to 192 samples.
- **Pricing** - Brown also announced "pay-as-you-go" sequencing with a "zero-hour" flow cell (although "full flow cells" are still available). It was released that for the MinION MkI flow cell, the first three-hour block is to be priced at $270, but for the MkII, available next year with the new 3,000-channel ASIC, pricing will begin at $20 for the first hour of sequencing. The cost will then drop for additional sequencing hours on the same flow cell.

Takeaway: Advancements in Oxford Nanopore's MinION are making real-time, field and bedside testing a seeming possibility in the not too distant future.

Test Offers Hope for Catching Ovarian Cancer Earlier, *Continued from bottom of p.1*

In mid-May, Abcodia announced raising an $8 million round of venture capital financing, which will allow it to launch the ROCA test in the United Kingdom this summer (following CE Mark) and in U.S. markets later in 2015 as a laboratory-developed test. In the United Kingdom, Abcodia says, the test will cost about $229.

Detecting changes in an individual, rather than relying on populational averages for cutoff thresholds, can "substantially increase the power of a test" by reducing both false positives and negatives.

The ROCA test, the company says, is more effective than traditional, single measurements of CA125, which raise clinical suspicions based on a fixed cutoff (35 unit per mL). Abcodia says that longitudinal algorithms allow the personalization of disease detection by measuring biomarker levels in an individual over time. Detecting changes in an individual, rather than relying on populational averages for cutoff thresholds, can "substantially increase the power of a test" by reducing both false positives and negatives. Previous modeling studies using data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial have suggested that up to a third of the ovarian cancer cases could have been detected earlier if CA-125 velocity (changes in the marker) had been used instead.

According to a study published May 11 in the *Journal of Clinical Oncology*, screening for ovarian cancer with ROCA doubled the number of screen-detected invasive epithelial ovarian or tubal cancers (iEOCs) compared with a fixed CA125 cutoff. The large study analyzed data from 46,237 women (aged 50 years or older) participating in the United Kingdom Collaborative Trial of Ovarian Cancer Screening. These women had been randomized to undergo incidence screening using the multimodal strategy (MMS). Measurements of annual serum CA-125 levels were interpreted with ROCA and women's predetermined screening and care was triaged based on their risk categorization.

The researchers found that from June 25, 2002 to Dec. 21, 2011, 296,911 incidence screens were performed and 640 women underwent surgery. Of those, 133 had iEOCs. Overall, 22 interval iEOCs occurred within one year of screening, of which one was detected by ROCA but was managed conservatively after clinical assessment. The sensitivity and specificity of MMS for detection of iEOCs were 85.8 percent and 99.8 percent, respectively. ROCA alone detected 87.1 percent of the iEOCs while fixed CA-125 cutoffs at the last annual screen (>35, >30, and > 22 U/mL) would have identified 41.3 percent, 48.4 percent, and 66.5 percent of the cases, respectively. At the last relevant annual screen, median serum CA-125 in the 133 women with screen-detected iEOCs was 33.6 U/mL, with more than half (52.6 percent) of these women having CA-125 levels within the normal range (35 U/mL or less). All cancer-free women were ruled out, when ROCA was used in conjunction with an ultrasound.

"Our current findings are of immediate importance because they highlight the need to examine serial change in biomarker levels in the context of screening and early detection of cancer," write the authors, led by Usha Menon, M.D., from University College London (United Kingdom). "Reliance on predefined single-threshold rules may result in biomarkers of value being discarded."

Takeaway: ROCA, with its serial measurements of CA125, represents a significant opportunity to improve earlier detection of ovarian cancer. G2

Inside The Diagnostics Industry

Confusion Hampers Adoption of New Prostate Cancer Screening, Biopsy Strategies

The goals are clear. New testing strategies are needed to identify men at greater risk of prostate cancer (PC) who would benefit from routine screening. Then, test development needs to focus on assays that can better distinguish men who have low-grade PC from those with aggressive, high-grade cancer—those who need a biopsy and those that can be monitored with active surveillance versus those who need immediate intervention.

While the goals are universally accepted, strategies for PC screening and testing for those with suspected PC have grown increasingly murky following the United States Preventive Services Task Force's (USPSTF's) 2012 final guideline against prostate-specific antigen (PSA) screening. The recommendation stems from the realization that PSA screening, while possibly contributing to a trend of decreased PC mortality, was also leading to an overdiagnosis and overtreatment of low-risk, non-aggressive disease—men who would die with, but not from PC.

PSA Testing Guidelines

While revised guidelines tend to reflect the need to consider both the benefits and downstream harms associated with PSA screening, testing guidelines vary by organization.

USPSTF recommends against PSA-based screening for PC in all age groups.

AUA recommends shared decisionmaking for men age 55 to 69 years that are considering PSA screening, and proceeding based on men's values and preferences. In this group, a routine screening interval of two years or more is suggested. The group does not recommend routine screening in men between ages 40 to 54 years at average risk, in men 70 years or older, or any man with less than a 10 to 15 year life expectancy.

American Cancer Society emphasizes the need for shared, informed, decisionmaking. Screening should be considered for men 50 years and older with a life expectancy of at least 10 years with an average PC risk; men 45 years of age at higher risk (African American men and those with a family history of prostate cancer before age 65 years); men 40 years and higher with a strong family history. Screening should be performed annually for men whose PSA level is 2.5 ng/mL or higher and every two years for men whose PSA level is less than 2.5 ng/mL.

Since the time the recommendation was made, PSA testing has definitely declined. What remains uncertain, though, is what are the best strategies to identify men at highest risk of PC who could benefit from ongoing screening, those who do not need a biopsy, and those who have low-grade disease that can be watched, rather than treated. Are there supplementary tests that can improve PSA test performance? Should molecular factors be incorporated into decisionmaking? The answer to both of these questions is yes. However, consensus remains lacking for the best approach going forward.

"There is confusion as to the many tests out there," Yves Fradet, M.D., co-founder and chief medical officer at DiagnoCure (Canada), tells *DTET*. "Upfront there are tests that may be a little more predictive than PSA to identify men at risk. Then there are companies offering biopsy-based assays to decide surveillance versus intervention. In between there are urine-based tests that give a completely different way of looking at the prostate."

DTET reviewed recent publications and conference abstracts to examine current trends in PC testing and how emerging evidence may shape future testing efforts.

PSA Testing Utilization

Evidence is emerging that there have been notable shifts in PSA testing utilization and higher stage of diagnosed disease since the issuance of USPSTF guidelines.

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- **Primary Care Physicians Ordering Fewer Tests** - PSA test ordering by primary care physicians has decreased significantly since the USPSTF recommendation, according to an abstract presented at the American Urological Association (AUA) 2015 annual meeting (New Orleans; May 15-19). Researchers from Oregon Health & Science University Hospital in Portland compared PSA testing frequencies in 12,345 men over age 40 years without a family history of prostate cancer, seen as new patients at the university's family medicine or internal medicine clinics (2008 through 2013). The researchers found that overall, 14 percent of men received a PSA test before the final recommendations (May 2012) compared to 7 percent afterwards. Differences in test utilization among men aged 50 years to 70 years accounted for the overall decreased utilization.
- **Less Screening May Be Tied to Higher-Grade Disease at Diagnosis** - Lack of regular screening may be leading to diagnosis of higher-grade disease, according to a study presented at the American Society of Clinical Oncology's 2015 Genitourinary Cancers Symposium (Orlando, Fla.; Feb. 26-28). Evaluating data from 87,562 men diagnosed with prostate cancer from January 2005 through June 2013, researchers from City of Hope (Duarte, Calif.) found the percentage of men with intermediate or higher risk cancer was stable (roughly 70 percent) prior to 2011, but rose by nearly 3 percent per year after 2011. The data consisted of merged tumor registries, representing cases from more than 150 U.S. hospitals.

Not Abandoning PSA

"The findings highlight the urology community's concerns about the USPSTF recommendations and underscore a need to pay close attention to our high-risk patients," said Sam Chang, M.D., AUA spokesperson, in a statement.

Despite the false positives associated with PSA screening, many in the urology field believe the test still has merit. Several abstracts presented at AUA 2015 suggest altering PSA testing strategies can be useful in better identifying men at risk for aggressive PC.

- **Longer Screening Intervals With Lower Baseline PSA** - For men with low baseline PSA levels (0.1 to 0.9 ng/mL), screening every 10 years with a PSA test may "dramatically" reduce the cost of screening as well as significantly reduce detection of "low-grade, potentially-inconsequential" PC. Researchers from University of Texas Health Science Center at San Antonio followed 2,923 men without previous PC and found that over a median of 7.4 years of follow-up, 302 cases of PC were diagnosed. Men with baseline PSA in the lowest three sextiles PSA (0.1 to 0.9 ng/mL) were at greatly reduced risk of being diagnosed with PC. There was a 2 percent to 5 percent 10-year risk of PC among those with PSA less than 1.0 ng/mL, compared to a 10 percent to 35 percent risk among those in the highest PSA sextile (2.3-9.9 ng/mL). Of the PC cases in the lower half of baseline PSA levels, 90 percent of the cancers were low-risk.
- **Combo of PSA Markers IDs Aggressive Cancer** - The Prostate Health Index (PHI), a new formula that combines three well-known biomarkers: total PSA, free PSA and [-2]proPSA, can discern aggressive prostate cancer from indolent or no

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cancer in a biopsy-naïve population, according to a multi-institutional group led by researchers from Emory University (Atlanta). Higher PHI values were significantly associated with Gleason 7 score or higher. At 95 percent sensitivity in detecting aggressive prostate cancer, PHI specificity was 36.0 percent versus 17.2 percent and 19.4 percent for total pre-biopsy PSA and free PSA, respectively. At 95 percent sensitivity, the PHI cut-off of 24 would prevent 41 percent of unnecessary biopsies.

PCA3, A Cautionary Commercial Tale

A test that measures overexpression of PCA3 (Prostate Cancer gene 3) can "substantially" reduce the number of men undergoing repeat biopsies without missing many high-grade cancers. The assay, approved by the U.S. Food and Drug Administration in 2012, can discriminate better than PSA between PC and non-cancerous conditions such as prostate enlargement or infection. The urine-based test scores the likelihood of a positive biopsy based on the ratio of PCA3 RNA molecules to PSA RNA molecules—a score of less than 25 is tied to a decreased likelihood of a positive prostate biopsy. A 2014 economic impact study showed that incorporating PCA3 testing into the decisionmaking process, cut unnecessary repeat biopsies by 37 percent.

DiagnoCure first in-licensed PCA3 in 2000, but the exclusive worldwide rights to the in vitro nucleic acid amplification Progensa PCA3 assay are now held by Hologic (formerly Gen-Probe), which pays DiagnoCure royalties for the test. However, according to DiagnoCure "a lack of a substantive marketing investment" by Hologic has led to royalty revenue shortfalls as the assay had not "penetrated the market at levels commensurate with DiagnoCure's expectations." In the fall of 2014, DiagnoCure announced layoffs, but continues to engage in "unrelenting efforts" to ensure Hologic "fully exploits the commercial potential" of PCA3 "as the clinical community continues to report positive results on DiagnoCure's PCA3 PC marker."

DiagnoCure says that 40 scientific papers were published on PCA3 since the beginning of 2014 and that the company pursued an "unsuccessful initiative" to purchase the entire prostate oncology business unit from Hologic in order to "regain all commercial PCA3 rights." While DiagnoCure is still hopeful a "beneficial outcome to the current situation could emerge," the company is focusing business development on the out-licensing of its PCP test, which does not include PCA3.

Incorporating Genetics

Increasingly genetics is playing a role throughout the entire PC testing process, from diagnosis to recurrence. In some cases molecular information is being used as a complement to refine PSA and other more traditional prostate-related measures. However, in other cases molecular results are being used to risk classify men in order to determine who should be getting screened, biopsied, or treated.

"Due to innovative genetic testing methods, we're getting smarter around not only the diagnosis of PC, but who needs surgery and which patients should watch their cancer. This is a huge shift in this field," said David Samadi, M.D., chairman of urology at Lenox Hill Hospital in New York City, in a statement. "We cannot treat each cancer, such as Gleason scores of 6, 7, or 8+, the same. With PC, we must individualize the care and now we can with new genetic testing diagnostic tools."

- **Germline DNA Can Risk-Stratify Screening** - Information from germline DNA is able to stratify men regarding their PC risk and may have implications regarding which men will benefit most from PC screening, according to a multicenter clinical trial presented at AUA. Patients were identified from the Cancer Genetic Markers of Susceptibility database (1,017 controls, 550 non-aggressive PC case, and 677 aggressive PC cases). The prostate genetic risk score (PGS) was calculated based on genotypes at 33 PC-associated single nucleotide polymorphisms, with a PGS of 1.0 indicating an average risk. The PGS, PSA, and family history were all significantly associated with PC diagnosis. Furthermore, when the PGS was divided into quartiles, there was a significant increase in the rate of prostate cancer detection beyond the PSA: 43.2 percent for quartile 1; 47.8 percent for Q2, 58.8 percent for Q3, and 69.4 percent for Q4, as well as an improvement in PSA performance when used with the PGS.

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Co-author A. Karim Kader, M.D., Ph.D., from University of California San Diego, tells *DTET* that he "wholeheartedly" believes genetic markers are the missing piece to improve PC testing and that within the next two years a test, such as the PGS, will be available to inform men and their physicians what their lifetime risk of PC is.

- **Genetic Markers Refine PSA Results** - Applying four genetic markers to clinical decisionmaking before a prostate biopsy can "correct" over-diagnosis of PC associated with PSA testing, according to an abstract presented at AUA by researchers from Northwestern University Medical Center (Chicago). A PC diagnosis could have been avoided "for a significant number of men with seemingly indolent PC" by dividing an individual's PSA value by his combined genetic risk. Analyses were used to compare the percentage of men who would meet commonly used biopsy thresholds before and after genetic correction. Among men who received PC surgery, genetic correction reduced the number of men meeting biopsy thresholds of ≥2.5 ng/ml and ≥4.0 ng/ml by 23 percent and 27 percent, respectively. Among men analyzed in the active surveillance cohort, the genetic correction could potentially reduce the number of biopsies and PC diagnoses by roughly 40 percent.
- **Multi-Gene Panel Differentiates Aggressive PC** - A urine-based multi-gene signature detected in first void following a digital rectal exam (DRE) provides more accurate detection of indolent versus aggressive PC than PSA, or two other molecular PC markers. Expression of these 11 genes quantified by reverse transcription-polymerase chain reaction. The study, presented by DiagnoCure (Canada) at AUA 2015, evaluated the multi-gene signature in men over 50 years referred for PC evaluation due to an elevated PSA or abnormal DRE. In 764 men (261 in the discovery study and 503 in the validation study) the test, the Prostate Cancer Panel Risk Score (PCP Risk Score) significantly outperformed PSA, as well as two genetic markers PCA3 and T2:ERG for the prediction of PC as well as high-grade PC. Men with a PCP Risk Score over 60 had 74 percent risk of PC, while men with PCP Risk Score less than 20 had only a 7 percent risk of high-grade PC.

  Co-author Fradet, tells *DTET* that the company is actively looking for a commercialization partner with "deeper pockets" that can help bring the test to market. Initial commercialization efforts in the United States will focus on the reference lab model, while in Europe the company will focus on a kit format for the test.

"These studies demonstrate progress toward more targeted approaches to prostate cancer screening and furthers our evolution from a 'one-size fits all' approach," said Scott Eggener, MD, a AUA session moderator from the University of Chicago Medicine (Illinois). "While more research is needed, these data show promise in new techniques to minimize unnecessary biopsies and more accurately identify high risk patients."

Takeaway: While there is no dominant testing strategy that will immediately replace routine PSA testing, the objectives are clear that tests capable of identifying men at greatest risk of PC can eliminate unnecessary biopsies and treatment, without missing aggressive disease.

Skin Test May Diagnose Alzheimer's, Parkinson's

Skin biopsies can be used to detect elevated levels of abnormal proteins associated with Alzheimer's and Parkinson's diseases (AD and PD), according to two studies presented at the American Academy of Neurology's annual meeting (Washington, D.C.; April 18- 25). The researchers showed that phosphorylated Tau (p-Tau) and α-synuclein (α-Syn) are present in the skin from patients with the conditions and that the protein levels can be quantified using image analysis techniques.

The current gold standard for the definitive diagnosis of neurodegenerative diseases is the demonstration of misfolded proteins in postmortem brain samples. Diagnosis in living patients is primarily based on clinical observations, but biomarkers of Alzheimer's and Parkinson's have long been sought.

"We hypothesized that since skin has the same origin as brain tissue while in the embryo that they might also show the same abnormal proteins," said study author Ildefonso Rodriguez-Leyva, M.D., from University of San Luis Potosi (Mexico), in a statement. "This new test offers a potential biomarker that may allow doctors to identify and diagnose these diseases earlier on."

The researchers presented findings from two related studies. First, they evaluated skin biopsies from behind the ear in 65 individuals (20 with AD, 16 with PD, 17 with non-neurodegenerative dementia, and 12 age-matched healthy controls). They measured the reactivity against antibodies for p-Tau and α-Syn both in sections of paraffin embedded tissue and in proteins extracted from tissue homogenates. Light and confocal microscopy identified protein aggregates by immunohistochemistry and their presence in the skin was confirmed through Western blots. Immuno-positivity was characterized by: percentage of positive cells, a semi-quantitative scale, and through image analysis software. The skin biopsies from AD and PD patients had significantly higher levels of p-Tau immunopositivity, compared both to controls and patients with non-degenerative dementia. Those with AD and PD had seven times higher levels of p-Tau protein. People with Parkinson's had an eight times higher level of α-Syn protein than the healthy control group.

In the second abstract, the researchers demonstrated quantification of the expression of p-Tau protein in skin cells by selective correction processing and image colorimetric analysis. Tau inmunopositivity obtained in pixels by selective correction in Adobe Photoshop and colorimetry in IPP were compared with the quantification estimated by manual counting of immunopositive cells assisted by a computer. Image colorimetric quantification of the immunopositive area, the authors say, allows for a more objective comparison.

Rodriguez-Leyva tells *DTET* that the test can be performed in a standard pathology laboratory with an immunohistochemistry area, but cautions that independent validation in broader ethnic samples of patients is necessary to prove the utility of the test. Clinical use of such a test could be five years away, he estimates.

Takeaway: With independent validation, the findings could represent a breakthrough in the ability to definitively diagnose AD and PD in living patients.

Testing Advances to Aid Rheumatoid Arthritis Management

There has been consistent recognition of the need for more objective, precise markers of rheumatoid arthritis (RA) disease progression risk to complement clinical assessments. Recent publications are indicating progress towards development of such tests that will improve monitoring and prediction of disease activity and can better inform treatment selection.

Markers of RA disease susceptibility are also indicative of disease severity and treatment response to tumor necrosis factor (TNF) inhibitor drugs, according to a study published April 28 in the *Journal of the American Medical Association (JAMA)*. The HLA-DRB1 locus, previously tied to disease risk, was found tied to disease outcomes and may be used to better inform RA management, experts say.

"The way forward in this area will be to use the current genetic findings as one component in the construction of updated prediction algorithms that consider additional genetic differences, environmental and life style factors, biomarkers, and clinical characteristics "

—David Felson, M.D.

The United Kingdom-based researchers utilized data from three large prospective cohorts for discovery as well as validation. Sixteen different HLA-DRB1 haplotypes (amino acids at positions 11, 71, and 74) were evaluated, but the researchers found that the strongest association with radiological damage was seen in RA patients with valine at position 11 of HLA-DRB1. By the five-year follow-up, the percentages of patients with erosions of the hands and feet were: 48 percent of noncarriers of valine at position 11, 61 percent of heterozygote carriers, and 74 percent of homozygote carriers. In patients with inflammatory polyarthritis, valine at position 11 also was associated with significantly higher all-cause mortality and better TNF therapy response (78 percent of noncarriers had moderate or good response versus 81 percent of heterozygote carriers and 86 percent of homozygote carriers).

"These findings may add to the ability to predict outcomes of RA, thus helping to optimize therapeutic strategies for different patients," writes David Felson, M.D., in an accompanying *JAMA* editorial. "The way forward in this area will be to use the current genetic findings as one component in the construction of updated prediction algorithms that consider additional genetic differences, environmental and life style factors, biomarkers, and clinical characteristics ."

Economics of Adding Markers of RA Activity into Care

A separate study evaluated the impact a multibiomarker disease activity (MBDA) test has in the management of patients with RA. The authors say that improvements in functional status and lower long-term costs for patients with RA due to treatment alterations based on test results contribute to the test's cost-effectiveness.

The Vectra DA test (Crescendo Bioscience [CB]; South San Francisco, Calif.) measures serum levels of 12 proteins and uses a validated algorithm to score the level of disease activity in patients with RA. In a study published April 15 in *Rheumatology*, researchers sought to quantify the downstream effect of using the test on RA-related functional outcomes and costs to private payers and employers. Clinical utility was measured by reported changes to anti-rheumatic drug recommendations following receipt of test results. CB funded the study.

Treatment alterations incorporating Vectra DA test results occurred in 38.0 percent of patient management decisions. Subanalysis indicated that almost 30 percent of these altered decisions increased treatment intensity (drug dosage, formulation, or

class), while nine percent of treatment changes resulted in decreased intensity. The researchers found that over 10 years, overall quality-adjusted life years (QALY) increased by 0.08 years and costs decreased by $457. Changes in treatment led to an increase in RA drug costs ($171 per patient in year 1 and $881 over 10 years). However, improvements in patient health status resulted in mean per-patient savings in other direct costs ($150 in year 1 and $661 over the 10 years). This cost savings increased to $6,011 when including both third-party costs and costs related to work productivity. When examining data by disease stage, for patients with early RA the test saved $3,073 and improved QALYs by 0.23 per patient versus adding $275 in costs and a QALY improvement of 0.02 in established RA patients.

"Over a 10-year period, the Vectra DA test costs approximately $22,000 per QALY gained, which is about half of what is considered to be cost-effective medicine," said Peter Meldrum, CEO of Myriad Genetics, on a May earnings call. (Myriad acquired CB last year.) "This ... is further evidence of the value that Vectra DA can bring to the health care system while significantly improving the quality of life for RA patients."

Takeaway: Emerging markers of RA disease severity and activity are improving clinical management, while possibly saving money in the long-term.

Novel Test May Be Able to Detect Drug Use From Fingerprint

Metabolites of drugs of abuse can be detected in fingerprints using mass spectrometry technology, according to a small study published May 1 in *The Analyst*.

Both fingerprints and oral fluid were analyzed from five patients attending a drug and alcohol treatment service. Oral fluids were tested using chromatography mass spectrometry, while fingerprints were analyzed using Desorption Electrospray Ionization (DESI) with Ion Mobility Tandem Mass Spectrometry Matrix Assisted Laser Desorption Ionization (MALDI-IMS-MS/MS) and Secondary Ion Mass Spectrometry (SIMS)."Natural" fingerprints were placed onto clean glass slides.

The researchers found that both DESI and MALDI were able to detect the cocaine metabolites benzoylecgonine (BZE) and methylecgonine (EME) in latent fingerprints. There was "good" correlation between fingerprint analysis and oral fluid testing, but the authors say that development of a quantitative test with a standard cut-off level is an important next step. SIMS' sensitivity was "insufficient."

> *"Companies are already working on miniaturized mass spectrometers, and in the future portable fingerprint drugs tests could be deployed."*
>
> —Melanie Bailey, Ph.D.

"The ability to detect excreted substances in latent fingerprints proves that surface MS techniques could provide the drug testing industry with an exciting new and complementary tool, potentially allowing differentiation between drug consumption and contact solely based on the presence of metabolites in the residue," writes lead author Melanie Bailey, Ph.D., from the University of Surrey (United Kingdom).

Fingerprints afford advantages over other noninvasive samples such as saliva, hair, and sweat including ease of sampling and transport and that the identity of the donor is captured within the sample, making the test impossible to falsify. MS-based analysis offers "a high level of selectivity," while only consuming a small portion of the sample fingerprint, allowing repeat analyses of a single sample. However, the authors caution that a sampling regime needs to be established to

address the lack of spatial uniformity among fingerprints. Additionally, a quantitative procedure is needed, as there is currently no accepted method for determining how much material was deposited in the fingerprint sample, as opposed to blood, urine or oral fluid, where the sample volume is known.

"Companies are already working on miniaturized mass spectrometers, and in the future portable fingerprint drugs tests could be deployed ," said Bailey in a statement.

Takeaway: With further validation and refinement of sampling procedures, analysis of metabolites of drugs of abuse through fingerprint residues may be possible in the field. G2

Genetic Pathway Explains Lack of Response to Asthma Treatment

By monitoring changes in gene expression, clinicians can identify children who will not respond to common asthma medications and may benefit from alternate treatment, according to a study published April 21, in the *Journal of Allergy and Clinical Immunology*. Nasal expression of the VNN1 gene may be a clinically useful marker to identify a biological cause for difficult-to-treat asthma.

Of the seven million children with asthma, it has been reported that nearly two-thirds have had at least one attack in the past year. While systemic corticosteroid treatment is considered the most effective medication for controlling chronic asthma and rescue during acute exacerbation, treatment efficacy varies.

In the current study, nasal epithelial cells were collected during presentation to the emergency department for an acute asthma attack and again 18 to 24 hours later. Genome-wide expression profiling was performed before and after treatment in 15 children as part of a discovery cohort. Gene expression ratios were analyzed to identify associations with corticosteroid treatment response phenotypes. These potentially discriminatory genes were then tested in a new cohort of 25 patients.

"These might be clinically useful biomarkers to identify children with a biologic cause for poor corticosteroid response who would benefit from a different treatment plan."

—Chang Xiao, M.D., Ph.D.

The researchers found that VNN1 mRNA expression was lower in the poor responder group versus the good responder group. After treatment, methylation levels at the CpG4 site significantly differed, with a decrease in methylation among the poor responders and an increase in the good responders. The researchers believe methylation at the CpG4 site might be a "crucial molecular event regulating VNN1 gene expression and modulating the response to corticosteroid treatment." Relatedly, changes in VNN1 gene expression after treatment were significantly inversely associated with hospital length of stay, with every unit increase in VNN1 expression tied to a decrease in stay by 7.7 hours.

"Nasal epithelial cells can be readily sampled safely during an asthma attack and reflect changes observed in the bronchial airways of asthmatic children," write the authors led by Chang Xiao, M.D., Ph.D., from Cincinnati Children's Hospital Medical Center in Ohio. "These might be clinically useful biomarkers to identify children with a biologic cause for poor corticosteroid response who would benefit from a different treatment plan ."

Takeaway: VNN1 gene expression may be a clinically important marker to improve suboptimal management of childhood asthma in difficult-to-treat patients. G2

G2 INSIDER

ASCO Calls for HBV Screen Before New Cancer Treatments

To address the low rates of hepatitis B virus (HBV) screening among cancer patients initiating anti-CD20 therapy or hematopoietic cell transplantation, the American Society of Clinical Oncology (ASCO) is updating a clinical opinion to incorporate a risk-adaptive strategy to better identify patients with HBV infection and reduce the risk of HBV reactivation. The provisional clinical opinion, published online May 11 in the *Journal of Clinical Oncology*, advises that screening tests for HBV should be performed on patients before starting anti-CD20 monoclonal antibodies and hematopoietic cell transplants. However, patients who have risk factors for HBV infection should also be screened before initiating systemic cancer therapy.

The ASCO panel found that since the 2010 release of national recommendations, HBV screening remains "suboptimal" among patients at high risk for HBV infection or HBV reactivation after cytotoxic or immunosuppressive therapy. Additionally, despite the U.S. Food and Drug Administration's 2013 box warning about the risk of HBV reactivation with monoclonal antibody products, "a small but substantial group" of patients with cancer receiving the therapy remain unscreened for HBV infection. While screening strategies vary by cancer center (with some employing risk-based screening and others adopting universal screening before cancer therapy), the panel concluded that they could not definitely recommend one strategy due to a "weak" evidence base. The panel recommends HBV screening should include both the hepatitis B surface antigen (HBsAg) test and hepatitis B core antibody (anti-HBc) test, because HBV reactivation can occur in patients who are HBsAg positive/anti-HBc positive or HBsAg negative/anti-HBc positive. Either a total anti-HBc test or an anti-HBc immunoglobulin G (IgG) test should be used to screen for chronic or resolved HBV infection before cancer therapy, but anti-HBc IgM should not be used as it can only confirm acute infection. Two panel members held "a minority viewpoint" advocating for a screening strategy of universal HBsAg and selective anti-HBc testing.

For patients found to be HBsAg-negative/anti-HBc–positive clinicians can monitor HBV DNA and ALT levels and initiate on-demand antivirals. Screening is not supported for patients without HBV risk factors and not planning cancer therapy associated with a high risk of reactivation. However, risk-based strategies are complicated, the group admits, by a lack of validated clinical tools to guide risk-based HBV screening.

"The ASCO panel acknowledges that there is wide variability in approaching HBV screening before cancer therapy," write the authors led by Jessica Hwang, M.D., from University of Texas MD Anderson Cancer Center in Houston. "Until clear and definitive evidence is available to guide patient selection, the consensus of the panel is that a risk-adaptive HBV screening and management strategy incorporating what is known about the risks of HBV infection as well as risks of cancer therapy–associated HBV reactivation is reasonable." G2

Company References

Abcodia +44 7810 650932

American Society of Clinical Oncology 571-483-1400

American Urological Association 410-689-3700

Crescendo Bioscience 650-351-1354

DiagnoCure 418-527-6100

Hologic 781-999-7300

Oxford Nanopore Technologies +44 (0)845 034 7900

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