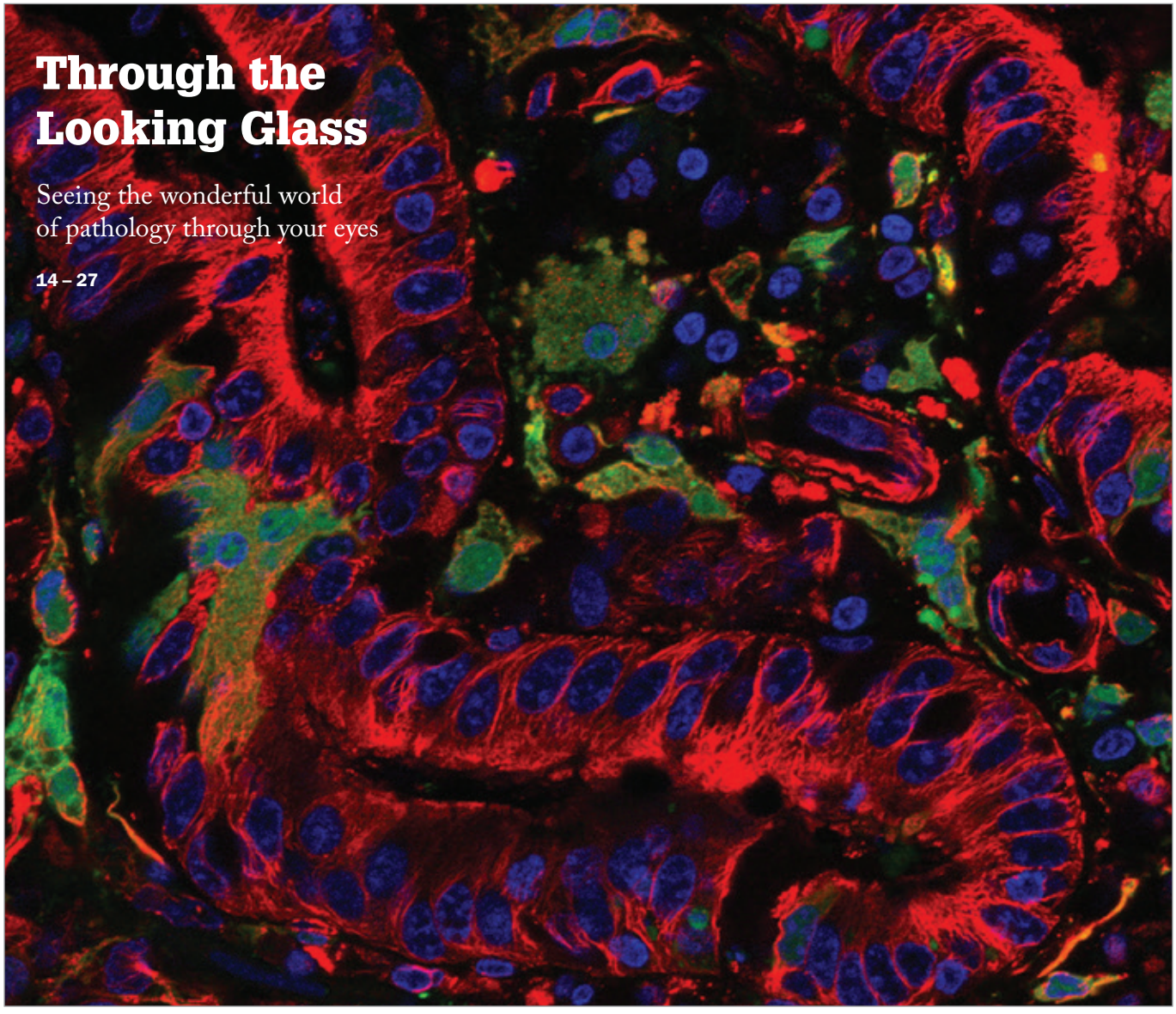
SEPTEMBER 2016 # 22

the Pathologist



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On the Conference Floor

The Pathologist visited the Great White North for the 2016 meeting of the Canadian Association of Pathologists – Association canadienne des pathologistes in Vancouver.

@CAPACPPresident presents @pathologistmag Michael Schubert with prize for social media @ #capacp2016 We're so proud!

http://bit.ly/2cMt9wD
11:12 AM – 13 Jul 2016

Karen Dallas: "Inappropriate lab test utilization is like weather; everyone talks about it, but no one does anything about it." #CAPACP2016
http://bit.ly/2dcWyyo
9:35 am – 11 Jul 2016

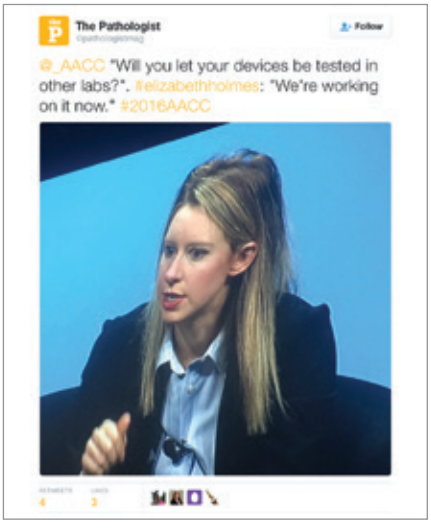
"What do we want our residents to leave on their last day knowing?" Chelsea Maedler-Kron says to begin with the end in mind at #CAPACP2016
http://bit.ly/2cVtDku
9:03 AM – 12 Jul 2016

We also attended the American Association for Clinical Chemistry's 2016 meeting and expo in Philadelphia and heard some industry-first presentations!

John McDevitt: "It's going to be difficult to digitize biology, but we need to do it!" #2016AACC
http://bit.ly/2cjd8uh
3:10 PM – 31 Jul 2016

Sir Richard Peto: "Halving death before age 70 in the next 20 years is achievable." #2016AACC
http://bit.ly/2cqMN0O
5:54 AM – 1 Aug 2016

And take a look at our full coverage of Theranos CEO, Elizabeth Holmes' widely anticipated lecture and Q&A session on Storify: *https://storify.com/pathologistmag/theranos-at-aacc-2016*



Last but not least, we joined Europe's microscopists at the European Microscopy Congress 2016 in Lyon for an extreme close-up look at advances in the science of small.

Pushing the limits of live-cell imaging resolution to new heights with Eric Betzig at #EMC2016

http://bit.ly/2d4tcll
12:21 AM – 29 Aug 2016

Xiaowen Liang proposes optical #biopsy using multiphoton #microscopy as method of histological #diagnosis. #EMC2016
http://bit.ly/2d0SPqY
3:09 AM – 30 Aug 2016

You'll find more coverage of conferences and other news from the world of laboratory medicine on our Twitter feed at @pathologistmag

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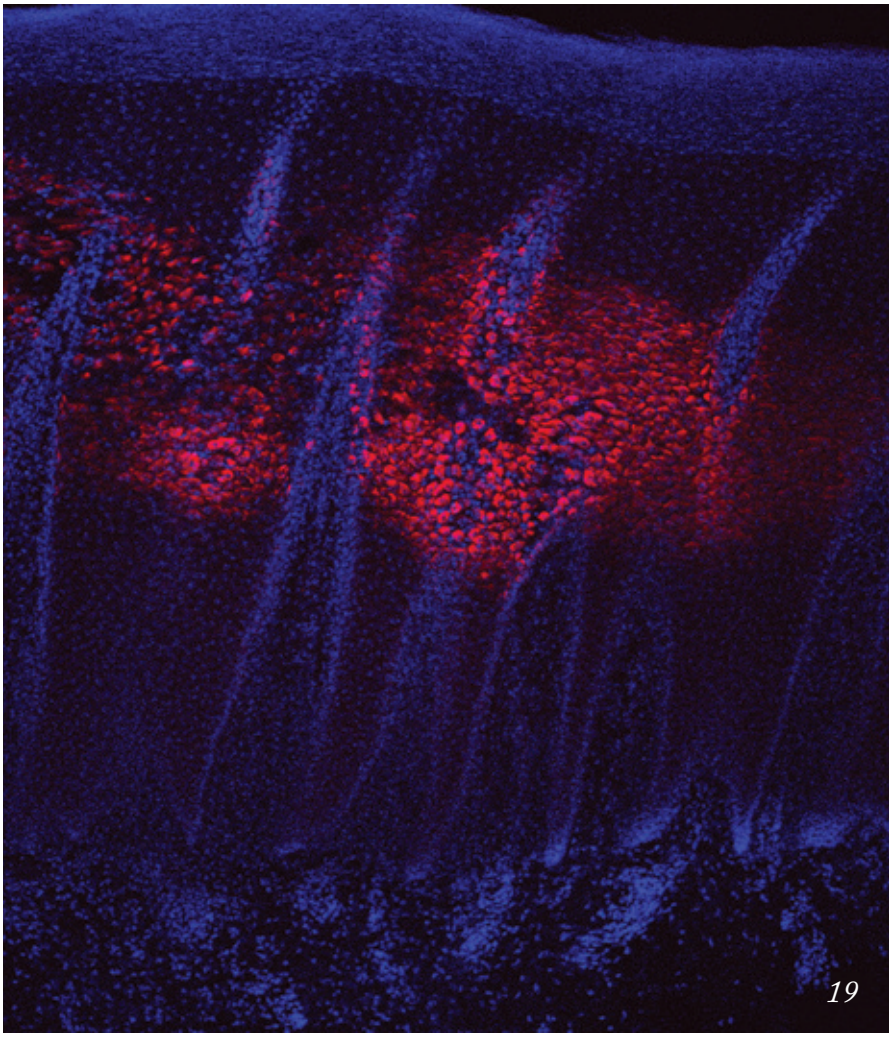

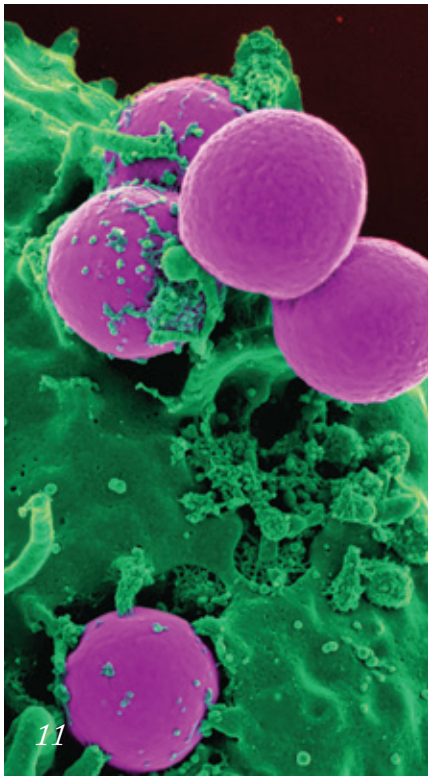

On The Cover

Section from bovine duodenum showing Rinderpest infection. Green shows GFP expressing the virus. Image courtesy of Jennifer Simpson.

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Sitting Down With

the Pathologist

ISSUE 22 - SEPTEMBER 2016

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Humble Versus Harmful

Laboratory medicine is saving lives every day through its efforts in humanitarian crises – so why is nobody talking about it?

According to the UN's Office for the Coordination of Humanitarian Affairs, in the last year alone, more than 76 million people from 31 countries needed humanitarian assistance and the number of people displaced by conflict exceeded 51 million. That's the highest number since World War II! But when you hear the words "humanitarian crisis," what's your initial association? Natural disaster? Disease epidemic? War? Does laboratory medicine spring to mind? I would hazard a guess the answer is probably "no." Imagine, then, the level of awareness among the general public, and even other medical professionals, of the lifesaving role that pathology and lab medicine play during these crises – such contributions certainly don't make mainstream press. Recent disease outbreaks have been in the headlines for months on end, and though lab medicine has been vital in helping bring both Ebola and Zika epidemics under control, the brave work of the doctors involved has not been recognized. Why? As always, it comes back to (you guessed it) communication – or lack thereof.

What I'm suggesting is this: when an opportunity presents itself to speak with the press or to get involved in social media campaigns, take it! Think of how much more recognition pathology will get if national or international news channels aired interviews with pathologists or wrote about a pathology-related hashtag that's "gone viral?" Just as the profession is struggling to entice new trainees because of diminishing pathologist educators, so too is it suffering because most don't talk about their work to people not immediately associated with their jobs. You know how valuable lab medicine is, and yet pathology services only make up around two percent of global healthcare spend – shocking!

I'm so passionate about this, as I'm sure you are too. So when the Royal College of Pathologists (RCPath) asked if The Pathologist would partner with them on two events they're hosting in November to highlight the crucial role of the laboratory in humanitarian disasters and public health emergencies, we jumped at the chance. The first, Pathology is Global (1), will unite experts from the pathology and healthcare communities with NGOs, including prominent figures involved in the refugee and Ebola crises in Iraq and Sierra Leone, respectively. And during the College's International Pathology Day (2), we will be co-hosting a roundtable – streamed live online – with a panel who will debate how pathology education and training can be strengthened in low and middle income countries. I can't wait to hear insightful and valuable discussions on topics that need more of our attention. Registration details are in the references. I hope to see you there!

Fedra Pavlou
Editor

References

1. *"Pathology is Global", November 1, 2016, Royal Society of Medicine, London. Register at http://bit.ly/2cjxNmR*
2. *International Pathology Day Roundtable, November 16, 2016. Register your interest at international@rcpath.org (use "IPD2016" in the subject line of the email).*

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?

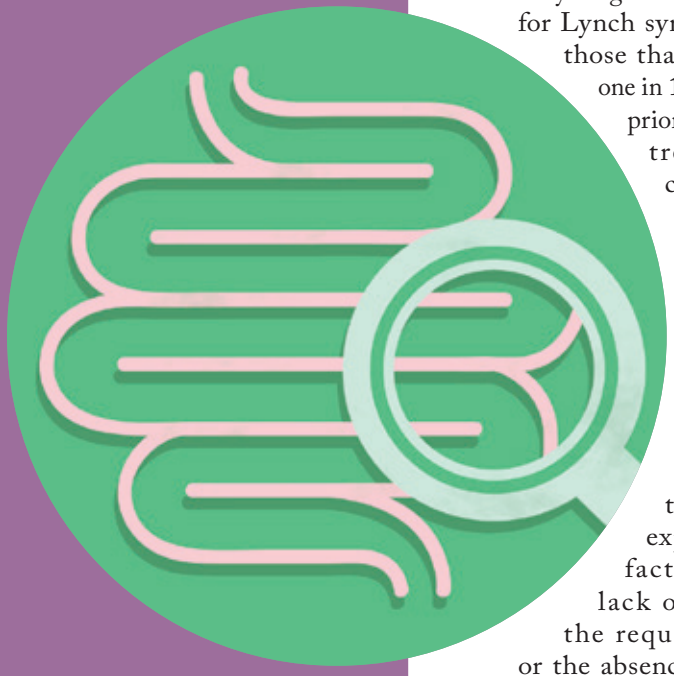
Email: fedra.pavlou@texerepublishing.com

Reflex Recommendations

RCPath and Bowel Cancer UK call for improved testing of Lynch syndrome in young bowel cancer patients

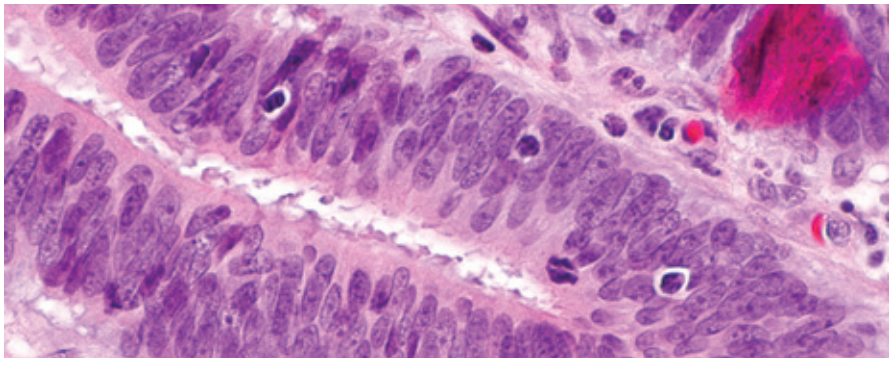
It's estimated that Lynch syndrome causes 1,000 cases of bowel cancer each year, many of which occur in patients under the age of 50 – but fewer than 5 percent of those with the condition have been identified. Why? At least in part, it's because these unusually young bowel cancer patients aren't being tested for the genetic disorder.

The test itself is simple – immunohistochemistry can reveal the presence of a defect in a mismatch repair gene. But recent findings (1) published by the Royal College of Pathologists (RCPath) and Bowel Cancer UK indicate that nearly three in 10 hospitals don't test young bowel cancer patients for Lynch syndrome – and of those that do, only about one in 10 perform the test prior to administering treatment. With current RCPath guidelines (2) in place for two years, why hasn't testing become automatic? "The main obstacles are financial, resource and capacity barriers," the researchers explained. "Other factors could be a lack of awareness of the requirement to test or the absence of a specialist gastrointestinal pathologist in some smaller units." But previous studies have shown that molecular testing for Lynch syndrome is cost-effective (3), allowing patients to be placed on surveillance and their cancers diagnosed and treated in their earliest stages.



"Some hospitals in the UK have developed local approaches to overcoming the obstacles," say spokespeople from RCPath and Bowel Cancer UK. "For example, Central Manchester Foundation Trust has developed a regional, centralized service – an approach that might alleviate pressure on smaller trusts to develop in-house testing." They also recommend that England and Wales consider following Scotland and Northern Ireland's example by taking a national approach to ensuring that all hospitals test patients under the age of 50. "It's important to carry this out as a reflex test at diagnosis because it can help detect people at greater risk of recurrence, inform treatment options and identify family members who may also have a high risk of bowel cancer. Furthermore, Lynch syndrome patients and their families can be offered regular colonoscopic surveillance, which can reduce mortality from bowel cancer by up to 72 percent."

RCPath and Bowel Cancer UK are working together to raise awareness of Lynch syndrome testing and to encourage all hospitals to carry out automatic molecular testing in bowel cancer patients under 50 at diagnosis. The UK's National Institute for Health and Care Excellence will publish new draft recommendations imminently, with final guidance expected in February. "It's important that the guidelines stipulate whom to test, when to test, and which test to use, as this will help reduce the current variation in practice. We're also optimistic that the guidance will encourage more widespread adoption of reflex testing, and we hope to see



Credit: Wikipedia user Nephron.

Colorectal carcinoma showing tumor-infiltrating lymphocytes suggestive of microsatellite instability common in Lynch syndrome.

a further increase in the number of patients for whom testing is offered."

The organizations' recommendations for pathologists? "To help encourage Lynch syndrome testing, increase awareness, and ensure that individual hospital trusts are implementing guidance, pathologists and other healthcare professionals can raise it as an issue at the multidisciplinary team level." RCPath and Bowel Cancer UK also encourage each hospital to identify a clinician with a special interest in genetic testing to oversee service delivery and ensure pathways for patients are instituted, and to carry out regular audits to verify that that Lynch syndrome tumor testing is taking place at the time of diagnosis. *MS*

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A New Angle on CKD

Measuring levels of amino acid D enantiomers may reveal which chronic kidney disease patients are at greatest risk of disease progression

Chronic kidney disease (CKD) is a rising health problem, with patients prevalent throughout the world and the number progressing to costly, life-threatening end-stage disease increasing every year. To tackle the problem at its root, nephrologists recommend early referral of CKD patients so that they can be treated before progression – but at the moment, there's no good way to predict which patients are at risk. Doctors use indications like decreased kidney function, proteinuria, and additional complications like age or other diseases – but all of these have flaws, and there's a great need for a more effective test.

Tomonori Kimura and his colleagues at Osaka University sought out just such an alternative – and discovered that levels of D-amino acids, normally present in only trace amounts in humans, could predict progression to end-stage disease (1). "All D-amino acids are measured simultaneously by 2D high-performance liquid chromatography (HPLC)," explains Kimura. "The first HPLC separates each amino acid, while the second separates D from L enantiomers (2). This method allows for absolute quantification, allowing us to directly compare our results to those of other studies." The researchers found that some D-amino acids, particularly D-serine and D-asparagine, were robustly associated with the progression of CKD; the risk was elevated two- to four-fold in those with higher levels.

"I'm certain that a D-amino acid test would change clinical practice," Kimura says, "but we need further studies to validate its utility in specific clinical situations, and measuring D-amino acids is still a challenge. Though our system's throughput increases year by year, we still need much higher throughput to meet clinical demand." In future research, he proposes to focus more on specific kidney conditions and to study the poorly understood physiology of D-amino acid metabolism to increase its utility as a biomarker. "The D-amino acid world is a mystery," he says, but he and his colleagues are working hard to solve it. *MS*

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2. *K Hamase et al., "Simultaneous determination of hydrophilic amino acid enantiomers in mammalian tissues and physiological fluids applying a fully automated micro-two-dimensional high-performance liquid chromatographic concept", J Chromatogr A, 1217, 1056–1062 (2010). PMID: 19767006.*

To Screen or Not to Screen?

Is childhood lipid disorder screening worthwhile? In the wake of updated guidelines, some argue that the benefits are worth the costs and risks – but others disagree

Money is always a hot topic in healthcare – where it's going, why it's being spent, and how we can get more value out of each cent. Indeed, diseases are often referred to by their economic burdens, and it is by regulators' weighting of cost benefit versus quality-of-life gains that therapeutics and diagnostic solutions are often assessed. Sometimes, not to intervene is deemed the favorable solution – and it is this strategy that has recently been suggested for the screening of lipid disorders in children.

After nine years, the United States Preventive Services Task Force (USPSTF) has updated its guidelines on screening for lipid disorders in people under 20. The screening received an I statement, meaning that "current evidence is insufficient to assess the balance of benefits and harms of screening (1)" – but not everyone agrees. Earlier recommendations from the National Heart, Lung, and Blood Institute (NHLBI) were strongly in favor of screening "unless a clear and compelling rationale for an alternative approach is present. (2)"

Is there such a rationale? The authors of a recent JAMA Internal Medicine editorial believe so (3). They argue that screening for low-likelihood issues like cardiovascular disease events in children results in costs and harms without accompanying benefit – especially if they receive treatments like statin drugs, which can increase the risk of diabetes mellitus. Furthermore, they add that if the USPSTF had considered the cost-effectiveness of screening in its evaluation, the outcome would most likely have been a grade of D: "Discourage the use of this service." It seems that an uncertain future is ahead for childhood lipid screening… *MS*

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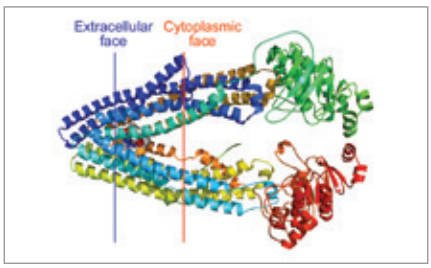
How the Bullet Lost its Magic

Changes in some leukemia patients' P-glycoprotein expression levels could explain their disease's resistance to treatment

When it was first approved for leukemia treatment, imatinib was hailed as a "magic bullet" in the fight against cancer – a drug that could turn a medical death sentence into a treatable condition. But not every patient sees the same effect; about one-fifth of patients don't respond well to imatinib treatment. Predicting who will benefit from the drug is tricky, as until now, doctors haven't understood what sets those one in five patients apart – but a discovery from the University of Adelaide is shedding some light.

Laura Eadie and her colleagues investigated the role of P-glycoprotein (P-gp) in imatinib therapy (1). The protein is a multi-drug transporter capable of removing imatinib from leukemia cells, so the researchers suspected it might play a role in treatment resistance. What they found is that it's not a patient's P-gp levels at diagnosis that determine their long-term response to imatinib; instead, it's the change in P-gp expression before and after treatment. The greater the increase in P-gp, the more likely the patients are to develop resistance.

So how can we test patients for potential resistance? "Our P-gp 'test' utilizes PCR," says Eadie. But although it relies on a well-known technique, P-gp testing is not yet ready for clinical implementation. "In order for our assay to be translated into the clinic, standardization would need to occur. Further investigations into the biological basis for the observed increase in P-gp levels are also needed." Eadie's group hopes to validate the initial findings in an independent cohort of patients undergoing imatinib treatment, and meanwhile, they're also investigating P-gp expression in patients undergoing frontline nilotinib therapy. *MS*



The structure of P-glycoprotein.

Reference

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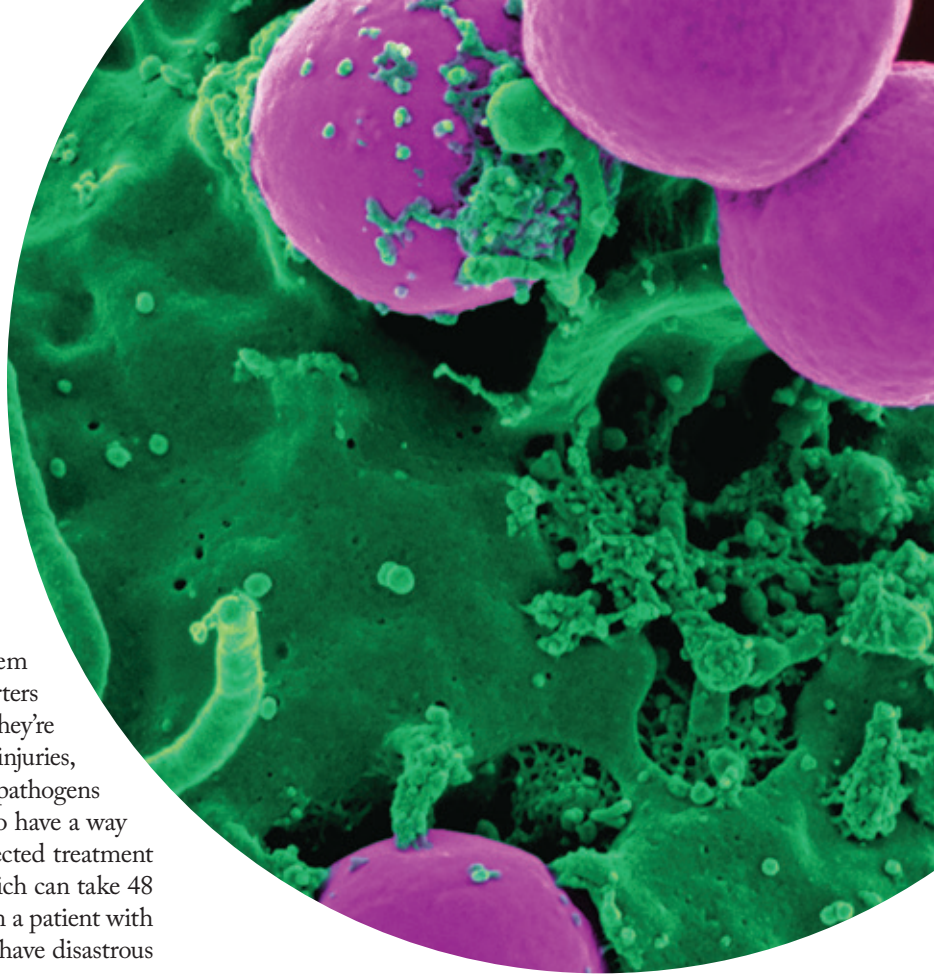

Waging War on Resistance

Automated digital microscopy can shave hours, or even days, off the time needed to diagnose multi-drug-resistant infections and initiate treatment

Multi-drug-resistant infections are a problem everywhere, but nowhere so much as in close quarters like hospitals, prisons and military environments. They're a particularly significant concern in combat-related injuries, where wounded soldiers often exhibit a range of pathogens that don't respond to antibiotics. Although we do have a way of tackling this challenge – namely, culture-directed treatment – it requires the growth of a positive culture, which can take 48 hours or more. Delaying treatment by this long in a patient with injuries and related drug-resistant infections can have disastrous consequences. So what's the alternative?

Connie Price, chief medical officer at Denver Health and a professor of medicine at the University of Colorado School of Medicine, has developed a new approach that may significantly speed up multi-drug-resistant disease diagnosis. The technique makes use of multiplexed automated digital microscopy (MADM), a type of imaging that can rapidly identify and even quantify pathogens. During their studies, Price and her colleagues have been able to spot a wide variety of treatment-resistant bacteria including *Staphylococcus*, *Klebsiella*, *Enterobacter*, *E. coli* and more – all within one hour, and with a sensitivity and specificity exceeding 97 percent (1). In fact, when applied to blood, respiratory and infected tissue samples from soldiers suffering multi-drug-resistant infections, MADM has shown an ability to analyze bacterial populations, differentiate between phenotypes, and characterize heterogeneous and inducible resistance mechanisms based on only four hours' growth (2). With detailed results in such short amounts of time, Price hopes that their MADM-based method can save hours – and, more importantly, lives. *MS*

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2. *CS Price et al., "Rapid antibiotic susceptibility phenotypic characterization of Staphylococcus aureus using automated microscopy of small numbers of cells", J Microbiol Methods, 98, 50–58 (2014). PMID: 24393790.*

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 editor at fedra.pavlou@texerepublishing.com

Undermined and Undervalued

Medical school curricula must change before it's too late for pathology. We have some practical ideas that will help…

By H. Cliff Sullivan, Chair of the American Society for Clinical Pathology Resident Council 2015–2016

I am writing this on behalf of the American Society for Clinical Pathology (ASCP) Resident Council, and in response to articles that have been published previously in The Pathologist regarding pathology education. I also want my colleagues within the pathology and laboratory medicine community to be aware that I have presented this letter directly to John Prescott, Chief Academic Officer of the Association of American Medical Colleges (AAMC) and the Council of Deans (COD) of the AAMC as it is imperative that our concerns are voiced. Specifically, I want to address the current state of pathology training in medical school and the consequences it may have on the field at large.

The ASCP is a nonprofit medical specialty society representing more than 100,000 members, including board certified pathologists, pathology residents, other physicians, clinical scientists (PhDs), certified medical laboratory scientists/technologists and technicians, and educators. We're one of the largest medical specialty societies in the US and the world's largest organization representing the field of laboratory medicine and pathology. As the leading provider of continuing education for pathologists and medical laboratory personnel, we place a great emphasis on enhancing the quality of the profession through comprehensive educational programs, publications, and self-assessment materials.

We appreciate the complexities faced by medical schools across the US. We understand the task of teaching the doctors of tomorrow is a great challenge; ensuring that medical students are exposed to and have a basic understanding of each specialty is a significant undertaking.

However, the ASCP Resident Council is concerned that the current trend in transitioning from a traditional two-year, course-based curriculum to a more integrative, systems-based curriculum, may inadvertently underrepresent pathology. As such, pathology education has gone from an intensive course with practical sessions, lectures, and gross pathology labs, to only a limited number of lectures integrated with other clinical subjects. Moreover, evaluation of knowledge has been reduced to a sprinkling of pathology questions lost in a milieu of pathophysiology, pharmacologic, and clinical questions; consequently, passing is easily feasible with little to no knowledge of pathology.

This diminution of pathology is unfortunate and undermines the importance of the field. The diagnoses and laboratory values that pathologists provide are absolutely crucial to patient management. The results provided help guide decisions about whether a patient will undergo surgery or a doctor will initiate a life-saving treatment. As pathologists, our job is to supply accurate results to ensure that medical decisions are based on correct diagnoses and valid lab values, as these results provide a platform to justify medical choices and ultimately a foundation for patient care. Unfortunately, despite pathology's central role in medicine and patient care, the field is undoubtedly undervalued by fellow clinicians and

patients alike. There are many reasons for this. A main contributor is a general lack of awareness of what pathologists truly do. In part, the nature of the profession physically isolates us: we spend large amounts of time sitting at our desks looking through a microscope or in the lab interpreting data, troubleshooting problems, implementing new tests, etc.

The lack of exposure to pathology, coupled with the aforementioned deficiency in medical school pathology training, comes with deleterious effects. Indeed, medical school preparation for pathology residency training is problematic. According to our annual national survey of pathology residents and fellows (1), approximately 80 percent of pathology residents feel that medical school pathology training did not adequately prepare them for residency and 45 percent of residents cite no exposure to pathology or no first-hand pathology experience during medical school. Furthermore, the dearth of pathology instruction in medical school parallels a declining interest in pathology residencies: The National Resident Matching Program reports approximately half of the pathology residency spots have been filled by US allopathic medical students for the last three years (2013–2015). Unmistakably, pathology is no longer viewed as a central component of clinical medicine. The decline in the knowledge of pathology is undoubtedly attributed, at least in part, to the revised curriculum, which has stripped pathology to a bare minimum.

Besides the negative effects on pathology training, the transition to the new, integrated curriculum has also negatively impacted the perception of pathology. For instance, among medical students, fewer students consider pathology to be central to medicine (49 percent in new curriculum vs. 96 percent in old curriculum) or believe knowledge of pathology will be useful in their future careers (52 percent vs. 96 percent) (2). This poor perception persists beyond training and into practice, leading to a decline in communication between pathologists and clinicians as well as a negative impact on patient care. To that end, the Institute of Medicine released a report highlighting an inappropriate utilization of diagnostic testing by clinicians (3). The report emphasizes that pathologists have much to offer in test utilization from test selection to interpretation. Especially with the expansion of molecular diagnostics, pathologists' role is going to become even more critical in diagnosis, monitoring, risk assessment, prognosis, and predictive aspects of disease process and cancer. Thus, enhanced teamwork among pathologists and treating physicians can only improve diagnostic testing and patient care. However, in order to achieve this goal, the value of pathology needs to be emphasized and taught early on in training.

The time and resources put forth by medical schools across the US to develop and implement the new integrative curriculum are substantial. As such, we realize that drastic revamping or redesigning of the curriculum is not feasible, nor necessarily desirable. However, we hope you agree that the state of current pathology education is in a precarious position. Unless deliberate and conscious steps are taken to ameliorate the situation, the field of pathology as a specialty may be negatively impacted, not only in terms of decreased enrollment of medical students into pathology residencies, but also in perpetuating the perception of pathology within medicine. We encourage the AAMC to revitalize and improve pathology education in medical schools across the nation. Although bringing back traditional pathology courses may not be possible in the current integrative model, we do have some practical ideas that can be implemented to help improve pathology education:

- Increase in the extent of exposure to pathology
- Ensure that pathology lectures and courses are taught by pathologists
- Review sessions devoted to basic and systemic pathology
- Include gross anatomy laboratories
- Run microscopic/histological sessions
- Incorporate a mandatory pathology clerkship or a "mini" clerkship within another clerkship (e.g. a week of frozen sections or transfusion medicine during surgery clerkship, laboratory week integrated into internal medicine, cytology week within the obstetrics/gynecology clerkship, a week of biopsy service during dermatology clerkship, spending a week with hematopathology during pediatric clerkship, a week of pathology signout during radiology clerkship to reinforce radiologic-pathologic correlation, etc.)
- Work in concert with pathology and laboratory medicine department(s) to support a pathology student interest group
- Recruit pathologists to be small group leaders or lead problem based learning sessions within the integrated curriculum
- Encourage clinicopathologic correlation early in clinical training prior to clerkships
- Solicit pathology and laboratory medicine department(s) for ideas on how to integrate pathology within the current curricular structure at your institution.

The ASCP Resident Council appreciates the opportunity to present our concerns and our ideas, and we are more than willing to address any questions or concerns that the AAMC or others might have.

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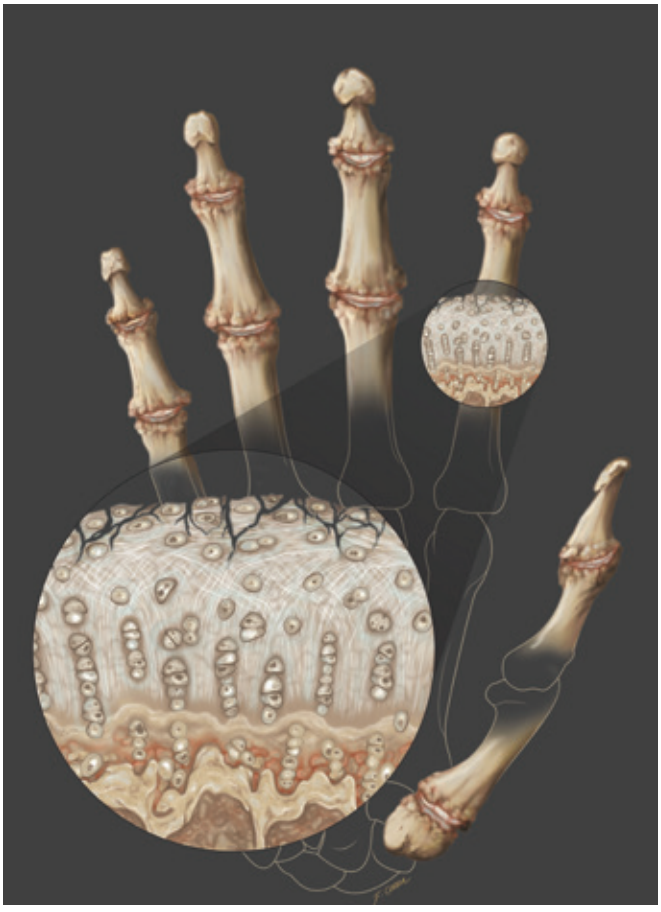
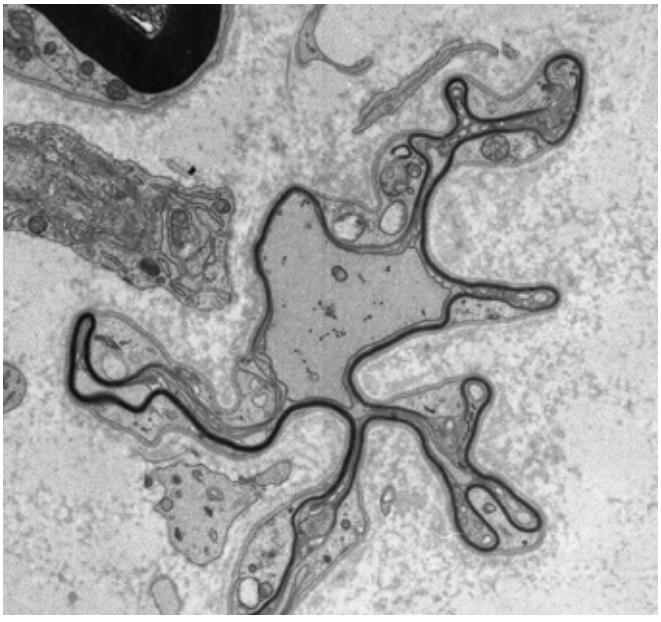
2. *S Holck et al., Human Pathol, 38, 384–385 (2007). PMID: 17234470.*
3. *E Balogh et al., Institute of Medicine (2015).*

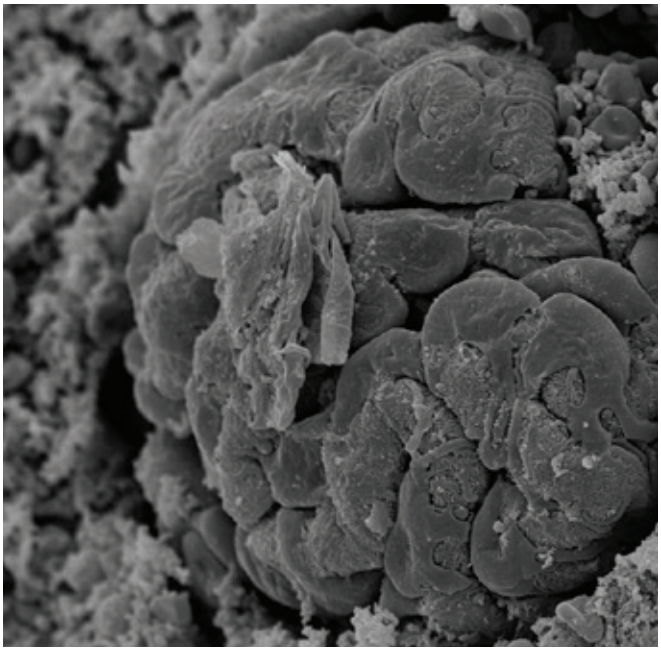
THROUGH THE LOOKING GLASS

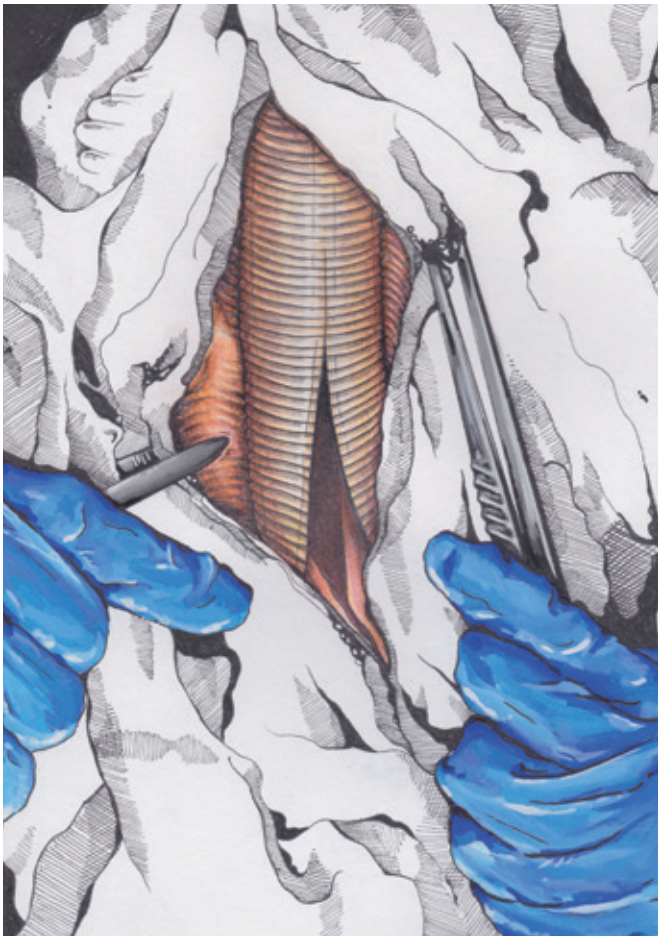
You take us on a journey through the beautiful world of pathology – the small, the stained, the simulated – through your own eyes in this gallery of images from all walks of laboratory medicine.

Boom!
An explosive image of an intranasal fungal infection.
Alberto Berjón (@otromicroscopio)









Opposite Page

Clockwise from top left:

Hand Osteoarthritis

A medical artist's representation of osteoarthritis within the joints of the hand.
Francesca Corra

Abn Myelin

This electron micrograph shows remyelination in a case of chronic demyelinating polyneuropathy (biopsy taken from a 57-year-old woman).
Rosalind King

Dissection in Blue

This mixed media image on paper shows surgical treatment of an acute aortic dissection.
David R S Evans, Cardiff University, Affiliate Member of the Medical Artists' Association

Rat Glomerulus

This sample was fixed with glutaraldehyde, dehydrated with alcohol, and then critically point dried. After mounting on stubs, it was sputter-coated with gold for electron microscopy.
Glenn M Harper, Plymouth Electron Microscopy, University of Plymouth

This Page

Top:

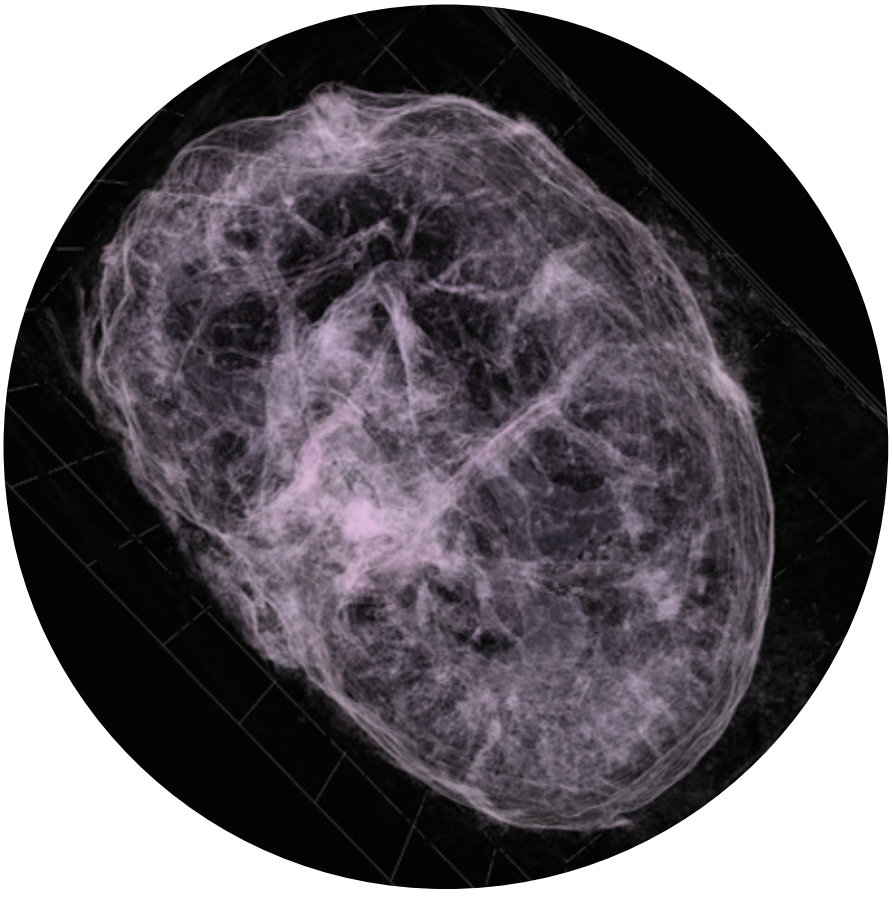
Rat Kidney

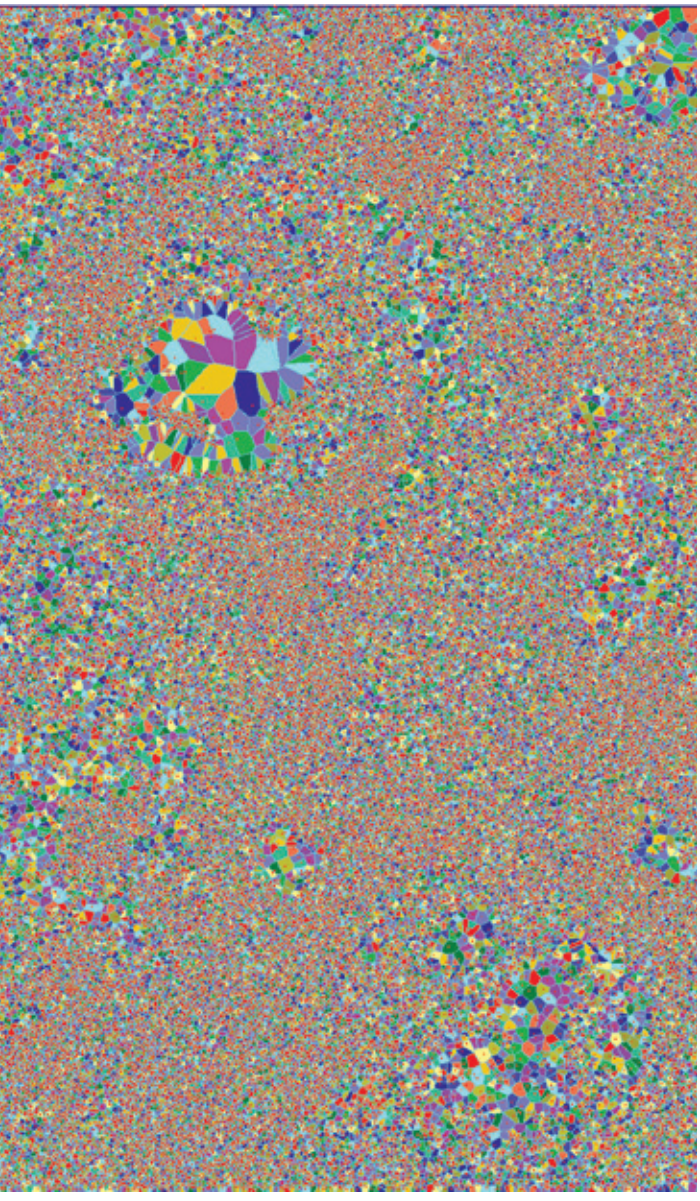
3D reconstruction from 651 hematoxylin and eosin stained sections, in semi-translucent view, generated with microDimensions Voloom®.
microDimensions

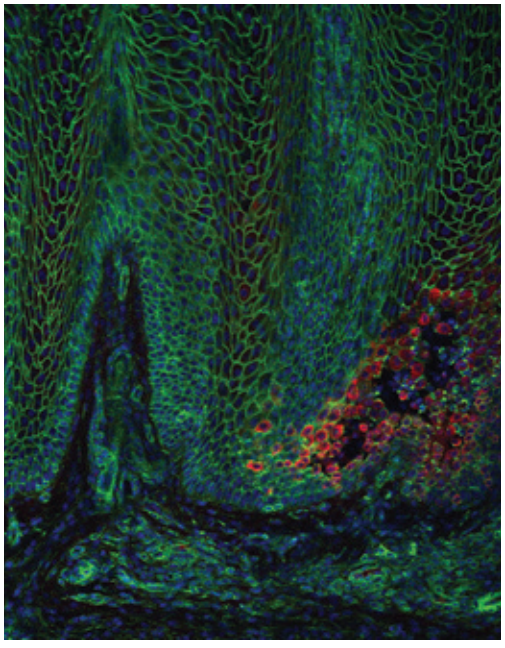
Bottom:

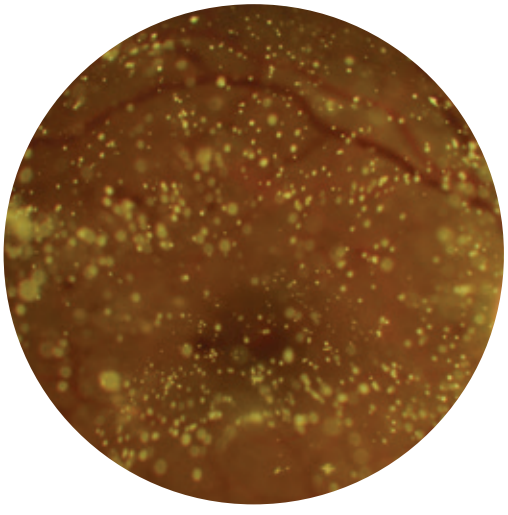
Giardiarium

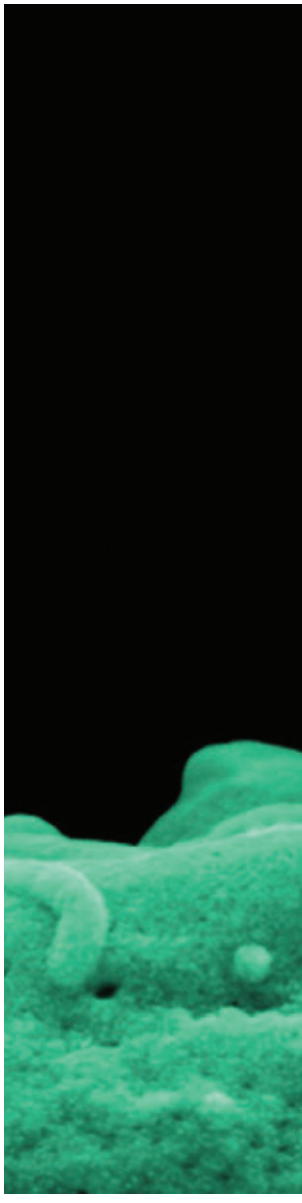
A small collection of electron micrographs of a photogenic Giardia lamblia selected from enterobiopsies.
Josef Špaček









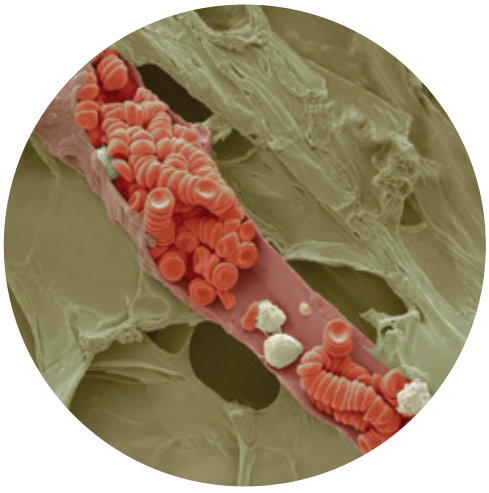


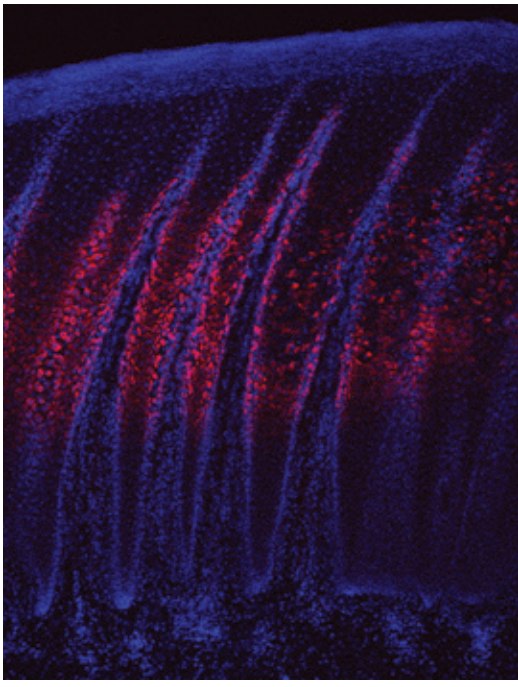
Clockwise from left:

Voronoi Mosaic
This tissue section of an oral squamous cell carcinoma was scanned with whole-slide imaging and segmented, followed by Voronoi diagram calculation. Small polygons are mostly stromal cells, whereas larger polygons are epithelial cells from glands.
Martial Guillaud

Foot and Mouth
A low power image showing a foot and mouth disease lesion in a section of bovine tongue. Blue: cell nuclei, green: actin filaments, red: foot and mouth disease virus.
Jennifer Simpson

Asteroid Hyalosis
This degenerative ophthalmic condition occurs when "asteroids" – calcium-lipid globules – accumulate in the vitreous humor of the eye, giving the appearance of stars in the night sky.
Natalie Cook





Clockwise from left:

Broccoli Bacteria
Enteropathogenic *Escherichia coli* grows actin "feet" to attach itself to the wall of the intestine, causing infection. Taken on a ZEISS field emission scanning electron microscope.
Manfred Rohde, HZI Braunschweig; ZEISS Microscopy

Ruptured Venule
Coloured scanning electron micrograph of a ruptured venule running through fatty tissue. Stacked red blood cells (rouleaux formation) and white blood cells are seen within the venule.
Steve Gschmeissner (theworldcloseup.com)

On the Move
A low power image showing the spread of foot and mouth disease virus in a porcine interdigital lesion. The necrotic region of the lesion is on the right; on the left are infected cells with normal epithelium. Blue: cell nuclei, red: foot and mouth disease virus.
Jennifer Simpson

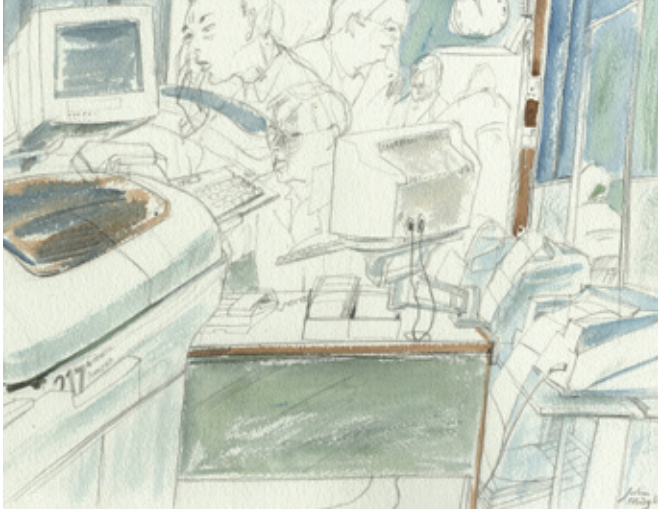



TABLE
1
DANGER

Opposite Page

Pathology Reportage

Clockwise from top left: the rectification room, where plaster casts are carefully refined in the prosthetics department; the busy clinical chemistry laboratory; the histopathology laboratory; the cut-up room, as pathologists examine biopsies; PAPNET, a new cytologic screening technology under trial.

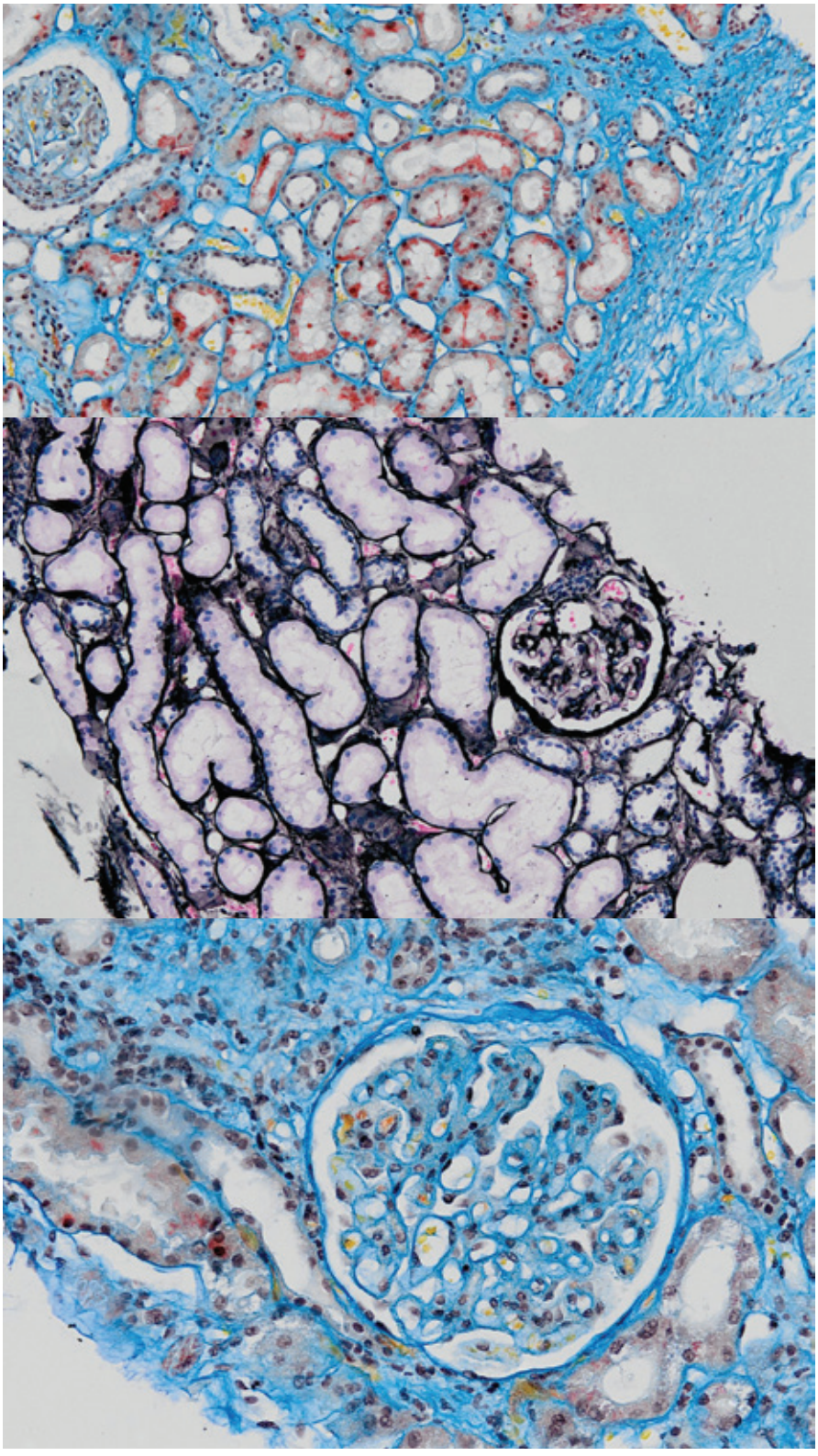
Julia Midgley; images from "Drawn From Experience" project except top right, from "War Art and Surgery" project

This Page

Renal Biopsies

Top to bottom: renal biopsy with Martius scarlet blue (MSB) staining for fibrin, collagen and muscle tissue; renal biopsy with Jones' stain for basement membrane; renal tissue control with MSB staining.

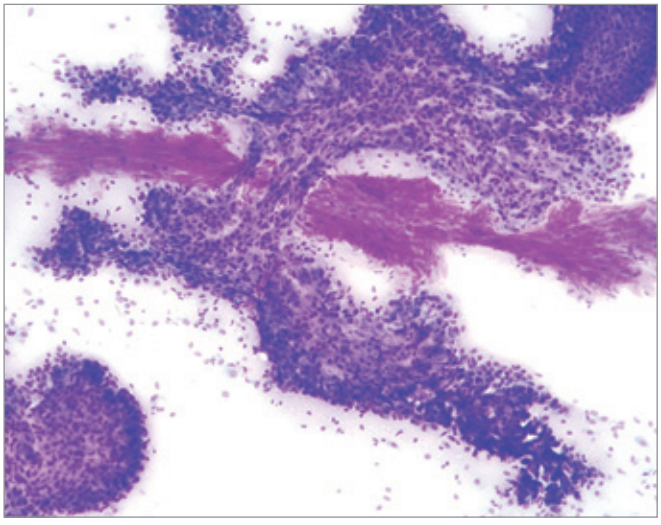
José Bernardino

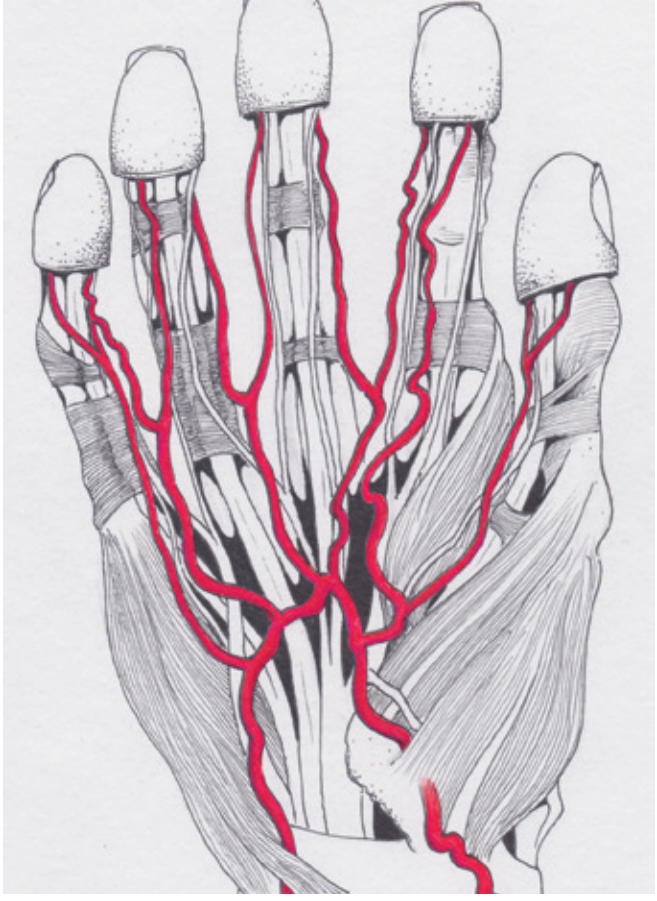


Fire-Breathing Dragon

This image of a breast fibroadenoma shows branching cohesive ductal epithelial cell groups with associated intrinsic smaller and somewhat spindled myoepithelial cells, scattered stripped bipolar nuclei, a magenta strand of hypocellular fibrous stroma – and a fearsome beast that belies the benign nature of the condition.

Charles Sturgis, Cleveland Clinic





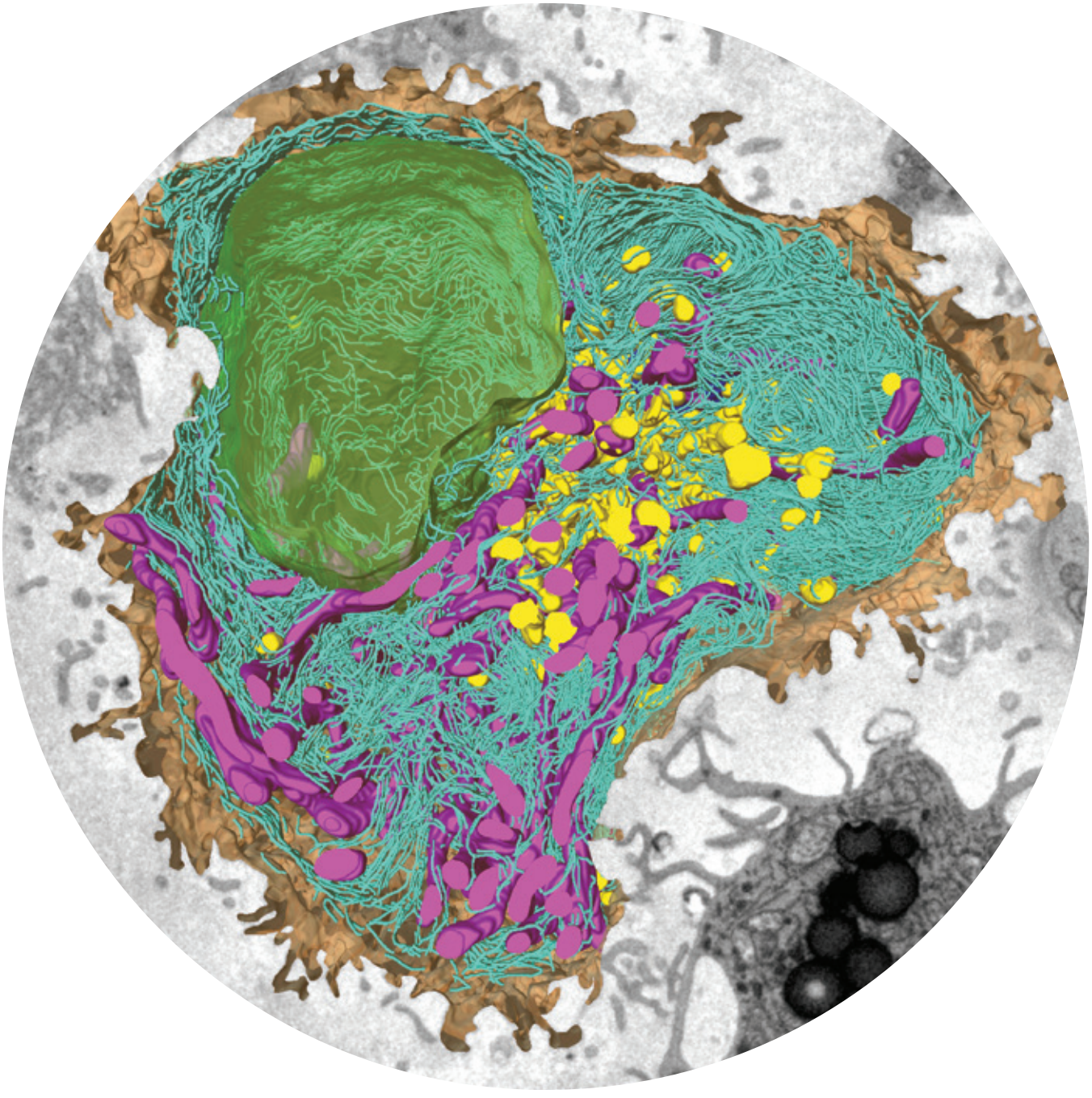
Arterial Supply

The image, in pen and ink on paper, shows the arterial supply of blood to the hand.

Credit: David R S Evans, Cardiff University, Affiliate Member of the Artists' Association

"The coroner wants an autopsy to confirm the cause of death!"

Merlyn Harvey



Dendritic Cell

Serial block-face scanning electron micrograph with 3D reconstruction of a dendritic cell shown in its ultrastructure.

Peter Munro and Hannah Armer, UCL Institute of Ophthalmology; ZEISS Microscopy

Right:

Step By Step

Walking through a breast tissue fine needle aspiration. The lighter-colored cells are ductal cells, whereas darker ones are myoepithelial cells from a benign lesion.

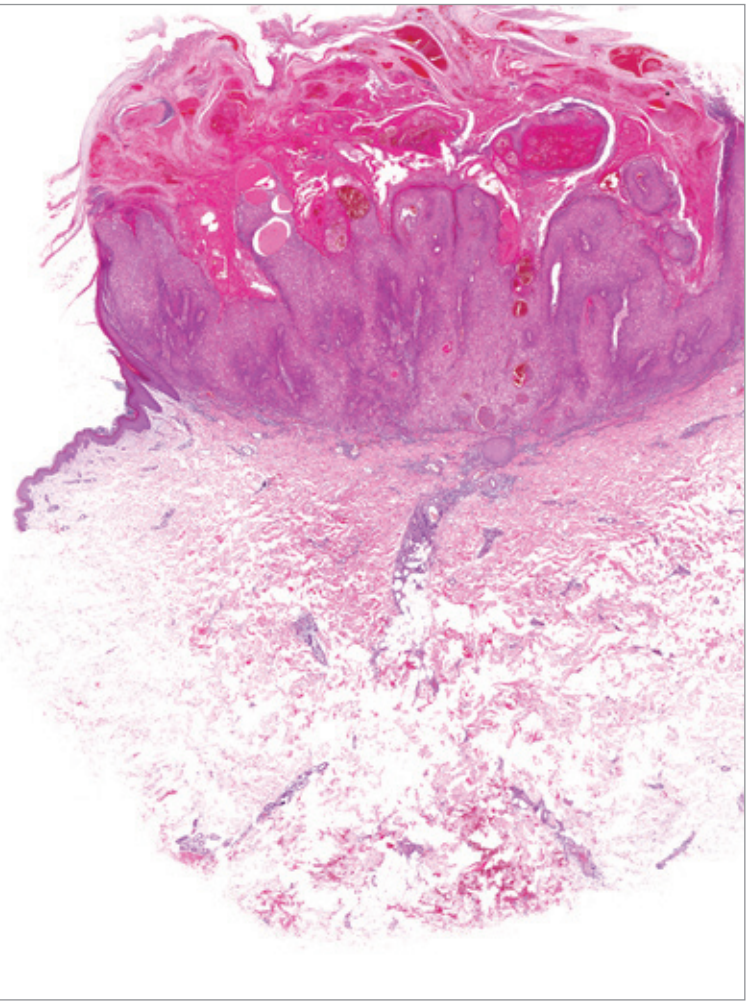
Lara Pijuan

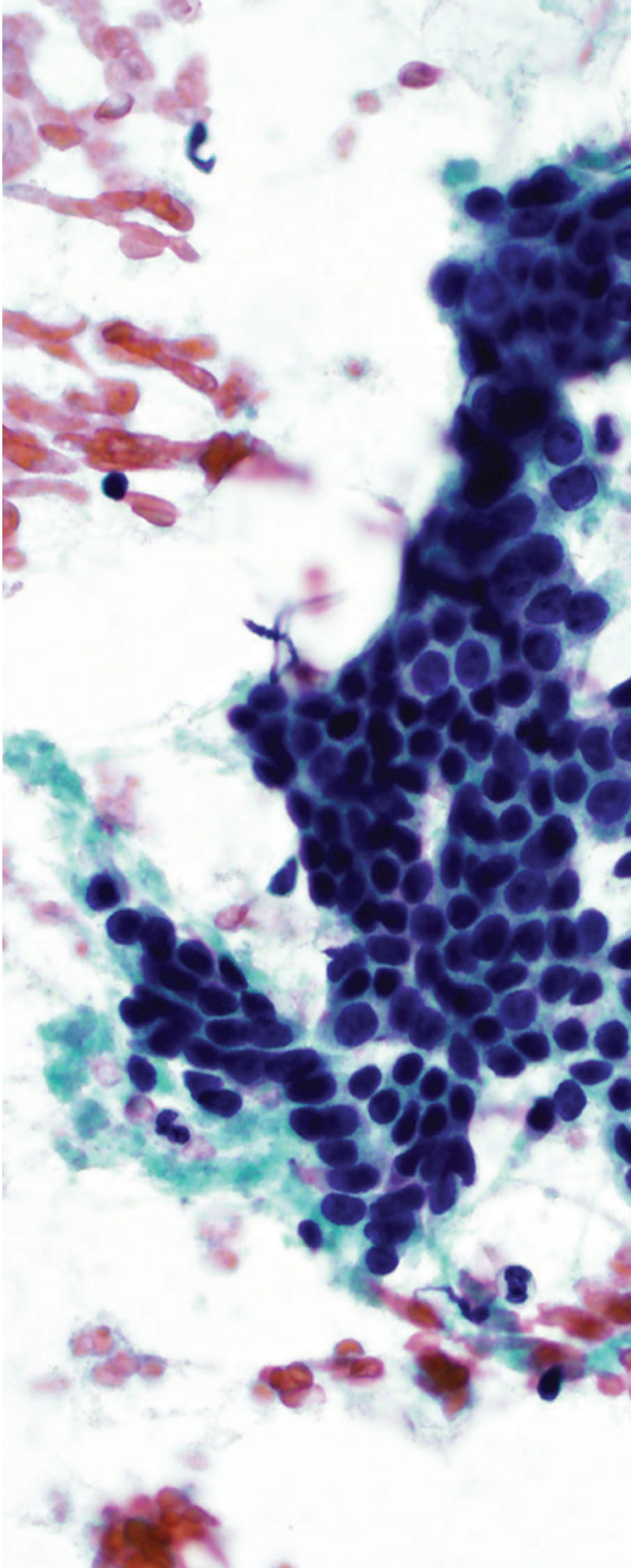
Below:

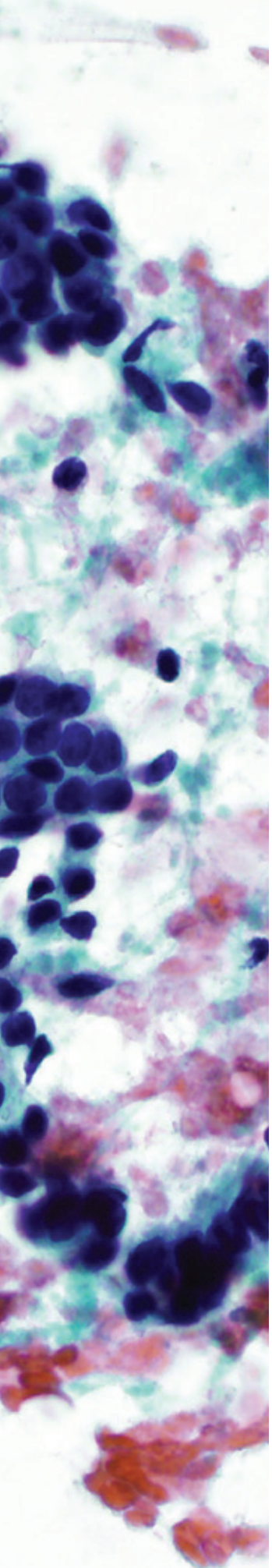
Cancer Crater

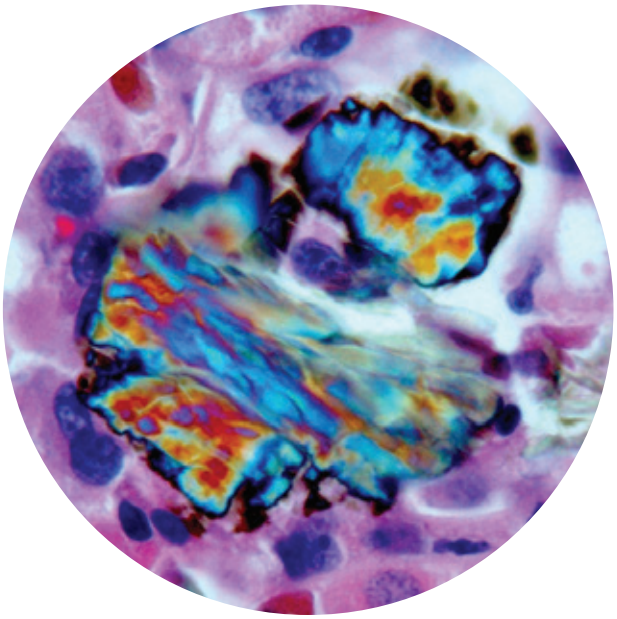
Keratoacanthoma in the abdominal skin of a male Caucasian patient. This image was captured on a MetaSystem Metafer VSlide scanner.

Lukas Lacina, Declan P Lunny, Ildiko Szeverenyi, Sarah Zulkifli, Graham D Wright, Institute of Medical Biology, A*STAR, Singapore









Top:

Taking Wings

There are those who label behind-the-scenes medicine "boring" – but show them this rainbow in a tissue stain and they'll see how pathology can reveal hidden beauty.

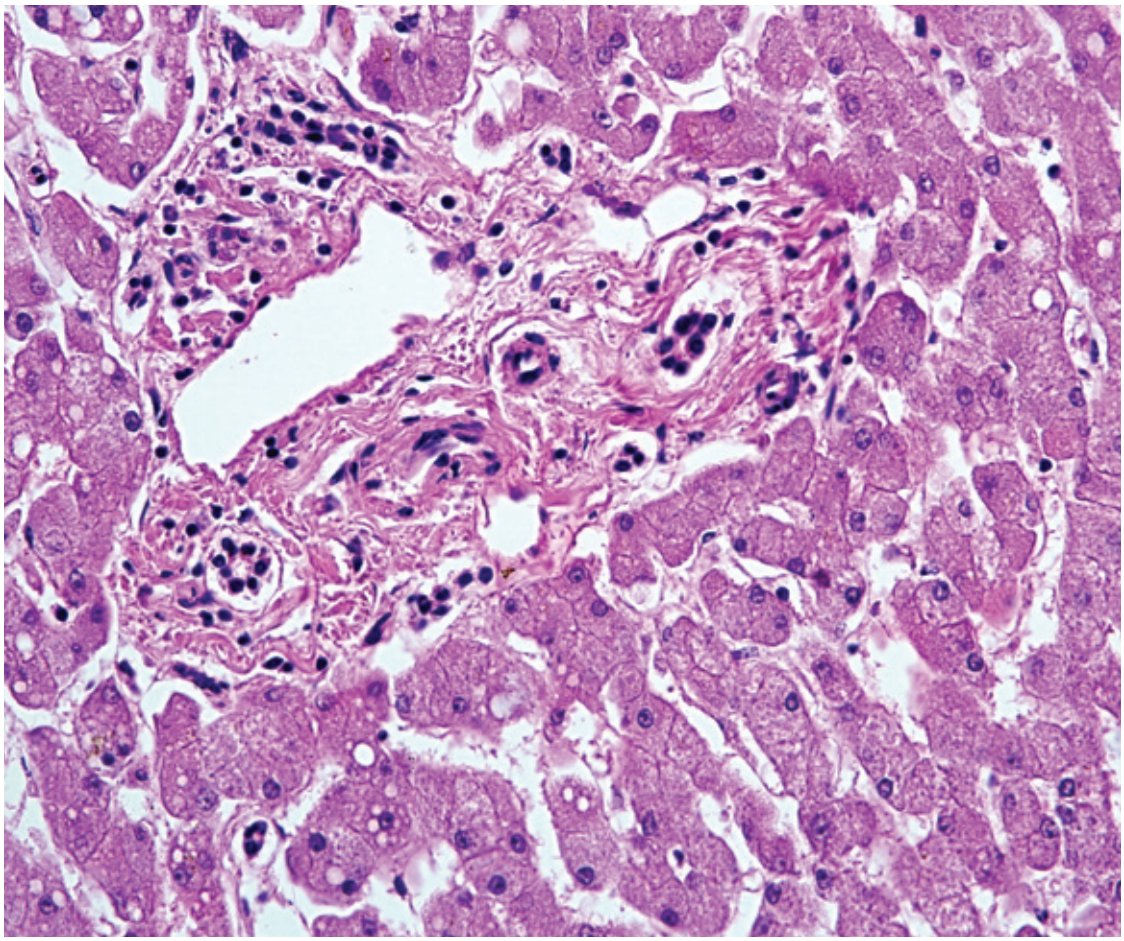
Priti Lal

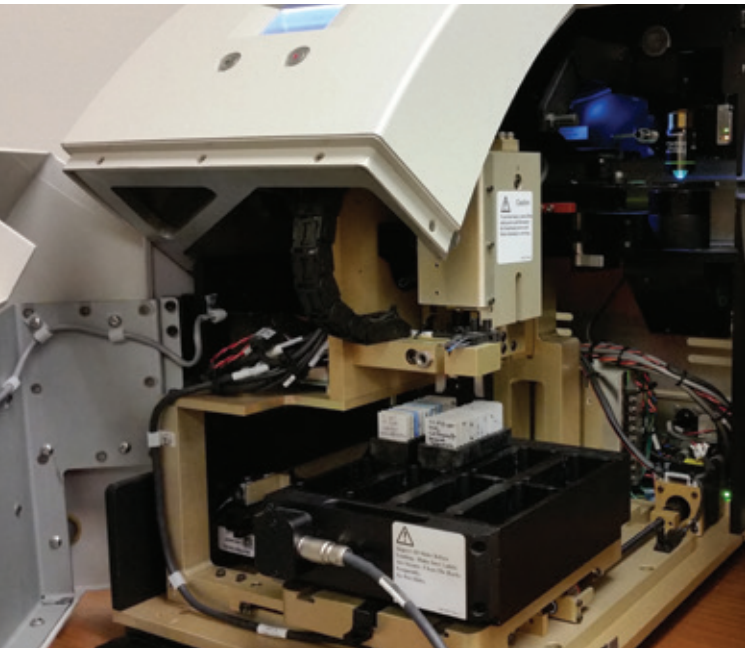
Bottom:

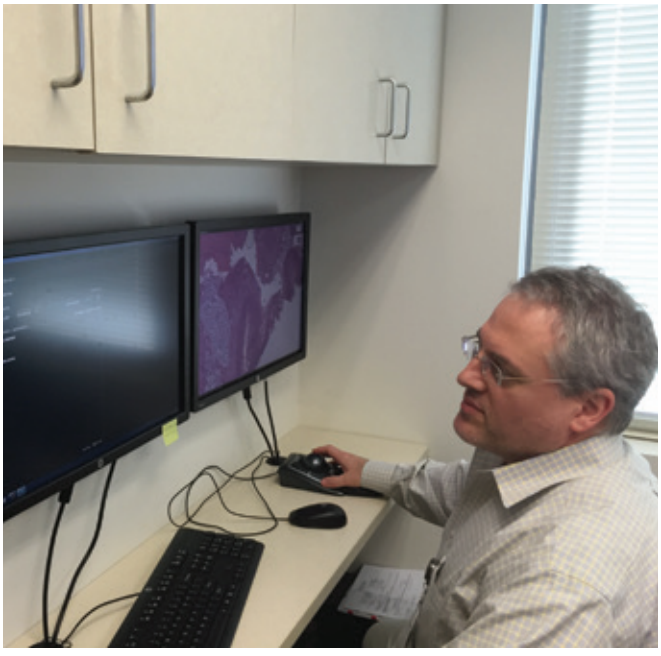
Driving a Car Up the Hill

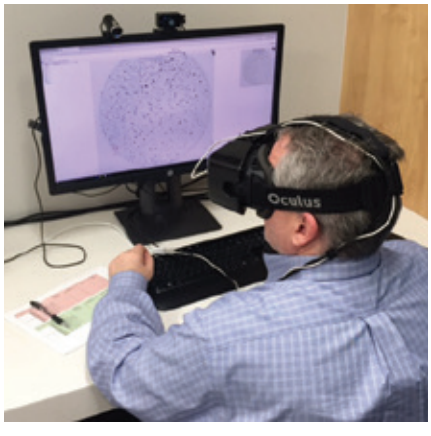
You can find art and humor in your day-to-day work, proving that pathology can be both funny and functional.

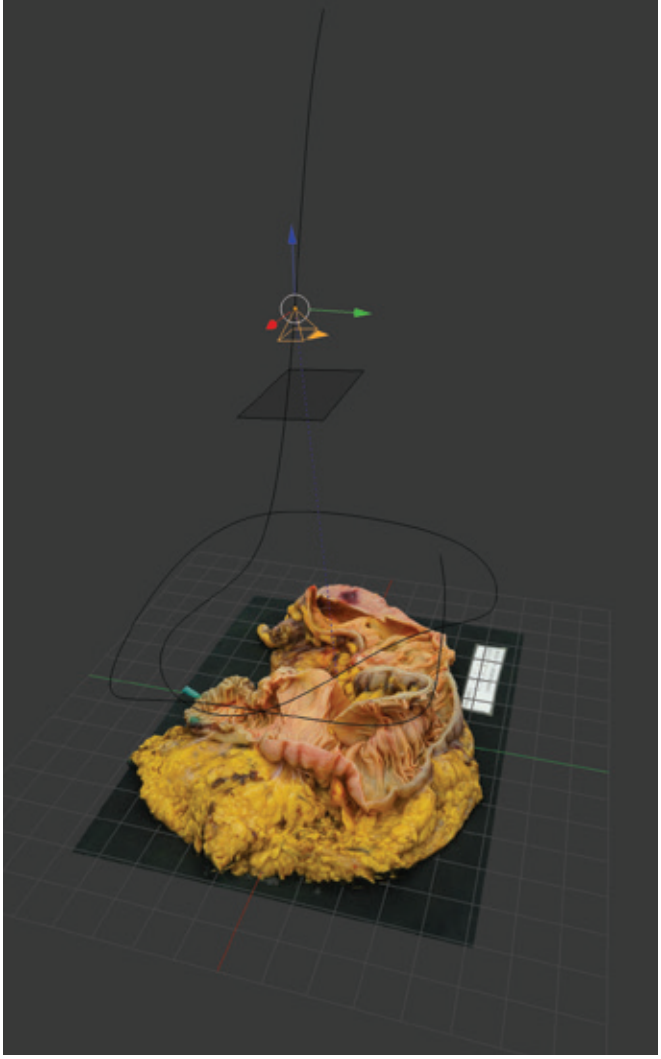
Lenka Bartonova








Oculus



Opposite Page

The Secrets of Digital Imaging

Clockwise from top left: An open whole slide scanner, revealing what's inside the box; using a digital pathology workstation to check case management and workflow; the digital imaging research laboratory at UPMC; using a virtual reality headset to screen and interpret digital Pap tests; testing smartphones for telepathology using real-time viewing.

Liron Pantanowitz on behalf of the Division of Pathology Informatics at the University of Pittsburgh Medical Center (UPMC)

This Page

Top:

The Reconstructed Gut

A 3D reconstruction of a right hemicolectomy specimen composed of about 100,000 texture mapped polygons. This image is a screenshot of the flight plan for a flyover animation; the orange pyramid indicates a virtual camera and the black line the path it will follow. See the video at http://bit.ly/2cFxDHE. "My hope is that such videos will spur interest in anatomy and anatomic pathology in young adults using a powerful medium that they're familiar with."

Shane Battye

Bottom:

Sprinting

This wax écorché anatomical figure was based upon the Paralympian athlete Richard Whitehead and shown as part of an exhibition entitled "Anatomy of an Athlete" at the Huntarian Museum in London.

Richard Neave and Denise Smith

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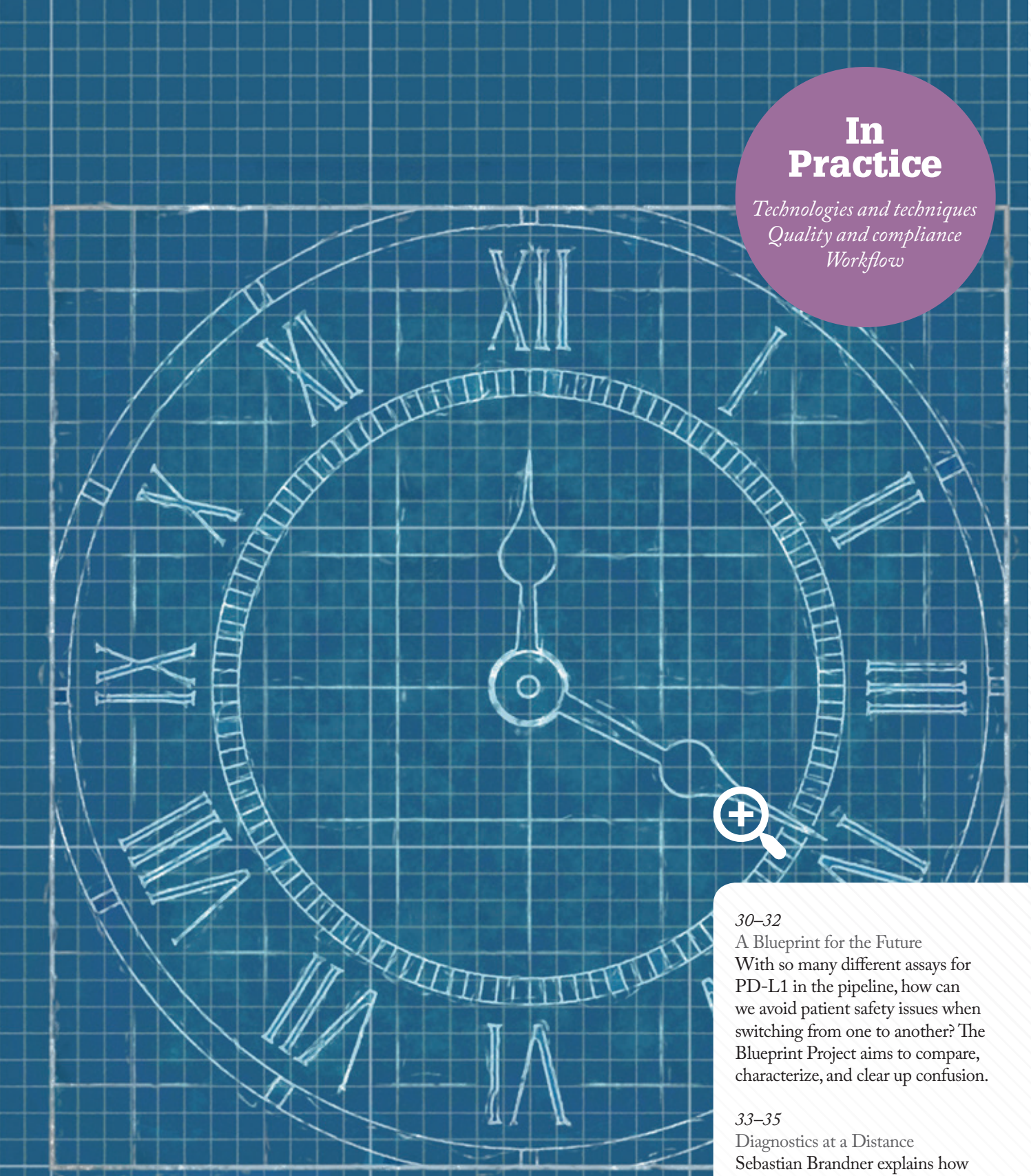


A Blueprint for the Future

A unique collaboration between industry and regulators is aiming to understand what possibilities may exist to harmonize or consolidate PD-1/PDL-1 assays

By Henrik Winther and Hans Christian Pedersen

There's no doubt in people's minds that immunotherapy is an up-and-coming area of cancer treatment. In fact, some even think certain pathways – like the PD-1/PD-L1 immune checkpoint – are receiving an unfair share of the collective attention. Whether or not that's true, one thing is for sure: there are a lot of PD-L1 expression assays currently under development. Surely, though, that's a good thing – the more research, the better? Not necessarily – and in this case, with two existing PD-L1 therapies and at least four distinct companion assays in the pipeline, the US Food and Drug Administration (FDA) has expressed concern over the potential for future market confusion and, as a result, patient safety issues. But what can be done to avoid this from happening? Six companies (four pharmaceutical; two diagnostic) currently competing to bring their assays to market have taken an unusual step – they're working together on an initiative known as the Blueprint Project to compare and characterize their tests.

At a Glance

- *Immunotherapy – and in particular immune checkpoint targeting – is a rapidly growing area of cancer treatment*
- *Because of this rapid growth, multiple assays for pathways like the PD-1/PD-L1 checkpoint are under simultaneous development, but are not interchangeable*
- *Members of the pathology community have expressed concerns over the negative impact that recommending a single therapeutic, based on a single companion diagnostic, might have on patient care, laboratory costs, and workload*
- *To address concerns, the Blueprint Project, which is an unusual collaboration between industry and regulators, was set up to characterize and compare four PD-1/PD-L1 companion diagnostics*

Why do it?

In February of 2015, the six Blueprint sponsors (Bristol-Myers Squibb, Merck & Co., AstraZeneca, Roche, Dako/Agilent and Ventana/Roche Tissue Diagnostics) met with the FDA and the American Association for Cancer Research (AACR) to discuss the concerns. After a public workshop to examine the issues, the sponsors put forward a proposal for the Blueprint PD-L1 Assay Comparison Project.

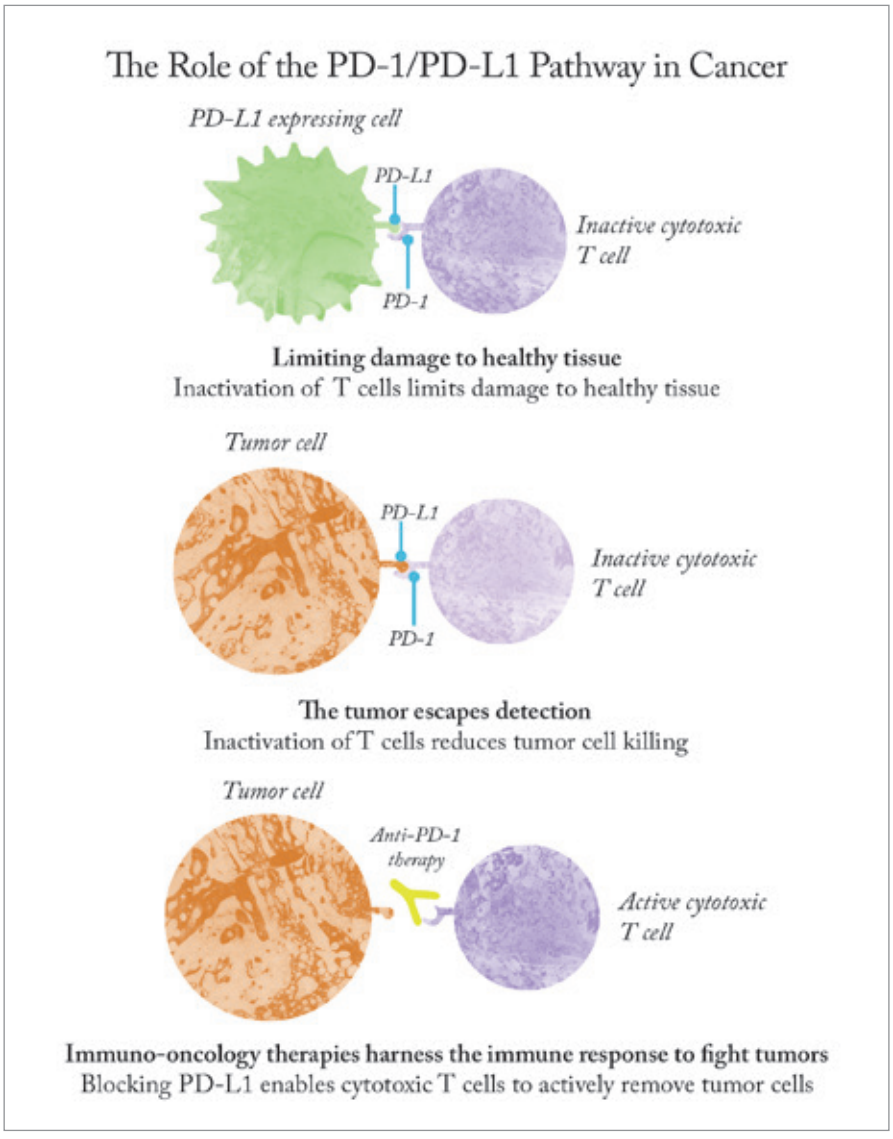
Why was it so important to establish this kind of comparison? It was clear from clinical trials that each assay had unique scoring guidelines and cutoffs used to identify responding patient populations – a fact that further fueled FDA concerns over the potential for patient safety issues if the assays were used to identify patients for cross-matched therapies. It's important to be able to transpose results from one assay onto another – but at the time the Blueprint Project was initiated, no analytical comparison studies existed between any of the PD-L1 tests going through drug-diagnostic co-development. Obviously, companies generally don't collaborate on pre-market evaluations like this one. The PD-L1 situation is unique, though; never before have so many companion diagnostic combinations been developed for the same biomarker at the same time. The Blueprint sponsors agreed that the situation – and the concerns that went along with it – required an uncommon approach.

"Never before have so many companion diagnostic combinations been developed for the same biomarker at the same time."

What is the Blueprint Project?

The project involves a number of parties, each playing a unique part. The FDA is the overall observer and monitors the process; the AACR facilitates conversations and provides project updates; the pharma and in vitro diagnostics companies provide support in the form of resources and technical expertise; and the International Society for the Study of Lung Cancer acts as a neutral observer and provider of pathology expertise.

To begin, the FDA and AACR identified the sponsors who were most advanced in the clinical trials process with "Investigational Use Only" PD-L1 assays and drugs. During initial meetings, the scope of the project was refined to include only non-small cell lung cancer (NSCLC), as those tended to be the most advanced trials. Other scope refinements meant that only assays that would eventually be submitted



for FDA review and approval via the Premarket Approval (PMA) process were included – excluding lab-developed tests. Ultimately, assays for four agents – nivolumab, pembrolizumab, durvalumab and atezolizumab – were selected.

In Phase One, teams from Ventana and Agilent selected procured NSCLC specimens to demonstrate the full dynamic range of each assay, then stained the slides using their own solutions with each of the four PD-L1 IHC assays. The analysis was fairly detailed. First, with no prior training or pre-alignment, two pathologists from Ventana and one from Agilent (experts in interpreting their respective assays) independently evaluated 156 IHC slides (39 cases for each of the four assays) for percentage of tumor cells and percentage of immune cell expression of PD-L1. Each expert pathologist also independently evaluated each case using only the clinical algorithm associated with their own assay of interest; for example, the 22C3 expert pathologist read all slides using the 22C3 selected cutoff. Then, the validated clinical diagnostic PD-L1 determinations for each assay were compared on a cohort of cases to demonstrate how hypothetical treatment decisions might be made for the four therapeutics. And, finally, the agreement rates of various combinations of assays and cutoffs were examined.

Phase One of the Blueprint Project was originally defined as a premarket study. One condition of its existence was that it would not delay pivotal studies or patient access to critical new therapies. In March of 2015, no PD-L1 assays or drugs had been approved – but during the course of the Phase One study, Agilent had two PD-L1 tests receive three premarket approvals (PMA), and several drug registrations in different indications occurred. Patient selection criteria were changing, both in the market and in clinical trials based on new data. Additional drug and diagnostic filings were in process. All of these activities took priority in, but also created challenges for the Blueprint Project, as all of these dynamics created a "moving target" for analytical assay comparison. That made it more complicated to reach agreement on the data analysis approach and factors to be compared. Eventually, however, initial results were delivered showing that three of the four assays evaluated demonstrate similar PD-L1 expression in tumor cells, while all four were shown to be variable (with lower reproducibility) in immune cells.

Onto the next phase

The primary purpose for Phase Two of the project is to validate the initial findings of Phase One using a much larger set of samples that better reflect "real-world" conditions. Phase Two will also likely assess variability between test sites and observers to better understand the magnitude of those variables and their impact on testing robustness. The International Association for the Study

"Given its significance in many cancers, I envision that PD-L1 testing will continue to play a major role in identifying patients who may benefit from immune checkpoint inhibitors. The hope is that testing can be simplified in the future as we learn more about PD-L1 expression in cancer."
– *Henrik Winther*

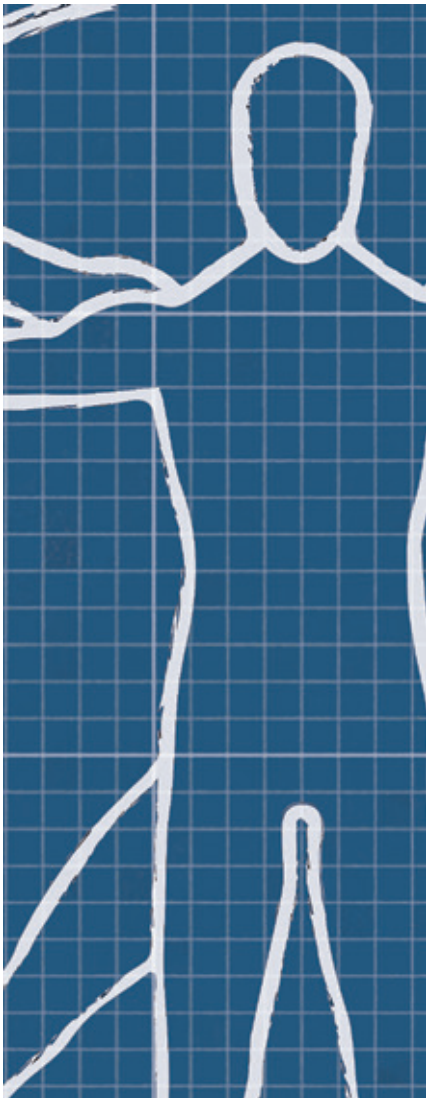
"Pathologists should be aware that, when they use PD-L1 IHC tests to inform therapy decisions, it's important to follow the validated assays' intended use. Despite the analytical similarities between some assays, the conclusion so far is that they are not interchangeable when selecting patients for PD-1 or PD-L1 therapies. Each test is developed and validated independently to identify corresponding populations for individual drugs, and they should only be used for their specific drug. As long as the tests are used appropriately, they could lead to significant improvements in treatments not just for NSCLC, but eventually for other diseases as well."
– *Hans Christian Pedersen*

of Lung Cancer (IASLC), which was originally selected to participate in Blueprint as a third party observer, is well-positioned to plan and execute the Phase Two study with support from the project's sponsors. Together, they're working out the study scope and design, but at the moment, that remains a work in progress.

PD-L1 immunohistochemistry isn't limited to NSCLC, though; it's being explored in many indications beyond lung cancer, and Blueprint serves as proof-of-concept for this kind of collaboration within industry. It will definitely be valuable for companies to continue working together to show analytical correlation between tests and data on assay performance in NSCLC. The Blueprint Project and its successors will likely influence how future indications for PD-L1 IHC are designed, both in terms of assay development and showing clinical utility.

Entry into the field of complementary diagnostics adds a new category for approval, the influence of which is yet to be determined. New testing technologies and platforms will require new paradigms to determine clinical utility and gain regulatory approval, but no matter what happens next, the future of companion and complementary diagnostics promises to be a dynamic one.

"Concerns [of the laboratory medicine community] were the main drivers for this initial public workshop in 2015."



Words to the wise

Many members of the laboratory medicine community have expressed concerns over the negative impact that recommending a single therapeutic, based on a single companion diagnostic, might have on patient care, laboratory costs, and workload. These concerns were the main drivers for the initial public workshop in 2015, and for the project that ultimately arose. The analytical performance comparison of PD-L1 assays is the first step to understanding what possibilities may exist to harmonize or consolidate – but this will be an ongoing discussion for some time between pharmaceutical companies, diagnostic companies and regulatory agencies.

Henrik Winther is Vice President and General Manager of Companion Diagnostics in Agilent's Diagnostics and Genomics Division.

Hans Christian Pedersen is Head of Companion Diagnostics and Immunohistochemistry Reagents for Agilent Pathology Solutions.

Diagnostics at a Distance

How a London neuropathology institute provides full diagnostic pathology services to a remote hospital – but could full digitization work for all?

By Sebastian Brandner

Digital pathology is a hot topic right now. The transition to computer-based services is on everyone's mind – to speed up service delivery, to aid in teaching and training, or to provide long-distance consultation on unique or challenging specimens. But although tales of success (and discussions of difficulties) abound, we don't often hear the behind-the-scenes stories of implementing and running a digital telepathology laboratory. At The National Hospital for Neurology and Neurosurgery, one of the specialist hospitals of University College London Hospitals (UCLH), we have become the sole provider of diagnostic neuropathology to Brighton and Sussex University Hospital (BSUH), meaning that we offer regular long-distance services via digital technologies. We began providing routine neuropathological services on an ad hoc basis in 2013. Then, Brighton went for tender with a detailed service specification and we applied as a bidder – a contract we won in 2015. Ever since, we've been providing them with a full diagnostic neuropathology service.

At a Glance

- *Since 2013, University College London Hospitals has been providing long-distance neuropathology diagnostic services to Brighton and Sussex University Hospital*
- *Since the end of 2015, remote intraoperative diagnosis has been provided through telepathology*
- *In this scenario, digital pathology is a suitable alternative to having an on-site pathologist; although the workflow is more involved and can take more time, the benefits of specialty service access can outweigh the challenges*
- *It is difficult to predict how digital pathology adoption will progress in future – though unlikely to gain full adoption any time soon, the better and cheaper the technology becomes, the more likely its success will be*

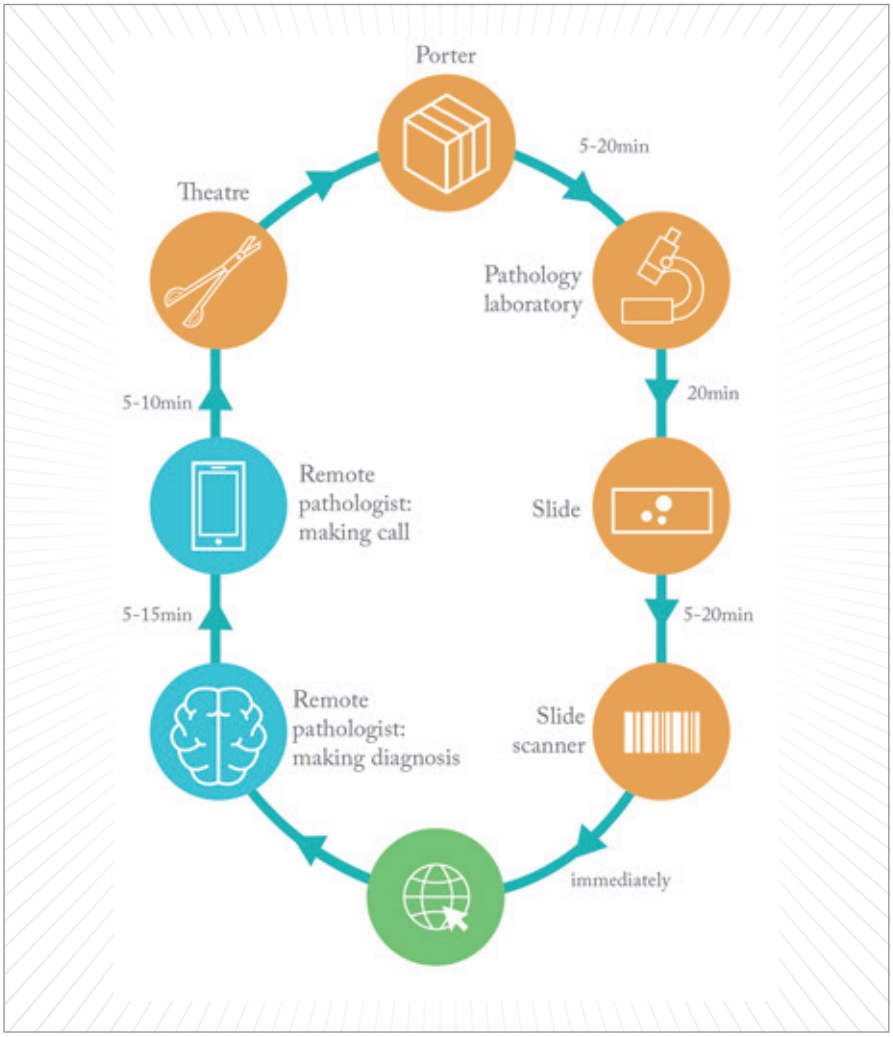
"We make the diagnosis on our screen, pick up the phone, and discuss our findings directly with the surgeons."

How our system works

Most of the specimens we examine don't require intraoperative assessment, so they are shipped directly to our laboratory in fixative. Upon arrival, we treat them the same way we treat all other referred and local specimens – that is to say, they are booked in, cut up, processed, stained and reported. After reporting, the only difference between these and local cases is the electronic transfer of reports. Whereas our hospital's cases and other ones are transferred directly onto the hospital information system and are immediately visible, Brighton's go to a centralized email actioned by office administrators and forwarded to the doctors.

When we need to handle intraoperative specimens from BSUH, it's always done digitally so that we can give rapid feedback. The surgeons usually notify us by email or telephone that they're working on a patient who will need intraoperative service. Then, during the procedure, they submit their material directly to the local pathology department at BSUH. A trained biomedical scientist on-site performs a smear (the most common method of intraoperative microscopic assessment of neuropathological specimens), scans it, and notifies us that it's ready to be reported. We log onto the system via the NHS N3 network (a private data network designed to ensure patient confidentiality and availability at all times) and view the specimen remotely. Our report is typed into the appropriate section of the slide management software and locked to be compliant with ISO standards. We make the diagnosis on our screen, pick up the phone, and discuss our findings directly with the surgeons (see Figure). We want our service to feel as close to having an on-site pathologist as possible – and I think we're succeeding; the only difference right now is the additional scanning time.

In theory, the remaining part of the tissue pathway – its processing, sectioning and staining – could also be done at BSUH, but we talked it over with their pathology department and decided that it would be preferable in terms of quality assurance, cost and turnaround to perform those tasks in our specialist center. Neuropathological specimens need an entirely different, specialized spectrum of immunohistochemical and molecular tests to those available at BSUH, and it would be expensive for them to set up, maintain and validate such a complex test portfolio for a relatively small number of samples. We routinely do these specialist tests in large quantities at our center, so we can offer them a solution that's not only cost-effective, but provides better quality



The workflow of intraoperative remote reporting for neuropathology. During surgery, a specimen representative of the lesion is taken to the pathology department. Upon arrival in the laboratory, a small (~1 mm) fragment is pressed between two object slides; a smear is prepared and stained with a blue dye to visualize the nuclei and cell structure. The slide is then scanned (time varies depending on magnification and specimen size, but we always scan the whole slide to ensure that we capture the entire cell spread). The data are stored on a local server and the remote pathologist is informed (usually by a phone call) of its presence, logs onto the system, and examines the slide remotely. Importantly, only the area that is viewed requires data transfer, so there is no need to download the entire slide. Once the diagnosis is made, the pathologist informs the surgeon by telephone.

control. As most intrinsic brain tumors require additional molecular diagnostics, we can initiate these tests immediately upon examining the H&E sections, ensuring that most molecular tests are back within two to three weeks of the operation.

For a fully integrated digital pathology remote reporting system, we could easily report sections prepared at BSUH, which would eliminate the two to three working days needed to transport specimens to our center. Until we implement that, though, I anticipate that our system will continue in its current form for some time, because it works very well – the way intraoperative sections are reported is ideal for a remote or digital pathology setup.

The good and the bad

The high quality of modern digital pathology has made this kind of remote consulting much easier. We now have rapid, high-quality communication with surgeons across a distance of 60 miles even as they operate on a patient. This is nearly equivalent to having a pathologist available locally. That's not to say that it's without its drawbacks, though; the workflow, of course, is slightly more involved. We have to establish communication between the remote laboratory and the operating theater staff. We do a good job, though – many of the neurosurgery trainees in Brighton aren't even aware that the pathologists reporting on their patients are at an entirely different center!

The most time-consuming element of establishing a digital telepathology service isn't necessarily the communication process, though – in our case, it was coordinating the IT departments and setting up the logistics of a fast, secure network connection. To ensure data could be transmitted securely, we needed to establish a connection accessible through a restricted number of computers in our department – which meant limiting it to a range of IP addresses. There are pros and cons to this – the advantages, of course, are the speed and security, but the downsides are a lack of access when not on site and a limited choice of hardware. Nonetheless, now that the system is fully established, it runs so smoothly that we may as well be on the ground in Brighton!

Who would benefit most from the transition?

There are two fundamentally different scenarios in which a need for telepathology might arise. The first is a

department that is staffed with a team of pathologists, but lacks specialist expertise in a few areas. In this scenario, digital pathology would help with the provision of second opinions on both a regular and an ad hoc basis. This option is well-suited to district general hospitals with a limited spectrum of expertise, but a wide range of pathological specimens, some of which may need a specialist opinion. It works especially well if the immunity chemical tests for such cases are available locally. The other potential scenario is the concentrated expertise that can be found in a center's specialist area. These areas are what I call the "recipients" of digital pathology services – that is, they don't need to scan any slides, but instead receive scanned slides from remote centers that require expert opinions.

There is one other possibility – a transition to digital reporting in the department where the sections are prepared. The business case for such a transition would look entirely different, and would mean a complete changeover from glass slides to computer screen-based reporting. Few departments have made this transition because of the obvious obstacles – chiefly the addition of work steps (consistent barcoding, automated scanning, automated slide and case management software, connecting to LIMS) that would be needed to enable digitization. That's why I think telepathology is the obvious area to get started with digital pathology. In fact, it has already been available for many years, though its first incarnation was more primitive; it used to be delivered through remotely controlled microscopes. The limitation with those was the bandwidth of the Internet connections at the time – but now, our advanced software capabilities and better bandwidth make digital pathology a safe and effective reporting tool for remote pathology.

Is a fully digital future realistic?

Although the transition to digital telepathology seems like an obvious path, making the same move for the entire pathology department (for instance, digitizing all slides for exclusively online reporting) is a different scenario, and it's very difficult to predict how well a rollout would go. If we look at more widely known technologies that seemed futuristic a decade or two ago there are failures and successes. Time will tell, for example, how successful electric cars are, or what the future of driverless cars really is. Under well-defined road conditions, these systems may work very well… but the devil is in the detail (like snow, rain, unexpected – but harmless – objects, or worse, harmful objects misidentified as "road signs"). Pathology is much the same. Fully digitized reporting must be properly field-tested, and in order to do so, the providers of integrated solutions must be open to discussing the pitfalls and risks. Are they? I'm not yet convinced that all of the potential pitfalls, errors, and lack of business continuity are really openly and transparently discussed and disclosed.

Generally, novel solutions will be adopted quickly if they are time- and cost-saving, especially if they allow users to simplify or omit parts of a complicated process. Digital cameras are a good example; adoption started relatively slowly because the resolution was inferior to film and the cost for a camera higher than conventional devices. But once resolution improved and price decreased, the demise of film-based cameras was very rapid. This development had the biggest impact in radiology, where digitization reduced a number of expensive steps – and now, many radiology departments are partially or entirely digital. These examples are in stark contrast to digital pathology, which requires additional equipment, IT infrastructure and software. Those are the obstacles we need to tackle now – because in my view, adoption of novel pathology technologies works better if they make people's lives easier, cheaper and more convenient.

Timeline

May 2013 – retirement of only neuropathologist at Brighton and Sussex University Hospital (BSUH)

May 2013 – start of interim telepathology service by University College London Hospitals division of neuropathology

June 2013 – interim contract to provide comprehensive neuropathology services

October 2014 – tender for provision of comprehensive neuropathology services, including surgical pathology reporting and remote pathology using digital slides

June 2015 – award of contract to Queen Square neuropathology, followed by BSUH purchase of digital pathology scanner and software

December 2015 – first live remote reporting of an intraoperative slide by Queen Square neuropathology to BSUH

Sebastian Brandner is Professor of Neuropathology at the University College London Institute of Neurology and Head of the Division of Neuropathology and honorary consultant neuropathologist at the National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust.

Micro Sample, Maximum Outcome

Next-generation microsampling technologies are overcoming the limitations of traditional DBS and offer advantages over typical wet sampling as well. It's time to open your mind to it...

There are clear advantages to dried blood spot (DBS) sampling compared with routine wet sampling in many situations. However, DBS – as a technology with potential to replace wet sampling – has fallen out of favor with clinical laboratories because of its inherent limitations. Technology is advancing, though, and what were once obstacles to an accurate result, are no longer issues of concern – next-generation blood microsampling devices now have a clear place in clinical labs. Hitesh Pandya, Senior Lecturer in Pediatric Respiratory & High Dependency Unit Medicine, and Bikalpa Neupane, Clinical Research Fellow, University Hospitals of Leicester NHS Trust, UK, tell us why.

What has driven the development of microsample technology?

There are some obvious advantages to any system that will allow us to acquire and test very low blood volumes. A particular urgency for this type of technology was stimulated around a decade ago by changes to procedures for new drug applications to the FDA and EMA. Where new drugs had a pediatric indication, it was now mandated that these drugs be "tested" in children and not just in adults. Resultantly, a substantially higher number of pharmacokinetic (PK) studies, and therefore blood samples, during clinical development were needed. The solution at the time was to use GUTHRIE (DBS) cards – basically cards that collect blood droplet samples for testing. While these cards filled an unmet need, there were two vital boxes they didn't tick – accuracy and precision, and spoilage and loss. These cards were impractical in real-life settings – "hematocrit issues" were substantial, and each dried blood spot had to be "punched" out manually, so staff time and costs were high. There was also a lot of wastage, too; in the population of new-born infants routinely screened for congenital disorders, such as cystic fibrosis, I would estimate that around 10 percent of GUTHRIE cards needed to be repeated as they were unacceptable for analysis. That number is not insignificant, in particular when you're dealing with huge populations – the UK birth rate, for example, is ~700,000 per annum. It's clear that an alternative was needed.

Aren't wet samples good enough?

As we know, most routine sampling in hospitals is performed using wet samples, which is fine in most cases, but there are intrinsic issues associated with them. The requirement for high sample volumes (a minimum of 200 µl) makes testing, in particular regular testing and screening, near-impossible in some pediatric patients. Ongoing monitoring of chronic diseases and general screening are hampered by the high workload that accompany high-volume wet sampling too. The preanalytical error that we are exposed to as a result of routine blood sampling are also well-known – errors that can creep in at the acquisition, storage, transport and testing stages. These are processes that have to be tightly controlled if we are to have confidence in the accuracy of our results.

How do you currently use microsampling?

We use it (specifically Mitra® microsampling devices, Neoteryx®) for research purposes only, in particular for PK studies, but we see a clear need for its expanded use clinically; in our opinion it overcomes the key limitations of traditional DBS testing.

Our experience after using hundreds of Mitra tips has been very positive, the obvious instant advantage being volume – we only need 10 µl of sample to conduct a test. We can collect a sample very quickly, simply with a fingerpick – no venepuncture is required – and we've never had a test case denied because of the inadequate blood volume. Our team required minimal training, in fact, we would go as far as to say it's foolproof and its accurate. By that we mean that blood collection is very simple, there is little to no risk of spoilt samples and, importantly, storage requirements are straightforward and minimal; the ability to store and transport tips in normal room temperature is extremely useful and cost-effective too. Plasma processing steps are eliminated, which is a great advantage.

To date, we have not encountered any specific issues relating to collection of blood samples, storage or transportation of tips. From an end-user's perspective, we can confidently say that microsampling shortens and simplifies our work considerably, its automatable and the risk of infection is minimized too. A specialist team of doctors or phlebotomists aren't needed either; literally anybody, including patients, could take a sample.

Could you please outline any challenges to introducing microsampling into a clinical laboratory?

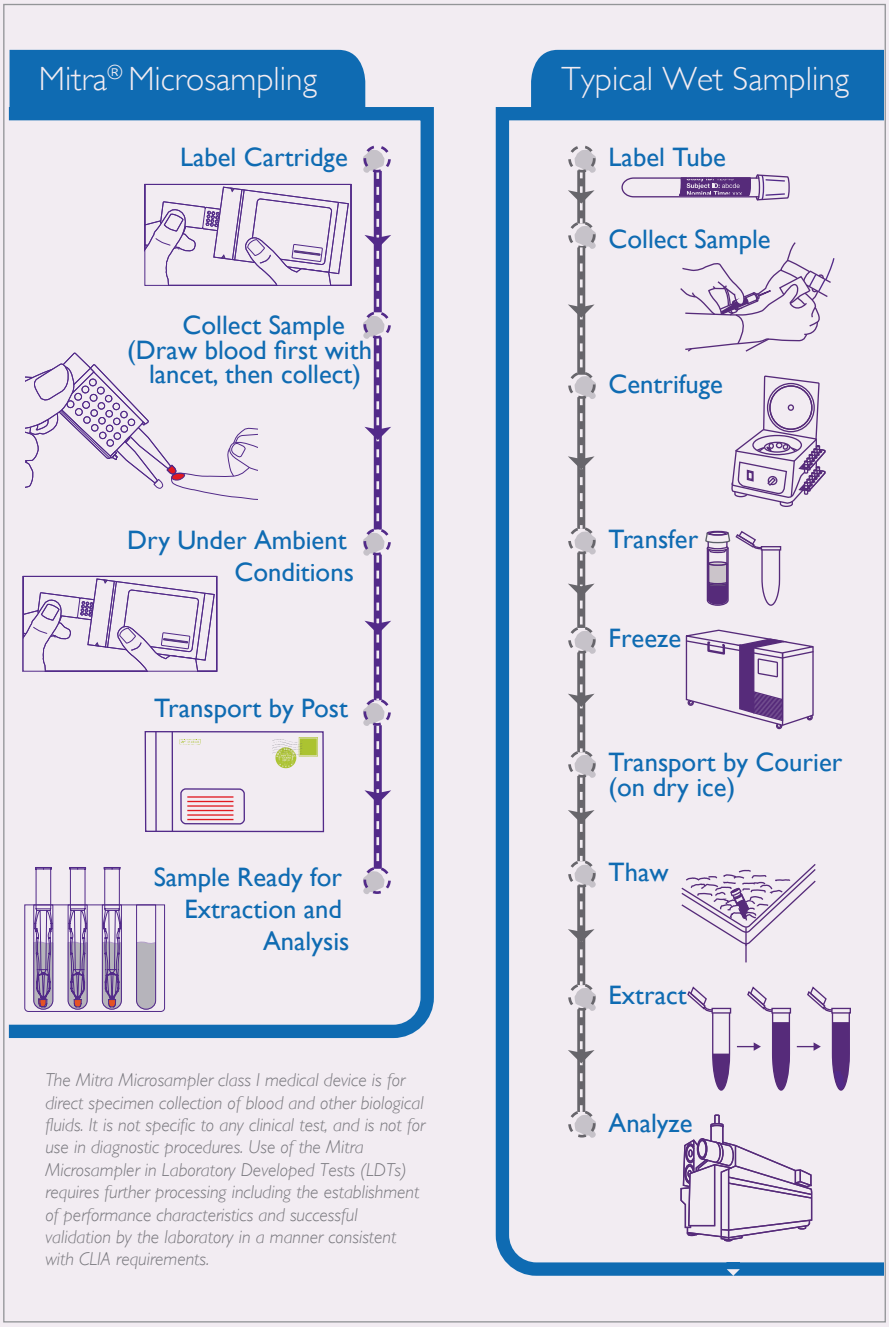
The biggest challenge with any dried blood microsampling technology is to develop a uniform collection method and establish normal ranges / values for blood parameters. So much of what we do is on plasma so we do need to define normative values for our blood samples. User economics also presents an obstacle – microsampling technology requires an initial financial investment to integrate it into a lab. With resources already being stretched in most labs, this

may be a difficult argument to win, but we can say that the long-term economics of the introduction should be favorable. We feel the quality benefits are huge, too. And although microsampling is not the solution for every analyte, we would remind that a universal wet sample system doesn't exist either (EDTA sample bottle, plasma sample bottles, etc.).

What is your prediction for the future role of microsampling in the future?

There are many potential uses. First and foremost, in clinical trials, in particular for pediatric patients as explained, but also in preclinical testing – think of how many fewer animals you would need if only tiny amounts of blood were required for testing. We also see a big role for it in screening and at-home testing. For example, screening young children for disorders, like cystic fibrosis, that are disabling if not picked up early. The same could be applied to adults too. We already have good biomarkers for very common conditions, in particular in aging populations. Imagine a patient testing themselves for vitamin D deficiency, COPD, heart failure markers, and also to manage their diabetes, through HbA1c testing at home. A Mitra tip can be given to patients to do their own sampling, and they post it to the lab. The result is then waiting for them at the clinic when they arrive. There are important applications for this type of sampling in research too. When it comes to the search for new diagnostics, often you don't know what you're looking for so high volumes of samples are needed. Think of how much more simple and cost-effective the process will be if microsamples were used to support the discovery of new analytes or biomarkers.

While liquid chromatography/mass spectrometry (LC/MS) equipment is already capable of effectively analyzing low blood volumes, imagine trying to get some mass spec equipment out to Mount Everest, for example. It's not going to happen. This technology ought to be a winner for the developing world where standard wet sample acquisition and testing is difficult, while offering big advantages for developed countries too.



The benefits of microsampling for pediatric patients, for screening and chronic disease management are clear, but is its use commonplace in the clinic?

Not yet, but it should be. In our opinion, microsampling will supersede current collection methods and this will be driven by the revolution in at-home testing by patients.

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From Blood to Breath

Circulating microRNAs in the blood may serve as noninvasive biomarkers for the diagnosis and characterization of asthma and other respiratory disorders

By Faoud Ishmael

Asthma is a very common and well-known disease, but unfortunately, it's also a disease that can be very difficult to diagnose. At the moment, we use lung function tests that rely on measuring how fast we can blow air out of our lungs – but these tests are often unable to make a definitive diagnosis, and it's difficult for children, and even some adults, to perform them. That's not all that complicates the matter; we now also know that asthma is very heterogeneous. It has a number of subtypes, including allergic and non-allergic disease and high and low eosinophil presence. It's not always easy to determine what type a particular patient has, and that means that we can't always decide which treatments might make a difference – not everyone benefits from inhaled corticosteroids, the standard medication, and we don't always understand why. As a result, there is a great need to find tests that can both diagnose asthma and characterize the disease further.

Ideally, for patients who present with respiratory symptoms, we'd be able to conduct a simple test – perhaps with a blood or saliva sample – to get the information we need. Until now, there have been no such tests. Recently, though, we've discovered a collection of microRNAs (miRNAs) present in human body fluids that can give us better insight into asthma, both in terms of absolute diagnosis and individual phenotype.

At a Glance

- *Despite its prevalence, asthma can be difficult to diagnose and even trickier to treat, because not all subtypes respond to the same medications*
- *Current asthma tests rely on spirometry, but completing these tests can be a challenge for some patients and may not yield reliable results*
- *A subset of circulating microRNAs is differentially expressed in patients with asthma compared with healthy controls, and a further subset can distinguish asthma from allergic rhinitis*
- *In the next decade, these microRNAs may translate to a quick, cost-effective, noninvasive asthma diagnostic without the limitations of current methods*

Asthma answers

Our research project kicked off when we discovered that RNA species were present in exhaled breath condensates – basically, cooled breath vapor harboring particles that originate in the lungs. After making that discovery, we cloned the exhaled RNA and found that miRNAs were enriched in lung fluid. We then went on to show that a number of miRNAs were differentially expressed in patients with asthma, in comparison with healthy control subjects. It turned out that those miRNAs were differentially expressed in the blood of asthmatics compared to non-asthmatic subjects, suggesting that blood could serve as a source of miRNAs capable of describing inflammation in the lungs.

Initially, we identified a panel of 30 miRNAs that were different in the blood of asthma patients compared with healthy controls (1). Many of these had similar expression patterns, though, so we were able to come up with a final panel of seven that could differentiate between asthma, healthy controls, and subjects with allergic rhinitis (an upper respiratory disease; asthma, in contrast, is a lower respiratory disease). The ability to narrow our options down to a limited panel is a plus because it means that we can make a simpler, cheaper diagnostic test.

> *"As we expand the numbers of subjects we study, we'll be able to uncover even more asthma subtypes based on miRNA profiles."*

How do the expression profiles of various asthma subtypes differ? We performed a cluster analysis on the original 30 differentially expressed miRNAs in asthma, and we found that they clustered primarily into two groups. One group of asthmatics had a high level of eosinophils, whereas the other cluster had low eosinophils. Emerging research shows that patients with non-eosinophilic asthma may not respond to typical asthma therapies, so our hope is that we can start to predict whether or not patients are likely to respond to treatment by measuring their miRNA profiles. We think that, as we expand the numbers of subjects we study, we'll be able to uncover even more asthma subtypes based on miRNA profiles – sort of a "fingerprint." This would be a huge stride toward personalizing asthma therapy!

We're beginning to understand that the miRNAs we've identified may play important roles not just in asthma, but in allergic and inflammatory diseases. Some of the miRNAs (miR-155, miR-570,

and miR-1248, for example) have pro-inflammatory roles and may be necessary for the development of asthma. Others, like Let7a and miR-146a, seem to be anti-inflammatory. So, in addition to the diagnostic potential of these miRNAs, they may have therapeutic potential as well. So far, we've found that miR-146a works in parallel to glucocorticoid medications, and that when the two are combined, they have additive effects. We currently have some difficulty treating patients who fail to respond properly to glucocorticoids, so adding miR-146a to their treatment regimens may help increase their sensitivity to the drugs.

Partnering with pathology

It's interesting to note that such tests are being referred to as "liquid biopsies." One day, we may be able to find out more about our patients' lung pathology and airway inflammations simply by examining the miRNAs in their blood – or even other fluids like exhaled breath condensates. We're collaborating with the Milton S. Hershey Medical Center's pathology department on our current work, as they may be able to apply the assays we develop to other diseases. Tests like that would really open up our ability not only to diagnose diseases, but also to learn about their pathology. And because the current diagnostic workflow involves simple, PCR-based tests, we anticipate that our new assays could be readily integrated.

Right now, we're in the process of acquiring larger numbers of subjects to validate our initial results and see how good the miRNA test is at diagnosing asthma in an unknown population. As the technology we use to measure miRNA levels in the blood is sensitive, reproducible, and cheap, we hope that our recent findings can soon be translated into a diagnostic test – hopefully within the next five years. We'd eventually like to develop a chip-based assay that would allow physicians to take and analyze saliva or fingerstick blood samples at their patients' bedsides.

Towards personalization

Moving forward, we have three main goals. First, we'd like to reduce the size of the miRNA testing technology so that it can fit on a chip for analysis by a cell phone. Next, we're trying to understand the biology of the miRNAs; we think that some of them are crucial to the development of asthmatic inflammation, so we're working on understanding what they do. Third, we're moving toward using this technology to really personalize asthma treatment. We will determine whether or not we can use miRNA profiles to predict treatment responses. Putting all three of these goals together, in a decade or less, we'd like to see this happen:

1. A patient with wheezing enters the office.
2. A fingerstick blood sample is analyzed by a chip the size of a credit card, plugged into a cell phone.
3. The software not only confirms an asthma diagnosis, but also tells you what form of asthma is present, and may even make a recommendation about which medication to initiate.

The ability to diagnose asthma quickly, conveniently and noninvasively – perhaps even on a patient's first visit to the clinic – would offer significant advantages over current tests. Patients unable to perform respiratory tests could still receive a diagnosis and begin treatment rapidly, and characterizing their disease at the same time has the potential to prevent overtreatment in situations where steroids won't be effective, and to eliminate the period of uncertainty as patient and physician wait to see whether or not the drugs will help. And we might even gain a better understanding of the molecular pathogenesis of asthma and allergic rhinitis, knowledge that may allow us to identify new therapeutic targets and continue to improve patient care.

Faoud Ishmael is Associate Professor in the departments of Medicine and Biochemistry and Molecular Biology at Penn State College of Medicine and a physician at the Milton S. Hershey Medical Center, Hershey, USA.

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Diagnosis Ex Machina

A new software tool may improve upon current infectious disease diagnosis methods to return faster, more accurate results

By Robert Schlaberg

When faced with an unidentified infection, doctors typically pursue two approaches – they apply treatment (for instance, a broad-spectrum antibiotic) based on the suspected cause of disease, or they can proceed with diagnostic testing to determine the cause of the infection, and therefore the best treatment. Both approaches have limitations. The former might result in inadequate treatment and risks prescribing antibiotics where none are needed. The latter is often time-consuming, delaying the patient's treatment, and may provide inconclusive results, hampering decisive action. This lack of good options becomes even more unconscionable when one considers the high morbidity and mortality from infectious diseases, especially in the elderly, young children, the seriously ill, and in resource-limited settings. My colleagues and I felt it was clear that a better solution was needed – so to tackle the problem, we developed methods that use next-generation DNA sequencing for the rapid, unbiased detection of all known pathogens. These efforts led to the development of Taxonomer (1,2), an ultra-fast NGS data analysis software package that can identify pathogens from millions of DNA sequences within minutes.

At a Glance

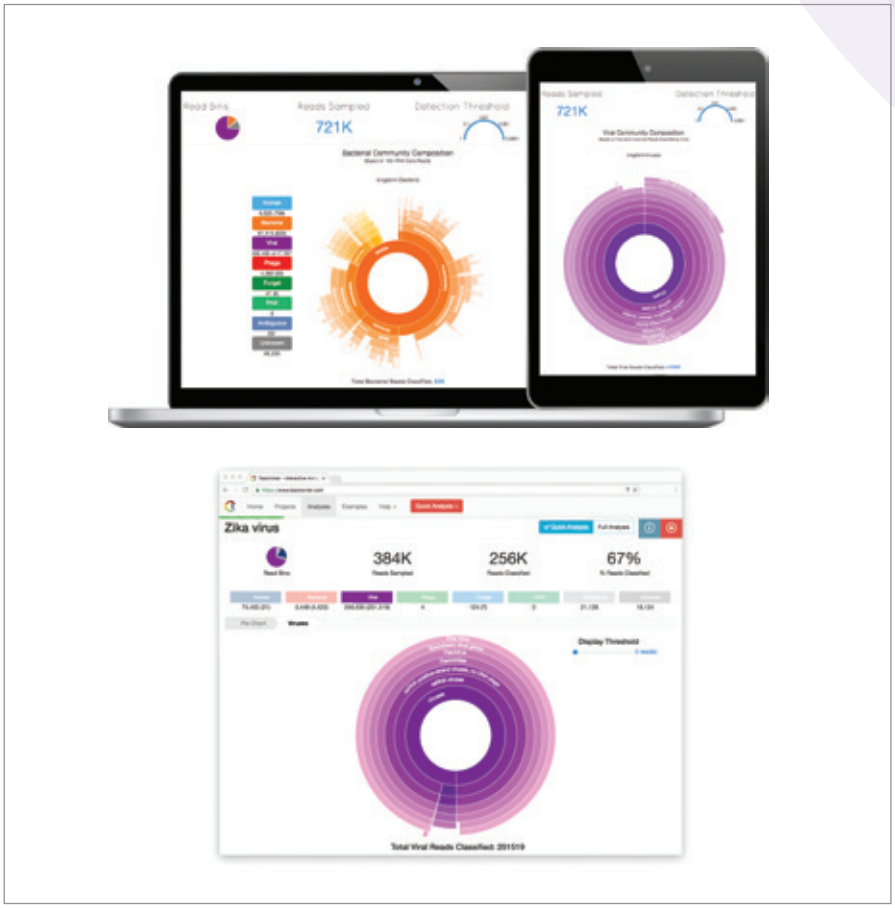
- *Infectious diseases are one of the world's biggest killers, but doctors still lack rapid, conclusive ways of identifying pathogens*
- *Current methods may be time-consuming, inconclusive, have limited scope, or require equipment and skills not available in all settings*
- *A new type of software, Taxonomer, may be able to speed up clinical diagnostics by examining pathogens' genetic material*
- *Unbiased pathogen detection combined with fast, accurate, and easy-to-use data analysis will, in the future, provide rapid answers to the question, "What's wrong with my patient?"*

How does it work?

After DNA from a patient's sample is converted to millions of short sequences, the user simply uploads the data to Taxonomer, which can be accessed through a web browser without the need for the large computers and programming skills usually needed to analyze NGS data. It even works from mobile devices, empowering physicians and scientists to analyze their NGS data interactively and in real time. Users can analyze DNA sequences that are stored on their own devices, in the cloud, or use public data by simply selecting the files they want to access. All the heavy computing happens on a server that can be located anywhere in the world – which frees physicians and scientists of the need to invest in expensive computers and bioinformatics resources and lets them interact with their data immediately and directly.

How does Taxonomer analyze the information? By using search algorithms to compare short DNA sequences from patient samples to millions of reference sequences. The software classifies each sample sequence to the organism from which it most likely originated – but it completes the task automatically and within minutes, rather than requiring extensive input and hours or days of time as would have been needed only a few years ago.

Taxonomer compares DNA query sequences to both DNA and protein reference sequences. This makes it possible to identify new organisms more effectively, because there is usually greater sequence conservation at the protein level. The end result is a catalog of all known viruses, bacteria, and fungi detected in a given sample; not just their presence, but also their relative abundance. And it's not only a tool for identifying pathogens – it can also identify which of the patient's genes are turned on. This helps determine the way a patient reacts to an infection. When combined with the right interpretive information, this may be a method that laboratory medicine professionals use in



Taxonomer's interactive display presents the vast genomic data extracted from pathogens found in a patient.

the future to differentiate patients who need antibiotics from those who don't – among other things.

Benefits of unbiased detection

Patients whose symptoms may be caused by any one of a long list of pathogens, or in whom an infectious disease needs to be ruled out, will benefit most from unbiased pathogen detection. This includes cases of suspected pneumonia, encephalitis, meningitis, or sepsis, especially in transplant and other immunocompromised patients, the elderly, infants, the severely ill, or patients with unusual symptoms. Often, we're not sure what to test for. Unbiased pathogen detection lets us explore all of the possibilities without having to guess.

Many diagnostic laboratories now have access to next generation sequencers within their departments, in core facilities or through commercial laboratories. Sequencing itself is only the first step, though. Quickly, accurately, and consistently analyzing NGS data is still a challenge, especially for diagnostic laboratories. We believe we've developed software that can help physicians and scientists close this gap. And with next generation sequencers becoming ever smaller and even portable, the software holds great promise for unbiased pathogen detection in remote locations and in outbreak settings. The technology's interactive web interface enables access from a smartphone, tablet computer, or laptop, which means laboratories around the world can stream their data to the program's server for analysis and view the results in real time. It's my hope that, by providing accessible and easy-to-use software, we can speed up the adoption of NGS for improved diagnosis of infectious diseases.

Getting the clinic on board

I'm convinced that unbiased pathogen detection combined with fast, accurate, and easy-to-use data analysis is heading rapidly toward widespread adoption for infectious disease testing. Sample preparation workflows are still challenging to perform in diagnostic laboratories, but improvements are made continuously. Our vision for Taxonomer's development was to provide rapid, accurate, and complete answers when doctors ask, "What's wrong with my patient?" – and to achieve that without having to first assume a suspected pathogen. I wouldn't be at all surprised if multiple laboratories offered tests based on Taxonomer or similar tools within the next few years.

Robert Schlaberg is Medical Director of the Microbial Amplified Detection, Virology and Fecal Chemistry Laboratories and Assistant Medical Director of the Virology and Molecular Infectious Disease Laboratories at ARUP Laboratories, Salt Lake City, USA. He is also an Assistant Professor of Pathology at the University of Utah School of Medicine and a co-founder of IDbyDNA Inc.

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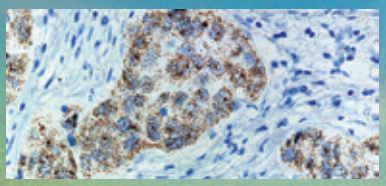

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Quis Custodiet?

Evaluating the evaluations of medical tests

By Patrick Bossuyt

My group is interested in methods of establishing whether diagnostic tests are really useful – that is, whether patients benefit from them. This is an under-developed area, as it's always been easier to establish the utility of a new drug than it is to assess whether patients actually benefit from a test.

Fit for purpose?

One of the main problems is that patients don't directly benefit from the test; the benefit typically arises from how the test results are used to guide clinical management. That's different from other interventions, such as drugs, where there is a direct link between the intervention and the patient outcome. So to evaluate a medical test, you have to understand how it is used in the clinical pathway – how the results are communicated and how they guide decisions and influence outcomes. But lab professionals usually focus on the tests and the results, and don't always consider how these results affect clinical management and patient outcomes.

Another factor is that the medical testing field receives far less attention than pharmaceuticals and other interventions; for example, it is given scant attention during the training of medical doctors. In particular, it receives less emphasis from a regulatory and reimbursement perspective, and in consequence the barriers for marketing are worryingly lower for medical tests than they are for pharmaceuticals. There is accordingly less pressure on companies to produce direct evidence that their tests improve outcomes. Indeed, if a new test provides the same results as an existing one, it can replace the existing test without going through the expense of a randomized trial. So the need for a more complex evaluation – one that requires consideration of patient outcomes – in combination with lower regulatory hurdles has resulted in the field of medical test evaluation being slightly disadvantaged.

A pragmatic approach

This is not to say that every new test should be validated by a randomized trial – that would be an extremely exaggerated position to take – but we should consider a middle ground. Clearly, some personalized and precision medicine tests really must be evaluated in randomized trials before the clinical community can accept that they are effective in improving patient outcomes. For example, if you propose using a marker to identify patients likely to respond to therapy, you should provide evidence that marker-based stratification in combination with treatment gives better outcomes than the alternatives (i.e. either no treatment or treatment of all patients). But many other tests could legitimately rely only on diagnostic or analytical accuracy studies for approval. So I'd like to see a staged approach in which some types of test require randomized trial evidence while others do not.

> *"There is accordingly less pressure on companies to produce direct evidence that their tests improve outcome."*

In any case, however, the methods by which tests are evaluated deserve close consideration. Typically, one assesses three features of a lab test: analytical performance, clinical performance and clinical effectiveness. Evaluation of analytical performance determines the trustworthiness of the test – does it reliably give results that correspond with the true value? Evaluation of clinical performance indicates whether the test result is meaningful – does it distinguish diseased from non-diseased patients, or patients who progress from those that don't? But the bottom-line evaluation is that of clinical effectiveness – is the test useful, that is, does it guide management better than not relying on the test? So these three types of evaluation answer different questions,

At a Glance

- *Evaluation of diagnostic tests receives far less attention than, for example, pharmaceuticals; as a result, tests receive less regulatory scrutiny and barriers for marketing are low*
- *Analytical performance, clinical performance and clinical effectiveness of a test should be considered in the context of its intended application*
- *Unfortunately, many published evaluations of medical tests do not include the key information necessary for a valid assessment of the test, and indeed may be over-optimistic in the interpretation of the data they report*
- *The STARD checklist is changing this situation by helping readers, authors and editors to verify that critical data are included in reports of the evaluations of medical tests*

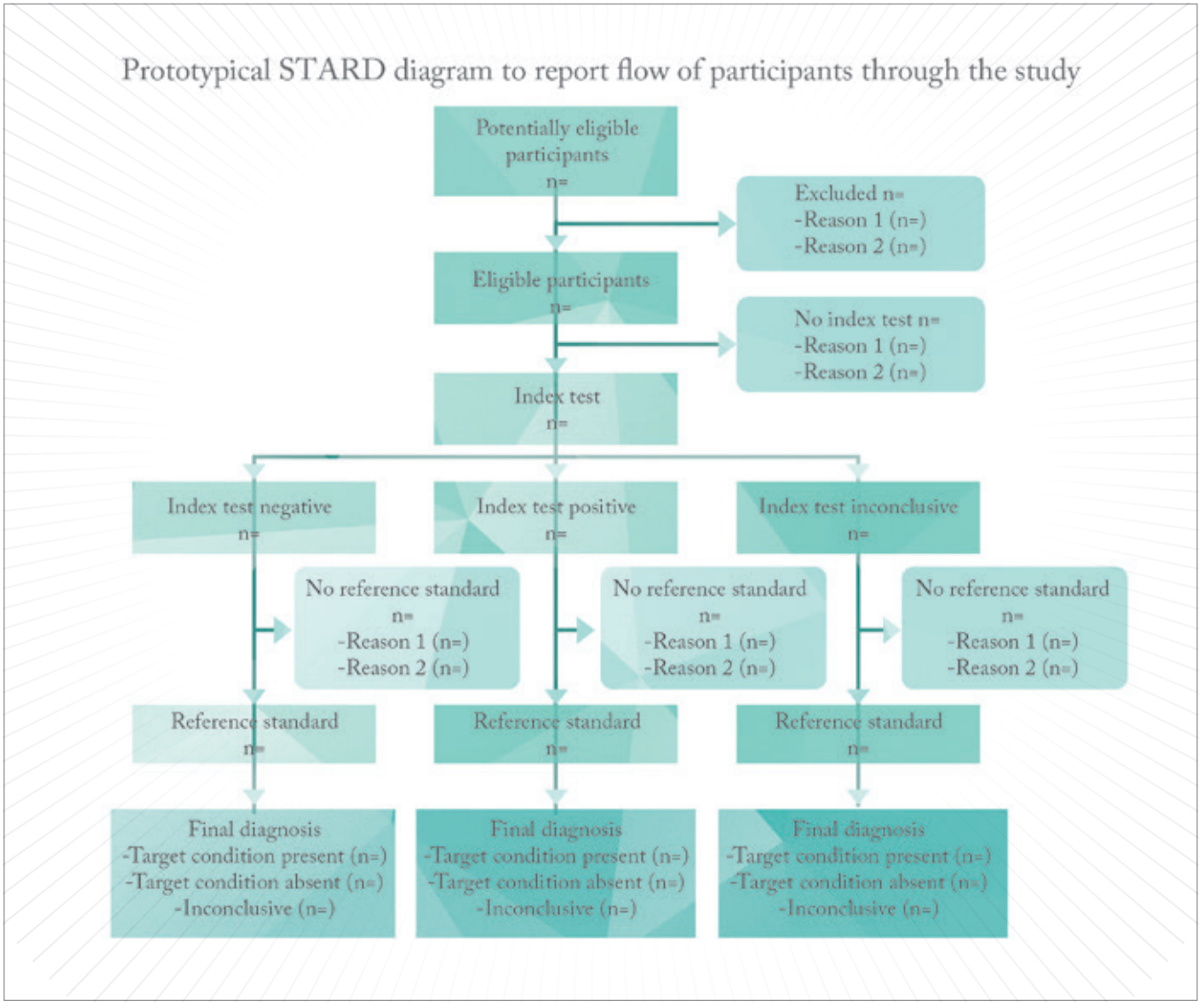

Figure 1: STARD flowchart

The STARD flowchart and checklist tools (1) have been developed to support the robust evaluation of medical tests intended for various purposes (diagnosis, screening, staging, monitoring, surveillance, prediction and prognosis), and for various clinical roles (for example, to use before an existing test, to replace an existing test, or to use after an existing test).

A typical study evaluates the ability of a medical test (the "index test") to correctly classify study participants as having a target condition – for example, disease presence, disease stage or response to therapy. This is usually done by comparing the distribution of the index test results with those of the "reference standard", i.e., the best alternative method for establishing the presence or absence of the target condition.

Cross-tabulation of index test results against those of the reference standard can be used to estimate the sensitivity of the index test (the proportion of participants with the target condition who have a positive index test), and its specificity (the proportion without the target condition who have a negative index test). This approach permits derivation of other statistical measures, such as the positive and negative predictive values of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical uncertainty of the measurements.

Standardization of the evaluation of medical tests through wider use of STARD tools, such as the flowchart illustrated, is expected to improve the quality and reliability of reported evaluations of medical tests.

and each should be answered satisfactorily before releasing a test into clinical care.

Furthermore, each of these three evaluations should be guided by the intended application of the test, in particular with regard to the kind of patients who are to be tested in clinical practice. Thus, there is some interdependence between these three types of evaluation. For example, the evaluation of analytical performance should be connected to the clinical effectiveness evaluation, in that once the test is sufficiently developed, you should anticipate the required level of clinical effectiveness in the context of the intended use of the test. So a proper evaluation should take into account the link between the test and its intended application in healthcare.

The test of time?

Unfortunately, medical test evaluation suffers from silo thinking – evaluations of analytical performance and clinical performance are disconnected. Often, manufacturers and healthcare professionals develop tests without a proper understanding of how the test will provide a level of effectiveness sufficient to persuade payers or clinicians to use the test in the first place. And this is not the only problem in the field of medical test evaluation. Like other areas of biomedical research, the test evaluation literature suffers from widespread problems, such as failure to report negative results and presentation of results with an optimistic 'spin'. Commonly, this is a result of researchers feeling that they must emphasize positive findings in order to achieve publication. And this, in combination with the fact that some studies will generate positive findings by chance or through poor study design, may explain why reports of medical test performance are not replicable, or differ significantly from performance estimates in systematic reviews and meta-analyses. But by the time systematic reviews have been generated, it may be too late, in that the initial positive and encouraging publications have received a lot of attention, and may be prompted by the premature introduction of the test into clinical care. Of course, introduction of suboptimal tests is hardly ever due to fraud or misconduct; rather it is a reflection of the fact that we have an abundance of studies that are not always well-conducted and which present their results in an over-optimistic way. And this contributes to waste in medical research, because these findings generate other studies which then fail to replicate the initial finding – and which could have been avoided if the prevailing culture supported publication of negative findings as well as positive findings.

"The test evaluation literature suffers from widespread problems, such as failure to report negative results and presentation of results with an optimistic 'spin'."

Don't be blinded by spin

So how can we improve the evaluation of medical tests? I believe that there are a number of improvements that can be made. Clearly, it would help if editors and peer-reviewers were better trained to recognize spin, but it's a difficult situation – the journal's reputation is built on the number of citations it gets, so it too is incentivized to publish optimistic findings. More fundamentally, I think that we should raise awareness among

manufacturers that useful tests must improve patient outcomes – and that manufacturers are partially responsible for providing the evidence that tests are not only accurate but also useful. This would encourage manufacturers to undertake more trials and effectiveness studies than they currently do.

Another helpful advance would be to increase the understanding and appreciation of the methods for evaluating medical tests. This would benefit not only statisticians – who are usually less familiar with methods for evaluating medical tests than they are with methods for evaluating randomized trials of drugs – but also many healthcare professionals, whose understanding of medical tests is not as extensive as it should be and who would benefit from additional training. For example, clinicians often have high expectations for precision of medical tests but only limited understanding of intrinsic variability, and a poor appreciation of the links between test results, management actions and patient outcomes.

Finally, I expect additional changes to be forced by the growing reluctance among healthcare payers to support expensive drugs and automatically reimburse new markers and new tests. Increasingly, payers are expecting proof of effectiveness for these new tests before they are willing to support their use, especially if they are expensive multimarker panels or completely new forms of testing like clinical mass spectrometry. And that implies that the community will have to improve its understanding of the link between medical tests and management, and between management and patient outcomes.

In the short-term…

In the near-term, however, there are pragmatic actions we can take that will improve the quality of reports of the performance of medical tests. Our group, like several others, has found that crucial items of information are often lacking in published studies. For example, the age and gender of the study subjects may not be disclosed, details of how and where subjects were recruited may be omitted, and the actual results may not be provided in full; for example, the report might give no more than the number of subjects and the percent of correct classifications provided by the test! That's why I have collaborated with an international group of researchers, editors and authors to develop a one-page checklist; the intent is to help verify that essential information is included in studies reporting the results of medical test evaluations. That checklist, known as STARD (from 'standards for reporting diagnostic accuracy studies'; see Figure 1), was initially published in 2003, and updated in October last year (1).

STARD can be used by authors to verify that they have included all the essential information in the study report, and by peer reviewers, editors and readers to see if the study report can validly answer the question of whether the evaluated test is useful for a specific application. Since the initial release of STARD, we've seen a small but significant increase in the number of reported items per study, and we're very much encouraged by the fact that some journals have started to use STARD systematically in their peer review process for evaluations of medical tests. We're not there yet – I want to see STARD adopted even more broadly – but we are at least starting to make a difference.

Patrick Bossuyt is Professor of Clinical Epidemiology at the University of Amsterdam, the Netherlands.

Reference

1. *PM Bossuyt et al., "STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies", BMJ, 351, h5527 (2015). PMID: 26511519. Also accessed in full at http://bit.ly/10UMKRk*

The Changing Man

Sitting Down With... Ivan Damjanov, Professor of Pathology,
The University of Kansas School of Medicine, Kansas City, Kansas, USA.

You've had a long career as an educator and won many awards for your teaching. How did you get involved in pathology education?

At first, reluctantly. When I entered academic pathology in the US in the 1960s/1970s, the common adage among the key thought leaders at the time was that those who can't do research do hospital pathology, and those who can't do that, teach. Teaching was relegated to the lowest branches of the academic tree; even popular teachers who did not do any research were considered "losers" by their peers. So it took some courage to become a "professional teacher."

I was genuinely interested in research, though, so I applied for grants and luckily managed to establish an NIH-funded laboratory, which I ran for 25 years in Philadelphia. I now have over 300 published papers in peer reviewed journals, over 12,000 citations and an "h factor" of 50, and some 30 books to my credit. In spite of these achievements and my numerous academic qualifications, some of my detractors insist on calling me "just a teacher." At my age of 75, though, I don't care how they label me anymore.

When did you start considering yourself a professional teacher?

My major switch to medical education occurred in 1986 after I joined the staff of the Jefferson Medical College in Philadelphia, where I took charge of the undergraduate student medical education. My first task was to reorganize the teaching of pathology and to improve the results of the students on the Pathology part of USMLE1 (US Medical Licencing Examination), which were in the lower 20th percentile at the time. In less than a year I managed to raise scores to the 90th percentile – through full engagement, teamwork and motivating students for active learning – and they remained that high during my tenure there. We extended our teaching program to a newly established post-sophomore program in which medical students worked as pathology residents for a year in our department and also helped us to teach. With six students enrolled, it became one of the largest post-sophomore programs in the country. Many of those students chose pathology as their lifetime career. We were elated by our success in graduate and postgraduate education! On behalf of our teaching team, I received the Golden Apple Award for Teaching and later the Tom Clark Award for Teaching Excellence.

You teach a pathology course in Croatia. Why?

Patriotism. I am from Croatia and I wanted to help my colleagues establish a pathology course in English. I have taught it now in the Universities of Zagreb and Split to Croatian students and those Europeans who are not admitted to universities in their home countries, and I can assert that those ex-pat students are as good as their peers in typical US medical schools. I am a bit appalled, though, that the European Union has not yet developed some rules to control English-taught schools to reassure the public that these graduates have knowledge equivalent to their peers graduating from the "regular" medical schools. The EU should, sooner rather than later, give an official stamp of approval to this form of medical school education.

What have you learned during your years of teaching?

We need to move away from lectures ex cathedra and teach in small discussion groups. Case-based learning is used in our residency programs and there's no good reason why it cannot be applied to all medical courses, including pathology.

Our trainees serve as our apprentices and that type of tutorial teaching of pathology has proven to be the most popular model in the US. As residents advance they also assume teaching roles, and I believe that's the best way of learning. We supplement our teaching with formal lectures, and some of us have even prepared formal, commercially available courses for pathologists in training. I'm very proud of the 22 courses, covering all of human pathology, that I have helped develop. Young pathologist in training love them, reflecting the fact that the new generation uses different learning techniques compared with the "older guys," who take such course begrudgingly for CME credits. As the Latin saying goes, "*tempora mutantur*" (times change), and we'd better adjust to our trainees' needs before it's too late.

I also believe that good medical teaching must include other professionals, such as educational psychologists, psychometricians and learning experts. I firmly believe that medical education is in need of major change, but the chances of change happening any time soon are slim. Inertia and self-complacency are deeply rooted in most medical education and, for the time being, traditionalists will be in charge, ensuring that nothing really changes.

What advice would you give to pathology educators?

A few years ago I gave a presentation entitled "Is it possible to practice medicine without teaching?" The short answer is emphatically: no. For a longer answer you may consult my web-based presentation of the same title. I also think that we need to further develop computer-based learning. Today, we have this incredibly powerful technology and all we do is use it as a substitute for the pencil and paper to complete multiple choice exams! We should develop computer-based national exams that would test students' real knowledge. The other day, my teenage grandchildren told me, "If you are not on the web you do not exist." I fully agree. In the 1967 film The Graduate, the protagonist was given the advice to go into "plastics." So today my advice would be, go "in silico."

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