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Replacing Tissue-of-Origin Cancer Classification With Molecular Taxonomy Has Prognostic Value

Researchers are calling for adoption of a molecular-based classification system of cancer rather than the current tissue-of-origin methods, according to a study published Aug. 14 in *Cell*. Utilization of the new molecular taxonomy would reclassify one in 10 cancer patients, which the authors say could lead to different treatment choices.

"The refined molecular taxonomy we describe builds on centuries of pathology and genetic research," write the authors, led by Katherine Hoadley, Ph.D., and colleagues from the Cancer Genome Atlas research network. "This initial Pan-Cancer-12 analysis lays the groundwork for a richer classification of tumors into molecularly defined subtypes unlike all prior cancer classification systems."

The researchers utilized data from six different "omics" platforms to look for molecular alterations shared across cancers arising from different tissues. This integrative analysis was completed on 3,527 samples from 12 tumor types referred to as the "Pan-Cancer-12" set. Cases were assayed by at least four of

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Shifting Cardiovascular Guidelines Focus Attention On Lab's Role, Emerging Tests

While results from laboratory testing continue to play a pivotal role in risk assessment and management of cardiovascular disease, some experts feel that new guidelines published by the leading associations do not go far enough in incorporating newer markers of cardiovascular risk.

The American College of Cardiology and American Heart Association (ACC-AHA) in July published the final version of the 2013 Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Risk in Adults and the 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk.

Those involved in the drafting of the guidelines defend the rigorous systematic review of randomized controlled trials with arteriosclerotic cardiovascular disease (ASCVD) outcomes utilized and say the resulting guidelines are not merely reaffirmation of previous recommendations. The guidelines include the development of new risk prediction equations and a new cholesterol guideline framework that is not simply an extension of previous Adult Treatment Panel III (ATP III) guidelines.

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▲ **Shifting Cardiovascular Guidelines Focus Attention,** *from page 1*

From the laboratory perspective, the fasting lipid panel remains the cornerstone of testing mentioned in the guidelines. The ACC-AHA panel recommends a fasting lipid panel prior to the initiation of statin therapy comprising total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and (calculated) high-density lipoprotein cholesterol (HDL-C), followed by a second lipid panel four weeks to 12 weeks after initiation of therapy to determine a patient's adherence. Additional lipid panels, as clinically indicated, should be performed every three months to 12 months.

Unlike the ATP III recommendations, the ACC-AHA cholesterol guidelines represent a "dramatic departure" from previously recommended risk-dependent LDL-C targets and rely upon the percent reduction of LDL-C. While the recommendations call for calculation of LDL-C using the Friedewald equation, research shows that the Friedewald equation might not be reliable in patients who have triglycerides more than 200 mg/dL and an LDL-C less than 70 mg/dL.

The ACC-AHA cholesterol guidelines represent a "dramatic departure" from previously recommended risk-dependent LDL-C targets and rely upon the percent reduction of LDL-C.

"In these patients, laboratories might need to reflex to either a direct method or to an ultracentrifugation method to quantify LDL-C," according to a piece on the laboratory's role in adoption of the new guidelines, written by Ishwarlal Jialal, M.D., Ph.D., from the University of California at Davis, in the *American Journal of Clinical Pathology*. "Also, laboratories should adhere to standardized protocols to minimize preanalytic variation such as the patient fasting at least 10 to 12 hours."

Should Additional Tests Have Been Considered?

Several aspects of the guidelines have been controversial, with one of the criticisms being that the ACC-AHA guidelines did not go far enough in endorsing alternative markers of ASCVD. Jialal cites apolipoprotein B and LDL particles as alternate measures of LDL and estimated glomerular filtration rate and albuminuria (measures of chronic kidney disease) as additional risk markers for cardiovascular disease as some of the tests not included.

In an editorial in the September issue of *Mayo Clinic Proceedings*, Jennifer Robinson, M.D., vice chair of the ACC-AHA cholesterol guideline development committee, says the group did review the epidemiologic evidence for apolipoprotein B, creatinine and glomerular filtration rate, and microalbuminuria but found a lack of evidence to determine whether they improved risk prediction using the new 5 percent and 7.5 percent 10-year risk of nonfatal and fatal stroke or myocardial infarction. She writes that while this is a direction for future research, there is not presently "compelling evidence" for the general use of these tests.

The ACC-AHA found the strongest predictors of the 10-year risk of ASCVD events (first occurrence of nonfatal myocardial infarction, coronary heart disease death, or fatal or nonfatal stroke) were age, sex, race, total cholesterol, HDL-C, systolic blood pressure, blood pressure treatment status, diabetes, and current smoking status.

But a task force of Mayo Clinic experts feels adjunctive measures should also be considered for risk stratification, including genetic determinants of ASCVD, according to a special article published in the September issue of the *Mayo Clinic Proceedings*.

To refine risk, the group, led by Iftikhar Kullo, M.D., also recommends a blood test to measure lipoprotein(a), or Lp(a), a type of LDL cholesterol (reimbursed $17 by Medicare).

The Lp(a) level is determined by genes, and high levels are associated with increased cardiovascular risk. Unlike hs-CRP, which the authors say is a marker of risk, the genetic variants associated with high Lp(a) levels are a causal risk factor. They say elevated levels of Lp(a) would reclassify 4.1 percent of intermediate-risk individuals to higher risk.

"A one-time measurement of Lp(a) should be considered when there is uncertainty in the estimates of 10-year ASCVD risk, particularly in those with a family history of ASCVD," write Kullo and colleagues. Mayo researchers are pushing ahead on improving the understanding of genomic information to further refine cardiovascular risk with a pilot trial called the Myocardial Infarction Genes (MI-GENES) study.

"The suboptimal performance of available ASCVD risk algorithms needs to be acknowledged, and research efforts to improve risk assessment in asymptomatic adults should be intensified," Kullo writes. "Although the variants have modest effect sizes, most are not associated with conventional risk factors and therefore provide an orthogonal means of risk assessment, in contrast to several existing biomarkers such as hs-CRP whose incremental predictive utility is diminished because of its correlation with factors such as obesity, diabetes, and hypertension."

To Fast or Not?

One additional criticism of the ACC-AHA guidelines is the issue of fasting. The guidelines, while recommending fasting lipids, do not specify the length of time for fasting. Researchers of several recent studies are calling on national and international professional organizations to re-examine the necessity of fasting for LDL-C based in part on the argument that nonfasting lipids may actually be superior in predicting cardiovascular outcomes because the nonfasting state may more accurately reflect the body's exposure to circulating lipids.

"The suboptimal performance of available ASCVD risk algorithms needs to be acknowledged, and research efforts to improve risk assessment in asymptomatic adults should be intensified."
—Iftikhar Kullo, M.D.

The latest study to question the benefits of fasting was published online July 11 in *Circulation*. The researchers from New York University (New York) found that over 14 years of follow-up in a national cohort of National Health and Nutrition Examination Survey III participants (1988 to 1994), mortality (all-cause and cardiovascular) was similar between 4,299 pairs of adults undergoing fasting (eight hours or more) and nonfasting lipid panels (less than eight hours). Fasting did not improve the prognostic value of LDL-C values, and the authors urge a reassessment of the fasting necessity, which they say can become a practical issue standing in the way of hyperlipidemia detection.

Takeaway: The assessment of cardiovascular risk is an evolving area of clinical care that will likely incorporate adjunct tests into more traditional assessments. While recent guidelines have generated some controversy, laboratory test results will continue to play an important role in informing clinical discussions.

Gut Microbiome Emerging as New, Noninvasive Screening Tool

Analysis of the gut's bacterial composition may aid in the noninvasive screening for early-stage colorectal cancer (CRC), according to a study published Aug. 7 in *Cancer Prevention Research*. The ability of gut bacterial markers to differentiate healthy individuals from those with early- or advanced-stage CRC can be used as a complement to existing screening methods, the authors say.

"The feasibility, lack of invasive procedures, ability to be [a] complement [to] existing screening methods (e.g., gFOBT), and the strength of signal seen in this study support the further investigation and application of microbial biomarkers from stool as a method for colorectal cancer screening," write the authors, including senior study author Patrick D. Schloss, Ph.D., from the University of Michigan in Ann Arbor. He adds in a statement, "We don't think that this would ever replace other CRC screening approaches, rather we see it as complementary."

The researchers used sequencing techniques to characterize the gut microbiome from stool samples in patients across the spectrum of CRC, including those that were healthy (n = 30), with adenoma (n = 30), and with carcinoma (n = 30). Using the Illumina MiSeq platform the researchers analyzed the V4 region of the 16S rRNA gene from the feces of each individual. During the discovery analysis, sequences were categorized into operational taxonomic units (OTU) using a similarity cutoff of 97 percent and then the relative abundance of each OTU was calculated.

"My dream is that every year at a checkup you take a stool sample, it is sent for sequencing and analysis, and in two weeks the physician calls you and says that you have a high level of this bacteria which is associated with XYZ, and therefore we should consider some more follow-up."

—Patrick Schloss, Ph.D.

Analysis of 25,953 sequences per stool sample showed both an enrichment and depletion of multiple bacterial populations for both adenomas and carcinomas. Data from the gut microbiome (five OTUs for adenomas and six OTUs for carcinomas) when combined with known clinical risk factors of colorectal cancer (fecal occult blood testing [FOBT] results) and demographic (body mass index, age, race), significantly improved the ability to differentiate between healthy and adenoma individuals (4.5-fold), and healthy and carcinoma individuals (5.4-fold), compared to utilizing risk factors alone.

"Interestingly, when we looked at each patient, we rarely observed significant enrichment of every bacterial population among the OTUs incorporated in the logit models," the authors write. "This strongly suggests that there may be multiple underlying mechanisms by which the microbiome is involved in colorectal cancer."

Incorporating age-specific incidence rates of colorectal cancer into Bayesian models, the authors found that using gut microbiome data as a screening tool improved the pretest-to-posttest probability of adenoma more than 50-fold. For instance, the pretest probability in a 65-year-old was 0.17 percent, but by incorporating the microbiome data, this probability increased to nearly a one in nine chance of having an adenoma.

The authors say that the significant difference in the gut microbiome of people with colonic adenomas compared with those with healthy colons is of "considerable importance" for screening for early-stage colorectal cancer.

Schloss tells *DTET* that within the next five years he anticipates microbiome analysis to move into the clinical diagnostic realm, even outside of cancer.

"My dream," Schloss says, "is that every year at a checkup you take a stool sample, it is sent for sequencing and analysis, and in two weeks the physician calls you and says that you have a high level of this bacteria which is associated with XYZ, and therefore we should consider some more follow-up."

Schloss says right now the biggest limitation to that scenario is that knowledge of the natural biology of the microbiome is limited and clinicians don't know how to get rid of a certain deleterious bacteria or why it is there.

Takeaway: Analysis of microbiome markers from stool samples is feasible as a noninvasive screening test for CRC.

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Inside the Diagnostics Industry

Laboratory Industry Split on Anticipated Impact Of FDA's LDT Regulation Framework

The laboratory industry has now had a few weeks to mull over the U.S. Food and Drug Administration's (FDA's) framework for increasing its regulation of laboratory-developed tests (LDTs), and the reaction is decidedly mixed.

Commercial diagnostics companies are projecting a much more optimistic tone, with analysts noting public testing companies have been factoring an increased regulatory environment into their strategies and are well-positioned to make the transition. Directors in the academic and hospital laboratory sector, on the other hand, have said the outlined framework is a "nightmare" that will have a "crippling" effect on their operations and will negatively impact clinical laboratory care.

DTET spoke to stakeholders throughout the industry to better understand the anticipated impact of the expanded regulation.

Evolution of LDTs Raised FDA Concern

"While CLIA oversight has played an important role in ensuring clinical labs are operating appropriately, it has not necessarily ensured that LDTs are properly designed, consistently manufactured, and are safe and effective for patients," writes Philip Desjardins, former FDA lawyer and current counsel at Arnold & Porter (Washington, D.C.) in an August advisory.

The need for oversight, the FDA believes, stems from the perceived increasing risk patients face resulting from the expanded complexity and availability of LDTs since the agency was given explicit authority over diagnostic tests over 35 years ago. Desjardins says that the FDA's previous decision to exercise enforcement discretion stemmed from the fact that LDTs were initially low-volume tests or limited to a small population, similar to "standard diagnostic devices," required manual techniques by lab personnel, and were typically used and interpreted directly by clinicians working within a single institution that was responsible for the patient.

However, Desjardins notes that is not the case today. Now LDTs are often used in laboratories that are independent of the health care delivery entity, are "frequently" manufactured with components and instruments that are not legally marketed for clinical use, and tend to rely on automated instruments and software to generate results, he says.

What Is Known

As expected by many in the diagnostics industry, the outlined framework includes provisions for LDTs to be evaluated using a risk-based approach, which will rely upon the existing medical device classification system. Some factors the FDA will consider in assessing risk include the disease and population of intended use, whether the device

Lyme Disease Assay Highlights Questions About LDT Quality

A public back-and-forth between the U.S. Centers for Disease Control and Prevention (CDC) and Advanced Laboratory Services (ALS; Sharon Hills, Pa.) highlights concerns over the level of validation needed for LDTs.

This past spring, the CDC issued a clear warning to patients and clinicians to use only the 83 FDA-approved diagnostics for Lyme disease. The CDC recommends a two-step testing process: first, an FDA-cleared enzyme immunoassay, followed by confirmatory Western blot in immunoassay-positive or equivocal cases.

ALS offers an LDT that uses a novel culture method to identify Borrelia burgdorferi, the spirochete that causes Lyme disease ($595 for the basic culture and $695 for the monoclonal Immunostaining for Borrelia burgdorferi only). The CDC reviewed this LDT and has "serious concerns about false-positive results caused by laboratory contamination and the potential for misdiagnosis."

will be used for screening or diagnosis, the nature of clinical decisions affected by results, and consequences of erroneous results.

The FDA intends to continue to exercise enforcement discretion for LDTs used solely for law enforcement purposes and certain LDTs used for transplantation. Light regulatory requirements, including registration and listing and adverse event reporting, will be expected for low-risk LDTs, LDTs for rare diseases, "traditional LDTs" (similar to those available in 1976 when enforcement discretion first began), and "LDTs for Unmet Needs," when no FDA-approved or cleared equivalent device is available. Registration and listing, adverse event reporting, plus premarket review and quality systems regulation (QSR) requirements will be expected for moderate- and high-risk LDTs, although the timeline for enforcement will differ based on risk.

***"All stakeholders on every conceivable side of this issue have tried to define just what is* high-risk.**"**
—Mark Grodman, BioReference Laboratories

Stakeholders were pleased that the FDA addressed some previous concerns expressed by the laboratory and diagnostics industry, namely that notifying the FDA of an LDT does not trigger the medical device tax and that the FDA intends to continue its enforcement discretion for currently available tests undergoing agency review of premarket submissions. Also encouraging was that the FDA "generally intends to rely on the scientific literature to support clinical validity if appropriate."

Outstanding Questions

But despite some clarification, many outstanding questions remain.

"The new LDT notification documents address but do not answer several critical issues," writes Bruce Quinn, M.D., Ph.D., senior health policy adviser at the law firm Foley Hoag, in a life science alert. "While high-risk tests would be first to be subject to premarket review, there is little to illuminate precisely how FDA intends to assess risk, which intended use and diagnostic claims will be permissible or feasible, or what types and amounts of evidence will be required in premarket review."

The definition of *high-risk* has large repercussions, not only in designating the regulatory pathway but also the timeline for initial enforcement.

"All stakeholders on every conceivable side of this issue have tried to define just what is *high-risk*, and I have been party to this for over a decade of conversations," said Mark Grodman, chief executive officer of BioReference Laboratories, on an August earnings call. "I will tell you that there hasn't in this entire time ever been a concurrence, consensus, or resolution of this definition."

Laboratory experts have also raised the question about the need for additional submissions if modifications to the test or operational process changes are made following FDA-approval or clearance. Edward Ashwood, M.D., CEO of ARUP Laboratories (Salt Lake City), cites the rapidly evolving space of clinical sequencing as a particular area of concern for this.

"Is what database you compare the sequence to part of the test?" questions Ashwood. "Are improvements to the database considered changing the test even if you are not changing the instrument or the chemistry?"

Additionally, there are serious questions if the notoriously slow FDA can handle the dramatic increase in submissions expected to come with LDT regulation. Grod-

man calculates that there are about 11,000 laboratories in the country that perform complex testing in the United States that may perform as many as 100,000 to 200,000 discrete assays. This volume of submissions, he says, is a "huge undertaking" for an agency that typically clears 25 discrete assay premarket approvals annually.

Industry Split on Impact

Critics cite the costs of additional compliance work, lengthier time to market, and challenges to implementing test improvements as potential negative effects expanded regulation could have on the diagnostics industry, all of which could ultimately impact care and stifle research and innovation.

Concern is perhaps greatest among academic and hospital laboratories. Weeks before the FDA's congressional notification, laboratory directors from 20 academic centers sent a letter to the Office of Management and Budget in opposition to FDA regulation of LDTs. The group—collectively representing thousands of LDTs—questions the FDA's authority to regulate the tests, calling them "services, not devices," which are part of the "practice of medicine."

"This is a very difficult framework to work in," Ashwood, the lead author of the letter, tells *DTET*. "They are treating us as manufacturers and are not considering that we are clinical labs."

The clinical care aspect of testing, Ashwood believes, will suffer as a result of the regulation. He says the approval-related costs will make it unfeasible for all current tests to be translated into the FDA regime, thereby limiting access to testing. For other tests, the increased "lag time" from submission to approval will effectively withhold advances in technology and biological understanding from clinical care.

"An academic laboratory may have a test menu of 100 tests that require a premarket submission, and that number is not out of the realm of possibility," explains Ashwood. "Then there is the cost of that submission, which could be tens of thousands of dollars per test. Who is going to bear the cost of that submission?"

It will also take extensive time to prepare submissions. Ashwood says academic laboratories run LDTs in a "service mode" and don't systematically review outcomes for all patients tested. Doing so requires institutional review board approval and patient consent, which may become necessary to demonstrate clinical validity to the FDA. Given the expenditure of effort, time, and money for submissions, make-or-buy decisions will need to be made for each test.

The decision to buy a kit is not always decided by price, Ashwood tells *DTET*. Utilization of kits can be limiting in terms of the rapidity with which new variants can be added to molecular tests. As an example, Ashwood cites BRAF. The first FDA-approved BRAF test, which Ashwood says

Timeline for LDT Enforcement

If implemented as the framework indicates, enforcement will be phased in over a nine-year period. The clock will start ticking following issuance of the final guidance, which is now estimated to be January 2016. Milestones include:

- Descriptions of class I, II, and III tests will likely take 18 months to 24 months after guidance is finalized.
- Enforcement of premarket review and QSR requirements for the highest-risk LDTs may begin in 2017.
- 2019 through 2021—Phased-in enforcement of premarket review and QSR requirements for Class III LDTs.
- 2021 through 2025—Phased-in enforcement of premarket review and QSR requirements for Class II LDTs.

While designation of risk affects the timeline, William Quirk from Piper Jaffray said individual company filings can accelerate the timeline for the entire sector. For instance, Myriad is expected to accelerate the timeline for other BRCA test providers (like Ambry, Counsyl, LabCorp, and Quest) by filing its BRACAnalysis as a companion diagnostic for olaparib. Similarly, Illumina's expected filing for the verify assay will accelerate the timeline for other prenatal test providers like Natera and Sequenom.

cost $18 million to develop, is limited to assessment of one variant. LDTs can detect more variants and can more quickly incorporate new variants as the understanding of clinical significance grows. Kit manufacturers are incented to sell the test for as long as possible to recoup research and development (R&D) costs. While the FDA's regulation of LDTs is touted by in vitro diagnostic device manufacturers as leveling the playing field, Ashwood says it holds the potential to create an environment of "monopolies for obsolete FDA-approved tests."

"The FDA stamp freezes a test in time," Ashwood says. "Three of every four R&D dollars in my lab are spent on improvements of older assays, and some of them aren't that old. With this framework we won't be able to take advantage of rapid improvements in technology. Can you imagine if we receive approval, launch a test, and then have to submit another 510k in six months and wait for review before we can use the improved test?"

Analysts for the commercial laboratory sector are more optimistic, saying the framework allays uncertainty and that many large companies have already factored an increased regulatory environment into their strategies.

Illumina, for example, already had plans to ramp up the number of products it brings through the FDA process, says Bryan Brokmeier, vice president and senior equity analyst of life science tools and diagnostics at Maxim Group. The company was pursuing the strategy in advance of the LDT regulation plans in order to accelerate clinical adoption of sequencing.

"The FDA stamp freezes a test in time." —Edward Ashwood, M.D., ARUP

Nationally available, widely used LDTs from companies such as Myriad Genetics, Genomic Health, and Foundation Medicine may be best positioned to enter the new regulatory paradigm. Experts say these existing tests, with established clinical utility and payer coverage, will be able to fairly easily file FDA submissions and may commercially benefit from a higher bar for new entrants to enter the market.

This sentiment was echoed by CEOs from these companies during quarterly earnings calls held in August.

Foundation Medicine's CEO, Mike Pellini, said his company welcomes the adoption of rigorous standards for LDTs. "From the early days, our company has been working in anticipation of an increased FDA oversight of LDTs. . . . We are already designing and building our QSR compliance lab, which will support the development and processing of FDA-approved tests and is expected to be completed next year," he said.

"A hidden benefit might be that as we transition to value-based pricing, it is easier to achieve with the clinical validation that comes from FDA approval," says William Quirk, senior research analyst at Piper Jaffray & Co. "I am optimistic based on payer comments that there will be improvement in reimbursement for tests that have clinical utility and I would argue also economic utility. The up-front premarket costs [with increased regulation] are higher, but companies will need that clinical validation anyways to convince physicians to use the test. For those that can keep an eye on costs and the economics of the test, innovative companies will continue to push diagnostics forward."

Takeaway: Reaction is mixed to the FDA's proposal to regulate LDTs. While many labs are concerned about the impact on their ability to develop and modify tests, others say they have already factored increased regulation into their business models.

▲ **Replacing Tissue-of-Origin Cancer Classification,** *from page 1*

the six possible methods: whole-exome sequencing, DNA copy number, DNA methylation, mRNA expression, microRNA expression, and protein expression.

Statistical analyses of the molecular data (both individually from each platform and integrated cross-platform) divided the tumors into clusters. Eleven integrated cancer subtypes were identified through cluster-of-cluster assignments (COCA). While five of these subtypes were consistent with tissue-of-origin classifications, several newly identified subtypes were seen across tissues, and some tissue-of-origin categories were split into multiple different molecular subtypes. Importantly, approximately 10 percent of cases were reclassified by the molecular taxonomy, with the COCA subtypes providing important molecular and tumor biology information that is significantly associated with prediction of clinical outcomes beyond tumor stage and primary tissue of origin.

"We're just appreciating the tip of the iceberg when considering the potential of this multi-platform type of genomic analysis," co-senior author Christopher Benz, M.D., from the Buck Institute for Research on Aging (Novato, Calif.), said in a statement. "It could be that as many as 30 or 50 percent of cancers need to be reclassified" when more tumor samples and 20 tumor types are included in the next round of analysis.

Benz believes that even when looking at the 12 current tumor types, the 10 percent reclassification rate in the current study is likely an underestimate due to the unequal representation of different tumors. "If our study had included as many bladder cancers as breast cancers, for example, we would have reclassified 30 percent," Benz said.

Although there were only 120 bladder cancer samples included, it proved to be the most diverse tumor type, with samples clustering into seven of the 11 COCA subtypes. Among the most dominant bladder subtypes, one was remarkably similar to lung adenocarcinomas and another was similar to head and neck squamous-cell cancers. Survival differences were dependent on subtype classification.

Other specific findings included:

- The two most commonly mutated genes in the overall data set were TP53 (41 percent) and PIK3CA (20 percent), which were both prognostic, even across different tumor types.

- Regardless of molecular platform, cancers of nonepithelial origin appear most different from epithelial tumors. This confirms previously established differences between breast cancer subtypes (basal-like and luminal). In this study, the breast basal-like subtype exhibited similarities between lung squamous cell carcinoma and serous ovarian cancers.

While further validation of this taxonomy is needed, the authors are hopeful that the results will further propel clinical trial design to rely on genomic classification of tumors for eligibility.

Takeaway: Classification of cancers based on a new integrated taxonomy, rather than tissue of origin, reveals common molecular features across tissues of origin and may be useful in reclassifying patients for treatment purposes. G2

Variability in Lab Charges Hampers Rational Consumer Behavior

There is wide variability in outpatient hospital charges for 10 common blood tests, according to a study published Aug. 14 in *BMJ Open*. While hospital-level factors, including ownership and teaching status, explain some of that variation, the authors say the lack of transparency in establishing charges hampers patients paying out of pocket from making informed consumer choices.

"These findings highlight the lack of predictability facing Americans paying full charges for healthcare, limiting their ability to act as rational consumers," write the authors, led by Renee Hsia, M.D., from University of California, San Francisco.

The researchers examined variation in charges between general acute care, medical/surgical hospitals (average of 177 hospitals per test) for 10 common blood tests (basic and comprehensive metabolic panel, lipid panel, complete blood cell count [automated and with differential white cell count], thyroid-stimulating hormone, creatine kinase, troponin assay, prothrombin time, and thromboplastin time [partial]).

The researchers found significant variation in charges for blood tests. For example, while the median charge for a lipid panel was $220, there was a thousandfold difference in charges, with a range from $10 to $10,169 per test. The median charge for a basic metabolic panel was $214, but again charges ranged from $35 to $7,303. Even when extreme cases were excluded and charges from the fifth to 95th percentile were analyzed, there was still large variation. The largest was for a comprehensive metabolic panel ($79 to $948), while the smallest variation was for an automated complete blood count ($37 to $278).

"As employers are switching to more consumer-directed health plans with higher deductibles and co-pays, the out-of-pocket costs of even insured patients can be affected by these charges."
—Renee Hsia, M.D.

Given that the line item outpatient charges should have no variability in patient characteristic or clinical presentation and should just represent institutional differences in charges, the researchers sought to understand the influence of hospital-level and market-level factors on charges.

Government hospitals and teaching hospitals charged significantly less than their counterparts for many blood tests, but no other hospital characteristics and no market-level predictors significantly predicted charges for blood tests. For seven of the 10 blood tests, teaching hospital status was associated with a significantly lower charge (i.e., charge for a troponin assay is 65 percent lower at a teaching versus non-teaching hospital). Additionally, five of 10 blood tests had lower charges at government hospitals than nonprofits. These models explained, at most, 21 percent of the variation between hospitals in charges for the blood test in question, the authors say.

"It is notable how few characteristics were significant predictors of the charges patients faced," the authors write. "A hospital's case mix and labor costs (wage index) do not affect charges for these common procedures. Market level characteristics, including competitiveness of the hospital market, percent uninsured in the hospital's county and county poverty rate, also showed no significant effects on charges for any of the 10 blood tests."

Do these charges really translate into patient bills? The authors believe yes.

"You may hear people say that, 'Charges don't matter' or that 'No one pays full charges,'" said Hsia, in a statement. "However, uninsured patients certainly face the full brunt of raw charges, especially if they don't qualify for charity care discounts. And as employers are switching to more consumer-directed health plans with higher deductibles and co-pays, the out-of-pocket costs of even insured patients can be affected by these charges."

Hsia tells *DTET* that this variability is not isolated to laboratory charges but is systemic throughout the health care system. According to a study from the California HealthCare Foundation and the Robert Wood Johnson Foundation, some efforts are being made to legislate price transparency, especially in New Hampshire, a pioneer in the area, where transparency efforts were enacted to support cost-conscious consumer behavior and spur competition increasing efficiency among health care providers.

Takeaway: At a time when a growing number of patients are faced with footing an increasing portion of their own health care bills, wide variation in charges for common blood tests is a concern for patients, especially in situations when price transparency is not available.

miRNAs Emerging as Markers of Heart Injury

An increase in certain microRNAs (miRNAs) circulating in the blood indicates injury to cardiac muscle, according to a study published online July 10 in the P*roceedings of the National Academy of Sciences*. While protein biomarkers, namely cardiac troponin, have served as the basis for diagnostic and prognostic evaluation of patients with heart muscle injury, the authors say that use of mRNAs may prove to be more sensitive and in the future may provide the basis for a "universal" test, rather than current assessment of single proteins.

The researchers compared the composition of miRNAs in a cohort of patients with stable and advanced heart failure (HF; n = 35) to the composition of eight normal adult and five fetal samples. Heart failure patients were examined both before and after treatment with a left ventricular assist device (LVAD).

The researchers identified three RNAs, known to originate in the heart, with potential for use as markers of heart injury. Heart- and muscle-specific circulating miRNAs (myomirs) increased up to 140-fold in advanced HF, which coincided with a similar increase in cardiac troponin I (cTnI). Three months after initiation of LVAD support, these extracellular changes nearly completely reversed. There was less than a fivefold difference in levels of circulating miRNAs among stable HF patients, compared with normal patients, while myomir and cTnI levels were only captured near the detection limit.

While disappointed at the relatively low levels of heart-specific circulating miRNAs, the authors do believe miRNAs afford advantages over cardiac troponin, including that the protein complexes containing miRNA are fully soluble and may be more rapidly released into circulation, compared to troponin, which is heavily bound to heart muscle filaments. Additionally, they believe enough evidence exists to justify further research in the diagnostic value of miRNAs, which they believe will lead eventually to a more "universal" test, encompassing a cluster of miRNAs, rather than the current evaluation of single proteins.

"Our findings provide the underpinning for miRNA-based therapies and emphasize the usefulness of circulating miRNAs as biomarkers for heart injury," writes senior author P. Christian Schulze, M.D., Ph.D., from Columbia University Medical Center (New York). "The translation of this type of RNAseq assay into clinical practice is currently limited by the time required for cDNA library preparation and sequencing, however, single-molecule direct RNAseq or targeted RT-PCR assays may overcome some of these limitations."

Takeaway: While miRNAs are not currently ready for use as a marker of heart muscle injury, researchers believe that within the next five years they will be integrated in panel formats, rather than relying on current tests of single protein markers.

G2 Insider

Outpatient Serum Folate Testing Unnecessary. . . Serum folate testing is significantly overused given the "exceedingly low" rates of detected serum folate deficiencies, according to a research letter published Aug. 11 in *JAMA Internal Medicine*. While serum folate testing is proving financially beneficial for laboratories and medical centers, it should be significantly cut or eliminated in the name of providing high-value care, the authors suggest.

The researchers retrospectively reviewed all outpatient serum folate tests performed at Beth Israel Deaconess Medical Center (Boston) from 2003 through 2013. The researchers found that over the study period, 84,187 serum folate tests were performed in 77,627 individuals. Levels were classified deficient (less than 3 ng/mL; 0.056 percent), low-normal (3-3.9 ng/mL; 0.197 percent), normal (4-19.9 ng/mL; 68.195 percent), and high (more than 19.9 ng/mL; 31.552 percent). Ordering habits have not changed over the last decade relative to routinely ordered tests like creatinine, the authors found, despite the significant reduction in folate deficiency since folic acid fortification of processed grains began in 1998.

Indicators are that folate testing is widespread nationally. The 2014 College of American Pathologists Ligand (General) K-A Proficiency Testing Survey found that 2,137 laboratories report results of serum folate and 225 laboratories report red blood cell folate. This total of 2,362 is essentially identical to the 2,340 laboratories that reported vitamin B12 test results from this same survey.

"Eliminating folate testing and other antiquated clinical laboratory tests is the responsibility of the laboratory itself and requires cooperation of the clinical staff," writes Alan H. B. Wu, Ph.D., in an accompanying editorial. His own clinical chemistry laboratory at San Francisco General Hospital eliminated in-house red blood cell and folate testing, which has greatly reduced the volume of test requests. For those patients in whom folate deficiency is strongly suspected, samples are sent to an outside reference laboratory for testing.

Eliminating folate testing may actually pose a financial challenge to many institutions. In the Beth Israel Deaconess Medical Center study, the authors examined 2014 institutional costs and charges (less than $2 per test and $128 per test, respectively), as well as a reimbursement assessment (based on the Medicare fee schedule of $20.02 per test). Costs totaled $168,374 ($3,582.43 per deficient result), while charges totaled $10,775,936 ($229,275.23 per deficient result). Reimbursements totaled $1,685,423.74, yielding a net surplus of $137,913 per year for the medical center, which the authors call "an interesting financial dilemma."

"Every physician and medical center should aim to provide high-value care," conclude the authors, led by Jesse Theisen-Toupal, M.D. "In the case of serum folate, this would mean a significant reduction, or perhaps elimination, of testing, which in fee-for-service payment models would result in a significant loss in revenue to the medical center."

Company References

ARUP Laboratories
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American Heart Association
800-242-8721

BioReference Laboratories
800-229-5227

Foundation Medicine
617-418-2200

Genomic Health
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