MAY 2017 # 30

the Pathologist

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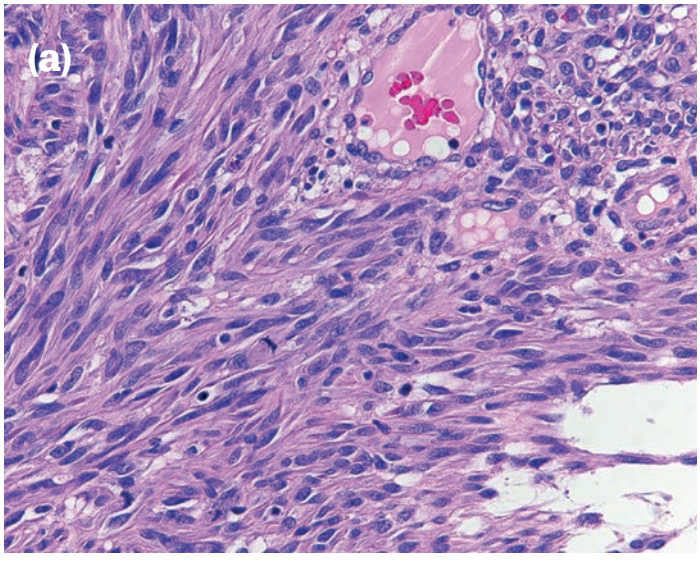
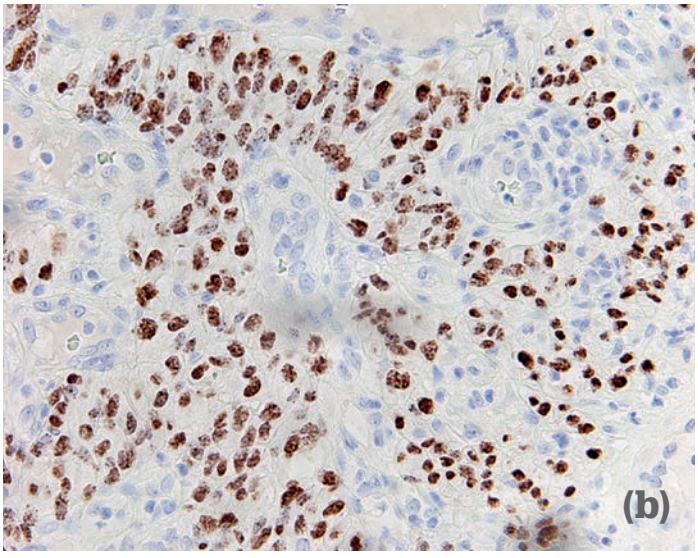
Case of the Month





Recurrent Skin Tumor

Hematoxylin-eosin section (a) demonstrates a recurrent tumor from the leg of a 91-year-old man. The tumor was diagnosed two years previously as an angiosarcoma and treated by radical excision. The recurrent tumor avidly expressed CD31 and was nonreactive for smooth muscle actin, SOX-10, Melan A, and CD10. It was positive for HHV-8 (b).

What is the most likely diagnosis?

a Angiosarcoma

b Kaposiform hemangioendothelioma

c Kaposi sarcoma

d Spindle cell hemangioma

e Spindle cell hemangioendothelioma

Answer to last month's Case of the Month…

D. Yolk sac carcinoma.

The tumor shows several histologic growth patterns, forming among others reticular, tubular and papillary structures, and even the so called Schiller-Duval bodies. The blood submitted to the laboratory after surgery contained increased amounts of alpha-fetoprotein.

Submitted by Da Zhang, The University of Kansas, Kansas City, USA.

To register your guess for this month's case, please go to http://tp.txp.to/0317/case-of-the-month
We will reveal the answer in next month's issue!

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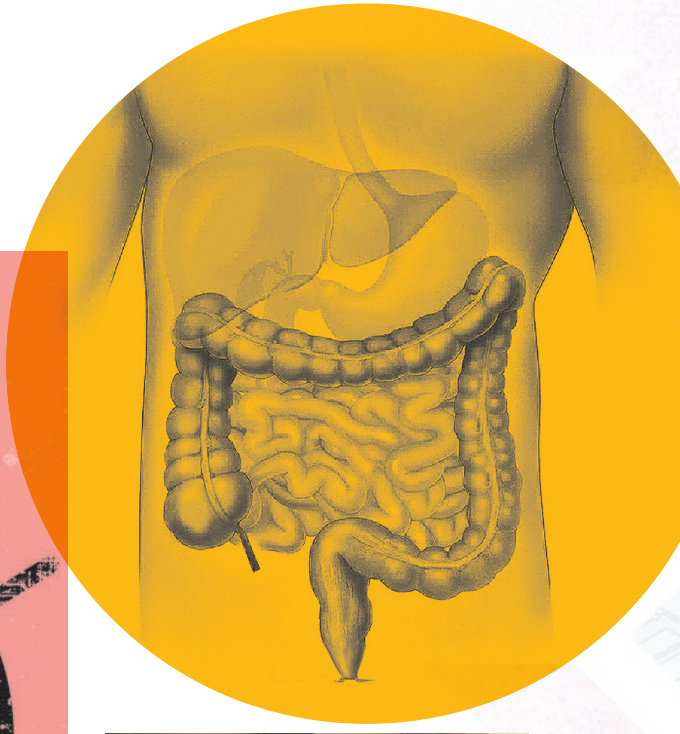

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An artist's representation of the pathologist as Atlas, with the burden of an ever-growing workload on his shoulders.

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the **Pathologist**

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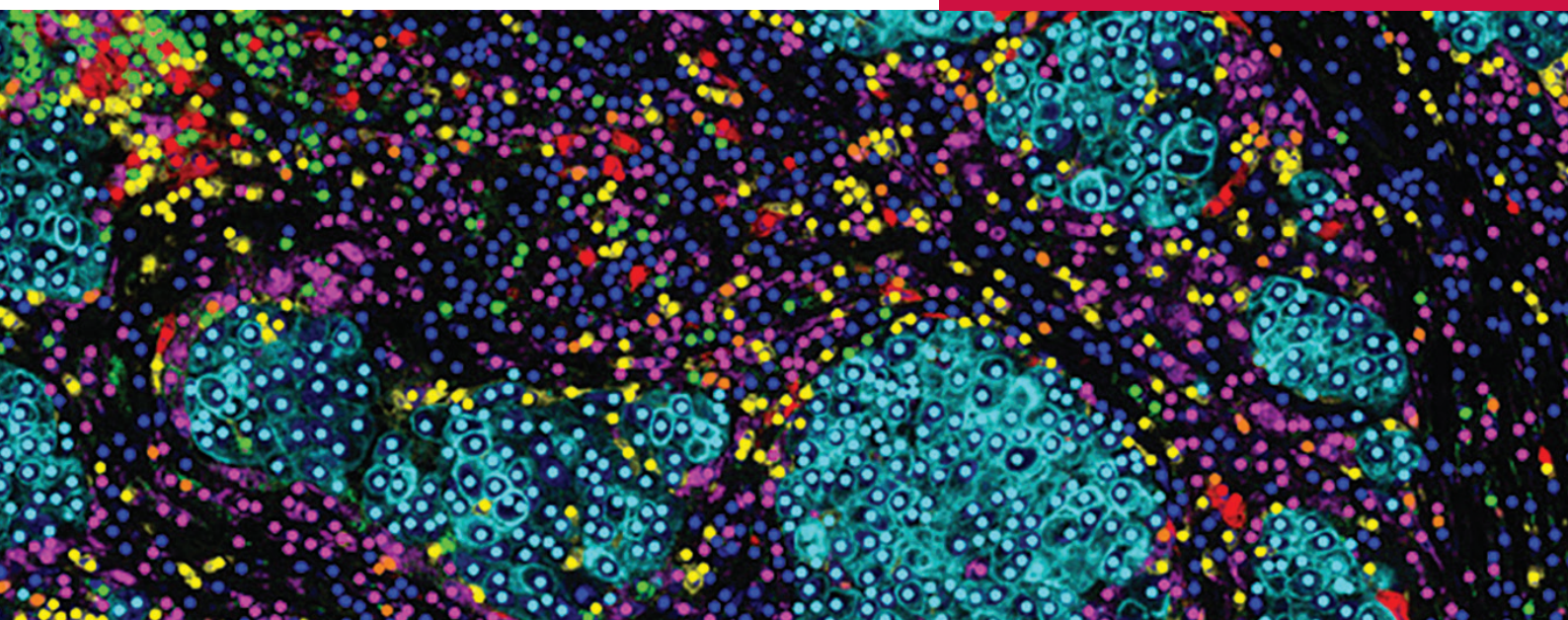


DIY Pathology Panic

Can genetic home test kits ever be a good thing?

"We just found out that she's at a high risk of getting cancer. And she's not even 40 yet," said a gentleman ahead of me in the supermarket line. He was loud, so I couldn't avoid listening to the rest – but I'll admit I was also curious. The gist was this: his wife had surgery to remove a brain tumor last year, and decided to order a genetic home test kit to be aware of any other conditions that she might expect later in life. The gentleman didn't specify the cancer type, but was adamant that his wife would be afflicted by the disease at some point in the future. Thinking back, I wish I had intervened and reassured him that the test was in no way definitive. Instead I kept silent, believing it was not my place to say anything.

The conversation took place just a few weeks after I read that 23andMe had gained the green light from the FDA to market its genetic tests direct to consumers for 10 conditions (1). It was the very first such approval and a huge win for the manufacturer – and I honestly felt unnerved by it. Not because I was wary of the strength or validity of the trial data presented by 23andMe in support of the approval, but because of the impact that widespread marketing of these tests could have on the general public. In the regulator's press release, the director of the FDA's Center for Devices and Radiological Health reminded, "It is important that people understand that genetic risk is just one piece of the bigger puzzle, it does not mean they will or won't ultimately develop a disease." Will they really understand that? And will the manufacturer make it abundantly clear that its test is not a disease predictor or diagnostic but simply a disease-risk indicator?

The gentleman in the supermarket went on to say that his wife had made dietary changes to try and improve her chances of avoiding the dreaded "C." If the test has encouraged someone to opt for a healthier lifestyle, that's a good thing, isn't it? But we're approaching Mother's Day here in the US and a TV commercial re-awoke the pessimist in me. It began: "This Mother's Day, enjoy $20 off when you order your kit at 23andMe.com," (with free gift wrapping!), and concluded with a little girl gushing, "I love you, Mom." Terrifying. Let's see what the long-term outcome is – public panic or healthier lifestyle choices? I'm hoping the "CynicalMe.com" is proven wrong on this occasion.

Fedra Pavlou
Editor

Reference

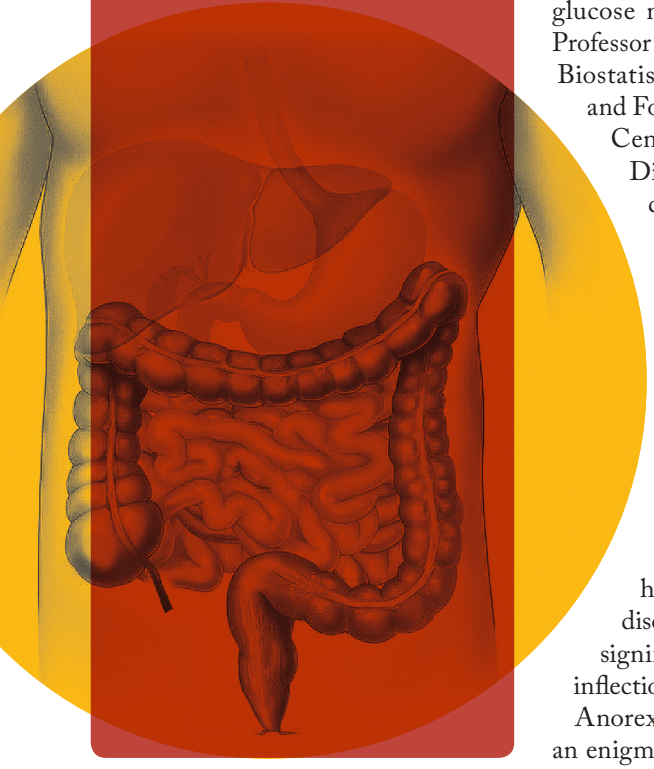
1. *US Food and Drug Administration, "FDA allows marketing of first direct-to-consumer tests that provide genetic risk information for certain conditions" (2017). Available at: http://bit.ly/2oIy6gc. Accessed May 15, 2017.*

Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

Email: edit@thepathologist.com



Metabolic Mystery Revealed

A new genome-wide study shows that anorexia nervosa is not purely psychiatric – metabolic factors also play a role

Anorexia nervosa is a devastating disorder – psychologically and physically damaging, tenacious in its grip on those diagnosed, and sometimes even fatal. It is usually diagnosed and treated by a psychiatrist – but now, new research asks: is the disorder exclusively a mental illness? A genome-wide association study (GWAS) conducted by researchers at the University of North Carolina School of Medicine has discovered strong correlations with psychiatric traits like neuroticism and schizophrenia – but, unexpectedly, also with metabolic features, such as insulin-glucose metabolism. Cynthia Bulik, Professor of Medical Epidemiology and Biostatistics at Karolinska Institutet and Founding Director of the UNC Center of Excellence for Eating Disorders, discusses her team's discovery of a significant locus for anorexia nervosa on chromosome 12 (1).

What's the importance of the newly discovered locus?

It's the first significant locus discovered for anorexia nervosa – in an area that has been previously associated with type 1 diabetes and autoimmune illnesses. As we have seen in other psychiatric disorders, the discovery of the first significant locus tends to mark an inflection point in genomic discovery.

Anorexia nervosa has always been an enigma. Especially puzzling is how these individuals can reach and maintain such low body mass indices (BMIs). Moreover, we have had no explanation for how or why, after therapeutic re-nourishment, their bodies rapidly rebound to the previous low BMIs. It makes me wonder whether what we are seeing is, in essence, the opposite of obesity. Individuals who are obese and diet down to a lower weight are known to regain that weight (and more) – a phenomenon that has been described as a "high set point." It's possible that what we see in anorexia nervosa is essentially the opposite – the body returning to a low set point. To date, we have primarily turned to psychological explanations for this repeated loss of weight. Now, our data suggest that we need to explore metabolic factors as well. That was the biggest eye-opener for us. We hadn't anticipated that the associations with anorexia nervosa would be so strong.

Will this help diagnose or stratify patients with the disorder?

That's our hope. We have been notoriously ineffective in treating anorexia nervosa, especially in adults. There are no medications that effectively treat the illness, nor any that target the underlying biology (because, until now, it has been poorly understood). Of course, we hope that genomic discovery will lead us in the direction of biologically or genetically informed therapeutic options.

In the future, using other genomic techniques, we may discover that some cases of anorexia nervosa are more strongly metabolic than others – or more strongly psychiatric. The ability to distinguish between different "subtypes" could potentially help guide our therapeutic approach.

First, though, we need a much more thorough understanding of the disorder's genetics. The next step is to increase sample size – right now,

we have over 13,000 cases queued for genotyping – and conduct additional analyses. We expect, based on GWAS for other disorders, that we will discover additional significant loci.

How did you bring together such a large collaboration?

The Psychiatric Genomics Consortium (med.unc.edu/pgc) was founded in 2007. It first focused on schizophrenia, major depressive disorder, autism, ADHD, and bipolar disorder. I watched their progress and decided that it was essential to develop an eating disorders working group. I could see that it was important to rapidly unite researchers and clinicians around the world in a quest to discover the genes that contribute to eating disorders.

What we've achieved so far is a brilliant example of what can be accomplished through global collaboration. It's so clear that we are scientifically stronger as a team than we could ever be individually. I hope other laboratory medicine professionals take the same route – together, we can accomplish so much!

Reference

1. *L Duncan et al., "Significant locus and metabolic genetic correlations revealed in genome-wide association study of anorexia nervosa", Am J Psychiatry, [Epub ahead of print] (2017). PMID: 28494655.*

A PCR Technique Out of Left Field

Circulating tumor DNA profiling can yield new insights into early-stage lung cancer evolution

Biomedical engineers at Vanderbilt University have designed a new handheld device that uses left-handed DNA (L-DNA) to monitor and control the molecular reactions that take place in PCR. The L-DNA is fluorescently tagged, and provides information on the reactions taking place – a spectrophotometer detects the varying levels of fluorescence in the sample. Based on the hybridization state of the DNA, the device adjusts its thermal cycling, allowing it to adapt to variations in the reaction and compensate for errors.

The aim of the innovation is to improve the reliability of PCR and shrink the required equipment down to improve accessibility. Dubbed "adaptive PCR," the approach removes the need for thermal calibrations and cycling programs and reduces the impact that environmental conditions have on the success of the reaction. Its small size also means it can be transferred easily from the lab to the clinic. *JC*

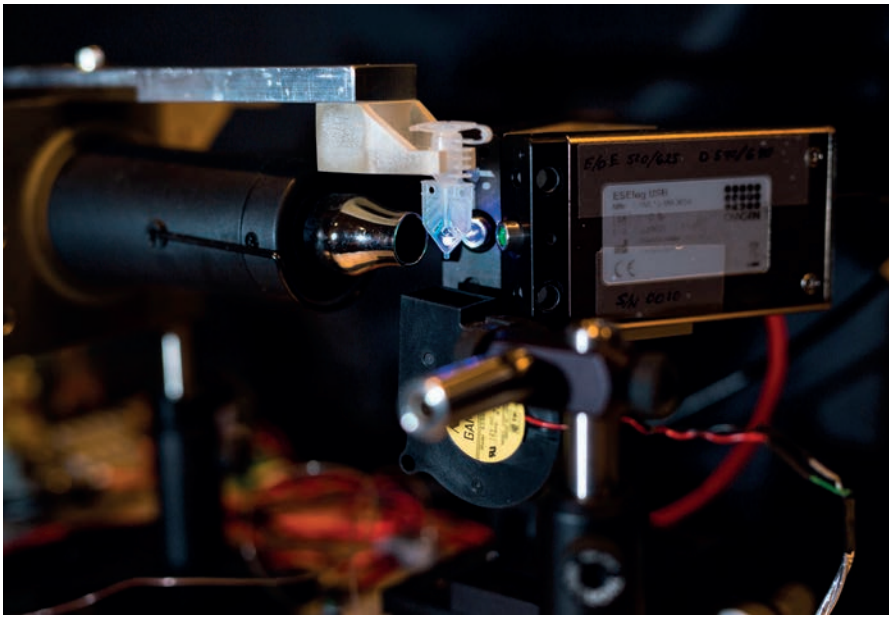
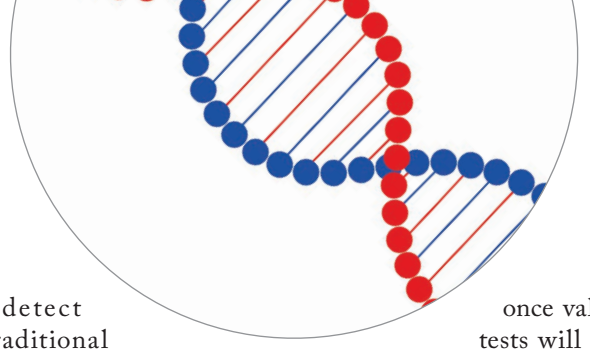


Figure 1. The handheld "adaptive" PCR system. The spectrophotometer (left) detects fluorescence in the sample (center). Image credit: Anne Rayner, Vanderbilt University.

Reference

1. *NM Adams et al., "Adaptive PCR based on hybridization sensing of mirror-image l-DNA", Anal Chem, 89, 728-735 (2017). PMID: 28105843.*



The Rise of ctDNA, Part One

New ctDNA assays could make more metastatic melanoma cases detectable

A new blood plasma test that detects circulating tumor DNA (ctDNA) could help identify mutations in metastatic melanoma that are tough to spot using current methods, according to researchers at NYU Langone Medical Center, USA (1).

BRAF and NRAS mutations account for over half of the 50,000 melanoma cases diagnosed in the US – but what about the rest? Though telomerase reverse transcriptase (TERT) promotor sequence mutations appear in up to 85 percent of all metastatic melanomas (2), the high G-C content of the TERT sequence can make such mutations difficult to detect using more traditional sequencing technology. The problem – and potential – prompted David Polsky, senior investigator of the associated study, to try an alternative technology – mutation-specific droplet digital PCR – and successfully developed a pair of tests that can detect changes in two mutation hot spots in the sequence. The assays were able to detect TERT mutations with high sensitivity and specificity; in tumor and plasma samples from patients with and without metastatic melanoma, all cases were detected successfully, with no false positives – even with as little as 1 percent of the mutated ctDNA present in a 5 mL blood plasma sample.

The blood tests could offer an alternative to CT scans – and the resulting radiation exposure – and allow more convenient and frequent testing that covers a wider range of melanomas, explained Polsky (3). He is hopeful that, once validated, the tests will quickly see widespread use. "Our goal is to use these tests to make more informed treatment decisions and, specifically, to identify as early as possible when a treatment has stopped working, cancer growth has resumed, and the patient needs to switch therapy," he added. *RM*

Reference

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2. *2. S Horn et al., "TERT promoter mutations in familial and sporadic melanoma", Science, 339, 959–961. PMID: 23348503.*
3. *3. EurekAlert!, "New gene-based blood tests identify more skin cancers", (2017). Available at: http://bit.ly/2qjrUfb. Accessed May 15, 2017.*

The Rise of ctDNA, Part Two

Circulating tumor DNA profiling can yield new insights into early-stage lung cancer evolution

What do we know about the early stages of lung cancer? Not much, because most cases are only diagnosed in late stages, once the symptoms have become unmistakable – and even relapses are often missed at first. Given that lung cancer is both the most common cancer worldwide and the leading cause of cancer death, it's vital that we learn as much as we can about how the disease evolves – and what we may be able to do to detect and stop it early.

To that end, a group of researchers have performed circulating tumor DNA (ctDNA) profiling on the first 100 participants in the TRACERx (Tracking non-small cell lung Cancer Evolution through therapy) study, taking a tumor-specific, phylogenetic approach (1). What does that mean? The team were able to spot early predictors of ctDNA release, detect resistance to adjuvant chemotherapy, and identify patients likely to experience a relapse. But the method's power doesn't stop there – researchers were even able to keep track of the molecular profiles of recurrent and metastatic tumors, allowing them to observe the cancer's evolution and potentially opening the door to future personalized treatments.

The science isn't quite ready for prime time yet. Its sensitivity is constrained by tumor volume; the smallest tumors visible by standard imaging correlate with plasma ctDNA levels at the very extreme of current detection limits – and the cost of targeted ctDNA profiling is still a significant burden. But there's a clear need to improve current treatments, whose success rates are low and toxicities high. If ctDNA profiling can provide insights into which patients are most likely to relapse and which cancers are most susceptible to chemotherapy, then as technologies improve and costs drop, we may one day be able to offer every lung cancer patient the treatment most likely to yield a cure. *MS*

Reference

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Computers Catching Cancer

A deep-learning network that accurately detects invasive breast cancer may lighten the load for overly busy diagnosticians

If there's one thing on which all pathologists agree, it's that their workloads are becoming increasingly untenable. It is a discussion of increasing importance (see "All in a Day's Work," page 18) – and with a growing patient population and a shortage of trainees entering the profession, solutions are difficult to find. Enter a promising pathology assistant: the computer.

With the rise of digital pathology, fewer and fewer pathologists are strangers to computer-aided diagnosis, but a new deep-learning computer network developed by researchers at Case Western Reserve University significantly ups the ante. The network demonstrated 100 percent accuracy in detecting and delineating invasive breast cancers in whole biopsy slides, and made the same determination in each individual pixel 97 percent of the time – exceeding the accuracy and consistency of the four pathologists against which it was tested (1).

So is it time to replace the human brain at the microscope with a digital one? Not just yet. "The network was really good at identifying the cancers, but it will take time to get up to 20 years of practice and training of a pathologist to identify complex cases and mimics, such as adenosis," said Anant Madabhushi (2), study co-author and Director of the university's Center of Computational Imaging and Personalized Diagnostics. Instead, he proposes that the network could triage cases for review by pathologists, saving time and allowing them to focus their attentions on the samples – and the patients – who need it most. "If the network can tell which patients have cancer and which do not, this technology can serve as triage for the pathologist, freeing their time to concentrate on the cancer patients." And best of all, the software can be set to run independently while pathologists work (or sleep), alleviating the intensifying burden on pathology department staff. *MS*

References

1. *A Cruz-Roa et al., "Accurate and reproducible invasive breast cancer detection in whole-slide images: A Deep Learning approach for quantifying tumor extent", Sci Rep, 7, 46450 (2017). PMID: 28418027.*
2. *"Computer accurately identifies and delineates breast cancers on digital tissue slides" (2017). Available at: http://bit.ly/2pBV6dh. Accessed May 11, 2017.*

More Than a Gut Feeling

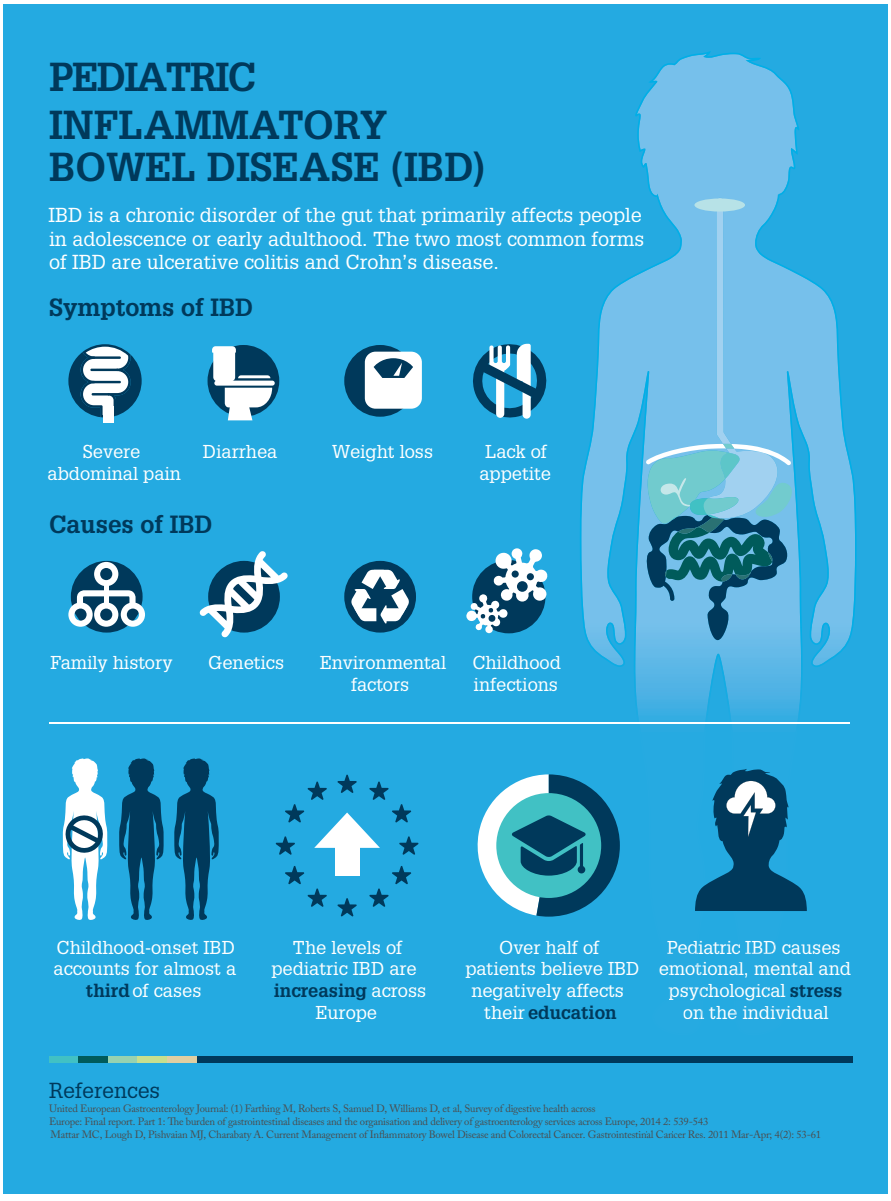
A noninvasive test to profile intestinal microbiota could help diagnose and predict the course of inflammatory bowel disease

Inflammatory bowel disease – what does that diagnosis mean? For many patients, it can be hard to say. The disease is highly variable, and it's extremely difficult to predict its course –especially in children, where IBD can be far more aggressive than in adults. But with so little ability to forecast the behavior of the disease, how can doctors decide which young patients are likely to need more extensive monitoring and treatment?

Until now, there has been no effective way to stratify IBD patients for intervention. Enter a team of researchers from Oslo, Norway, who have developed a genetic test to profile the intestinal microbiota of newly diagnosed pediatric IBD patients. After examining fecal samples from 235 children, they discovered that the microbiota profiles of those with more extensive disease differed significantly from those with limited or no disease (1).

Lead researcher Christine Olbjørn explained that the intensity of the probe – indicating abundance of fecal microbiota – was significantly lower in symptomatic children (whether diagnosed or not) than in those with no IBD symptoms (2). Olbjørn added that, when compared with children with limited disease, those with more extensive IBD had significantly more Clostridiales, whereas those with extensive Crohn's disease had more Proteobacteria, which she described as "intriguing." Furthermore, children who were ultimately deemed to need TNF inhibitor therapy exhibited lower pre-treatment microbial diversity than those for whom conventional treatment sufficed.

What does that mean for the clinic? Genetic profiling of fecal samples is a noninvasive way of establishing the extent of gut dysbiosis in newly diagnosed IBD patients – and may help to predict the course of the disease and identify patients who could benefit from more aggressive interventions. *MS*



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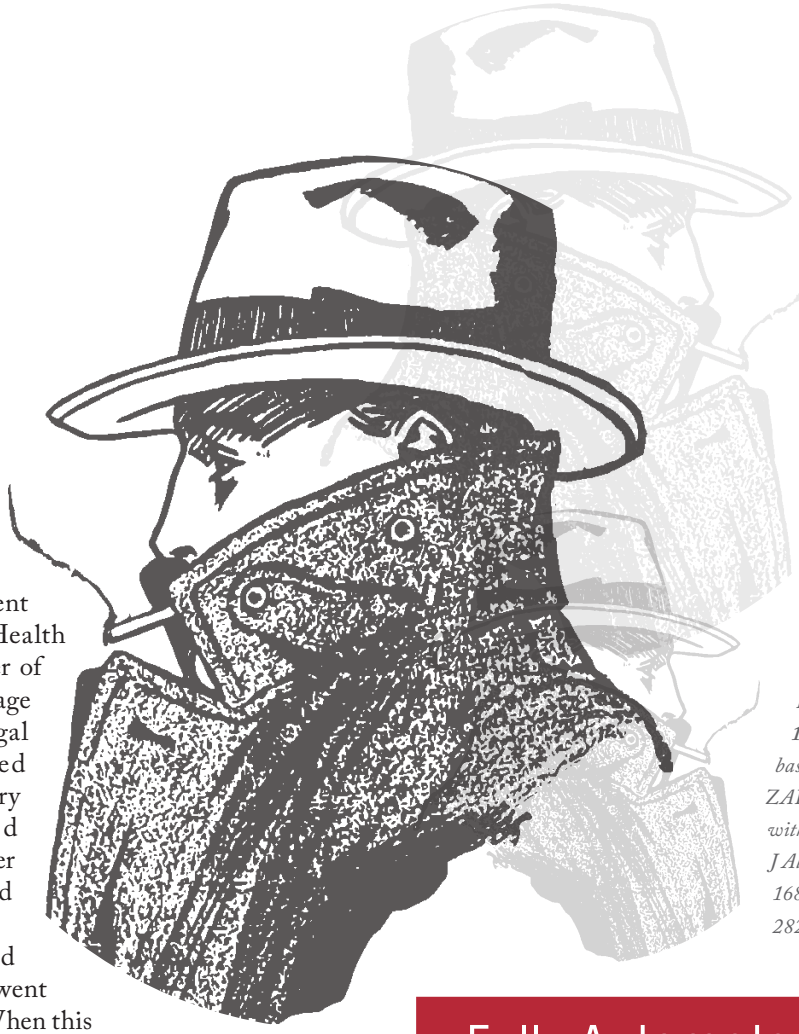
Cracking a Cold Case

A 30-year-old medical puzzle leads researchers to develop a new molecular therapy



Steven Francis, a patient at McGill University Health Centre, was at the center of a mystery. From an early age he had experienced fungal infections, an inflamed colon, shingles, respiratory problems, impeded growth, and a host of other problems. But no one could explain why.

At 33, he was referred to Donald Vinh, who went searching for answers. "When this patient was referred to me, I went over his entire file in detail, covering some 30 years and literally filling two large cardboard boxes. I also looked at his family history. Since the 1980s, many new immune deficiencies have been identified, and I was able to apply the knowledge from these advances to solve the case," he says.

And solve it he did – discovering that Steven had a mutation in *ZAP70*. The ZAP-70 protein helps to activate T cells and is critical for immune system function – and usually, mutations of the gene require a hematopoietic stem cell transplant for the patient to survive beyond early childhood. "Leaky" deficiencies in the gene are less common, with only a few cases reported in the literature. As stem cell transplants could prove risky for older patients, Vinh and his colleagues looked at a different approach: mutation-targeted molecular therapy.

Steven's specific mutation affects the splicing of ZAP-70, so the team designed an antisense morpholino oligonucleotide that targets the splice site generated by the mutation. This allowed the protein to be successfully synthesized ex vivo. If the treatment can be translated to humans, it could potentially improve immune system function.

Vinh is hopeful that the discovery of *ZAP70* mutations in adults, and the proof-of-concept study of a potential treatment, could lead to great advances in the field. "There are definitely more steps to take before we can test this treatment. For one thing, we have to convince the industry to support us. When Steven can finally get the benefit of the treatment, I'll be able to count this as a victory," he adds. *RM*

Reference

1. C Gavino et al., "Morpholino-based correction of hypomorphic ZAP70 mutation in an adult with combined immunodeficiency" J Allergy Clin Immunol, 139, 1688–1692 (2017). PMID: 28216435.

In My View

In this opinion section, experts from across the world share a single strongly held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of laboratory medicine. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@thepathologist.com

Foreseeing a Delectable Land

Open minds and eyes are the requirements for the future of pathology.

By David Booker, Owner, Laboratory Medicine PC, and Founder/CEO, BasePath LLC, Augusta, Georgia, USA.

"I need it fast, cheap, and right!" Pathologists get a lot of this, and we usually respond, "two out of three ain't bad." as the song goes. But, with Health Information Technology (HIT), including lab systems – "money for nothing" hits closer to the mark.

In the USA, we spend US$4 trillion annually on healthcare with dubious quality return on investment, and widespread HIT adoption brought about by government incentives has not improved on the 1,000 hospital deaths annually due to preventable error. This has saddled hospitals with technical debt, poor usability and interoperability, and poor productivity due to shortcomings of legacy HIT. And, even if hospitals had the funds to change, no comprehensive solutions exist that can offer significant improvement.

Hospitals are facing challenges on three fronts: first, the onslaught of data from the "Internet of Things" (IoT), genomics, and digital imaging; second, the entry of large retail business and enterprising startups into primary care; and third, the transition from fee-for service to value-based pay and coordinated care models. The first pushes legacy HIT past the breaking point; the second and third will require hospitals to demonstrate quality and customer satisfaction at low cost. This is because hospitals and specialists will become "suppliers" to companies controlling primary care that seek to use technology and innovative business plans to "Uberize" healthcare (by combining real-time data, mobile payments, instant fulfilment and dynamic pricing). This is also beyond current enterprise HIT capabilities. At the same time hospitals are taking a major productivity hit for their increasingly employed physician base and lose good physicians to EHR-induced burnout.

> *"Hospitals, clinics, and enterprise HIT companies must embrace the same mobile technologies and apps that are driving the disruption of primary care".*

To remain relevant, hospitals, clinics, and enterprise HIT companies must embrace the same mobile technologies and apps that are driving the disruption of primary care. According to McKinsey, a global consulting group, the next decade will see US $78 trillion in global economic impact from these technologies, greater than all

other economic sectors combined. The "disrupters" are well aware of this and their sights are set on a remarkably inefficient US $4 trillion industry that they believe they can make fast, cheap, and right.

Companies like Amazon and Netflix have solved their own versions of this problem using mobile technologies and related "microservices" architecture to replace the weak links in their monolithic enterprise systems gradually with small, agile, focused apps and modules using free or almost free, widely supported Web technologies. One of the key shifts is away from point-to-point interfaces to open web application programming interfaces (APIs) and the use of standards to ensure semantic and functional interoperability. Layered atop legacy HIT, these fix the pain points, make HIT more agile and responsive to customer needs, and decrease enterprise HIT risk, and lower costs. Such systems also offer the potential to improve the poor data quality of most HIT systems, which rely on unsustainable medical coding systems.

So, why are developments like Amazon and Netflix important? The answer is that open APIs merit additional focus because they leverage content and data, which is particularly important in healthcare. Outsiders will freely improve an enterprise's products by improving or adding apps using its APIs for mutual benefit – like a modern-day version of Tom Sawyer's friends whitewashing his fence. App stores like Apple's discovered this long ago!

"As patients use these tools to engage and collaborate on their care, a mass market of new health services will become available."

These technologies also allow end users to create and customize their own workflows with little or no programming. Just as you can use WordPress to create a professional web site very inexpensively without programming, this means doctors and other healthcare workers can quickly and easily adapt HIT to their workflows, rather than the opposite situation that now exists. And, since these are, after all, Internet technologies, they are built for interoperability. Healthcare can follow this path too, whereas wholesale replacement of legacy HIT is not feasible; and, fortunately, it is not necessary.

This combination of ability to crowdsource, manage and distribute content, and communicate are wonderful opportunities for hospitals and HIT companies, which are showing early movement in this direction by embracing Apple's HealthKit, HL7 FHIR (a web health exchange standard), and others. As patients use these tools to engage and collaborate on their care, a mass market of new health services will become available to them.

What does that mean for pathologists? It means keeping an eye out for emerging pathology and lab apps on platforms that can link with other apps to provide the benefits listed above. It means influencing colleagues and health systems to integrate these with their legacy HIT. It means embracing customer-driven healthcare. If we can learn from Tom Sawyer, it might mean that work will be more rewarding for all of us!

Seeking the Killer App

An examination of domain-specific companion diagnostic testing

By David Rimm, professor of Pathology, Yale University School of Medicine, New Haven, Connecticut, USA.

Immunohistochemistry (IHC) was the first companion diagnostic test for estrogen receptor (ER) and then human epidermal growth factor receptor 2 (Her2). Used in this context since the late 1990s, the tests have not changed much since then. Newer IHC tests, like those for EGFR (epidermal growth factor receptor) have more recently been introduced, but others, such as that for MET (mesenchymal epithelial transition factor) have been tried but failed, most likely due to lack of standardization, controls and reproducibility. And, there have been numerous efforts to quantify expression, including automated measurement of both traditional IHC and quantitative immunofluorescence. While these tests have been widely published, and even show significant improvements over subjective testing, levels of evidence or political factors have prevented any quantification from becoming "standard of care."

In my view, this is a missed opportunity

for the field of pathology. Arguably, the reason for this is that it is not necessary. That is, the quantification of protein expression on slides has not been "required" for any assay. Instead, we have been able to get by with semi-quantitative subjective measurements for ER and HER2, and with the appearance of newer drugs requiring companion diagnostic tests, the companies introducing them were afraid of asking for a measurement method that the majority of routine pathology IHC labs could not easily adopt. As a result, big pharma have been making deals with large diagnostic companies to develop companion diagnostics that can broadly deliver conventional IHC. Because of such deals, we may never know whether onartuzumab (anti-MET), for example, would have been more effective if targeted with an accurate, sensitive and specific IHC method.

Is this a lost opportunity? In my view, it is not, but it will require a "killer app". That is, a test that explicitly requires careful measurement that requires performing on select patients for a highly effective drug. The killer app may be around the corner in the detection and measurement of specific protein domains, in a manner that requires quantification or at least standardization. The test would be specific HER2 domains that may be required for the drug trastuzumab emtansine (TDM1 or Kadcyla) (1).

In a recently published a paper, our research shows that different antibodies that bind to different domains of the HER2 molecule display variable expression in some breast cancer cases (2). We demonstrated that antibodies like CB11 that bind to the intracellular domain (ICD) are not equivalent to those that bind to the extracellular domain (ECD) like SP3. This is biologically sound because proteases can cleave the ECD of HER2 and release it into the serum (3). Also, a cytoplasmic domain may also be produced by alternative ribosomal start sites resulting in short HER2 molecules that have the kinase domain (in the ICD) but not the ECD. Our hypothesis was that patients that had only ICD could respond to the standard regimen of trastuzumab with chemotherapy, but would not respond to trastuzumab alone. It was difficult to find a trial that offered this test, but we described an improved benefit corresponding to the presence of ECD in The Hellenic Cooperative Oncology Group (HeCOG) 10/05 – a trial in which patients received trastuzumab months after their chemotherapy.

"The quantification of protein expression on slides has not been 'required' for any assay. Instead, we have been able to get by with semi-quantitative subjective measurements…"

Soon, there will be a test of TDM1 on patients in the absence of chemotherapy to try to spare patients the associated toxicity (the GEICAM [Spanish Breast Cancer Research Group] trial). We hypothesize that patients with insufficient ECD, will not benefit from TDM1. Therefore, we think that the killer app could be a quantitative measurement of ECD and ICD to find patients that have enough ECD (probably 70 percent of the cases) that can benefit from TDM1 versus those with predominantly ICD, who will still require chemotherapy, and perhaps other non-trastuzumab therapy (such as lapatinib, the small molecule HER2 kinase inhibitor). It is too early to tell whether this will be a killer app and if it will be required to prescribe TDM1. It will require a number of studies, and perhaps the development of a simpler application than that described in our first paper (2).

In my view, this may be the tip of the iceberg. Many transmembrane proteins are processed and detection of domain specific activity could go beyond HER2. The next candidate is PD-L1. While the detection methods for PD-L1 are currently highly controversial and in development (4), it should be noted that both ICD and ECD antibodies for this molecule are commercially available and that we and others have suggested that cases show variable expression of PD-L1 as a function of the domain specificity of the antibody. This test also has potential to be the killer app.

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Beyond Stone-Age Sample Prep

Miniature detection technologies are evolving fast – but unevolved sample preparation is holding us back.

By Rodrigo Martinez-Duarte, Multiscale Manufacturing Laboratory, Department of Mechanical Engineering, Clemson University.

Miniature detection technologies have matured over the last decade thanks to significant investment from industry, funding agencies and investors. We can accurately identify target compounds using myriad technologies, including biosensors, spectrometers, PCR and sequencing. Highly abundant molecules, such as sodium and glucose, can now be monitored from a single blood drop using handheld systems, such as the i-STAT.

Unfortunately, when the target is of low abundance or contaminated with other substances, we're still struggling. Prevention of sepsis, food poisoning and water contamination, as well as the diagnosis and monitoring of cancer, all depend on the timely detection of rare targets – pathogens and circulating tumor cells. In these cases, we still rely on a series of cumbersome processes to convert the sample we gather into suitable fractions for analysis. Sample preparation currently relies on a suite of instruments for centrifugation, re-suspension, lysing, filtering and sorting; cue extensive labeling, wet chemistry and endless pipetting – all carried out manually so that reproducibility is too often dependent on experience...

Detection of one pathogen or tumor cell in a 10 mL sample is commonly required in clinical diagnostics (and in environmental monitoring, there can be as little as one target per liter). To obtain statistically valid results in these applications, we have to process large samples. It is unrealistic to expect the accurate and precise detection of a low abundant target when sampling only a few microliters of sample from a patient or water supply. Hence, preparation of large sample volumes is quite often a necessary step to enrich a target and enable analytical techniques. For example, lateral flow assays can only detect targets at a concentration of 100 nM. Even analytical technology with sensitivity of 1 attomolar would require at least one target per microliter of sample.

Is there a solution to this "needle-in-the-haystack" problem? Well, transforming samples retrieved from a real-world scenario into ideal fractions for analysis is by no means a trivial task. But the reward is worth the effort, and a number of promising technologies for sample handling, particle and molecular sorting, and lysis are being developed. These include contained platforms such as centrifugal microfluidics and digital microfluidics; and label-free bioparticle sorting, such as dielectrophoresis, inertial microfluidics, deterministic lateral displacement, and acoustophoresis.

There is much work to do before these next generation techniques can prepare a sample at the touch of a button. I foresee a combination of these techniques gradually enriching a target in a decreasing sample volume over time. Multi-scale fractionation of sample components will allow us to tailor the fraction depending on the analysis to be performed. Importantly, we will also need standards that enable the integration of modules developed by different companies; for example, standardizing the connector for transfer of a given sample type.

"Transforming samples retrieved from a real-world scenario into ideal fractions for analysis is by no means a trivial task."

Improving throughput, efficiency and reproducibility of a technology, and its integration with others, are not incremental advances. They are enablers of a practical platform that can have tremendous impact on clinical diagnostics, as well as disease diagnostics in rural, space, battlefield and wilderness scenarios. Investors and funding agencies first need to understand the challenge of sample preparation – and then do more to reward our efforts.

All In a Day's Work

Just how much should pathologists be working – and what happens if we exceed our own limits?

By Raymond Maung

We've all seen newspaper headlines that make sensational statements: "Overwork blamed for medical error!" "As hospital probe widens, are pathologists overworked?" "Pathology errors spark concern over hospital laboratories!"

But how sensational are these claims really? In my opinion, not especially. Many pathologists are indeed overworked – which can cause diagnostic and medical errors and be detrimental to both doctors and patients. As an anatomic pathologist in active practice for nearly three decades, I've worked in a number of medium- to large-sized, hospital-based laboratories. At one point in the late 1990s, my colleagues and I were trying to convince our administration to hire additional staff members – but because there were no good workload models, our needs were compared to institutions that, although similar, were not identical, and as a result we weren't granted the support we so desperately needed. Comparisons were done based on accessioned surgical cases, which was like comparing apples to oranges. Our department had a significant exclusion list (specimens removed in the operating room, but not submitted to pathology by mutual agreement) that decreased our accessioned number by up to 25 percent, whereas our counterpart had no such issue. At the time, the Royal College of Canada had a guideline for tissue pathologists' workload that was based on population served. In contrast, the Royal College of Pathologists in the United Kingdom and Kaiser Permanente in the United States based their guidelines on total accessioned surgical and autopsy cases, with modification for academic and non-academic centers. Why were their approaches so different? My curiosity prompted me to do a study. The question: what is the best and most practical way to determine the workload of anatomic pathologists?

The study evaluated readily available parameters, namely population served, total accessioned cases, number of specimens, blocks and slides, the Royal College of Pathologists' model, and a metric we called "level 4 equivalent," or L4E (weighted based on specimen type). Any of the parameters measured could be used but the L4E was statistically the best, and was adopted by the Canadian Association of Pathologists. I did a comparative study and showed that the different workload models, though using different units of measurement, gave almost identical recommendations (annual workload/FTE).

How does the L4E model work? Each specimen is weighted based on the time it takes to sign out, medical value to clinicians and patients, clinical urgency, degree of difficulty, and medico-legal responsibility. Specimens are categorized into six types (levels). Many biopsy specimens are categorized as level 4, which we use as a baseline and which is assigned a weight (or L4E) of 1. Other levels are weighted against that baseline; for example a radical resection will be at least one level 6 (L6) and will be weighted as the equivalent of ten L4s – that is, 10 L4E. The latest version of the model, published in 2014 (1), also integrates quality assurance, academic activities (teaching, training and research) and administration (regular and medical oversight). Last, but not least, the 2014 update defined nine simple rules for workload coding that cover about 95 percent of all cases. These are rules for:

1. Regular biopsies,
2. Core biopsies,
3. Curettings,
4. Tissue resections (other than radical resections),
5. Mandated synoptic reports,
6. Extra-levels and blocks,
7. Diagnostic stains,
8. Special studies, and
9. Radical resections.

Coding can be adjusted for the presence or absence of pathologist assistants, cytotechnologists, and resident trainees to meet the needs of different practice patterns.

How fatigue hides

My former department head once told me something I've never forgotten: "Administration understands dysfunction only." At the time, we were discussing the need for new pathology positions in our department. Pathologists are unique among the various medical disciplines, because unlike others, we don't have obvious rate-limiting factors that set an upper bound on our workload. In most disciplines, the main factor is time – whether that means consultation hours, availability of facilities or equipment, or scheduling around other specialties like radiology or anesthesiology. But in pathology, most samples can be processed within a few hours. The rate-limiting factor is the pathologists' ability to review and sign out the case – which is why so many pathologists work after hours or on weekends. That allows them to avoid undue delays in reports and hide any "dysfunction," but the chance of error increases as fatigue sets in.

> **"Obviously,** not every pathologist can review **and sign out cases** at the same pace."

Worst of all? Fatigue and overload are often only noticed when there is a crisis – an unhealthy situation for not only the patients, who bear the brunt of any errors, but for the pathologists themselves.

Obviously, not every pathologist can review and sign out cases at the same pace. Variations can stem from an individual's speed, degree of training, experience in a particular area, or general factors like the laboratory information system (LIS), use of voice recognition software, and technical and secretarial support. So with all these factors to consider, what is a safe workload for a pathologist? Despite plenty of discussion (2), there is no consensus yet. My own study using L4E (3) indicated that recommended workload ±7 percent (two standard deviations) will give a reasonable guideline for the minimum and maximum annual workload. It may be possible to increase that amount (up to as much as a 25 percent excess) for temporary situations like sick leaves or recruiting periods, but only if the timeframe is short and careful attention is paid to pathologist well-being.

A multifaceted workload

Pathologists have a vast array of duties. Not only do we engage in direct patient consultation via surgicals, cytology, autopsies, bone marrows, protein electrophoresis, and infection management, but we also have a medical oversight function that other medical disciplines don't – and our administrative duties are more demanding than most.

Many people view pathology as similar to our sister diagnostic discipline, radiology. But unlike radiologists, who review almost every procedure before sign-out, pathologists and even lab technologists don't have direct input into the finalization of

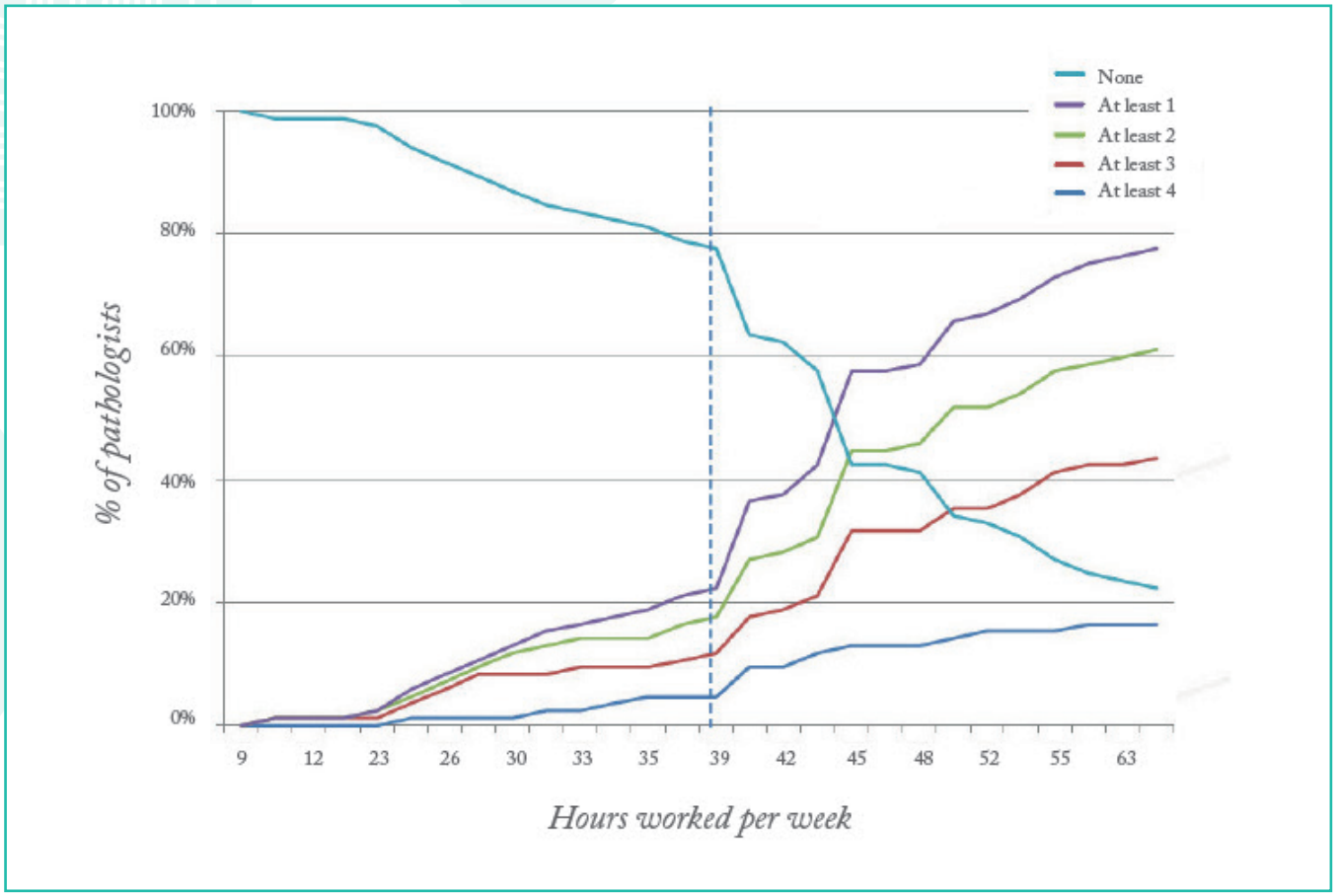


Figure 1. The percentage of pathologists experiencing adverse events increases significantly with a workload greater than 39 hours per week.

many laboratory reports. This makes our "medical oversight" duties essential to ensure that reports are accurate, timely, consistent, and delivered to the right individuals. The degree of oversight varies with each laboratory discipline – highest for biochemistry, followed by microbiology, hematology and tissue pathology – but in all cases, inadequate oversight can lead to disaster. In fact, the Commission of Inquiry on Hormone Receptor Testing (4)indicated the lack of medical oversight and quality assurance as major contributing factors to delays and errors.

Administrative duties are closely linked with medical oversight. It's to the peril of the institution when pathologists become advisors rather than participants in administration. For example, take the LIS. In cases where administrators have chosen systems without input from – or against the advice of – pathologists, those systems have sometimes needed to be abandoned or replaced at great cost to the institution. In cases where inadequate systems are implemented, essential activities like synoptic reporting and quality assurance, must often be carried out manually or by expensive add-ons. But the LIS isn't the only potential pitfall; the same principle holds true for budgets, equipment purchase, and more.

The function of the medical laboratory is to produce accurate, timely and consistent laboratory information for patient management – from screening to diagnosis to autopsy – so it's hard to overstate its ultimate impact on patients. My recent survey of the practice patterns of Canadian pathologists (5) showed that, like all physicians, our first priority by a large margin is patient care. So what happens when our total workload becomes unmanageable? We devote the time we have to patient care – at the expense of quality assurance, medical oversight and administration. That inevitably leads to system failures in the laboratory test cycle, which in turn have a greater impact on patient care than even diagnostic error. Whereas an error usually affects only a single patient, a system failure in something like fixation, sample collection,

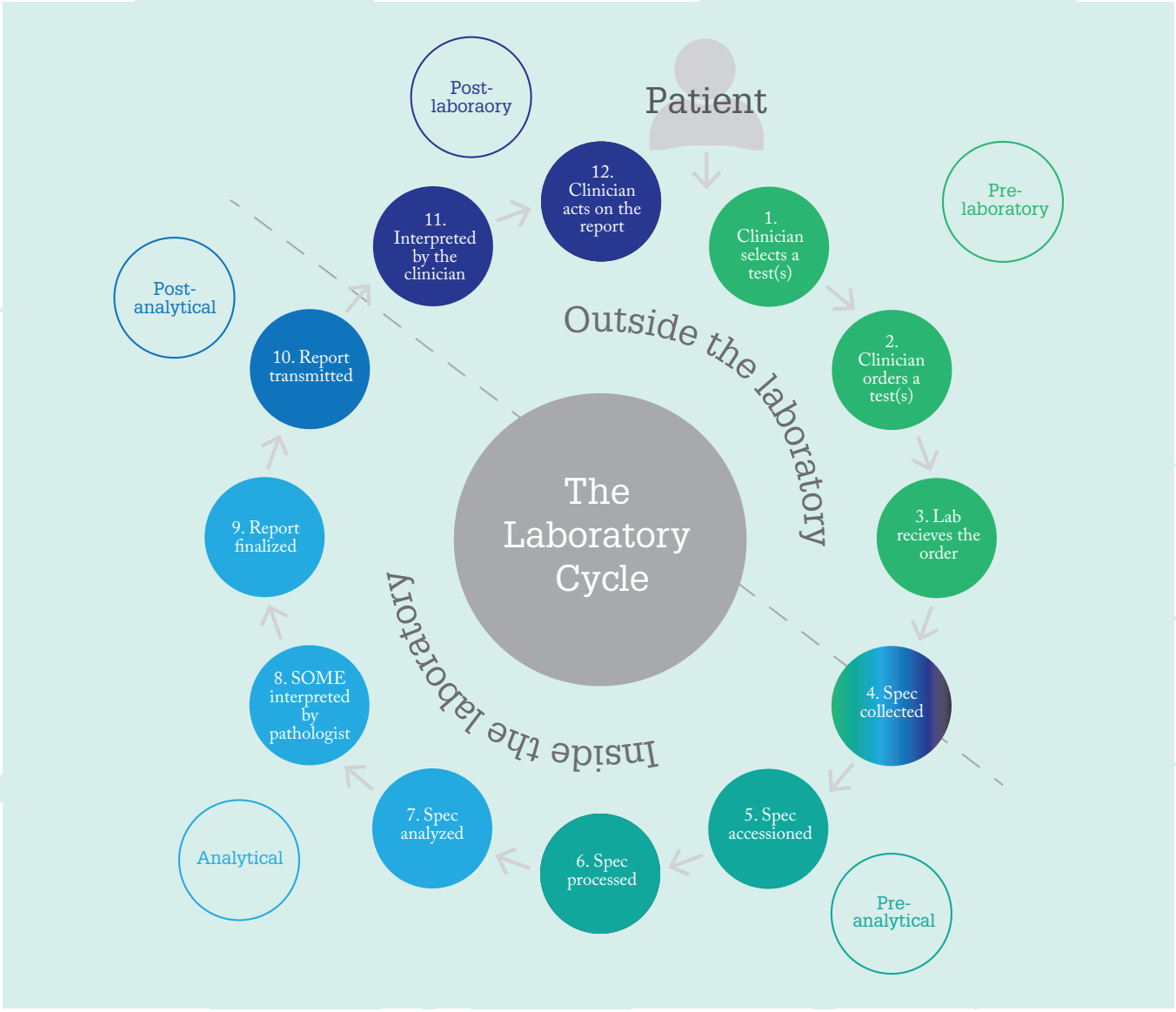


Figure 2. A detailed breakdown of the five-phase laboratory testing cycle.

labeling, or equipment function will continue to affect people until it is recognized and corrected.

Our own worst enemy

I think that we ourselves are our own worst enemy. Most pathologists will work overtime, without compensation, to ensure that we sign out the work that arrives on our desks within a reasonable timeframe. But laudable as that may seem, it's not good news for patients. We end up working while tired and ignoring our medical oversight and administrative duties in the process. In the short term, though, there's no apparent dysfunction in the laboratory – which makes it easy for administrators to ignore the situation even as pathologists ask for more resources. For example, one group of pathologists working in a rapidly growing city functioned with the same number of staff members for 15 years, with requests for more staff denied. Finally, contract negotiations broke down and the contract for the whole group was terminated. Now, a decade later, the department is approved for over three times

the original number of staff! That's how easy it is to miss the signs of pathologist overload, and how easy it is for pathologists to slip into habits that put both us and our patients at risk.

To avoid this, we need a workload model that takes into account the multiple duties of the pathologist – direct patient care, medical oversight, administration, and in some cases, academic activities like teaching, training or research. It also needs to adapt to our evolving profession, with technologies, standards, and expectations leaping ahead. The Canadian Association of Pathologists (CAP-ACP) is making an effort to do just that by updating the workload model every three to four years, and by trying to ensure that it can accommodate the wide variations in pathology practice between institutions with vastly different attributes and needs.

The great majority of pathologists – at least in Canada – are on fixed payment schemes linked to work hours. Thanks to our "professionalism and sense of responsibility," many of us work longer hours without pay or postpone the duties we deem least pressing. I've kept an eye on workloads throughout my career, and my experience tells me that the average workload increases by about 5–10 percent annually. That may not seem like much, but the compounding effect is significant – and in larger groups (with 20 or more full-time staff), it can mean two or three new staff members every year!

The side effects of overwork

Although there are no good double-blind studies for pathologists, plenty of studies in other medical disciplines and occupations have shown that fatigue is detrimental to job performance and results in increased errors. This is borne out by one of the best studies regarding pathologists (6), which discovered that adverse events – defined as increased turnaround time, quality compromises, patient care compromises, or damage to pathologist wellbeing – increase exponentially when we work more than 39 hours per week (see Figure 1).

The burden of responsibility

Most pathologists separate the laboratory test cycle into three phases: pre-analytical, analytical and post-analytical. I think it is best divided into five phases (see Figure 2) each of which has unique issues and requires unique solutions:

1. Pre-laboratory
2. Laboratory, pre-analytical
3. Laboratory, analytical
4. Laboratory, post-analytical
5. Post-laboratory

"Multiple studies have shown that what the pathologist means **and what the clinician understands** can be quite different!."

Most discussions combine the first two phases into "pre-analytical" and the last two into "post-analytical." But the pre- and post-analytical laboratory phases are directly under the control of the laboratory and solutions to problems are laboratory-based. For instance, frequent contamination of blood collections can be solved by altering laboratory practices. The pre- and post-laboratory phases are out of our control; they take place either before the specimen reaches us, or after our report leaves the laboratory. These issues – things like labeling errors, fixation issues, or misinterpretation of surgical reports – can involve multiple departments, and require cooperation and coordination to address. That's not to say that we don't have a role, though; in fact, we're in an ideal position to lead remediation initiatives especially in the pre-laboratory phase. Most errors in the testing cycle occur before the specimen arrives in the laboratory (see Figure 3). Issues in that phase come to our attention regularly during the different laboratory phases, so we can collect and analyze the errors. It won't be easy, but with a systematic approach, we can tackle these problems.

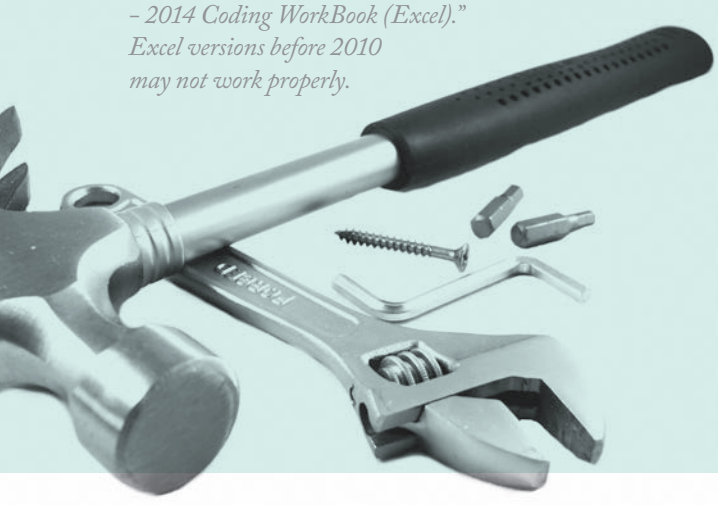
And tackle them we must, because with personalized medicine on the rise, the issue is more critical than ever. Nowadays, the collection, fixation and processing of specimens are vital for accurate analysis of the biomarkers we use for prognosis and treatment. Problems with these processes are difficult to detect unless they're being systematically monitored and analyzed – and yet, we know that they can significantly affect treatment decisions. For instance, improper fixation and processing can yield false negative results in estrogen receptor, progesterone receptor and HER2/neu status in breast carcinoma. The same is probably true for most of the molecular tests we use to guide cancer treatment, so it's clear that we need to be on the lookout for not only our own potential errors, but also those that may occur before patient samples ever reach us.

Unfortunately, we have less information on the post-laboratory phase. Multiple studies have shown that what the pathologist means and what the clinician understands can be quite different!

Tools of the Trade

If you are interested in documenting your own department's activities, CAP-ACP's Workload and Workforce Committee has developed a spreadsheet for doing so. The sheet automatically performs background calculations that yield the recommended FTEs needed to provide comprehensive service.

To download the spreadsheet, visit cap-acp.org/wkload.php and select "AP-ACP Workload Measurement Guidelines – 2014 Coding WorkBook (Excel)." Excel versions before 2010 may not work properly.



Understandably, that can negatively impact patient care and follow-up. Other post-laboratory phase issues include rolling out new TNM staging (with increasing frequency), new reporting systems (e.g., Paris urine cytology), new concepts (e.g., non-invasive follicular thyroid neoplasm with papillary-like nuclear features), and new classifications for various tumors. In addition, test results may be delayed, lost, or missed by the ordering physician if they receive several reports at once. According to Walz and Darcy (7), "The breakdown in the handoff of information in the care of patients is a common underlying cause of medical error." The researchers identify communication breakdowns as the most common cause of treatment delays, and failure to follow up test results as a common cause of malpractice suits. I have personally been told by administrators that the laboratory's responsibility ends as soon as the result has been signed out and reported through the LIS – which may be legally accurate, but doesn't necessarily lead to the best possible patient care. We all need to work as a team to prevent miscommunications and keep our patients from falling through the cracks.

"If they knowingly allow their pathologists to work overtime, then they should be held responsible for any errors that may occur."

My confession; my advice

I am one of the "stupid" pathologists who believe that they can work when fatigued without making errors – so I frequently catch myself putting in extra hours to finish my work. This has been the ethos for most physicians in the past, but now that the dangers of fatigue and overload are widely recognized, residents and house staff in most specialties are limited to more reasonable working hours. Not pathology, though! Administrators often fail to recognize the high volume of work that arrives on our desks – and our desire to finish in a timely fashion leads us to work overtime, neglect other duties, become fatigued, and potentially make dangerous errors.

Nevertheless, experience has taught me that I am not superhuman. I tend to hurry late in the afternoons when I am tired and may not be at my best for working with difficult cases. In the past few years, I have learned to stop looking down the microscope when I am tired; instead, I review and sign out reports or catch up with correspondence late in the day. Every pathologist is different, and what works for me may not be the right solution for someone else – but it's vitally important for each of us to recognize our own weaknesses and fatigue points, and to learn to accommodate them.

At least at the national level – if not internationally – we need to agree on a workload model. It's the only impartial way to determine the appropriate safe output for the average pathologist. And once that safe output is determined, how do we ensure that pathologists don't exceed it? That's down to the individual institutions. If they knowingly allow their pathologists to work overtime, then they should be held responsible for any errors that may occur – whereas right now, it's the pathologists themselves who bear that burden.

We need administrators to understand just how important our many duties are and to give us the resources and authority we need to deliver good laboratory information. It's easy to overlook the medical oversight and administrative aspects

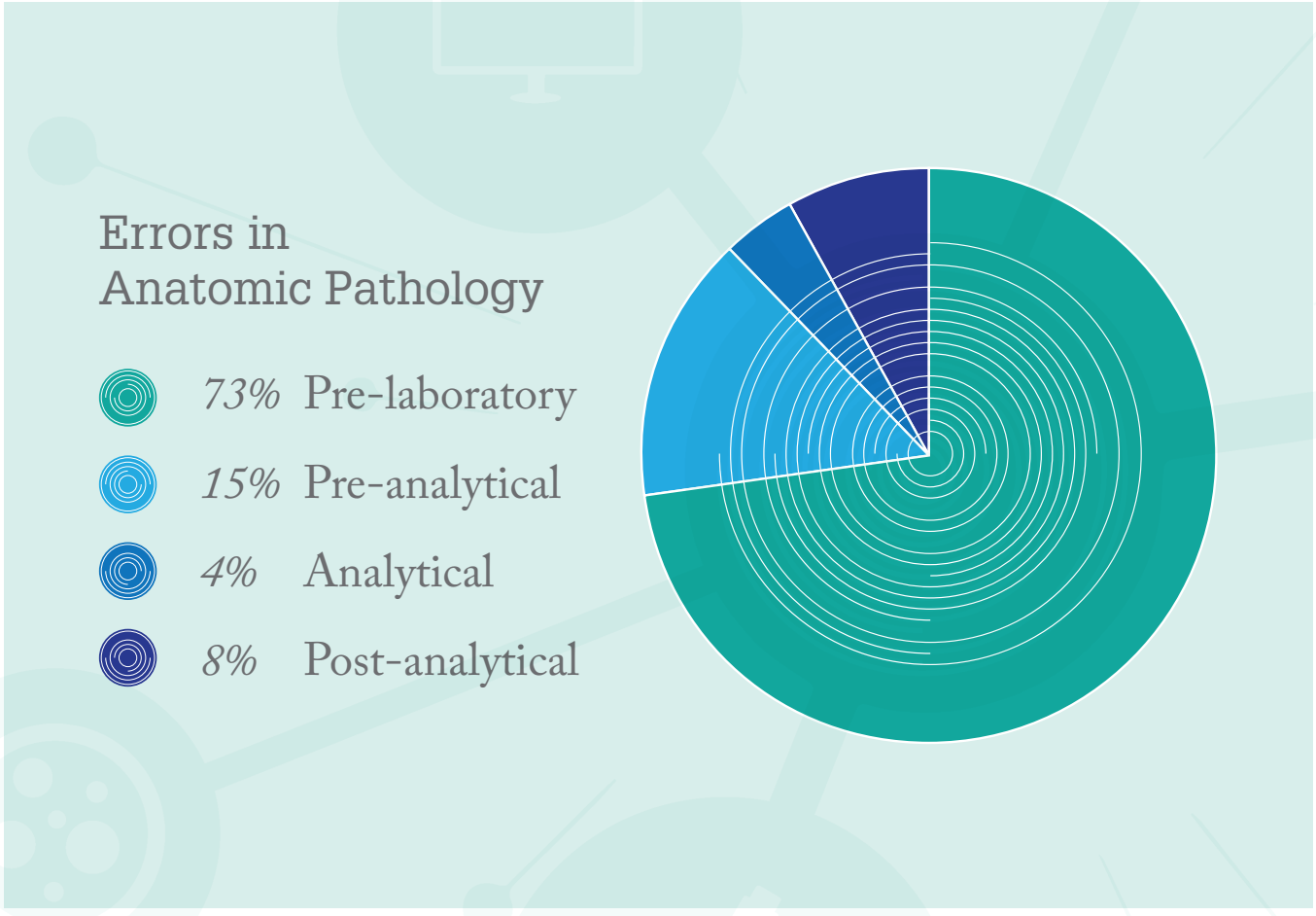


Figure 3. A breakdown of errors in anatomic pathology by testing phase (8).

of our work, but in the long run, that will negatively impact laboratory services. Our unique and multifaceted role must be clearly understood, and our workloads adjusted to ensure we can carry out every part of it to the best of our abilities. That's how we ensure the best possible health – for our patients, and for ourselves.

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A Clinical Calculus

Tools that quantify the (increasing) workloads of (increasingly) busy pathologists can help with staffing decisions

By Carol Cheung

The role of the pathologist is growing and changing. Precision medicine has certainly brought about more biomarker testing from a volume perspective – but the nature of that testing is also evolving. Predictive biomarker testing in precision medicine is of the "high-risk" variety, especially when it is used to select patients for targeted therapies. Nowadays, pathologists are required not only to analyze and report more informational parameters for the same testing, but also to issue reports for more of the new, high-risk biomarker testing that necessarily accompanies precision medicine.

What does that mean for our day-to-day work? Precision medicine is an exciting development, but for pathologists "on the ground," it is important to recognize that new advances – not to mention an aging population – are often accompanied by increases in the volume, complexity and risk level of our workload. On a routine basis, this translates into longer and more complex reports, additional quality assurance measures for all phases of biomarker testing, and the often-overlooked need for better training and administration to support these changes. Pathology residents need to be taught the new tests and reporting parameters; pathologist assistants need to be introduced to increasingly complex duties; pathologists need to devote more hours to quality assurance activities, meetings with colleagues, laboratory managers and administrators… the hours just keep mounting up.

How much is too much?

In kinder and gentler times, one of the best ways to determine a safe workload – or tell when it was being exceeded – was the simple act of listening to pathologists. But in today's world of evidence-based medicine and data-driven decisions, that is unfortunately no longer a realistic option. Metrics and benchmarking now dominate such discourse – and in a world where numbers reign supreme, it can be difficult to establish qualitative boundaries like "safe" workloads.

Pathology is certainly one of the medical specialties where focus, attention, and mental fatigue need to be foremost in our minds. When considering the work we do in our everyday practice, we need to be aware of the level at which we are functioning. "Am I still capable of assessing these samples accurately? Am I experiencing fatigue? Am I having difficulty concentrating? Are my reports still of the same quality as they were when I began work?" Sadly, we cannot necessarily tell when we are beginning to exceed safe workload levels; often, that only becomes apparent after mistakes come to light.

Complexity at the office

The Automatable Activity-Based Approach to Complexity Unit Scoring (AABACUS), like any workload model, primarily captures work done in the clinical sphere. For pathologists, this includes not only activities relating to microscopic assessment and the generation of diagnostic medical reports, but also those around grossing, frozen section coverage, one-to-one resident teaching, and, of course, quality assurance activities like review of clinical charts or radiology images, and intra-departmental consultations from colleagues. Because it is an activity-based model, AABACUS takes information documented in one or more laboratory information systems (LIS) as part of usual clinical practice and translates it into workload activities. These activities are then counted, scored (by applying a complexity factor), and translated into the complexity units (CUs) – the base unit of AABACUS. What can we do with these clinical activity scores? We can attribute them to institutions, sites, practice groups or individual staff members, which allows us to appropriately filter the questions we ask. That way, we can conduct analyses of staffing levels, resource allocation, utilization, impact and case costing (see sidebar, "A Counting Frame for Workloads").

It is important to remember that the amount of data that can be extracted for AABACUS is directly proportional to the amount stored in your LIS. The more you use the LIS as part of your workflow, the more robust the AABACUS data capture will be.

Data for decisions

AABACUS' primary function is workload assessment for the purposes of staffing – helping to determine how many full-time equivalent positions (FTEs) an institution needs to perform the work it generates and receives. AABACUS accomplishes this by taking the total CUs for the target institution for a calendar year and dividing that by the total CUs per allotted (funded) FTE for a "benchmark" institution for the same time period. The result is the number of FTEs that the target institution would require to tackle a similar workload to that of the benchmark institution. We call this "relative benchmarking."

Why does AABACUS work this way? It was developed in a multi-institutional, multi-site environment with general and

"We cannot necessarily tell when we are beginning to exceed safe workload levels; **often, that only becomes apparent** after mistakes come to light."

subspecialty sign-out, incorporating both community and academic practices in anatomical pathology, cytopathology, neuropathology, and hematopathology (solid and liquid). This diversity made AABACUS robust and enabled us to isolate certain practices (for example, by institution) so that we could develop and apply relative benchmarks to staffing. It has also made the model a powerful tool – AABACUS can be applied to subspecialty staffing questions as well. For example, if overall staffing calculations determine that a new position is justified, which practice group would get the new pathologist? AABACUS can help answer that question by taking the total CUs from the work attributed to a practice group and dividing it by the total number of FTEs allotted to that practice group. But note that AABACUS only provides information that can assist with the decision; it cannot make the decision for you.

Workload models, including AABACUS, are a reflection of pathology clinical practice for the environment in which they are being used – not the other way around. Any workload model has underlying assumptions, and the most important underlying assumption AABACUS makes is that your pathology clinical practice is legitimate and appropriate. Workload models can provide data and information, but the significance of the information must be interpreted in the proper context and by the proper decision-makers. AABACUS should reflect appropriate pathology practice; it should neither influence nor dictate such practice. Again, it is not a decision-maker – it is a tool to support the people who have to make the tough decisions.

Unfortunately, there are no magic bullets to alleviate pathologist workloads. Fiscal realities will always affect the practice of medicine, especially in the face of an aging population and an increasing availability of novel therapies as precision medicine expands its remit. I hope we get to a point where pathologists no longer have to keep their own workloads under control as individuals – that's just one more challenge to be addressed during a busy workday. Instead, I hope that non-pathologists will become more aware of the importance of our work to clinical care – and between that awareness and the availability of tools like AABACUS, workload management will become integrated into institutional processes to provide the best – and safest – care possible.

A Counting Frame for Workloads

What? AABACUS is a new approach to workload measurement that addresses the increasingly complex analyses pathologists must perform. A single case may require far more time and effort with today's precision medicine demands than it would have a decade ago – and in another few years' time, that same case may mean even more work!

Why? Traditional workload assessment methods may yield inaccurate results, overestimating the amount of work needed for simple cases and greatly underestimating the requirements for more complex ones. Pathologists need workload assessment that accurately reflects the types of cases they receive and the time and effort required to properly analyze each one.

When? We evaluated AABACUS over a five-year period from 2008 to 2012. The results of that evaluation were published in 2015 (1), and we hope to see the model's popularity grow alongside the demand for precision medicine.

How? The raw parameters are collected in LIS databases and exported as raw parameter data files for AABACUS. The user imports those files into the AABACUS database. The tool then selects parameters relevant to pathologist workload, converts them to workload activities, and provides a score in CUs. Those CUs can then be attributed and filtered to different pathologists, departments or institutions before a final analysis yields valuable information for staffing, resource allocation and more.

Carol Cheung is Assistant Professor of Pathology in the Department of Laboratory Medicine and Pathobiology at the University of Toronto.

Reference

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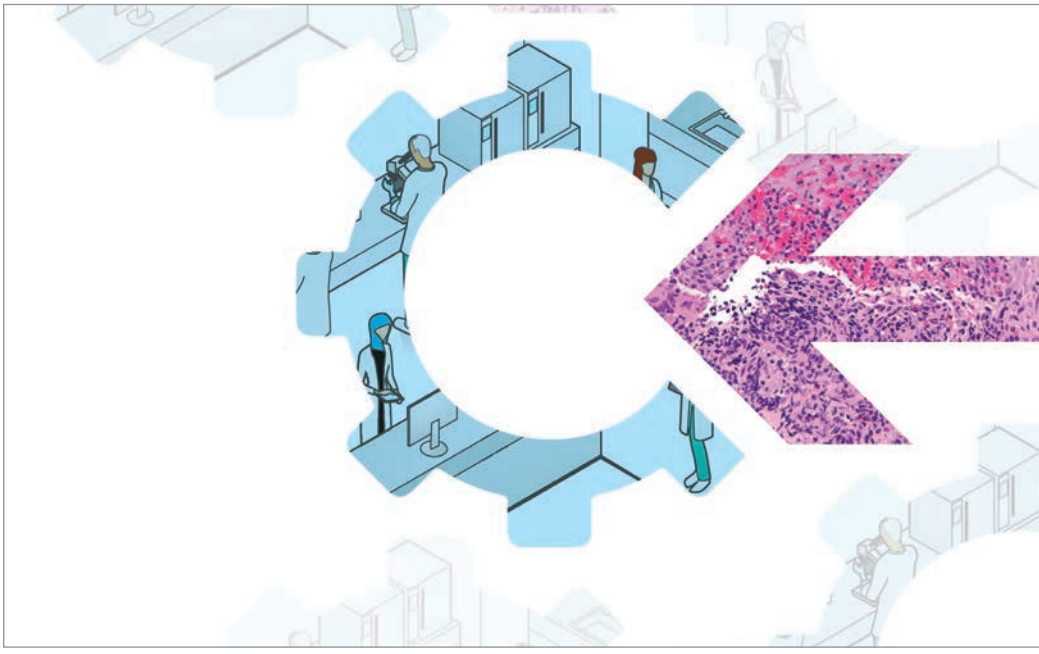
Gearing Up for Change

Can laboratories cope with the rising pressure to adopt new diagnostic technologies?

By Gene Elliott



Laboratories are under greater pressure than ever before to increase efficiency, but the challenges they face are also unprecedented. Reduced funding, altered payment models, escalating costs – all of these obstacles (and more) stand in the way of patient care, placing a significant burden on lab infrastructure and resources. But institutional hurdles aren't the only ones to overcome. The social and digital media explosion means that patients themselves are more aware of emerging trends and services, with everything from health apps to self-testing becoming increasingly commonplace. What does that mean for us? Today, we have to meet the demands of better-informed customers – with greater expectations around personalized care – who also present the challenge of complex conditions and multiple co-morbidities with ever greater frequency.

At a Glance

- *Despite a growing list of transformation demands, pathology services have been slow to adapt to diagnostic and technological advances*
- *We must urgently modernize the business of diagnostic laboratories, but not every lab has the same needs – or the same capacity to adapt*
- *Managing labs like businesses allows data collection to increase efficiency, demonstrate improvement, and drive further change*
- *Pathologists must lead the way to evidence-based laboratory transformation*

The concern that laboratories may not be able to cope with demand is growing. In the United Kingdom, for example, pathology pressures are now the subject of national newspaper headlines. One major cancer charity recently warned that laboratories are at tipping point; that soon, they may not be able to test the volume of samples they're expected to receive. Will that create knock-on delays in cancer care? Many people fear so, and unless our resources increase at the same rate as our workload, they may be right.

Patients aren't the only reason demand is increasing. New healthcare growth areas – genomic medicine, new techniques in disease screening and monitoring of chronic conditions, point-of-care testing, and more – are asking more than ever of the 21st-century pathologist, and I anticipate that the situation can only escalate. At the same time, though, skilled human resources are dwindling, not only because of budgetary constraints, but also because of the recruitment challenges pathology faces worldwide.

The urgency to modernize

Despite many years of calls to consolidate pathology services for increased efficiency, laboratories around the world still operate in much the same way as they always have. But now, the rising tide of pressures has created renewed urgency to modernize – starting with the acquisition of the tools and systems we need to make changes happen. Why are laboratories so slow to adapt to diagnostic and technological advances? And with change management skills a rare resource in public pathology services, what can we do to make sure labs are keeping up?

The first step is to make sure we know what our goals are. Laboratories need to maximize their existing resources, improve turnaround times of results and reporting, and reduce the costs of testing. These may seem like fairly obvious targets, but attaining them demands a

detailed understanding of workflows and procedures; we need to understand and tackle our own cost drivers and efficiency gaps. Current inadequacies in workflow visibility – often the cause of bottlenecks and delays in reporting and analysis – must be addressed if we want to shift our business objectives and allocate the right resources to speed up service.

One key skill we'll need to acquire is the ability to detect low-value tests, so that we can limit the number we perform. To get there, we need to give labs the power to ensure that patients receive the most appropriate testing right from the start. There is an urgent need for clinical context in the laboratory to help evaluate the appropriateness of tests, as well as for clinicians to have decision support so that they can avoid requesting inappropriate tests in the first place. Getting such services in place at the very beginning of the testing process should reduce pressure down the line. Clearly, the laboratory needs to step out of the "back office" and create a more clinically engaged service – one where pathologists and clinicians collaborate to decide which patients need which tests.

The transformation process

The whirlwind of factors, each requiring a tailored response, has made managing a laboratory extremely complex. As a result, legacy laboratory information systems (LIS) are no longer able to keep pace – to be effective in the modern lab environment, they must be able to support the logistics, measurement, planning and analytics we need to relieve staff burden, prevent bottlenecks, and provide business intelligence to support further transformation and consolidation.

Recently, we've seen a surge in the number of healthcare initiatives drawing on lean principles – including in the laboratory, where they're being adopted in response to increased demand. Why are lean strategies so important? They aim to reduce variations, eliminate waste, streamline workflow, and provide the means to improve service delivery – all vital characteristics for the "new generation" of pathology service provision. But as with any major change, adopting a lean approach invariably comes with organizational resistance, fears of job loss as efficiency increases, and the cultural challenge of moving away from long-held beliefs about how services should be delivered. For these kinds of changes to be accepted by pathologists on the ground, labs need to provide evidence of success – something legacy systems have not been able to do.

Beyond the LIS

A new breed of information system is emerging that will allow laboratories to evaluate the success of transformation initiatives, monitor compliance, and capture and analyze data to improve decisions for both patient testing and long-term strategic planning. If laboratories can measure improvements in turnaround times, costs, and the quality of test results, they can demonstrate the increase in operational efficiency to stakeholders and create a service thoughtfully designed to improve healthcare.

"We need to give labs the power to ensure that patients receive the most appropriate testing right from the start."

To that end, pioneering service providers are now starting to use laboratory business management systems (LBMS) to help them measure performance and gain an understanding of the costs, patient requirements and workflow needed to cope with unprecedented healthcare pressures. (For an example of these systems, see Figure 1.) I expect that, as the advantages of this entirely new generation of IT platforms become better-known, labs around the world will begin using them to support laboratory processes and to track the data they need to manage their work effectively – and to implement evidence-based transformation initiatives.

Why do we need such business-oriented laboratory technology? Modern service-level agreements demand improvement and require governance to monitor that improvement. LBMS platforms can capture and analyze the necessary data to show compliance (see Figure 2). They help the laboratory to transform from a reactive testing service

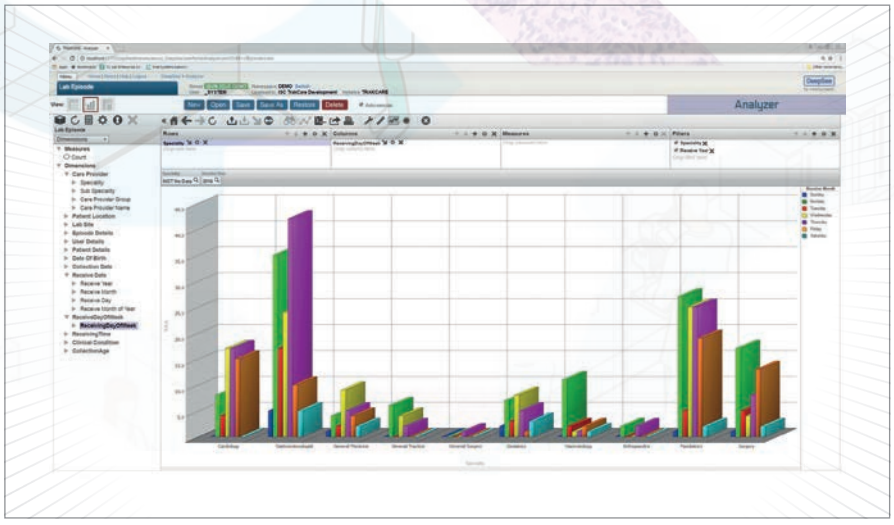


Figure 1. Requests received by each department, broken down by day of the week to facilitate resource planning.

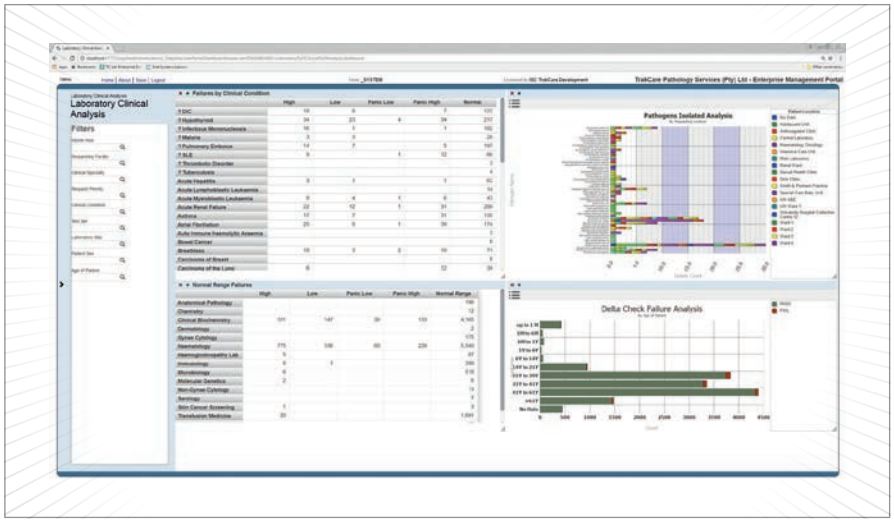


Figure 2: Clinical data can be presented as graphs or spreadsheets for analysis and export.

into a far more proactive part of the healthcare environment.

Diminishing resources, growing costs

In many parts of the world, pathology is receiving smaller and smaller slices of the healthcare budget. At the same time, costs – fueled by intensifying workloads and increasingly varied and expensive diagnostics – continue to rise. To respond, we need to start making changes to our services, and evaluating those changes against efficiency improvements and measurable clinical outcomes. I'd like to see laboratory results integrated into electronic patient records and used to inform clinical decision-making; in my opinion, that would go a long way toward minimizing unnecessary testing, targeting interventions, and optimizing overall patient care.

I don't think it's an exaggeration to say that the need for change is urgent.

"I don't think it's an exaggeration to say that the need for change is urgent. But that doesn't mean we should rush full steam ahead into those changes..."

But that doesn't mean we should rush full steam ahead into those changes; we need to harness the right information and provide the right results to ensure that we're making the right changes to the right services at the right times.

Emerging laboratory business models can help with that – but only if pathologists are ready to step up to the plate. What can we do right now to help facilitate improvements in patient care? Be open to new information; be ready to adopt new technologies; and, most importantly, don't be afraid to participate in the process of choosing and implementing change. It's our willingness to get involved that puts the best possible patient care within our grasp.

Gene Elliott is a physician executive for InterSystems, and has practised as a pathologist in private and public health sectors as well as studying lean management. Currently based in Johannesburg, South Africa, she advises a wide range of organizations on clinical and operational matters.

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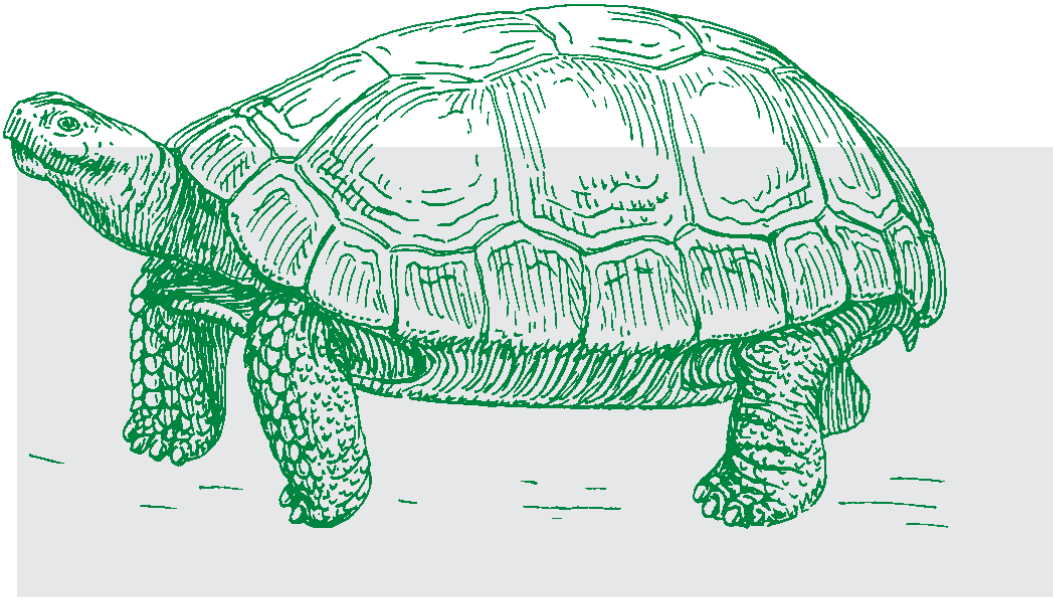

What if Aesop's Tortoise Were Smarter?

Rethinking the chromatography component of routine LC-MS bioanalysis

By Fred Regnier

The Problem

There is a growing need for routine analysis of small numbers of analytes in complex biological samples – and the world is increasingly turning to liquid chromatography-mass spectrometry for answers despite the challenges of low throughput and high cost per sample.

Background

There is great interest today in measuring small sets of biomarkers in biological samples as a means to assess biological function, health, disease, and treatment efficacy (1, 2), which is in turn putting new demands on the separation sciences.How can we separate, identify, and quantify relevant markers in samples that contain 10,000 other components within minutes – millions of times annually? Many believe LC-MS will play a role in this endeavor.

A major problem in routine analysis via LC-MS is that the LC column captures most of the substances in a sample; all of which elute into the MS over the course of the separation. With samples containing 10,000 or more components (see Figure 1), analytes and non-analytes will co-elute (3), making analyte differentiation difficult. Additionally, co-eluting non-analytes cause ion suppression, add background noise in spectra, and produce fragment ions of mass similar to analytes. Although MS is capable of millisecond analyses, analytes elute from LC columns over long periods of time; throughput is low, and elution times vary with instrument type, temperature, column lot, and column aging. As a consequence, the MS must continuously collect and examine huge amounts of useless spectral data to assure that analyte data is captured. Clearly, the MS is being poorly utilized.

The scenario brings to mind Aesop's tortoise and the hare; in an LC-MS/MS version of the fable, chromatography would be Mister LC Tortoise and the speedster would be Mister MS Hare. One realizes that, although illogical, this fable is played out millions of times annually in routine analyses. MS is fast and underutilized while LC trudges along. In the real world, LC Tortoise would have to be cleverer to survive – insisting on rules that include i) allowing him to finish in a few steps, ii) greater exploitation of his unique skills, iii) a course that is difficult for MS, and iv) requiring MS to do more work to finish. LC could dupe MS into accepting this rule change by telling him: "These new rules will make the race a little stressful but it will be over quickly, proving how fast you are. It will be more like a sprint than a marathon,

"There is clearly a problem with the way LC is being used in routine analytical applications."

At a Glance

- *Increasingly often, we need to measure small or low-concentration analytes like biomarkers in complex biological samples*
- *One common way to do so is LC-MS – but it's not ideal because slow, inefficient liquid chromatography results in poorly utilized mass spectrometry*
- *Newer techniques like mobile affinity sorbent chromatography can improve the efficiency of the first step*
- *There are a number of efficient chromatography options; all that remains now is to clear the financial obstacles to improving LC-MS*

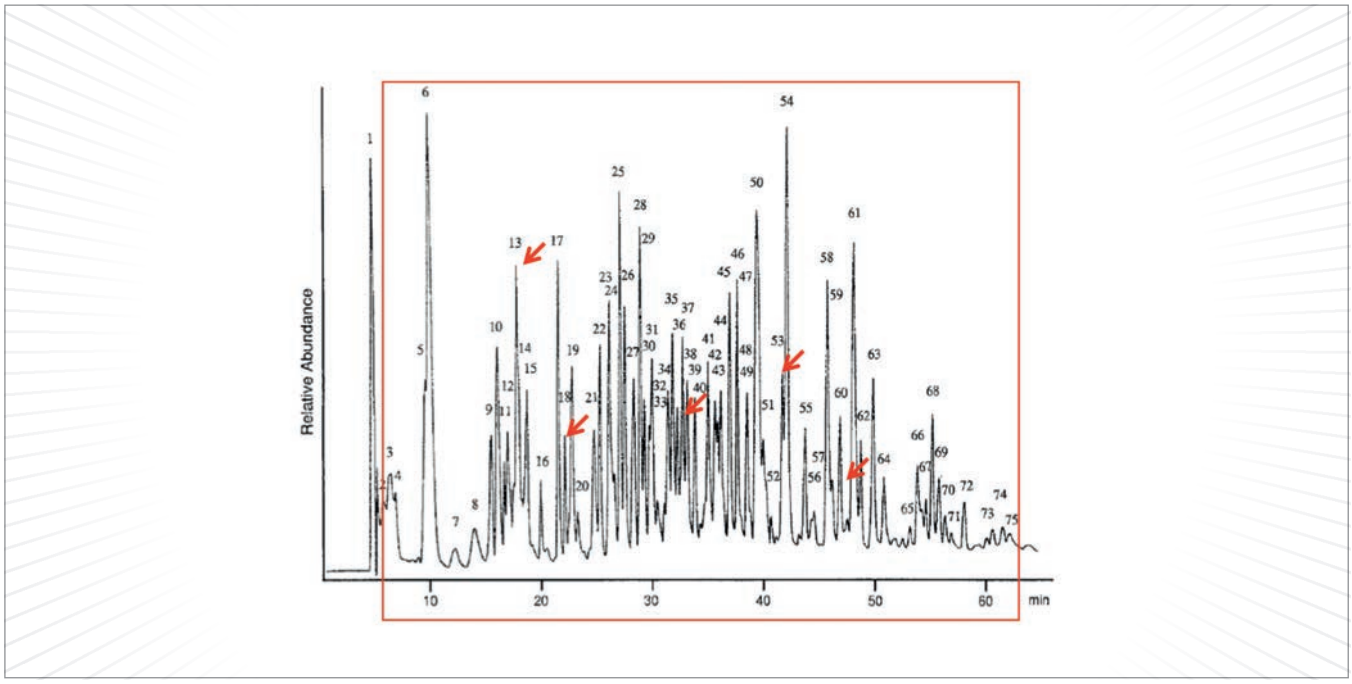

Figure 1. An illustration of problems encountered in LC-MS analysis of small numbers of analytes in complex biological samples. Red arrows show targeted analyte elution positions while the red box contains analytes that enter the mass spectrometer. Adapted from Reference 3 (highlighting added).

so falling asleep and waking up in time to win will no longer be a problem for you!" In actual fact, LC has skewed the rules far in his favor.

The solution

There is clearly a problem with the way LC is being used in routine analytical applications. Although MS can resolve the highlighted analytes (see Figure 1) a thousand times faster than LC, it cannot rid the effluent of non-analytes. The primary function of the LC should be to provide the MS exclusively with analytes of interest (devoid of non-analytes), rather than dividing complex mixtures into hundreds of fractions.

The problem could be circumvented if the small number of recurring analytes in the sample were structure-specifically selected and eluted concurrently in a cluster – unretained and relatively pure in a first chromatographic peak, and ahead of solvents and non-analytes – such that analytes could be rapidly transferred to an MS together (see Figure 2). The LC would be delivering a small group of highly purified, structure-specifically selected analytes to the MS for fractionation, identification, and quantification while discarding non-analytes. Being able to achieve this in two steps within minutes would have relatively large ramifications.

Structure-specific selection of analytes from complex mixtures is something that MS cannot do, whereas the LC could do so in minutes with high reproducibility. Moreover, nothing of analytical value would be eluted into the MS beyond the first chromatographic peak. The major work of the LC would be finished after delivering a single fraction to the MS. Non-analytes could be discarded by valve switching. Moreover, it would enable reduction of ion suppression, suppress background noise, and diminish fragment ion overlap in the MS. Structure-specific selection by the LC would be a critical component of the analysis, but the MS would be doing most of the work. The tortoise would be doing a critical thing MS cannot – quickly separating a small group of targeted analytes from the thousands of non-analytes in samples. Moreover, there would be no reason to fractionate all the non-analytes in a sample as shown in Figure 2, saving a huge amount of time. The tortoise would have crossed the finish line in two steps while the MS must do a large amount of work to finish. I think Aesop might have liked this "clever Tortoise" version of his fable...

We have achieved rapid, group-specific selection of a small number of analytes from complex mixtures as suggested above by developing a new type of chromatography. We call it mobile affinity sorbent chromatography

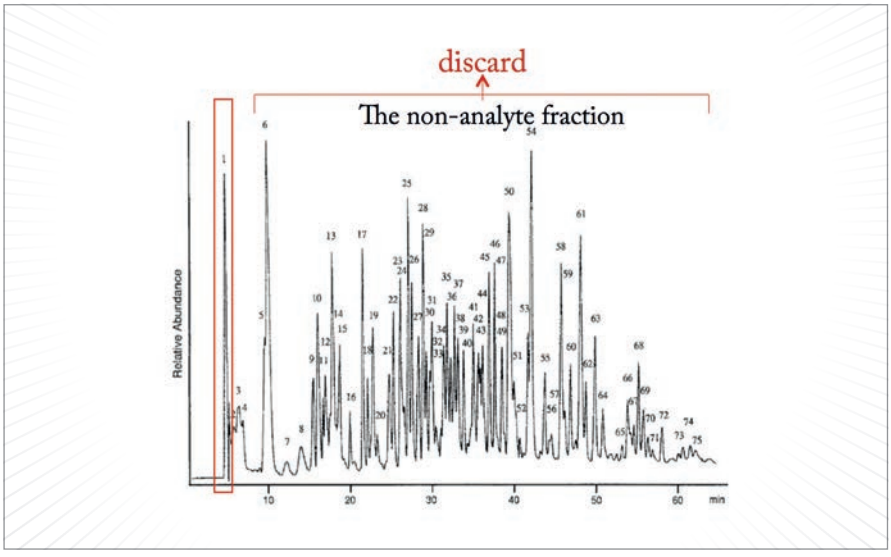

Figure 2. An MASC solution to the problem illustrated in Figure 1. The red box is where affinity selected analytes would coelute. All other substances would be discarded before entering the mass spectrometer. Adapted from Reference 3 (peaks highlighted in Figure 1 have not actually been removed from Figure 2).

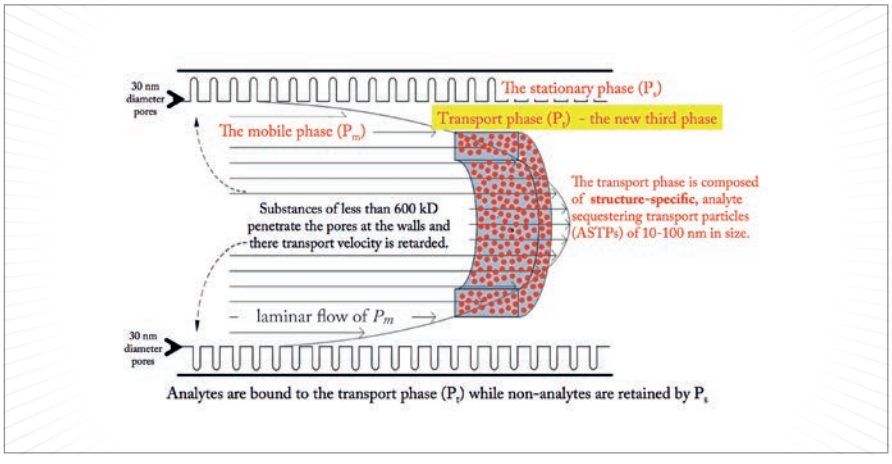

Figure 3. An illustration of the mobile affinity sorbent chromatography (MASC) model, demonstrating the three phases involved in separation processes. The function of the transport phase (P_t) is to sequester and accelerate the elution of analytes while that of the stationary phase (P_S) is to bind and retard elution of non-analytes. The mobile phase regulates partitioning between these two phases. This rapidly separates analytes from non-analytes.

(MASC) and first presented it at HPLC 2016 in San Francisco.

LC separations have long been based on differential partitioning of substances between two immiscible phases; one being an analyte transporting mobile phase (P_m) and the other a stationary phase (P_s) through which P_m is flowing. MASC is different, because a third, structure-specific analyte sequestering transport phase (P_t) is added to the conventional two phase LC system (see Figure 3). The function of this new transport phase is to i) sequester analytes of interest with high selectivity and affinity, based on their structure, ii) preclude their interaction with P_s, and iii) transport them though the column unretained. P_m still plays the role of mediating partitioning and transport.

"Although enormous gains have been made in structure-specific selection technology coupled to MS, adaptation in routine LC-MS has been disappointing."

The P_t used in our early MASC experiments is composed of 20-80 nm hydrocolloids with coupled affinity selectors. These analyte-sequestering transport particles (ASTPs) are larger than most substances in plasma while being sufficiently small to pass readily between the particles in a size exclusion chromatography (SEC) column or restricted access media (RAM) system. Single-structure specific-affinity selectors (Sa) were a component of each ASTP (see Figure 4) and, in our early studies, were antibodies (Ab) with an analyte association constant typically exceeding 106. Subsequent to association with the Ab, analytes are transported through MASC columns without desorption from the ASTP (4).

ASTP particles of ~60 nm eluted in the void volume of a 30 nm pore diameter SEC column designed for the separation of water soluble substances (see Figure 5). Non-analytes of less than ~400 kilodaltons (kDa) enter the SEC pore matrix and elute after

ASTPs; the retention time of ASTPs being in the range of a minute based on column dimensions and flow rate. Beyond enabling ASTP:analyte elution in the column void volume, a second advantage of MASC with an SEC column is that non-analytes have a short retention time, in contrast to the reversed phase chromatograms in Figure 1 and 2.

MASC was achieved in two ways; either by continuously adding ASTPs to P_m or by pre-equilibration of ASTPs with samples followed by injection of a small aliquot of the sample-bearing analyte:ASTP complexes. The latter of these two separation modes is referred to as zonal MASC in view of the fact that the small zone of analyte:ASTP complex acts like a short column from which weakly adsorbed substances are being continuously stripped as the particles move through the SEC column. Zonal MASC has multiple advantages, with the most important being that analytes bind to the ASTP before introduction into column, circumventing the need for in-column association of analytes with the P_t, which minimizes band spreading. Zonal MASC also minimizes antibody consumption and results in non-specifically bound substances being actively removed from equilibrations with ASTPs. Finally, fresh sorbent can be used in each analysis; minimizing carryover – equivalent to using a new affinity chromatography column for each analysis.

Analyte detection by MS in MASC is best achieved by dissociation of the analyte:ASTP complex after elution from the LC column. When the affinity selector is an antibody this entails antibody denaturation by heating or addition of an acidified organic solvent. With electrospray ionization-mass spectrometry (ESI-MS) high-temperature gas in the nebulizer spray was used to denature antibodies and desolvate the products before transport into the MS as seen in the detection of carbamazepine (see Figure 5).

Beyond the solution

The intent of the discussion above was to direct attention to three key facts:

1. The best solution to a problem may not be the most widely used,
2. The LC component of LC-MS for routine analysis is low-throughput and unoptimized,
3. Column parameters, such as particle size, theoretical plates, and peak capacity, are not always the dominant issues in an LC separation.

Although enormous gains have been made in structure-specific selection technology coupled to MS, adaptation in routine LC-MS has been disappointing. Structure-specific selection of analytes for MS analysis is actually an old

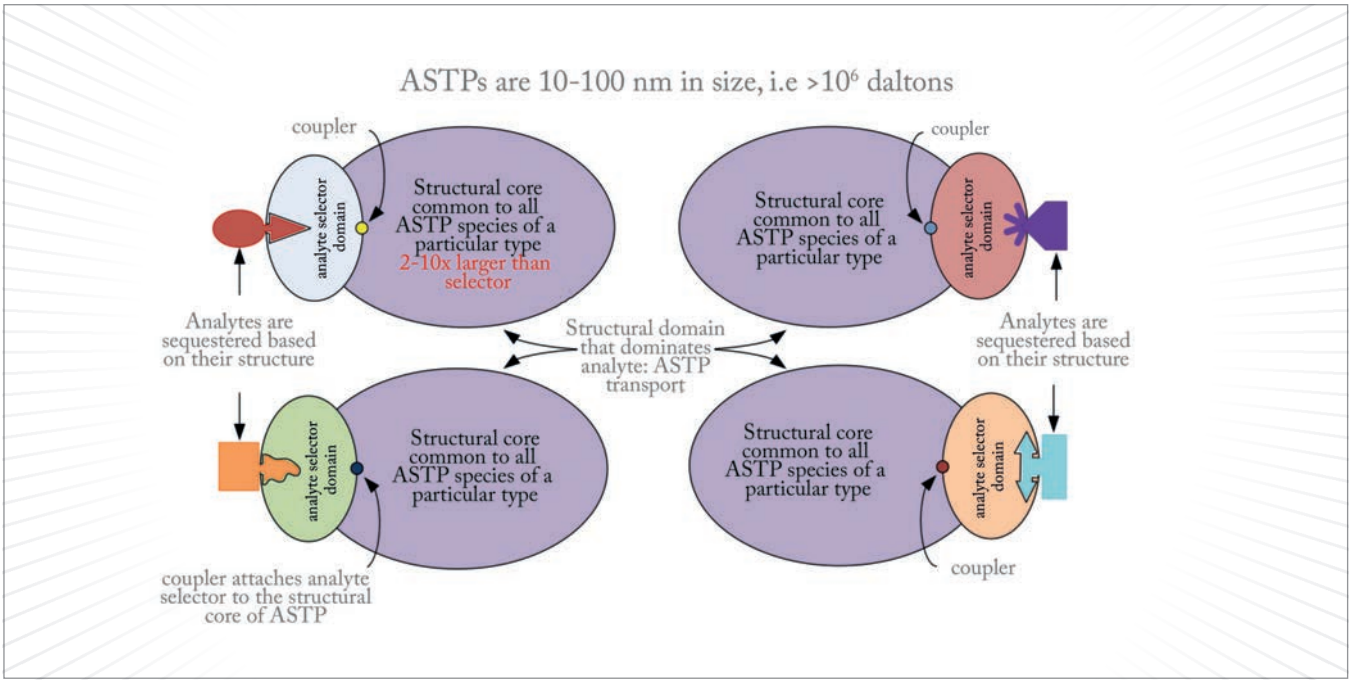

Figure 4. An illustration of the components in an analyte sequestering transport particle (ASTP).

technique – immunoaffinity assays using MS detection were first described in 1991 (6), followed by a host of MS-assisted assay methods ranging from mass spectrometric immunoassays (MSIA, 7), affinity-MS (8), and probe affinity mass spectrometry (PAMS, 9) to immunoMALDI (iMALDI, 10), surface-enhanced laser desorption ionization-TOF (SELDI-TOF, 11), and surface-enhanced affinity capture (SEAC, 12). These strategies all exploited either affinity chromatography with ESI-MS or affinity selection on MALDI plates as a means to simplify the purification of specific analytes – and they worked beautifully.

"It is often overlooked by the scientific community that economics towers above the other 'omics.'"

And yet, even with all of these powerful hyphenated-tools, reverse phase chromatography (RPC) still dominates sample preparation in routine LC-MS analysis of complex biological samples. As the LC-MS version of Aesop's fable suggests, the dominance of this old method is illogical. MASC with analyte-sequestering transport particles is simply another in a long list of structure-specific selection epiphanies (albeit one of the more powerful).

The future?

Scientists often seek – or expect scientific explanations for – puzzling phenomena such as the LC-MS conundrum noted above in routine analysis. But perhaps the dilemma is not of scientific origin. Clearly, the issues noted above obstruct the delivery of high-throughput, inexpensive diagnostics to millions of human subjects; removing these obstacles would be of massive value. So why hasn't the LC-MS/MS enigma in routine analysis been addressed?

It is often overlooked by the scientific community that economics towers above the other "omics." Finding the "right time" and mode of delivering a new technology often requires large investments. One skill of the investment community lies in guessing (or betting on) those elements and solutions that would provide the greatest return on investments, along with providing the requisite capital to back their bet. The manner and timeline in which a routine analysis revolution is triggered will more likely be a function of economic drivers than scientific issues.

As an afterthought, the "clever tortoise" version of Aesop's fable should probably have included a venture capital investor who would finance the contest and declare a winner based on investment returns. Looking at this as a sporting event with betting and a P&L bottom line is perhaps much more exciting than increasing LC-MS throughput, decreasing ion suppression, and eliminating background noise in spectra.

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NextGen

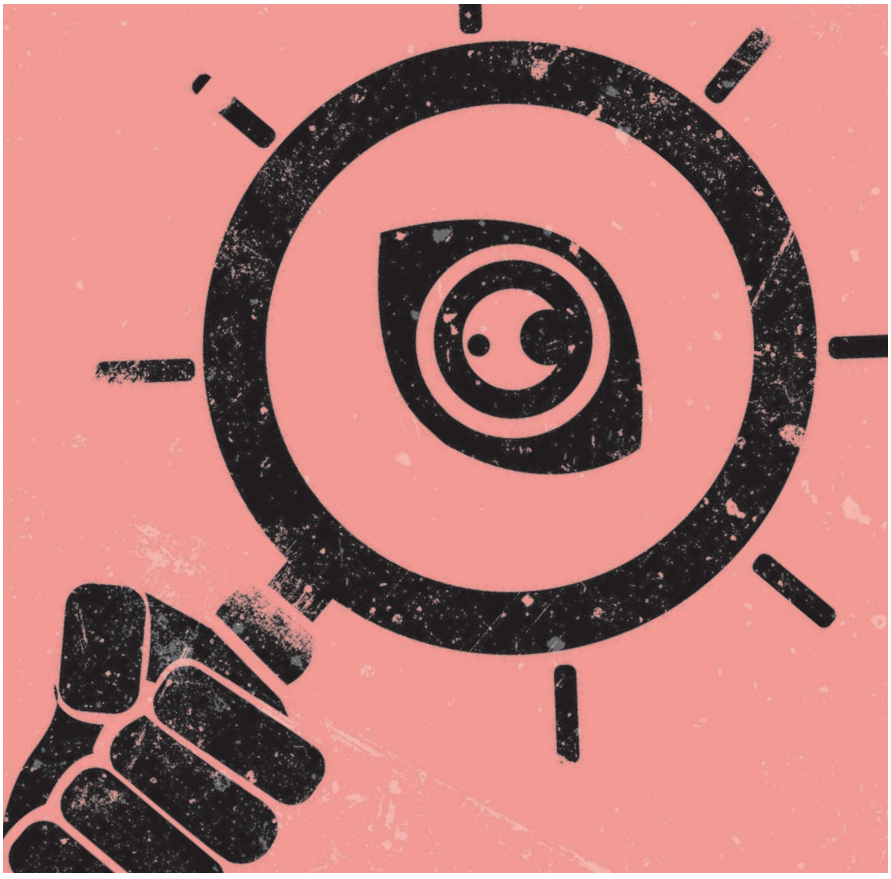
Research advances
New technologies
Future practice

40–43
Is Seeing Believing?
Automated digital imaging software could provide high-sensitivity in vivo detection of melanoma using visual biomarkers, and could serve as a pre-biopsy visual diagnostic for both dermatologists and non-experts.

Is Seeing Believing?

Digital imaging software could unlock a new realm of visual biomarkers for detecting melanoma

By Dan Gareau



The software will grow and improve as more lesion images and visual biomarkers are added.

Patients with skin anomalies often have only one question on their minds: mole or melanoma? Is this strange-looking freckle a benign dermatological feature or the frightening cancer they've heard about on television? Obviously, there are a few ways to tell the difference that even non-dermatologists can use – one of which is size. As a general rule, we know that melanomas tend to be larger than benign nevi... or do they?

Some time ago, a dermatologist colleague mentioned to me that a group of melanomas he and his colleagues were investigating may have actually been smaller, on average, than nevi in the same patients. To test their suspicion, they asked me to create an automated computer vision app to screen the sizes of the lesions. They selected a group of images of "difficult" lesions they had biopsied – nevi that looked highly suspicious, or melanomas that didn't. These are called "clinically equivocal" lesions. It turned out that they were right; on average, the melanomas were indeed smaller than the nevi in the group of images. Of course, that only holds for this study group, not the general population, and large moles should always be evaluated by a certified dermatologist. But at least in this instance, it seems that some cancers don't play by the "rules" – and that anomaly piqued my interest.

I wanted to be able to quantify and validate what we were seeing with a sensitivity and specificity analysis, so I took my first step towards creating digital imaging software that uses visual biomarkers to screen for melanoma (1). And there is a real need: at the moment, only 10 percent of excised skin lesions actually turn out to be melanomas (2), which means the vast majority of those patients are undergoing unnecessary biopsies that not only use up medical resources but also leave them prone to infection, scars, or even reduced mobility. Through the eyes of an expert dermatologist, lesions can be classified quite accurately – but current visual diagnostic methods (see "Current Classification Guidelines" on page 41) result in a much lower success rate for other professionals. Why? In the United States, there's one expert dermoscopist per 6.5 million people (see Table 1). No one can see that many patients in a year, so it's clear that not everyone who should be evaluated by an expert can be; demand far outstrips supply. I wanted to build a better mousetrap – a

At a Glance

- *Only one in 10 excised skin lesions are found to be melanoma*
- *New automated software allows high-sensitivity, in vivo detection of melanoma using visual biomarkers*
- *The software is intended as a pre-biopsy visual diagnostic for dermatologists and non-experts alike*

diagnostic that would be highly sensitive and specific no matter who was using it. Along the way, I created something even more valuable: a software program that not only delivered superior diagnostic accuracy in our study test group*, but has the potential to help the observer by showing what features it is analyzing and why.

Type of provider	*Patients per provider*
Dermoscopist	6,480,000
Dermatologist	32,400
Pharmacy	4,830
General practitioner	379

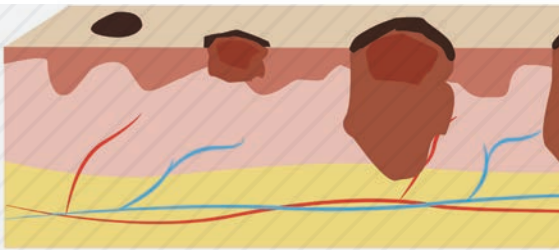
Table 1. Number of patients per type of practitioner (based on US population data).

Finding form

To create a visual biomarker algorithm, you first need to discover initial patterns. My journey began with the acquisition of 120 samples. Next, I sat down, brewed a cup of coffee, and spent 500 hours writing computer code to quantify patterns – fractal arrangements of pigment networks, little islands of the globular pigmented phenotype, and more. I tried to mathematically describe any deviations from "normal" nevus patterns and gave a basic visual estimation using those patterns.

Clearly, no doctor has that kind of time to spend analyzing each sample. Instead, I created a code to find, for example, every pigmentation island and its diameter, so that I could calculate a coefficient of variation (the standard

Current Classification Guidelines



ABCDE

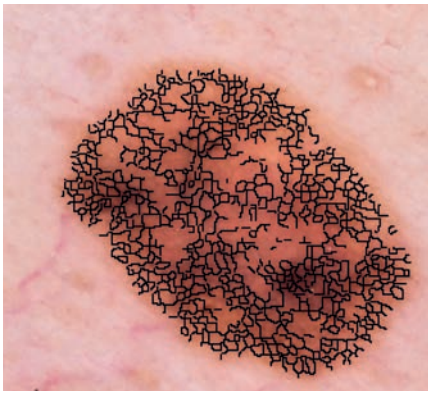
Asymmetry	Melanoma lesions are generally irregularly shaped, whereas benign nevi tend to be spherical or symmetrical
Border	Melanoma borders are usually uneven and irregular, making it difficult to establish where the lesion ends and normal skin begins
Color	Lesions displaying multiple colors (black, brown, blue, tanned) or various shades of a color may be indicative of melanoma
Diameter	Lesions greater than 6 mm in diameter are considered to be melanoma
Evolution	Lesions that undergo changes to any of the above points are suspicious for melanoma

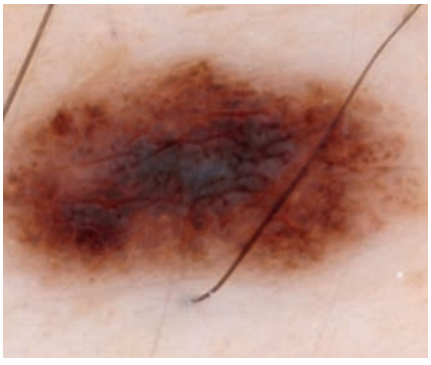
CASH

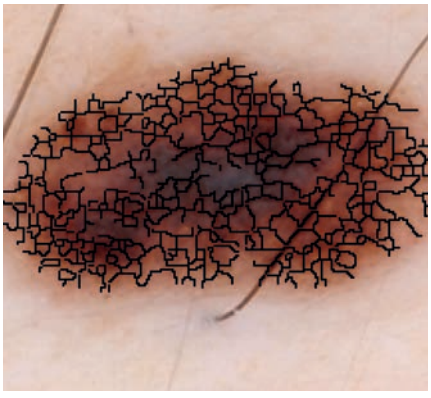
Color	As above
Architecture	Melanoma lesions have a disorderly pigment network and globule distribution
Symmetry	As above
Homogeneity	The more varied dermoscopic structures are present, the more likely a lesion is to be melanoma

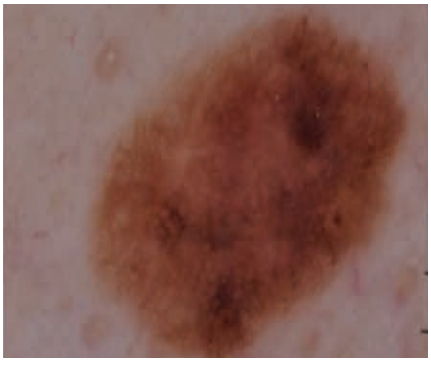
Menzies Method

Positive criteria (melanoma)	*Negative criteria (benign nevus)*
Multiple colors	Single color
Pseudopods	Symmetrical
Blue-white veil	
Multiple globular pigments	
Radial streaming	
Scar-like depigmentation	
Multiple blue/grey dots	
Broadened pigment network	









deviation of the island radii divided by the mean island radii). With the help of my colleagues, that coefficient – along with many other visual biomarkers – became the basis for a network of algorithms that generate a quantitative figure (Q-score) to differentiate melanoma from benign nevi. Visual biomarkers including pigmentation patterns and color contrasting with surrounding skin were taken into consideration, but of the 50 visual biomarkers collected, we found that the most significant was the lesion color variation – the number of colors that existed within the lesion.

The Q-scores range from zero to one; the higher the number, the more likely a diagnosis of melanoma. The software, used in conjunction with a dermatoscope, reached 98 percent sensitivity and 36 percent specificity in our initial publication; since then, we've improved the specificity significantly on that same data set and added additional data sets that confirm the reproducibility of the visual biomarkers as highly discriminant of melanoma versus nevus. Because this approach isn't based on cellular morphology, it's not poised to replace pathologists – it's a guide and a complement to tissue biopsy, not an alternative. What it can do is approach the same level of visual diagnostic ability an expert dermatologist has, with the added benefit of automation so that other medical professionals can use it.

Despite its highly sensitive and specific diagnostic performance, the current iteration of our technique has a few drawbacks. There's timing, for one. It can take between one and 10 minutes to image a single lesion, depending on its size and complexity – but I think all that's needed is a few optimization engineers cranking on the code to get the speed down to under 10 seconds for even the toughest lesions. Another concern is the technique's usefulness in patients with darker skin; our software takes into account color contrasting between the lesion and surrounding dermis, so we need to figure out how to handle different background skin colors. Finally, there are areas of skin that look altogether different – the palms of the hands, the soles of the feet – and the algorithm has yet to be tested on lesions from these areas.

"This approach isn't poised to replace pathologists – it's a guide and a complement to tissue biopsy, not an alternative."

One exciting aspect of ongoing work is the extension of these analytical imaging biomarkers beyond what the human eye (and standard cameras) can see. We're currently running a multicenter clinical trial to evaluate the melanoma advanced imaging dermatoscope, a hyperspectral camera; preliminary data on about 100 study participants indicates that the imaging biomarkers extend effectively into the ultraviolet and infrared imaging ranges.

The bigger picture

There are many different processes going on in melanoma of which we have only limited understanding from a biological and diagnostic perspective. Our study seems promising, but it's far from being the standardized technology we need so badly for melanoma detection. Our results are still relatively preliminary,

so we need to test the software in wider populations to establish its limitations and reduce the likelihood of significant false negatives. We really need 100 percent sensitivity and at least 50 percent specificity to be ready for prime time. In time, we may even be able to look into connecting these imaging biomarkers more strongly with the underlying biology of melanoma to create an even more powerful version of our tool.

Right now, we've taken the first step toward proving the principle that a "fingerprint" of imaging biomarkers can be used like a molecular biomarker. And as we present the software with more examples of known lesions, it will become more familiar with various cancer subtypes – and as a result, its effectiveness will increase. What do we need to take advantage of this technique? A community of people who can help us add as many cases as possible. That's where you come in.

Do you (or does your colleague) use dermoscopy to guide biopsy of suspicious pigmented lesions? If so, please feel free to contact me (dangareau.net); the next step in our investigations requires collaborators who have a folder of images with a spreadsheet of correlating diagnoses. We can provide a software program to prepare the images for our growing machine learning database. This venture is non-commercial, but academic credit and the satisfaction of contributing to a potentially life-saving research program are assured!

It may sound ambitious, but I truly believe that a collaborative team could hone the software's ability to the point where it creates a valuable visual diagnosis. Whereas other diseases may not be diagnosable without analyzing a plethora of factors, the unique visual biomarkers of melanoma – a disease that starts just a hair's depth from the skin's surface – may give us a fighting chance at a one-stop diagnostic tool.

Full-Body Diagnostic Imaging

The field of imaging biomarkers is a relatively new one, but complementary diagnostics like ours are advancing it rapidly. Numerous research groups are now working on total body photography – currently the best way to carry out machine-aided melanoma detection. Rather than generating a static evaluation of one lesion at a time like our system, their method involves taking multiple pictures of the entire body's surface. The method doesn't stop there, either; the photographs are updated at regular time points so that diagnosticians can spot the differences – allowing them to detect neoplasms and suspicious changes early on.

Dan Gareau is an instructor in clinical investigation at The Rockefeller University, New York, USA.

*This technology has not been reviewed by the FDA and should not be used in place of certified dermatologist evaluation.

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Profession

Your career
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Your life

46–49
What benefits could patient-facing pathologists bring to the profession? Ulysses Balis discusses introducing "office hours" to allow patients to discuss their disease with a pathologist.

Pathology for the People

Could patient-facing pathologists close the information gap, boosting disease awareness and the quality of service that pathology delivers?

By Ulysses Balis

Pathology: where morphology, gross anatomy, and microscopy intersect with disease. It's what initially drew me to the field – an opportunity to actually visualize disease processes with an anatomic frame-of-reference and the evolution of diseases in a physical form. But there's one aspect that's often overlooked from a pathologist's duty – the interface with patients. An error that may be a deficit to patients and pathologists alike.

I'm currently Director of the Division of Pathology Informatics at the University of Michigan, and our department has a long tradition of implementing quality systems into our infrastructure where appropriate. We've had a series of successful projects, the first of which involved generating tools that could mine and interface with pathology records. During that venture, we discovered that although critical results had been acted upon, there were many examples in which the right result was reported on the right patients, but there was no evidence that the clinician had either seen the report or acted upon it. Our quality system allowed us to recognize that, in many cases (because clinicians are so busy), there is less time and opportunity for patients to get complete – or even satisfactory – answers to queries about their disease. If we use cancer as an example, we could end up with patients who are not fully apprised of the biological potential of their cancer, what their complete management plan options are, or how they can actively participate in the resolution or cure of their disease. Our quality team felt there was a void that needed to be filled, and that the solution lay with pathologists.

Bringing pathology to the patients

The primary data for most disease diagnostics comes from pathology, and pathologists are often among the most informed about an individual patient's disease. We thought it would make sense, in some cases, for pathologists to be available to patients for a direct and interactive review of their case. A driving force leading to the creation of this process has been one of my colleagues, Jeffrey Myers, who is Vice Chair of Clinical Affairs and Quality and Professor of Pulmonary Pathology at the University of Michigan. Through his leadership, our department has been able to establish a simplified access model by which patients can easily contact the actual pathologist who reviewed their case to gain additional information and insight into the disease processes at hand.

The structure we have at the University of Michigan is relatively straightforward and informal. A patient uses a telephone or online portal to ask for a meeting with their pathologist. An anatomic pathology coordinator checks the pathologist's schedule and lets the patient know available times, free of charge. The pathologist is notified and given access to the patient's slides and reports to re-familiarize themselves. The pathologist and patient (and family members if desired) have access to a multi-headed microscope, with real-time cameras capturing the slides and projecting the images on a screen.

> *"Pathologists are often among the most informed about an individual patient's disease."*

The session is an opportunity for the pathologist to describe what's on the slide – the anatomic frame-of-reference – and then an opportunity for the patient to ask questions about survival, molecular underpinning of the disease, and so on, which in-turn informs the possible treatment options that are available. Generally, such conversations provide the patient with a much more concrete understanding of their disease than is available from a typical initial encounter with the clinician reading the pathology report. Indeed, patients have told us that the process provides much-needed insight into their disease, which better

At a Glance

- *Few doctors on the care team are more informed about that patient's disease than the pathologist*
- *Nonetheless, patients rarely have the opportunity to interact directly with their pathologists*
- *"Open office hours" let patients review their cases with pathologists, looking at slides and asking questions*
- *Not only does this improve quality of care, but it also helps pathologists become visible players in patient care*

prepares them for the "battle" ahead. Our reports suggest that when patients are about to embark on treatment, understanding what it is they are battling and being mentally prepared helps them to face what previously would be "the unknown" and the various consequences of treatment.

We've been doing this for about three years during which time we have served several hundred patients. We continue to gather anecdotal reports from our patients who participate in this process and it really has all been positive. To my knowledge, no one has complained or stated that the opportunity to meet with their pathologists wasn't meritorious. Therefore, we continue to offer the service and plan on growing it.

The positive feedback we've received hasn't solely been from patients. Currently, a growing number of institutions – including the Mayo Clinic and the Memorial Sloan Kettering Cancer Center – offer office-hours pathology to patients, and the reception from participating pathologists has been positive.

When Jeff Myers is on the national lecture circuit, he routinely notes that there is continued and enthusiastic interest from other institutions interested in initiating similar programs – which is fantastic. I would encourage as many care providers as possible to offer the service; I believe it should be the standard of practice.

"Pathologists make a monumentally important contribution to the decision-making process... and yet the patient often doesn't have direct contact."

No payday?

You may be wondering, as fellow pathologists have asked us, "How can you afford to do this, if you're not charging anything?"

The simple "high-level" answer is that it's not about money at all – it's about making sure that the patient has received the best possible care for the management of their disease. And especially now that we have operating evidence from running this program, we're convinced that this value-added service helps patients. We're doing it because we believe it is part of what should be a comprehensive care program for patients with severe and significant illness.

So far, the value added to the patient far exceeds the pathologists time so it seems to justify the expenditure of that extra effort, making the current model sustainable for us. However, as I mentioned earlier, we plan on expanding the service's scope, and I can imagine a time in the not-too-distant future when this becomes widespread, with the vast majority of patients wanting to take advantage of the service. When that happens, we may hit a threshold where the amount of pathologist resource required makes the service unsustainable without some type of reimbursement. At that point, we could conceivably petition the US insurance infrastructure to make it a reimbursable activity, as with a clinician's consultation.

Mutually beneficial

Fellow pathologist Mark Boguski said: "Pathologists are the most important doctors that most patients have never met" (1), and he's absolutely right. At the moment, pathologists make a monumentally important contribution to the decision-making process for patients, and yet the patient often doesn't have direct contact with a pathologist, which can result in certain mishaps.

What is not generally appreciated by patients – and even some clinicians – is that reports are not absolute metrics of truth. The reality is that they're our best approximation of the evidence available. Many diagnoses that we generate are nuanced, and there are complexities and ambiguities, which are best communicated via a conversation – but that unfortunately isn't current standard practice. And, in fact, it's not just one conversation – it

should be an ongoing dialogue between the pathologist and the clinician, the pathologist and the patient, or maybe the pathologist, clinician, and patient.

We're getting closer to that ideal; for example, in tumor boards, all the different specialties involved with the comprehensive treatment of a patient are part of the process. Yet I think there's a blind spot: patients may not always be in attendance at a tumor board, and I think there's a need for a simplified form that allows the patient to interact with a multidisciplinary team to attain a comprehensive understanding of their illness. Institutes such as MD Anderson, the Memorial Sloan Kettering Cancer Center have long recognized this truth and have very forward-thinking ways of engaging the patient as a participant of their treatment, but unfortunately it is anything but universal.

Going back to Mark's comment, we must seek areas where pathologists can actively participate in the management of patient care – both in clinically-oriented settings with teams of physicians and providers, but also in settings where the patient and their family might be present. Expanding the pathologist's role by using direct knowledge of the mechanisms of disease to improve treatment, but also to educate both patients and the clinician can only be beneficial. And a more collaborative process is better for all parties involved.

Overturning irony

Historically, the general consensus has been that pathologists should only communicate with clinicians – and never with patients. There are various states, such as New York, where pathologists are forbidden – by law – from directly talking to patients, which I think is indefensible – and ironic given my call here. Fortunately, I believe that the College of American Pathologists and New York State Society of Pathologists are in the process of attempting to repeal that law. In many ways, the specialty of pathology can, and should, serve in a very direct patient contact role. In a way, pathologists are clinicians that don't directly treat patients. Any rule that underscores the antiquated thought that pathologists should stay in the basement, read their slides, and not interact with anyone should be very quickly put aside. Ultimately, the office hours endeavor leads back to my initial point: we, as pathologists, should always seek out additional opportunities to elevate the level of quality that pathology delivers. I believe there a significant number of such opportunities – and offering office hours pathology to patients is an essential step in the right direction.

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Sequencing, Stratifying, and Standardizing

Sitting Down With... Susan Branford,
Associate Professor in the Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, Australia.

How did you come to focus on chronic myeloid leukemia?

When I first joined SA Pathology in 1997, I was tasked with developing quantitative molecular methods to assess residual disease in cancer. My first method measured the levels of *BCR-ABL1* transcripts in patients with chronic myeloid leukemia (CML). The somatic *BCR-ABL1* fusion is the primary genetic lesion that characterizes CML, and sensitive detection following stem cell transplant was a prognostic indicator. But to distinguish early relapse from residual disease, we needed a way to tell whether *BCR-ABL1* levels were rising or falling. We used real-time quantitative PCR with hydrolysis probes – new technology at the time – and joined the first international clinical trial of a tyrosine kinase inhibitor, imatinib, in CML. With over 1,000 patients across three laboratories, the study soon demonstrated that imatinib therapy led to very rapid clearance of leukemia, and that monitoring at the molecular level provided prognostic information. That study kick-started my interest in the connections between treatment response kinetics, drug resistance, and patient outcomes.

How have patient outcomes changed since you entered the field?

They've changed dramatically! The disease is invariably fatal without therapy. When I entered the field, less than 40 percent of recently diagnosed patients qualified for transplant – a risky procedure that carried the danger of long-term morbidity or early mortality. The alternative, chemotherapy, was not great; interferon α could extend survival for the one-fifth of patients who responded well – but many couldn't tolerate the side effects. It's the introduction of imatinib and other potent tyrosine kinase inhibitors in recent years that has changed this once-fatal disease to one most patients survive long-term.

But there are still many challenges ahead. We don't understand why some patients fail to respond to therapy or develop drug resistance; we can't reliably identify patients who might benefit from more potent kinase inhibition despite the increased risk of cardiovascular events; we don't know which patients might remain disease-free after drug cessation and which might relapse. Biomarkers measured at diagnosis may be the key to answering some of these questions – if we can determine which are most reliable, they may help us improve risk stratification, guide therapy choices, and provide patients and families with better peace of mind.

What's your next research target?

We have used whole exome sequencing, whole transcriptome sequencing, and copy number variation to identify genomic variants in CML patients at diagnosis. This integrative genomics has revealed that patients with poorer outcomes exhibit a higher frequency of (potentially) clinically relevant variants – and those who progressed to acute leukemia also acquired similar variants. There is a substantial overlap between the variants we have detected in CML and those recently discovered in acute myeloid and lymphoid leukemias through next-generation sequencing, but there are also novel recurrent somatic variants that may be drivers of CML progression.

We aim to define the genomic mechanisms beyond *BCR-ABL1* that are associated with treatment response, drug resistance, and early disease progression. The goal is to translate this new knowledge to the clinic by introducing a comprehensive biomarker testing panel at diagnosis that will reliably predict treatment response and guide decision-making. Integrative genomics may reveal the interplay between the different layers of genomic processes that regulate the kinetics of response and identify the most pathologically relevant genetic events.

You also lead international efforts for molecular method standardization…

Without standardization, it's not possible to compare results across different labs. International recommendations now incorporate molecular response levels achieved at milestone time points over the first year of therapy to establish response. Patients who don't reach these response levels are considered to have failed treatment and a change of therapy is mandated – but it's hard to determine which patients are responding appropriately if we can't compare one lab's results with another's.

Thanks to an extensive global effort, standardization has been largely successful – but the process has taken substantially longer than we initially anticipated. Molecular techniques are complex and many variables can impact the quality and consistency of final results. Standardized testing kits and newer, simpler technologies have aided the adoption of molecular monitoring for CML and benefited patients around the world. Our major challenge now is to introduce a reliable proficiency-testing program and – at the individual lab level – the routine use of affordable, appropriate quality control material.

Any advice for people starting out in molecular pathology?

Molecular pathology and genetic testing are moving rapidly forward. Because the volumes of data being generated are constantly increasing, I'd recommend that anyone seeking to enter the field prepare themselves with a thorough understanding of genetics and bioinformatics. I was lucky enough to hit the jackpot combination of fantastic guidance and mentorship, great opportunities, fascinating findings, a thirst for knowledge, supportive employers and colleagues, hard work, and serendipity. Some of those, of course, are luck of the draw – but others are readily available to those who look!

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