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Foundation Medicine's Reimbursement Woes May Signal Broader Industry Challenge

Foundation Medicine (Cambridge, Mass.) candidly reported in quarterly financial filings that reimbursement challenges are stymieing the company's growth. The company's lack of Medicare coverage decisions and commercial payer contracts delays reimbursements and has led to a backlog of payments for more than 30,000 completed tests.

The company acknowledged these backlogs are not only delaying revenue, but also leading to slower than anticipated growth in its clinical testing volumes, particularly in the community setting. Industry watchers are taking note and assessing the implications of these reimbursement challenges for other, less well-funded companies.

For the third quarter (ending Sept. 30) Foundation Medicine's revenue reached $25.4 million, 54 percent year-over-year growth for the period. This growth was driven by strong results from the company's pharmaceutical business, which accounted for $11.7 million of the quarterly revenue, an increase of 75 percent. Pharmaceutical testing is negotiated on a set price per test. The third quarter pharmaceutical revenue was tied to 2,676 tests. (The company notes quarterly revenue is based on when reimbursement is actually collected, not when the tests were performed.)

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Meaningful Drop-to-Drop Variation Seen in Blood; Study May Raise More Doubts for Theranos

Test results from a single drop of blood are highly variable, according to a study published in the *American Journal of Clinical Pathology*. This variation between successive drops of fingerprick blood may be even greater than variation between fingerprick and venous blood samples. As a result, the authors say, tests may need to be run on six to nine drops of blood, rather than one.

This study's findings may affect many aspects of clinical testing. It could pose a problem for clinical decisions based on fingerprick tests, like anemia testing, and it could impact the future development of point-of-care diagnostics. Finally, this variability may also add momentum to the swirling of questions disputing the capabilities of Theranos' micro-sample technology.

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Foundation Medicine's Reimbursement Woes, *Continued from top of p.1*

The company's clinical testing revenue for the quarter also grew (40 percent year-over-year) to reach $13.7 million. This revenue reflected payment for 4,300 clinical tests in the quarter, well below the stated quarterly test volume of 8,012 clinical tests (7,000 FoundationOne tests and 1,012 FoundationOne Heme tests).

"Our efforts to obtain payment for individual claims can take a substantial amount of time, there is typically a significant lag between the time the test is reported and the time we actually recognize the revenue from such test," the company writes in a quarterly report filed with the Securities and Exchange Commission (SEC) on Nov. 2.

In the filing, Foundation Medicine said that 17,235 FoundationOne and 15,375 FoundationOne Heme tests have been billed to commercial third-party payers and Medicare, but remain unreimbursed. The company says in the filing that it has not generated "any" revenue from Medicare to date and is in the process of appealing these unpaid claims. In contrast, the company says it has been "reasonably successful" in securing reimbursement from private payers.

Impact of Uncertain Reimbursement

Despite growth in test volumes (25 percent year-over-year growth), the company says quarterly test volume fell short of expectations and blamed the shortfall on slower than anticipated adoption due, in part, to the reimbursement challenges.

> *"We expect that our current lack of significant coverage decisions and the general uncertainty around reimbursement for our products will continue to negatively impact our revenue and earnings, both because we will not recognize revenue for tests performed ... and because the absence of Medicare or other significant coverage decisions may lead physicians to not order a meaningful number of tests."*
>
> —Foundation Medicine SEC Filing

"We expect that our current lack of significant coverage decisions and the general uncertainty around reimbursement for our products will continue to negatively impact our revenue and earnings, both because we will not recognize revenue for tests performed ... and because the absence of Medicare or other significant coverage decisions may lead physicians to not order a meaningful number of tests," the company explained in the SEC filing.

In the filing, the company reveals both a mix of confidence in the value of its products, along with a warning that achieving "material" market success is contingent upon securing coverage decisions and favorable reimbursement rates.

"The company believes an information-based approach to making clinical treatment decisions based on comprehensive genomic profiling will become a standard of care for patients with cancer," the SEC filing says. "We believe we have a significant first mover advantage in providing comprehensive genomic profiling and molecular information products on a commercial scale."

The company is encouraged by recent policy decisions by private payers. United Healthcare recently published a medical policy for coverage of highly validated genomic profiling in patients with non-small cell lung cancer. Additionally, Aetna became the first national insurer to publish a coverage policy supporting whole exome sequencing under certain clinical conditions.

Takeaway: Reimbursement challenges are not unique to Foundation Medicine. As molecular testing continues to expand in scope, more companies will feel the impact on revenue and test volumes, if payers do not address the lack of coverage decisions and reimbursement uncertainty. G2

Meaningful Drop-to-Drop Variation Seen in Blood Samples, *Continued from bottom of p.1*

"These data suggest caution when using measurements from a single drop of fingerprick blood," writes co-author Rebecca R. Richards-Kortum, Ph.D., from Rice University in Houston. "Our results show that people need to take care to administer fingerprick tests in a way that produces accurate results because accuracy in these tests is increasingly important for diagnosing conditions like anemia, infections and sickle-cell anemia, malaria, HIV and other diseases."

> *"It is important to understand how variations in fingerprick blood collection protocols can affect point-of-care test accuracy as well as how results might vary between different kinds of point-of-care tests that use fingerprick blood from the same patient."*
>
> —Meaghan Bond

Fingerpricks are often used in point-of-care testing and are preferred over venipuncture because of patients' dislike of needles. Over the years, many tests have addressed the accuracy of results obtained from fingerstick samples versus venous blood, but few have assessed the variation in results between the successive drops of blood obtained from one fingerprick. The researchers were actually working on developing novel, low-cost platforms for low-resource settings when they observed wide variation in benchmark tests being performed on hospital-grade blood analyzers.

"A growing number of clinically important tests are performed using fingerprick blood," co-author Meaghan Bond, said in a statement. "It is important to understand how variations in fingerprick blood collection protocols can affect point-of-care test accuracy as well as how results might vary between different kinds of point-of-care tests that use fingerprick blood from the same patient."

In the study, the researchers analyzed the hemoglobin concentration, total white blood cell (WBC) count, three-part WBC differential, and platelet count in six successive drops (20 µL each) of blood. Samples were collected from one fingerprick from each of 11 donors, using best draw practices. Blood components were assessed with a hospital-grade hematology analyzer. A point-of-care hemoglobinometer was used to measure the hemoglobin concentration of 10 drops (10 µL each) of fingerprick blood from each of seven donors. Arm venipuncture samples were used as controls.

The researchers found that compared to venous controls, the successive drops of fingerprick blood had an average percent coefficient of variation (CV) that was higher by up to 3.4 times for hemoglobin, 5.7 times for WBC count, three times for lymphocyte count, 7.7 times for granulocyte count, and four times for platelets. For hemoglobin (using a point-of-care hemoglobinometer) the average percent for CV for fingerprick blood was up to five times higher for hemoglobin than venous blood.

Fluctuations in blood parameters are within published instrument variability when using volumes of fingerprick blood between 60 to 100 µL or greater. Recommending caution in using the results of these tests for clinical decisionmaking, such as determining anemia status, the authors suggest clinicians either accept the inaccuracy of fingerprick blood as a trade-off for easy blood collection; collect, read, and average multiple fingerprick samples (suggested six to nine blood drops), which adds accuracy but increases cost and time; or utilize venous blood.

Takeaway: While the current study did not utilize Theranos technology, new findings demonstrating wide variability in successive drops of fingerstick blood samples—and the resulting accuracy of test results— raises further questions about the company's closely-guarded testing mechanisms. G2

Inside The Diagnostics Industry

Will Paired-Normal Sample Testing Become the Norm for Molecular Tumor Assessment?

As precision oncology is becoming the norm, genomic analysis of tumors is rapidly guiding clinical decisionmaking. As a result of both more targeted therapeutic options and the recognition of tumor heterogeneity, molecular assessment of tumors is also becoming more comprehensive.

But with the identification of increasing numbers of mutations in each tumor, comes the inevitable questioning of whether all of the mutations are in fact pathogenic. Paired-normal testing, comparing tumor mutations to normal tissue from that individual, can help hone in on the alterations that are most likely to be pathogenic. This comparison of tumor and normal samples can also reveal germline variants that hold potential clinical implications for both the patient being tested, as well as his or her family members.

"You can't have personalized medicine without precision genomics."

—Victor Velculescu, M.D., Ph.D.

Currently, most clinical tumor testing does not currently involve analysis of a matched germline samples, but experts predict that will change in the next few years. While paired testing may not be necessary when assessment is contained to just a few well-characterized mutations, like BRAF, matched testing becomes more important as the scale of sequencing increases.

Is Paired Testing Clinically Relevant?

When tumors are analyzed without a matched normal comparison sequence, germline variants are detected in the tumor sample, but it may be difficult to distinguish their origin as a germline mutation. Thus, the presence of germline variants can complicate interpretation and potentially misidentify the true mutational driver of a tumor. This misinterpretation could have clinical consequences, as most germline susceptibility variants are not targetable, experts say.

"You can't have personalized medicine without precision genomics," explains Victor Velculescu, M.D., Ph.D., co-founder and chief science officer of Personal Genome Diagnostics, whose PGDx's CancerSelect Targeted Gene Profiling Panel provides for sequencing both normal and tumor DNA to accurately identify true cancer-specific changes. "If you are getting inaccurate information, it defeats the purpose of the test. With tumor-only testing you are getting false positives—extra mutations from the germline that are not subtracted out from the somatic-only mutations. The germline mutations were present, but not specific to the tumor."

How Frequently Are Germline Mutations Identified?

Two new studies provide evidence of how frequently germline mutations occur in oncology patients not being assessed for hereditary cancers. In the first study, published online Nov. 18 in the *New England Journal of Medicine*, 8.5 percent of children and adolescents with cancer were found to have mutations in cancer predisposing genes. The authors say these findings suggest that comprehensive genomic screening may be warranted on all pediatric cancer patients, not just those with a family history of cancer.

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"This paper marks an important turning point in our understanding of pediatric cancer risk and will likely change how patients are evaluated," said the study's corresponding author James R. Downing, M.D., President and CEO of St. Jude Children's Research Hospital, in a statement.

Interestingly, family history did not predict the presence of an underlying cancer predisposition syndrome in most pediatric patients.

The St. Jude–Washington University Pediatric Cancer Genome Project used next-generation sequencing, including whole-genome (n=595) and whole-exome sequencing (n=456), or both (n=69), to analyze the genomes of 1,120 children and teens with cancer. Patients had a variety of cancers associated with poor clinical outcomes (leukemia, 52.5 percent and central nervous system tumors, 21.9 percent).

In total, 565 cancer-associated genes were analyzed, with an emphasis on 60 genes associated with autosomal dominant cancer-predisposition syndromes and 29 genes associated with autosomal recessive cancer-predisposition syndrome. The remaining 476 genes were chosen based on published evidence of their role in somatic mutations in cancer. Sequence coverage exceeded 10× for more than 95 percent of the coding exons and 20× for more than 85 percent of the coding exons in the genes of interest.

The researchers found 633 nonsilent germline variants in the 60 genes associated with autosomal dominant cancer-predisposition syndromes. Twelve percent were pathogenic, 3 percent probably pathogenic, and 36 percent of uncertain significance.

The 95 pathogenic or probably pathogenic variants were detected in 21 of the 60 genes in 94 patients. P53 was most commonly involved (in 50 patients), followed by APC (n = 6) and BRCA2 (n = 6). Eight children had germline mutations in the adult-onset cancer–predisposition genes BRCA1, BRCA2, and PALB2, which are currently not included in pediatric cancer genetic screening. The highest frequency (16.7 percent) of germline mutations was seen in children with non-central nervous system solid tumors.

Interestingly, family history did not predict the presence of an underlying cancer predisposition syndrome in most pediatric patients. Of 75 pediatric patients with mutations that were deemed to be pathogenic or probably pathogenic, a review of medical records showed that only 12 patients had previously undergone clinical genetic testing. Forty percent of the medical records with a family history indicated a history of cancer.

This study dispels prior belief that the presence of such germline mutations in pediatric cancer patients was extremely rare and tied to children with strong familial cancer history.

As a result of these findings, St. Jude is initiating a new clinical research study, Genomes for Kids, which incorporates next-generation sequencing into the medical workup of every eligible pediatric cancer patient who enters the hospital for treatment. Any identified germline mutations in a cancer predisposition gene will be referred to the new St. Jude Hereditary Cancer Predisposition Clinic.

In a second recently published paper, researchers found that germline variants are also common in adult patients undergoing tumor-normal sequencing. Nearly 16 percent of

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patients carried presumed pathogenic germline variants (PPGV) in a gene linked to an inherited human disease, according to the study published Nov. 10 in *JAMA Oncology*.

> *"Clinicians and laboratories should not shy away from the use of genomic technologies in the care of their patients because of the potential to uncover unanticipated information ."*
>
> —Clinical Sequencing Exploratory Research Consortium

Targeted tumor sequencing with matched normal DNA was undertaken using a panel of 341 genes (the MSK-IMPACT panel) in 1,566 individuals with advanced cancer. The majority of the PPGVs were identified in genes associated with cancer susceptibility (most commonly BRCA2 [n=31], CHEK2 [n = 23], MUTYH [n = 23], and BRCA1 [n = 21]). However, the hereditary cancer findings were associated with the individual's cancer type in only 81 of 198 cases (40.9 percent).

"These findings indicate that it will not be uncommon to detect unexpected actionable variants. Even restricting reporting to the ACMG-endorsed gene set would identify potentially actionable mutations in at least 5 percent of our patients," write the authors led by Kasmintan Schrader, Ph.D., from Memorial Sloan Kettering Cancer Center in New York. "[Additionally,] over 60 percent of patients carried six or more variants of uncertain significance in genes linked to genetic disease. … Based on the experience reported here, reporting laboratories will require substantial resources for manual variant curation to ensure the quality of their reports."

Prepping Laboratories

"Clinicians and laboratories should not shy away from the use of genomic technologies in the care of their patients because of the potential to uncover unanticipated information," write the members of the Clinical Sequencing Exploratory Research Consortium in an article published online Nov. 21 in the *Journal of the National Cancer Institute*. "Instead, oncology providers, including both ordering clinicians and testing laboratories, should acknowledge the fact that tumor-only testing may reveal actionable germline information and actively implement solutions that maximize the clinical utility of this germline information while minimizing patient misunderstanding and harm."

The consortium's Tumor Working Group offers recommendations for how laboratories can prepare themselves for the discovery of germline findings when performing tumor analysis on large panels or exome-/genome-scale sequencing. They say that even in tumor-only sequencing, tumor mutation patterns (hypermutated tumors or massive chromosome rearrangements) may indicate a germline origin to the variant.

"Given the collaboration necessary in variant analysis and clinical interpretation, we believe that both ordering clinicians and laboratories share the responsibility for identifying and managing the potential for germline findings and clinicians will need to appropriately prepare patients for this possibility," writes the consortium's lead author Victoria Raymond, from University of Michigan, Ann Arbor.

Among the consortiums recommendations are:

- Carefully construct sequence analysis and data filtering algorithms with an ability towards differentiating germline and somatic variants.

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- Decide whether and how to disclose potential germline variants in their reports. While the ACMG recommends a minimum list of 56 genes (half of which are cancer-susceptibility genes). These genes should be "actively interrogated," even if the germline DNA samples are serving only as a control.

> *"Those medical centers offering testing must establish appropriate pretest education tools to inform patients about the potential for identifying inherited susceptibility or previously undiagnosed genetic disease and consider mechanisms for eliciting patient preferences regarding the communication of such results."*
>
> —Kasmintan Schrader, Ph.D.

- Decide what downstream testing will be performed and how it will be performed, when potential germline findings are identified. "Does the lab that carried out the tumor analysis also perform this germline test, and if so how should a germline sample from the patient be collected and the germline test ordered?" Raymond prompts in the article.
- Consider when to recommend referral to a genetic counselor or medical geneticist.
- Recognize the potential for medical, legal, and ethical complications in instances of misclassification and/or misreporting of somatic versus germline variants.
- Develop a standardized approach to the reporting of potential germline findings. The consortium suggests including a precise description of the variant (the specific nucleotide change and the genome build utilized), information for the ordering clinician about the potential implications of a germline finding, and a plan for confirmation, as appropriate.

"Those medical centers offering testing must establish appropriate pretest education tools to inform patients about the potential for identifying inherited susceptibility or previously undiagnosed genetic disease and consider mechanisms for eliciting patient preferences regarding the communication of such results," writes Schrader from the Memorial Sloan Kettering study. "Medical centers offering such genomic analysis will also need to develop appropriate post-test result communication protocols, as patients currently undergoing tumor mutation profiling may be difficult to engage in traditional post-test genetic counseling owing to their advanced disease. The clinical cancer genetics community will be challenged to establish best practices for communicating results to family members, particularly if a patient with advanced disease who is undergoing tumor-normal sequencing dies before receiving his or her test results."

Raymond tells *DTET* that the majority of cancer centers are not currently running paired normal testing because of logistical, turnaround time, and cost considerations, as well as not wanting to deal with additional consent and variant reporting issues.

Takeaway: Analysis of the cancer genome is most informative when paired with a second, normal DNA sample. This practice of distinguishing between variations of somatic and germline origin is expected to be incorporated into comprehensive molecular analysis in the next several years and can impact both treatment decisions for the presenting cancer phenotype and for longer-term surveillance for possibly unrelated cancer syndromes in both the index patient and their relatives.

Rise in Congenital Syphilis May Indicate Inadequate Prenatal Screening

Increasing rates of congenital syphilis (CS) in the United States may indicate inadequate access to prenatal care, including screening among vulnerable women, according to a study published Nov. 13 in *Morbidity and Mortality Weekly Report*.

CS has serious consequences, including death, but is largely preventable with proper identification of maternal infection and treatment with penicillin before birth. Researchers from the U.S. Centers for Disease Control and Prevention (CDC) analyzed national surveillance data (2008 to 2014) to calculate rate and identify demographic and clinical characteristics of infants with CS and their mothers.

Data from the National Notifiable Diseases Surveillance System shows that the overall rate of reported CS decreased from 10.5 to 8.4 cases per 100,000 live births from 2008 to 2012. However, from 2012 to 2014 the national CS rate increased to 11.6 cases per 100,000 live births, the highest CS rate reported since 2001. This increase was seen in all U.S. reporting regions and all racial/ethnic groups. The rising rate of CS was expectedly accompanied by an increase in the rate of primary and secondary syphilis in women.

Among mothers of infants with CS in 2014, 21.8 percent received no prenatal care. Of the 135 mothers who received no treatment but had one or more prenatal visits, 21 women were never tested for syphilis during pregnancy and 52 mothers tested negative for syphilis in early pregnancy and subsequently acquired syphilis before delivery. The remaining 62 mothers tested positive, but were not treated.

CDC Syphilis Testing Recommendations

The CDC recommends that syphilis screening includes:

- All pregnant women at their first prenatal visit.
- Repeat screening at the beginning of the third trimester and at delivery for women at increased risk (including living in high-morbidity geographic areas).
- Rapid plasma reagin screening at the time that a pregnancy is confirmed, if access to prenatal care is sub-optimal.
- Determining syphilis serologic status of the mother before newborn discharge, if not assessed during pregnancy or delivery.
- Testing of any woman who delivers a stillborn infant.

"Addressing CS will depend upon health care providers and STD programs being aware of infectious syphilis among women of reproductive age… [and] instituting more thorough prenatal screening practices when warranted," write the authors led by Virginia Bowen, Ph.D., from the CDC in Atlanta, Ga. "STD programs might also consider enhancing surveillance efforts by determining pregnancy status on all reported syphilis cases in women and by monitoring the screening and treatment practices among prenatal care providers in communities at highest risk for delivering an infant with CS."

Takeaway: While barriers exist in access to prenatal care, more widespread prenatal syphilis screening may cut the rate of congenital syphilis cases.

Patients Prefer Test Results Through Password-Protected Portals

Patients increasingly prefer to receive laboratory test results via password-protected websites and portals, according to a study published in the November-December issue of the *Journal of the American Board of Family Medicine*. Password-protected websites and portals are even more preferred with increased perception of test result sensitivity.

"It is necessary for health professionals to deliver results using the most confidential and patient-oriented method possible," write the Georgetown University Medical Center-based authors. "Despite these needs, no standardized delivery method has been established, nor have preferences been clearly delineated."

"Communication with patients may need to be on a case-by-case basis—every individual may have a personal preference, and there may be a way to indicate those preferences in the patient's record."

—Jeannine LaRocque, Ph.D.

Determining patient communications preferences is difficult given both how rapidly technology is evolving and how quickly patients adapt to these new communication methods. The authors acknowledge that while return of results must be Health Insurance Portability and Accountability Act-compliant, Internet-and mobile-based communication have dramatically increased since the law's passage in 1996, and this changing technological environment may not be fully captured in regulations. For example, in 2014, the Department of Health and Human Services began requiring laboratories to provide patients direct access to results and permits use of patient portals; the agency does not require, however, results be returned electronically.

The Georgetown study utilized electronic surveys to evaluate patient demographics, familiarity with certain medical tests, and patient's hypothetical preferences for delivery of medical test results. Preferences were assessed for common tests (cholesterol and colonoscopy) and for more sensitive ones (sexually transmitted infections [STIs] and genetic tests). The seven communication methods analyzed were: fax, mobile and home voicemail, e-mail, letter, mobile phone text message, and password-protected website. In-person communication was not measured.

Results were based upon responses from 409 participants (255 women; average age 37 years old; 88 percent white). For return of common test results more than 50 percent of respondents reported feeling comfortable with four of the seven communication methods (password-protected website, personal voicemail, personal E-mail, and letter). However, for STIs, more than 50 percent of participants only felt comfortable with password-protected websites. For genetic test results, no method garnered majority support, although password-protected websites were most preferred (46 percent).

Overall, participants were least comfortable receiving any results (common or sensitive test results) via fax and home voicemail. Interestingly, preference for returning results via personal e-mail and password-protected website preferences were not affected by age. However differences in comfort for receiving results via mailed letter were related to age (71 percent of participants over age 55 versus 35 percent of participants ages 18 to 24 years).

"Communication with patients may need to be on a case-by-case basis—every individual may have a personal preference, and there may be a way to indicate those preferences in the patient's record," said lead author, Jeannine LaRocque, Ph.D., from Georgetown's School of Nursing and Health Studies, in a statement.

Takeaway: It is necessary to consider patient preferences for methods to return test results given the trends around the consumerization of health care, increasing interest in patient satisfaction metrics, and mandated direct return of results. G2

Common Blood Protein Predicts Kidney Disease

A common protein in the blood can reliably predict a person's risk of developing chronic kidney disease (CKD) years before symptoms develop, according to a study published Nov. 12 in the *New England Journal of Medicine*. Researchers believe the marker—soluble urokinase-type plasminogen activator receptor (suPAR)—will be used in the near future as a screening test, much like cholesterol, to identify at-risk patients who can make lifestyle changes to ward off development of CKD.

Currently two markers—estimated glomerular filtration rate (eGFR; based on measuring blood creatinine) and proteinuria—are used to monitor CKD, but they are not sensitive enough to detect the disease in its earliest stages.

"SuPAR promises to do for kidney disease what cholesterol has done for cardiovascular disease ."

—Jochen Reiser, M.D., Ph.D.,

"For the last century, doctors have relied on creatinine levels and urine protein levels to detect and monitor kidney disease," said lead author Salim Hayek, M.D., from the Emory Clinical Cardiovascular Research Institute in Atlanta. "These markers are useful in diagnosing kidney disease, but are not helpful in predicting whether a person might develop disease in the future. We need to find a way to identify those at risk, in order to prevent the disease or catch it in its early stages."

Severe organ damage can occur with CKD before symptoms even develop and it is a costly problem. Medicare, the authors say, spent $87 billion on kidney disease in 2012. CKD affects more than 15 percent of U.S. adults, with an estimated 4 percent requiring dialysis or transplant due to organ failure from disease progression.

In the *NEJM* study, 2,292 patients' samples were used to measure suPAR and eGFR at baseline and then again after five years of follow-up. The patients (average age 63 years) were part of a cohort who underwent cardiac catheterization between 2003 and 2009. suPAR levels were classified into four quartiles.

The researchers found that 320 participants (24 percent) developed CKD during follow-up. A higher suPAR level at baseline was associated with a significantly greater incidence of CKD at follow-up. Among participants with high suPAR levels (greater than 3,040 ng/mL) but normal eGFR levels at baseline, 40 percent developed CKD over the five years. But only 10 percent of those with low suPAR levels (below 2,373 pg/mL) at baseline developed the disease. Additionally, suPAR predicted eGFR decline (a sign of CKD progression) in patients with CKD at baseline, regardless of their age, gender or race.

The researchers say that suPAR seems to be a more powerful predictor of CKD than other previously known risk factors, including race and the APOL1 risk gene. When suPAR level was included in a model based on conventional risk factors, the change in risk was larger than that with all other variables combined.

"SuPAR promises to do for kidney disease what cholesterol has done for cardiovascular disease," said senior author Jochen Reiser, M.D., Ph.D., from Rush University Medical Center in Chicago, in a statement.

The researchers replicated results in a second patient cohort—participants in the Women's Interagency HIV Study. After controlling for HIV, which is known to elevate suPAR, the researchers confirmed that high suPAR levels predict decline in kidney function, although at a slower rate than in the Emory cohort.

"suPAR remained associated with a decline in renal function among younger persons, who have a significantly lower burden of risk factors for cardiovascular disease, which suggests that the effect of suPAR is truly independent of traditional risk factors for cardiovascular disease and CKD," the authors write.

Takeaway: suPAR appears to be a robust marker of CKD risk. Researchers believe it will quickly be incorporated into preventive care, much like cholesterol screening. G2

Metabolic Profile May ID Early Ovarian Cancer

A metabolomic profile may hold the key for screening for early-stage ovarian cancer, according to a study published Nov. 17 in *Scientific Reports*. Researchers identified a profile of 16 blood-based diagnostic metabolites that could distinguish early-stage ovarian cancer patients with 100 percent accuracy. The researchers say these markers may yield a "clinically significant" diagnostic test with further validation.

Ovarian cancer is notoriously difficult to diagnose early, often leading to unsuccessful treatment. Given the low prevalence of the disease in the general population (0.1 percent in the United States), a screening test must attain "stringent accuracy," which the authors define as a positive predictive value of at least 10 percent, a specificity of more than 99 percent and a sensitivity of 75 percent or higher to be of clinical relevance in the general population. Current screening methods (trans-vaginal ultrasound combined with serum CA-125 levels) achieve a positive predictive value of only 24 percent.

"People have been looking at proteins for diagnosis of ovarian cancer for a couple of decades, and the results have not been very impressive," said co-author Facundo Fernández, Ph.D., from Georgia Institute of Technology, in a statement. "We decided to look in a different place for molecules that could potentially provide diagnostic capabilities. It's one of the places that people had really not studied before."

In the current study, the researchers evaluated serum samples from 46 early stage (I/II) serous epithelial ovarian cancer patients and 49 age-matched normal healthy controls. The samples were analyzed using ultra-performance liquid chromatography, high-resolution mass spectrometry (UPLC-MS) and tandem MS (MS/MS). This technology separates heavier molecules from lighter molecules to determine the molecular signatures.

One thousand candidate compounds were leaned to 255 by removing duplicates and unrelated molecules. These 255 were then analyzed by a learning algorithm, which evaluated the predictive value of each one. Molecules not contributing to the predictive accuracy of the screening were eliminated. Ultimately, the researchers identified 16 diagnostic metabolites that together are able to distinguish early-stage ovarian cancer with 100 percent accuracy.

"Molecular features closely associated with the cancer phenotype, like metabolites, may be expected to be less variable across patients than the broader spectrum of individual mutations and disrupted pathways underlying the disease," the researchers write in the paper.

Takeaway: Metabolomic profiles in serum may be useful in screening women for early-stage ovarian cancer. While further validation in larger populations is necessary, the researchers say a clinical assay based on the 16 markers is technically feasible. G2

G2 INSIDER

12-Hour Urine Collection OK For Preeclampsia Diagnosis

A 12-hour urine collection performs similarly to a 24-hour urine collection for the diagnosis of proteinuria in women with suspected preeclampsia, according to a study published in the October issue of *Obstetrics & Gynecology*. The shorter collection time maintains high sensitivity and specificity, with the added benefit of convenience and improved clinical efficiency, the authors say.

Quantification of urinary protein remains an important diagnostic step in the evaluation of hypertension during pregnancy in the absence of severe symptoms, including end organ involvement (thrombocytopenia, elevated liver transaminases, renal insufficiency, pulmonary edema, or new-onset neurologic symptoms).

"Several studies have investigated urine protein-to-creatinine ratio as a rapid test to obviate the need for a 24-hour urine collection," write the authors led by Molly Stout, M.D., from Washington University in St. Louis, Mo. "Although the data show that extremely high or low urine protein-to-creatinine ratio values may be a substitute for a 24-hour urine collection, there are clinical circumstances that may still require a 24-hour urine collection."

Dipstick quantification of urine protein is also not recommended, according to the American College of Obstetricians and Gynecologists.

The researchers conducted a literature search to identify studies that compared results of both the 12-hour and 24-hour urine collection in the same female patients (at or beyond 20 weeks of gestation). Test performance characteristics from each study were extracted and a diagnostic meta-analysis was performed to determine summary diagnostic characteristics and to estimate the optimal cut-off point for the diagnosis of proteinuria using the 12-hour urine collection. Proteinuria was defined as 300 mg of protein in 24 hours. Included studies must have reported total protein, not concentration.

Analysis was based on a total of seven studies that met inclusion criteria (410 patients). The incidence of 24-hour urine protein greater than 300 mg ranged from 14 percent to 86 percent in the studies. There was also variation in the cut-off point used for a positive 12-hour urine collection, ranging from 100 to 165 mg.

The researchers found that 12-hour urine protein was overall "highly predictive" of proteinuria (area under receiver operating characteristic curve: 0.97). The optimal cut-off point was 150 mg of protein on 12-hour collection, which maximized sensitivity and minimized false-positives. Using this cut-off, the pooled sensitivity was 92 percent and specificity was 99 percent.

The authors note that while future study should evaluate standardization of collection (bed rest, day versus night), use of the 12-hour urine collection would be more convenient and expedite diagnosis and clinical management, and decrease cost.

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Foundation Medicine
617-418-2200

Georgetown University
202-687-0100

Georgia Institute of Technology
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Memorial Sloan Kettering
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Personal Genome Diagnostics
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