JUNE 2021 # 76

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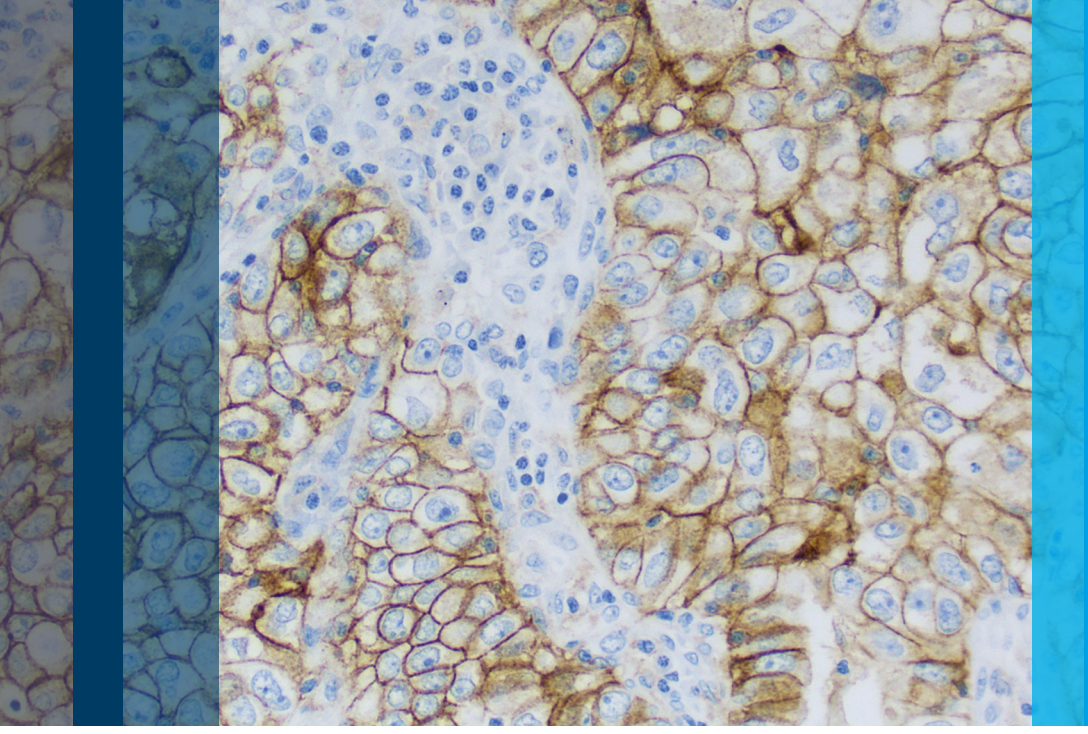
The Call of Coronal Duty

How proteomics became a crucial weapon in the fight against COVID-19



Agilent

Trusted Answers

PD-L1 Atlas of Stains Walkthrough

Build Confidence in PD-L1 Scoring by using the PD-L1 IHC 22C3 pharmDx Atlas of Stains

July 22nd 12pm ET/9am PT – Remote session

August 26th 3pm ET/12pm PT – Remote Session

Key learning outcomes:

- Understand how to navigate the PD-L1 IHC 22C3 Atlas of Stains
- View (H&E, NCR, PD-L1) of each case
- Learn to filter for tissue type and staining characteristic
- Read full case descriptions including scores
- Create notes or make comments on areas of interest
- Save as PDF or email cases of interest to colleagues

This is a virtual event. Click here to register.

Speaker: Allen Gown
Director and Chief Pathologist, PhenoPath Laboratories

Dr. Allen M. Gown is a pathologist-scientist recognized as one of the world's leading experts in the diagnostic and research applications of immunohistochemistry (IHC). His Pathology career started at the University of Washington in Seattle, where he developed many monoclonal antibodies still in use in diagnostic immunohistochemistry, and contributed extensively to the expanding horizons of immunohistochemistry. He then became the founder of PhenoPath, a national consultative reference laboratory specializing in IHC, flow cytometry, FISH, PCR, and cytogenetics testing. Dr. Gown is a member of the several pathology journal editorial boards and a co-founder of the International Society of Immunohistochemistry and Molecular Morphology (ISIMM). He is currently a Clinical Professor of Pathology at the University of British Columbia in Vancouver, BC. Dr. Gown has well over 300 peer-reviewed journal publications and numerous book chapters.

D66594/01/2021MAY28

A Season of Change

Has the veil lifted on pathology's place as the foundation of medicine?

"A season of change" – not spring this year, but summer (or winter for our southern friends). As early buds give way to full leaf, vaccination rates creep up, and academic years draw to a close, each day is different to the last.

For some, July's approach heralds the start of residency programs – new training and new opportunities. (And, if you're like me, a stack of exciting, fresh notebooks you don't want to start writing in because they're "too nice.") For others, it signals the departure of students and a few quiet months of research or clinic work before they return. And, for a lucky few, perhaps there will even be some remote signing out – from the beach!

But not all change is good – as exemplified by the recent course of the pandemic. With new variants arising, increases in transmissibility, and early signs of vaccine escape, it's not yet time to let our guard down. For most, the laboratory is that guard. We rely on laboratory medicine professionals to tell us where SARS-CoV-2 is, how it is evolving, and what it may do next. We rely on the lab to reassure us when we aren't infected and to sound the alarm when we are. We rely on the lab to let us know how well our defenses are working and what we can do to shore them up.

It has been said many times that COVID-19 has put the spotlight firmly on the lab. But what people may fail to realize is just how much of the world's pandemic response is underpinned by a quiet army of laboratory medicine professionals. From drug repurposing to vaccine development, rapid test design to genome mapping… without the lab (and the individuals skilled enough to make the most of it), there would be no fighting back.

When this truth is recognized – by other medical professionals, by administrators, by the public – it will herald a "season of change" for the perception of the laboratory. The stereotypes are outdated. The negativity is misplaced. Pathology is the root of all medicine – and it deserves to be recognized as such.

Michael Schubert
Editor

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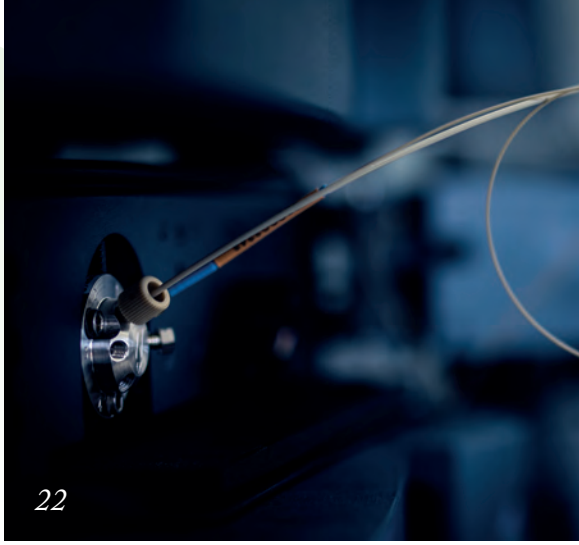

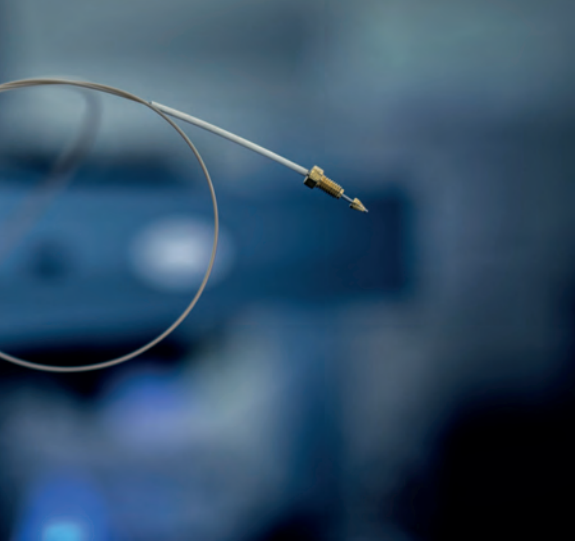

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Sitting Down With

the Pathologist

ISSUE 76 - JUNE 2021

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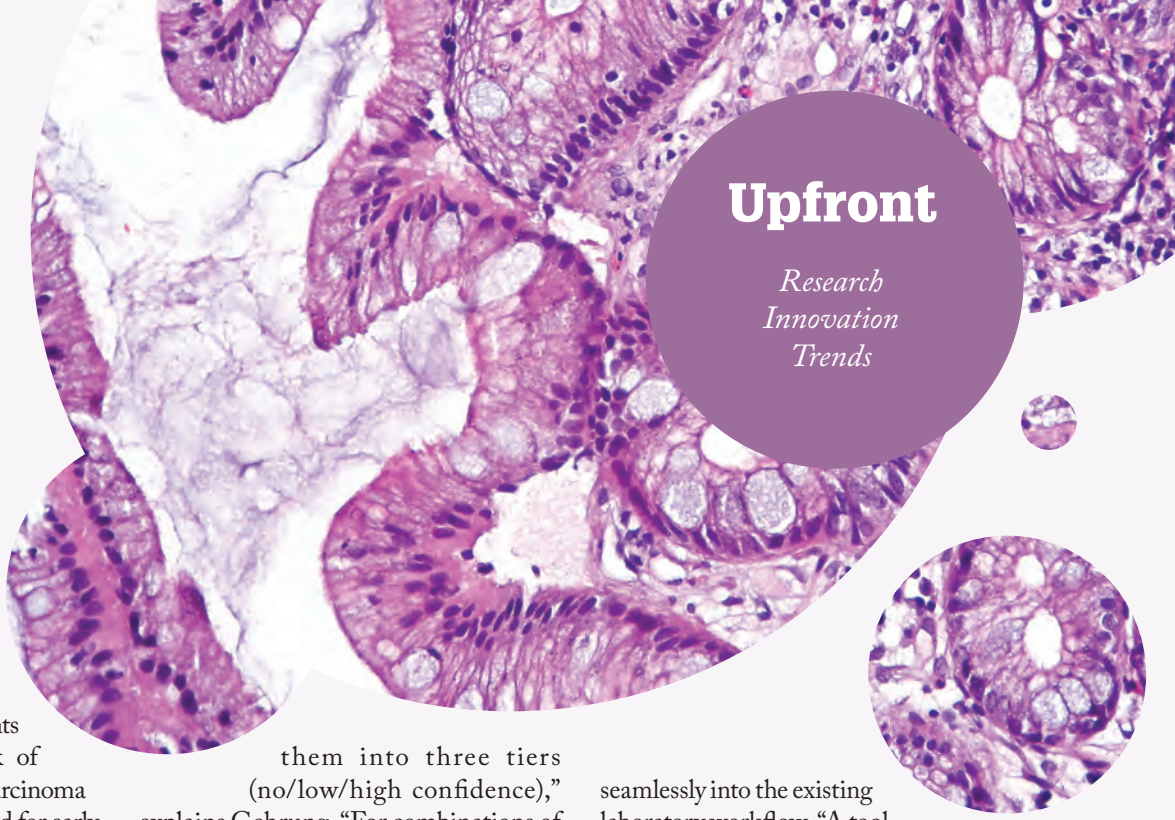


Upfront

Research
Innovation
Trends

Deep Learning Diagnostics

Triaging Barrett's esophagus diagnosis to detect early signs of esophageal adenocarcinoma

With Barrett's esophagus patients at a significantly higher risk of developing esophageal adenocarcinoma than the average person, the need for early diagnosis and monitoring is vital (1). To meet this need, researchers have developed and trained a deep learning algorithm to analyze samples from a Cytosponge-TFF3 test (2) – a device that collects cells from the lining of the esophagus, allowing patient triage for endoscopy. "The pathology analysis workflow for Cytosponge is laborious, but it consists of repetitive elements that can be automated using computational pathology to find cases where the automated analysis breaks down and human experts are required," says lead researcher Marcel Gehrung.

The system was trained to triage samples based on a two-variable, two-step process. "We defined two different scores (quality and diagnosis) and divided them into three tiers (no/low/high confidence)," explains Gehrung. "For combinations of no/low confidence between quality and diagnosis, we then decide that the sample should be analyzed by a human expert, rather than an automated algorithm."

The tool has clear benefits for laboratory medicine professionals, not least in reducing pathologists' workload by 57 percent while maintaining diagnostic standards (2). "It enables pathologists to focus more time on difficult cases – therefore reducing error rates due to increasing workload," says Gehrung. "It can also help to build more trust in (semi-)automated analyses because the 'easy' cases are those in which automated performance can best demonstrate its use."

But that's not all – it can also fit seamlessly into the existing laboratory workflow. "A tool like this can be used on whole-slide images immediately after scanning," says Gehrung. "The algorithm then decides whether a human is required or its own assessment is confident enough. If a human needs to review the case, then it follows the normal analysis pathway – but if the algorithm produces the report, then no human involvement is required."

Though the algorithm won't replace pathologists' valuable role in the patient pathway, it could help them deliver the best possible service for Barrett's esophagus patients whose risk of esophageal cancer might otherwise go undetected.

See references online at: tp.txp.to/deep-learning-diagnostics

INFOGRAPHIC

Testing for All

Uncovering hidden inherited genetic mutations in colorectal cancer patients

Colorectal cancer (CRC) patients

1 in 6 inherited cancer-related gene mutation

Sequencing panel used

>80 cancer-causing or predisposing genes

Standard CRC panels test **<20**

QUICK HITS

The latest research in pathology and laboratory medicine

Biopsies Be Gone

Researchers have developed a new urine test that measures the levels of both protein marker EN2 and 10 genes associated with prostate cancer risk (1). The test can predict whether a patient has prostate cancer and how aggressive the disease is – reducing the need for invasive biopsies.

Looking at Ligands

Artificial intelligence has been used to investigate whether amphiregulin and epiregulin immunohistochemistry (IHC) could predict treatment response in metastatic colorectal cancer patients (2). The model found that high ligand expression was associated with better response to anti-EGFR treatment, showing IHC's potential in routine practice.

Exposing Vulnerabilities

Hippocampal gene expression changes and associated hippocampal vulnerability have been examined to investigate disease-relevant gene expression in the brain (3). *SERPINA5*, *RYBP*, *SLC38A2*, *FEM1B*, and *PYDC1* were found to be associated with Alzheimer's disease neuropathology and *SERPINA5* was further associated with tau expression – a key characteristic of the disease.

35 Years On

Two new studies have investigated radiation-induced genetic changes caused by the Chernobyl disaster. The first found no evidence of de novo germline mutations in children whose parents were exposed to radiation – suggesting minimal transgenerational genetic effects (4). However, the second identified increased radiation-associated damage in papillary thyroid carcinoma patients who were exposed at a younger age (5).

Novel Nanodevice

Researchers have developed a design pipeline for DNA assemblies that allows users to build structures either top-down or bottom-up, enabling rapid construction of complex multicomponent structures while maintaining control over geometrical, mechanical, and dynamical components (6).

See references online at: tp.txp.to/bitesized-breakthroughs

Save the Spine

Predicting spinal cord injury severity with serum cytokine profiles

Despite modern medicine's rapid advancement, patients' chances of recovery after spinal cord injury (SCI) are still slim. But this is not just down to the lack of effective treatment – there's also a need for rapid, reliable diagnostic biomarkers to predict SCI severity and guide clinicians in their treatment decision-making.

A pilot study seeking to fill this gap has conducted a multiplex analysis of serum cytokines in patients two weeks post-injury – finding a significant increase of IFNγ, CCL27, and CCL26 (1). The researchers also found differences in patients with baseline injury grades A or B (according to the American Spinal Injury Association Impairment Scale) in CXCL5, CCL11, CXCL11, IL10, TNFα, and MIF.

Though the research is early-stage, the findings show potential for using serum cytokines to stratify SCI patients without the risk of complications commonly seen from repeated sampling of cerebrospinal fluid.

Reference

1. *S Ogurcov et al., Brain Sci, 11, 322 (2021). PMID: 33806460.*

Based on genetic findings

1 in 10 patients had medical or surgical therapy modifications

Clinically actionable findings

9.4%

would have gone undetected by practice guideline criteria or a CRC-specific gene panel

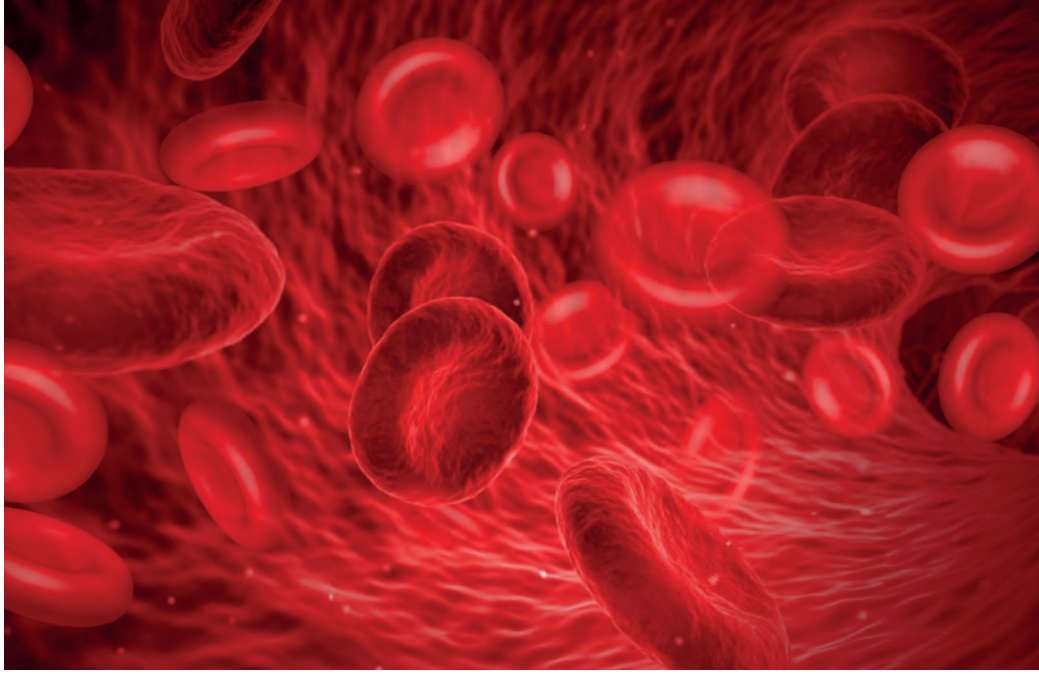
Cascade testing

16%

of family members underwent testing

Reference

1. *PLS Uson Jr et al., Clin Gastroenterol Hepatol, [Online ahead of print] (2021). PMID: 33857637.*



Rethinking Risk Stratification

An integrated approach to risk stratification and relapse prediction in pediatric leukemia

Researchers at St. Jude Children's Research Hospital have integrated genomic analysis with minimal residual disease (MRD) assessment to improve prediction of treatment response in pediatric patients with acute lymphoblastic leukemia (ALL). Up until now, medical centers have faced barriers to adopting this integrated approach. "Many are not able to perform both assays in house – especially the comprehensive genomic analyses," says Ching-Hon Pui, Chair of St. Jude's oncology department. "Even if they have the expertise, they need to perform the assays in a CLIA-certified laboratory and conduct the analyses in real time so they can be used clinically."

In the study, the team found that high-hyperdiploid and *DUX4*-rearranged B-ALL had the best five-year event-free survival rates; *BCR-ABL1, BCR-ABL1*-like, *ETV6-RUNX1*-like, and *KMT2A*-rearranged ALL had the worst. This was surprising to Pui. "Some investigators thought the *ETV6-RUNX1*-like subtype should have excellent outcomes because it shares the gene expression of the *ETV6-RUNX1* subtype – the most favorable subtype," he says. "But, based on our data, this subtype is actually unfavorable. Another notable finding is the excellent outcome of *DUX4*-rearranged ALL despite high rates of MRD positivity early in therapy."

When asked about how the findings will enhance the day-to-day work of pathologists working with pediatric cancers, Pui notes the positive effect pathologists can have on patients. "The cure and quality of life of our children with ALL are affected tremendously by pathologists," he says. "Many children inherited these cancer susceptibility genes – and so their family members will also require the expertise of our pathologists who perform germline genomic studies."

How long will it take until this integrated approach becomes available in the clinic? "We are routinely testing for more than 20 genetic subtypes in both our ALL patients at St. Jude and those participating in our clinical trial in several other collaborative medical centers – and using the information for risk-directed treatment," says Pui. "Other medical centers are screening their patients for selected high-risk subtypes and some can also perform comprehensive genomic analysis. I hope that, within the next three to five years, those tests will be readily available for all patients."

See references online at: tp.txp.to/rethinking-risk

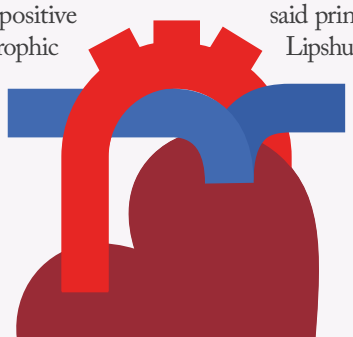
Small Patients, Big Hearts

Investigating the need for routine genetic testing in pediatric cardiomyopathy

Genetic screening can play a vital role in improving outcomes in pediatric cardiomyopathy – a disease burdened with high morbidity and mortality rates. And though guidelines for this testing exist, uptake between practices varies.

A study led by the University at Buffalo aimed to identify the genetic mechanisms underlying the disease and investigate current clinical genetic testing practices. Their findings? Genetic testing rates varied across sites, with positive family history and hypertrophic cardiomyopathy subtype increasing the likelihood of testing (1).

21 percent of children who did not undergo genetic testing were found to have a molecular cause; both familial and idiopathic cases were among the positive results. "Even in families without a family history of cardiomyopathy, we found that many children with cardiomyopathy have a genetic cause that we can establish," said principal investigator Steven E. Lipshultz (2), highlighting the need for routine genetic testing.



See references online at: tp.txp.to/small-patients

Deep Blue

A new stain offers beautiful possibilities in skin and gastrointestinal pathology

These images represent a new stain based on picro aniline, Ziehl's fuchsin, and acetic acid. The stain is useful in dermatopathology because of its utility in differentiating squamous epithelium keratinization (yellow/brown) and lymphocytes (dark red). It is useful in gastric biopsies to differentiate metaplastic glands.

See the protocol online at: tp.txp.to/deep-blue-stain

Submitted by Dmitry A. Zinovkin, pathologist at Gomel State Medical University, Gomel, Belarus.

Do you have a photo suitable for Image of the Month?
Send it to edit@thepathologist.com

QUOTE of the month

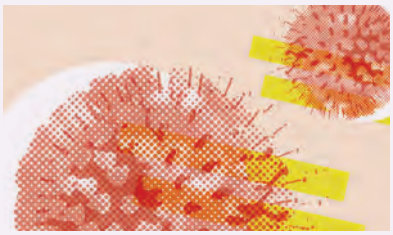
"I felt a lot of pressure because I thought maybe other women might not follow in my footsteps if I didn't succeed. I felt a huge responsibility to show that women can flourish in this field, and I would say that I have achieved that. Today, the ratio of male to female professors is more even, and it's great to see so many women on the programs for key conferences and so on, but – of course – there is still some way to go. To any women considering entering this field, I say go for it! It's a fantastic career with endless opportunities, and a little confidence can go a long way. In fact, if I could give my younger self any advice, it would be to have more confidence: you're capable of much more than you realize."

Carol Robinson, Professor of Chemistry at the University of Oxford, UK, and the first female chemistry professor at Oxford and Cambridge

SARS-CoV-2 Variant Testing

Guidance to laboratories for sequencing and identifying SARS-CoV-2 variants

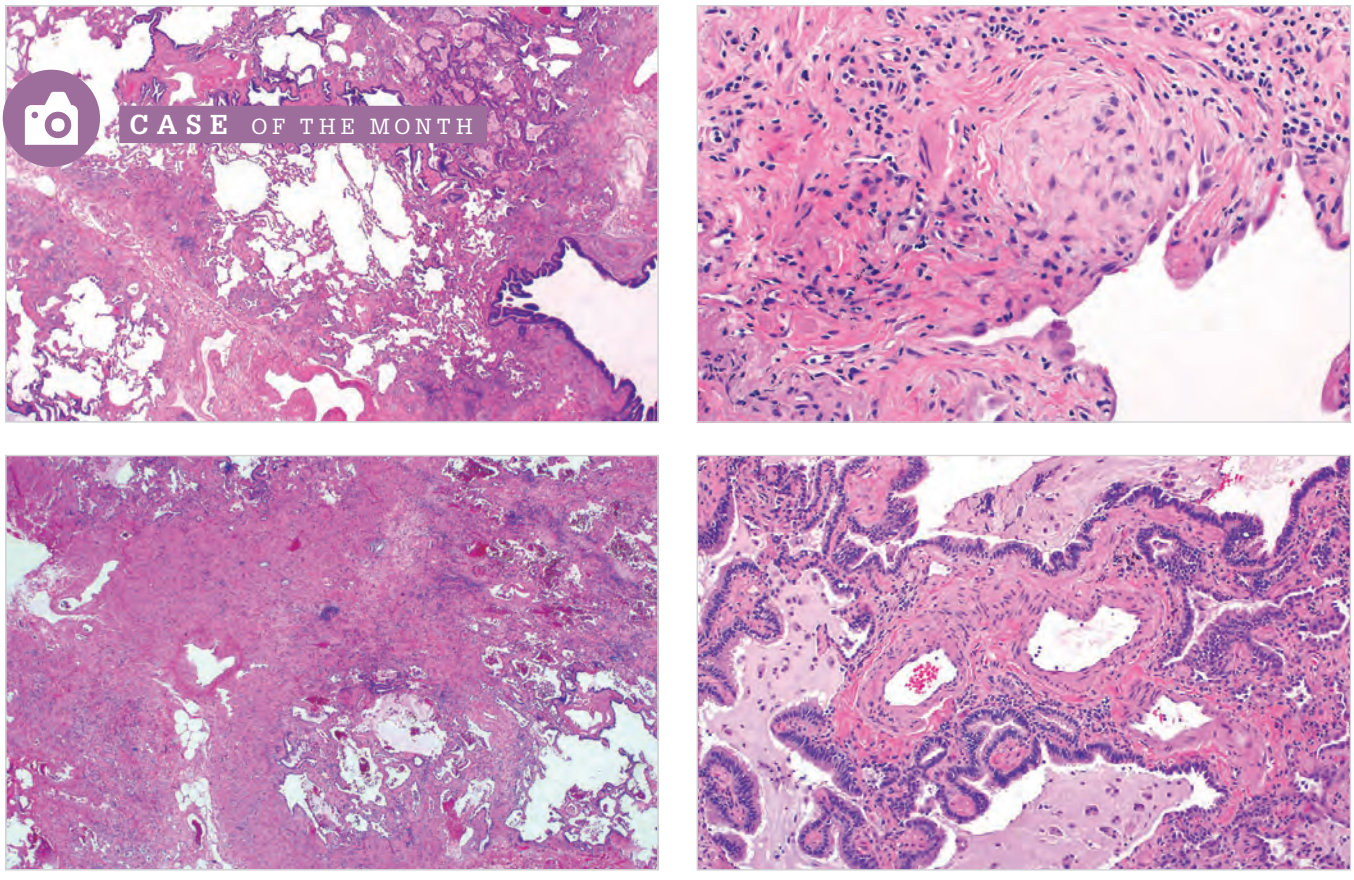
The Association for Molecular Pathology (AMP), Infectious Disease Society of America, and Pan American Society for Clinical Virology have released new guidance for laboratories on sequencing and identifying SARS-CoV-2 variants in clinical specimens (1).



"There are several situations in which genetic variants might be suspected and testing positive samples might be prioritized," says Blake W. Buchan of the AMP Clinical Practice Committee. "For example, samples collected from: patients with reinfection(s); patients who develop breakthrough infections; hospitalized patients with reinfection or vaccine escape; and patients with treatment failure."

However, Buchan highlights several technical barriers. "Variant identification can only be conducted on positive specimens collected in viral transport media and cannot be performed from the dry swabs used in some point-of-care testing methods; likewise, certain test methods use transport media that may interfere with sequencing reactions."

Interested? Read an expanded version of this article and see references online at: tp.txp.to/sars-variant-testing



A 60-year-old female presented with an intermittent cough and shortness of breath. A chest CT scan showed subpleural reticulation with mild bronchiectasis and peribronchial thickening. In addition, there were small, ill-defined ground-glass opacities in the upper and lower lobe, more prominent in the left lung. Pulmonary function testing documented a moderate restrictive lung defect. The patient's past medical history is remarkable only for a history of smoking. A wedge biopsy from the left upper and lower lobe was performed.

What is your diagnosis?

a) Non-specific interstitial pneumonia
b) Chronic hypersensitivity pneumonitis
c) Usual interstitial pneumonia
d) Cryptogenic organizing pneumonia

Answer to last issue's Case of the Month…

d) Syringocystadenoma papilliferum

Syringocystadenoma papilliferum, also known as papillary syringadenoma, is a warty tumor of the scalp, neck, and/or face that can occur at any age. Histologic sections show cystic epidermal invagination into which papillary structures project. A high-power view shows that the papillae are lined by two rows of cells; the luminal row is composed of columnar cells with decapitation secretions. Plasma cells are present within the stroma.

Submitted by San Yu Maung, Pathologist, Mandalay General Hospital, Mandalay, Myanmar.

To register your guess, please go to http://tp.txp.to/0621/case-of-the-month
We will reveal the answer in next month's issue!

Case of the Month is curated by Anamarija M. Perry, University of Michigan, USA.

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In My View

Experts from across the world share a single strongly held opinion or key idea.

An Ode to Autopsy

Without these vital medical procedures, doctors would still be working in ignorance

By Abdul Majeed Abid, Pathology Resident at the University of Texas Medical Branch, Galveston, Texas, USA

In sickle cell anemia, a point mutation in the *HBB* gene replaces one amino acid with another, leading to the formation of abnormal hemoglobin and deformed red blood cells. When oxygen supply is low or demand is high, the abnormal hemoglobin clumps and causes irreversible damage to the red blood cell membrane. The result is acute pain episodes, kidney disease, gallstones, anemia, splenic atrophy, stroke, and lung disease. The many blood transfusions most sickle cell patients receive cause excess iron deposition in the body, particularly in the liver. But the mutation is not all bad; like many others, it was a product of evolution. The sickle cell gene is prevalent in areas of Africa hit hard by malaria. One mutated copy of *HBB* is protective against malaria – but two will cause sickle cell anemia.

Born with two mutated copies of *HBB,* James was diagnosed with sickle cell anemia and had a stroke at age five that led to ongoing behavioral challenges. He was prescribed hydroxyurea and folic acid to reduce the frequency of painful episodes. After the age of 26, he was no longer able to remain on his parents' insurance plan and was therefore unable to afford his medication for three months. At the onset of winter, he developed upper respiratory tract symptoms and self-medicated with over-the-counter drugs. He felt better – but, a week later, he developed acute pain all over his body, which he rated as 10/10. His mother took him to the emergency department, where he was diagnosed with acute kidney injury. While medical management was underway, he coded multiple times and was pronounced deceased after several rounds of resuscitation. Because he had passed away within 24 hours of admission to the hospital, an autopsy was warranted by law and his mother agreed.

An autopsy is the final medical exam a patient receives – sometimes the only one. The ultimate purpose of an autopsy is to determine the cause and manner of death. In the early days of medical discovery, autopsy was the most useful way to learn about the anatomy and pathophysiology of diseases. Modern medicine owes a lot to early autopsies. In The House of God, an intern describes the value of autopsies in these words: "It's hard to describe the importance of the autopsy. Why, the autopsy is the heart – no, the flower, the red rose – of medicine. Yes, the great Virchow, the Father of Pathology, performed 25,000 d autopsies with his own two hands. It's crucial to our understanding of disease (1)." Autopsy, in summation, is the jewel in the crown of medicine.

An autopsy has performance and cognitive aspects. If you conduct an autopsy, you are supposed to write a report of your findings from the case after performing the dissection and a clinicopathological summary of the disease process affecting the patient. Once you've performed a few dozen autopsies, you realize that the cognitive element is actually the hard part. You discover many things when you start doing autopsies – for instance, the location of adrenal and parathyroid glands (they are not where textbooks say they are!). I am biased in saying this, but the joy of looking at a ruptured berry aneurysm or situs inversus with your own eyes is unsurpassed by any imaging modality. I learned about orthopnea and paroxysmal nocturnal dyspnea in heart failure patients in medical school. Still, the sight of lungs

drowning in a "mini-lake" in the thorax of a heart failure case was a different level of educational experience. In my experience, there is no better way to understand the pathophysiology of a disease than a complete autopsy.

"Autopsy, in summation, is the jewel in the crown of medicine."

Outside the medical realm, an autopsy also serves as a means of closure. Losing a loved one is hard enough; not knowing why can keep the wound open for years. Autopsies tell us not only how someone died, but also how they lived. Before opening the body cavities, an external examination reveals any scars, tattoos, or marks on the patient's body. These markings can tell you a lot about a person. Surgical scars on different parts of the abdomen can tell if the decedent had an appendectomy or a hysterectomy. Tattoos can include names, figures, or romantic associations and may reveal family, social, marital, or even criminal histories. Autopsies can also help in determining crimes committed against the decedent. Pathologists who perform autopsies are not only the decedent's final doctor, but also their final advocate.

Death is a terrifying prospect for most people and western culture is notorious for stigmatizing anything having to do with death. Medical schools don't emphasize the value of autopsies; the only exposure most medical students have to these procedures is through television shows – not well-known for their factual accuracy. The autopsy rate at most hospitals is less than five percent – so, for every 100 patients who die in the hospital, fewer than five get that close examination and the closure such a procedure brings. Efforts are even underway to eliminate the autopsy requirement from American hospitals that participate in Medicare – partly due to the dearth of medical examiners in the country and partly due to the devaluing of the autopsy in general.

But what of James? During his autopsy, we discovered a shrunken spleen, a liver full of iron pigment, lungs filled with blood, and kidneys with multiple scars and blood stasis in the glomeruli. It was hard for me to determine his cause of death. The suspects included evolution, genetics, societal failure to ensure health care for the needy, and acute kidney injury. I chose kidney injury because it was the easiest and most convenient way to give James and his family closure.

It's not only families like James' who benefit, though. The COVID-19 pandemic has spurred a recent increase in interest in autopsies due to our early lack of knowledge as to how the disease affects different organs and the demographics associated with severe disease and mortality. Autopsies on COVID-19 patients increased our understanding of the disease process and helped improve the management of future patients. Without these procedures, we would still be fumbling in the dark when dealing with this new threat. For this reason and many others, it's essential that we expose medical students to autopsies and medical laboratories – and not just those interested in forensics or pathology, but every student who is interested in the practice of medicine.

References

1. *S Shem, The House of God. Richard Marek Publishers: 1978.*

Advocates of the Underserved

Addressing healthcare inequalities as a core component of US medical education

By Cesar Padilla, Clinical Assistant Professor at Stanford University School of Medicine, Stanford, California, USA, and Chief Medical Education Advisor at MiMentor, Alliance in Mentorship

Let me take you back to the first day of medical school. We all remember our nervousness as we embarked on this new and exciting journey. We learned about the fundamentals of physiology and the Hippocratic Oath – but our memories are foggy and incomplete when considering our own history. As educators, we love to teach about the Greco-Roman origins of medicine, but we omit one of the most powerful stories – the story of the Parisian hospital that inspired the first such institution in the United States.

Hôtel-Dieu is considered one of the oldest actively operating hospitals in the world. Serving as a Catholic and charitable institution, it became widely known for combining high-quality education and medicine with free healthcare for the poor. The hospital survived the middle ages and, during the Age of Enlightenment, became a dialectical hub for doctors, scientists, and students from across the globe.

"The modern hospital was built on the ethos of providing free care for the poor and sick. The story of a lonely medical student inspired by the charitable hospitals of Paris deserves recognition."

During the 1730s, the US (known then as the Thirteen Colonies) was starting to become an independent nation – though still under British rule – and its founders began to look to Europe for ideas of progress. Thomas Bond, then a US medical student seeking to expand his medical education, traveled to Paris to spend time at Hôtel-Dieu. Hospitals did not yet exist in the US – the idea of providing free medical care to the poor and sick was indeed a radical idea. But Pennsylvania, Bond's home state, was a major trading port and often the site of disease transmission. Unsurprisingly, the need for a hospital was dire and Bond became feverish with the idea. He returned to the US inspired and, alongside powerful political friends (Benjamin Franklin), succeeded in building Pennsylvania Hospital. The first US medical school, the University of Pennsylvania, became an affiliated institution.

The inception of US medicine has blueprints from the charitable philosophy of Hôtel-Dieu, but where is the story in our textbooks? Why don't medical students learn about the most influential hospital in our history?

Hôtel-Dieu and Pennsylvania Hospital stood on a foundational theme that transcends time – our commitment to underserved communities. Over 270 years later, this ethos has renewed life. Influential think tanks are rediscovering the duty that corporations should have to society and the wellbeing of the underserved, given that their inception is only possible through the government's public infrastructure.

Medical schools are also beginning to understand the power of integration – helping maturing physicians understand underserved communities on a person-to-person level. During the COVID-19 pandemic, Harvard Medical School helped Latino and Hispanic medical students to launch "Quetzals of Health" – a program that connects students with undocumented members of the community. With little access to public support and healthcare, these people often live in the shadows of society.

In the US, maternal mortality is three to four times higher in Black women than in white women. In my first job after medical training, I helped launch a program to link medical students with pregnant minority patients. Our hope was, and still is, that the Maternal Minority Health Alliance will become an integral part of the medical school educational curriculum. Its purpose is to create meaningful relationships between medical students and community members who are at risk of worse health outcomes – just like Thomas Bond intended.

A reformed curriculum, in which integration with surrounding communities becomes a core component of students' learning, would signal the potential rebirth of US medical education. Working with legislators and community leaders to create tangible solutions for underserved groups would give medical students necessary experience of the fundamentals of healthcare – understanding human health from a translational, cell-to-society level.

The modern hospital was built on the ethos of providing free care for the poor and sick. The story of a lonely medical student inspired by the charitable hospitals of Paris deserves recognition. Let's honor it by teaching medical students our history as doctors, scientists, and healers – and, most importantly, by educating them to become advocates of the underserved.

Further Reading

1. *B Collins, "Care for Undocumented Immigrants" (2021). Available at: http://bit.ly/38kYVwM.*
2. *Penn Medicine (2017). Available at: http://bit.ly/2OaHAQ8.*
3. *G Melillo, "Racial Disparities Persist in Maternal Morbidity, Mortality and Infant Health" (2020). Available at: http://bit.ly/2OBzM9T.*
4. *MiMentor (2021). Available at: https://bit.ly/32ZI4g1.*

Clinical Testing has Changed – Should CLIA?

Is CLIA falling behind the ever-changing landscape of laboratory medicine?

By Robin E. Stombler, President of Auburn Health Strategies, LLC, Virginia, USA, and Vice Chair of the A2LA Board of Directors.

Much of my professional life in the mid-1990s was spent uttering the mantra, "A test is a test is a test."

It meant that the Clinical Laboratory Improvement Amendments of 1988 (CLIA) should not be site-specific – a test is a test no matter where it is performed. Back then, there was a constant cry for physicians' offices to be exempt from the law – arguing that their in-office laboratories would cease to exist under the regulation. Physician office laboratories lost their exemption battle; however, they may have won the war.

Today, these labs make up the bulk of testing sites. In fact, according to the Centers for Medicare and Medicaid Services (CMS) March 2020 CLIA database, over 45 percent of all CLIA laboratories are described as physician office laboratories (1). In comparison, only 3.42 percent of laboratories are in hospitals.

Just over 30 years have passed since the first regulations implementing CLIA were finalized. Since then, CLIA has improved clinical laboratory performance – from analytical oversight to personnel credentials and quality markers. A federal advisory panel, the Clinical Laboratory Improvement Advisory Committee (CLIAC), was established to "provide scientific and technical advice and guidance to the Secretary" of Health and Human Services (2). To this day, the panel of experts, along with staff from the Centers for Disease Control and Prevention (CDC), CMS, and the Food and Drug Administration (FDA), deliberate on laboratory practice and standards and propose modifications to the regulations alongside scientific and technological advancements.

By many measures, CLIA has worked successfully. Laboratories are now held to standards – for instance, limiting the number of Pap smear slides a laboratory technologist may screen in a day or mandating that all cytology slide preparations be evaluated on the laboratory premises. An external, objective measure of laboratory accuracy, known as proficiency testing, must be treated in the same manner as patient specimens for proper assessment. The basic act of obtaining registration, certification, or accreditation is now required for accountability – and so it is no surprise that damning newspaper headlines such as "Medical Labs, Trusted as Largely Error-Free, Are Far from Infallible" (3) are long gone.

The CLIA triumvirate of the CDC, CMS, and FDA collaborate well and make regulatory adjustments as the law allows – but is it enough? Does CLIA have the capability to respond not only to the different types of laboratory facilities, but also to the life cycle of a clinical laboratory test and its advancements? The answer is a resounding "maybe."

Clinical testing has changed; there is no doubt about it. CLIA places laboratories into categories based on the type of testing performed – waived, provider-performed microscopy, moderate-complexity, or high-complexity. A laboratory may qualify for a certificate of waiver if the testing performed "employ(s) methodologies that are so simple and accurate as to render the likelihood of erroneous results negligible" or "pose no reasonable risk of harm to the patient if the test is performed incorrectly" (42 CFR 493.15). Like the volume of physician office laboratories, the number of waived laboratories has exploded.

Monique Spruill, Director of the CMS Division of Clinical Laboratory Improvement and Quality, reported at the CLIAC 2021 meeting, 220,862 CLIA laboratories hold a certificate of waiver and 29,883 are designated as provider-performed microscopy (1). Together, these laboratories make up a whopping 85 percent of all 296,815 CLIA laboratories. In the midst of the COVID-19 pandemic, the number of laboratories enrolled in CLIA grew by 37,989 (March 2020 to March 2021). Of those, 92 percent were designated as waived.

To maintain certificate eligibility, waived laboratories must follow manufacturers' instructions for performing the test. They are subject to inspection if: there is a substantive reason to believe the lab is operating in a manner that creates "imminent and serious risk" to human health; there are complaints from the public; or to determine if the laboratory is conducting only waived tests. With the sheer volume of these labs and waiver from oversight, it is no surprise that they are not regularly reviewed. Waived laboratory testing is important – but can be fallible. Think about how a laboratory test, even a simple one, impacts medication dosing, coagulation monitoring for blood clots, or diagnoses of HIV or hepatitis C.

"lapses in quality were identified at certain sites, some of which could result in patient harm."

A report published in 2005 on Good Laboratory Practices for Waived Testing Sites shared CMS survey findings from waived testing sites (4). While most surveyed sites followed accurate and reliable testing practices, "lapses in quality were identified at certain sites, some of which could result in patient harm." Quality deficiencies included: not possessing current manufacturer instructions; not checking product inserts for changes; not performing quality control or required confirmatory tests; and not documenting the name and expiration date for all tests performed, among others. The report also provided an educational guide to improving the quality of waived testing laboratories.

With the growing number of waived laboratories and point-of-care technologies, ensuring accuracy in this area is paramount. As foretold by Jeffrey Shuren and Timothy Stenzel, national investment in point-of-care technologies and assessments will allow for rapid expansion of testing capacity and patient access (5).

An updated CLIA may provide an opportunity to assess the effectiveness of existing educational efforts, oblige accountability for improvement, and expand on data collection and analysis. It may also create a mechanism for ensuring the reliability of tests delivered at point-of-care and in settings not currently covered by CLIA.

Proficiency testing evaluates laboratory accuracy and provides an objective tool for assessing the competency of personnel and the proper operation of equipment, specimen handling, and reporting functions. CMS maintains a list of analytes for which proficiency testing is required (6); however, it was last updated in 2003. An updated CLIA may establish a mechanism for adding and deleting regulated analytes in a more timely manner. Furthermore, if non-laboratory healthcare professionals assume clinical testing roles in the future, CLIA might consider offering proficiency testing for individuals performing moderate- and high-complexity testing, rather than for the whole laboratory.

Qualitative demands for the clinical laboratory have increased in complexity – making the case for standardized methods for new technologies. Proficiency testing is an important component of these standards and should be treated with more agility under CLIA. Quality assessment tools must also keep pace with the acceleration of genomic technologies.

CMS last published its list of top 10 CLIA deficiencies in October 2018 (7), including the percentage of labs with poor storage of reagents (4.8 percent), lack of proficiency testing (4.3 percent), and no quality assessment program (4.0 percent), among other concerns. The two preceding decades counted similar CLIA deficiencies – from proper storage of reagents and specimens to lack of analytic systems/quality assurance; from failure to follow manufacturer instructions to personnel concerns. Although the types of deficiencies remain somewhat similar in recent years, the percentages fluctuate. It's a game of whack-a-mole in which some laboratories make improvements and others do not.

CMS gives private nonprofit accreditation organizations the authority to accredit clinical laboratories under CLIA. Accredited laboratories must meet specific CLIA standards and comply with the accreditation program's requirements. Under CLIA, an accredited laboratory with a deficiency might provide a corrective action plan; however, this does not necessarily mean systemic change is occurring. An updated CLIA might seek incentives for laboratories to achieve ISO 15189 accreditation (an international standard) to implement an effective management system. ISO 15189 integrates review of pre- and post-analytical testing activities – where most errors occur – and includes system-wide processes for mitigating laboratory errors and improving performance. CLIA already recognizes ISO 15189 accreditation for clinical laboratories, but it is not widely used in the US.

The old "a test is a test is a test" mantra is no longer necessary for this regulation; there are tools, such as CLIAC, available to improve and update CLIA. The federal advisory panel has been a sustaining voice in requiring the three agencies – and a variety of laboratory experts – to discuss ongoing issues and recommend solutions. Now, the time is right for it to consider the next generation of CLIA.

References

1. *Centers for Medicare & Medicaid Services (2020). Available at: https://go.cms.gov/2TIoNhJ.*
2. *Federal Register, 57, 40, 6832 (1992). Available at: https://bit.ly/3g9vwIZ.*
3. *W Bogdanich, "Medical Labs, Trusted as Largely Error-Free, Are Far from Infallible," Wall Street Journal (1987).*
4. *Centers for Disease Control and Prevention (2005). Available at: https://bit.ly/3piuDSE.*
5. *J Shuren, T Stenzel, "Bending The Arc Of COVID-19 Test Development To Increase Access And Ensure Reliability—Now And In The Future" (2021). Available at: https://bit.ly/3z5dYWW.*
6. *Centers for Medicare & Medicaid Services (2003). Available at: https://go.cms.gov/3vYig0I.*

Labs in the Lead

Spearheading research for better patient care

By E. Blair Holladay

Since the outbreak of COVID-19, the laboratory has been driving research associated with SARS-CoV-2. Pathologists and medical laboratory professionals have taken the lead on discovering the causes of the disease, the virus' effect on the human body, how it manipulates underlying conditions a person may already have, and much more. The knowledge we have gained is unprecedented; without the timely and essential research laboratories have conducted – and continue to pursue – patient health would be in a tailspin.

The need for original research has always been important to patient health, but we have seen its influence carry much more weight over the past year. Our journals (*Laboratory Medicine and the American Journal of Clinical Pathology*) alone have published 79 papers in the past year on the topics of SARS-CoV-2 or COVID-19 and, if you search the United States' National Library of Medicine, the number of research articles on these topics in scientific journals worldwide is well over 100,000. Knowing that data was essential to combating COVID-19, our journals created rapid, streamlined editorial and production processes to publish high-quality, peer-reviewed original research in almost real time.

What's more, with such rapid expansion and attention to research, we have seen an evolution since the start of the pandemic – one that has changed how we diagnose and treat patients. From serologic tests to convalescent plasma, the laboratory has contributed a wealth of knowledge and critical information that has helped patients recover – and helped develop the vaccines that are pushing this pandemic toward its end.

When the pandemic does end – and we can resume our lives without the dark cloud of a highly contagious and potentially deadly virus hanging over us – the need for research will remain. We need to understand and dissect everything we can about SARS-CoV-2 so that we aren't faced with another pandemic like the one we are experiencing. And so that, if we do, we will have research on our side that gives us a better starting place to determine what works (and what doesn't) when it comes to patient testing and care.

Scientific research is the lifeblood of healthcare. It advances us and enables us to provide better options for our patients. As caregivers – and yes, pathologists and medical laboratory professionals are caregivers just like any other member of the medical team – we are committed to giving our patients what they need when we can and finding answers for them when we can't. We find those answers in research – and we find those answers in the lab.

"Scientific research is the lifeblood of healthcare."

The New Second Opinion

Diagnexia brings expert consults and algorithmic support to pathologists around the world

Over the last few years, diagnostic medicine has experienced a revolution: molecular pathology and precision medicine. The next revolution, which comes hot on the heels of that one, is computational pathology – which includes everything from digital imaging to artificial intelligence. In a world where pathologists are increasingly reinventing themselves as informaticians in the laboratory space, Dr. Runjan Chetty and colleagues have developed a service that puts simplicity, efficiency, and expertise at the heart of digital pathology. Here, we speak to him to find out more about Diagnexia – and about the future of digital and computational pathology.

Tell us a little bit about yourself…

My name is Runjan Chetty. I'm an anatomic pathologist who has trained in South Africa and worked in Australia, the UK, and Canada. I've been an academic all my life, having worked in academic institutions and held chairs of pathology in South Africa, Scotland, and here at the University of Toronto, where I have worked for the last 20 years. My research interests lie in the gastrointestinal tract and the pancreas.

What is Diagnexia?

Diagnexia is a pioneer in digital pathology. The company has harnessed the "best of breed" in terms of the way diagnostic algorithms work, the way they employ software, and reporting templates to enhance pathologists' ability to provide efficient, objective diagnoses using digital pathology.

We've built a systematic reporting template that will allow seamless assessment and review of digitized slides or whole-slide images and allow for incorporation into a customized report. The system easily facilitates synoptic reports based on the Royal College of Pathologists, College of American Pathologists, and International Collaboration on Cancer Reporting designated reporting templates.

What subspecialty areas does Diagnexia cover?

Diagnexia intends to cover all subspecialties eventually. However, for the initial phase, we are concentrating on eight or nine key anatomic pathology subspecialties – high-volume, high-complexity areas, including gastrointestinal pathology, genitourinary pathology, dermatopathology, and pulmonary pathology. With this approach, we're hoping to cover the vast number of cases that a general practice might encounter.

Our primary goal is to establish a remote second opinion expert consultation service whereby designated individuals in each subspecialty, who are well-recognized and well-versed in their areas of expertise, will provide second opinion consults. Additionally, we'd like to offer primary diagnostics as well – another area in which subspecialty services will be provided.

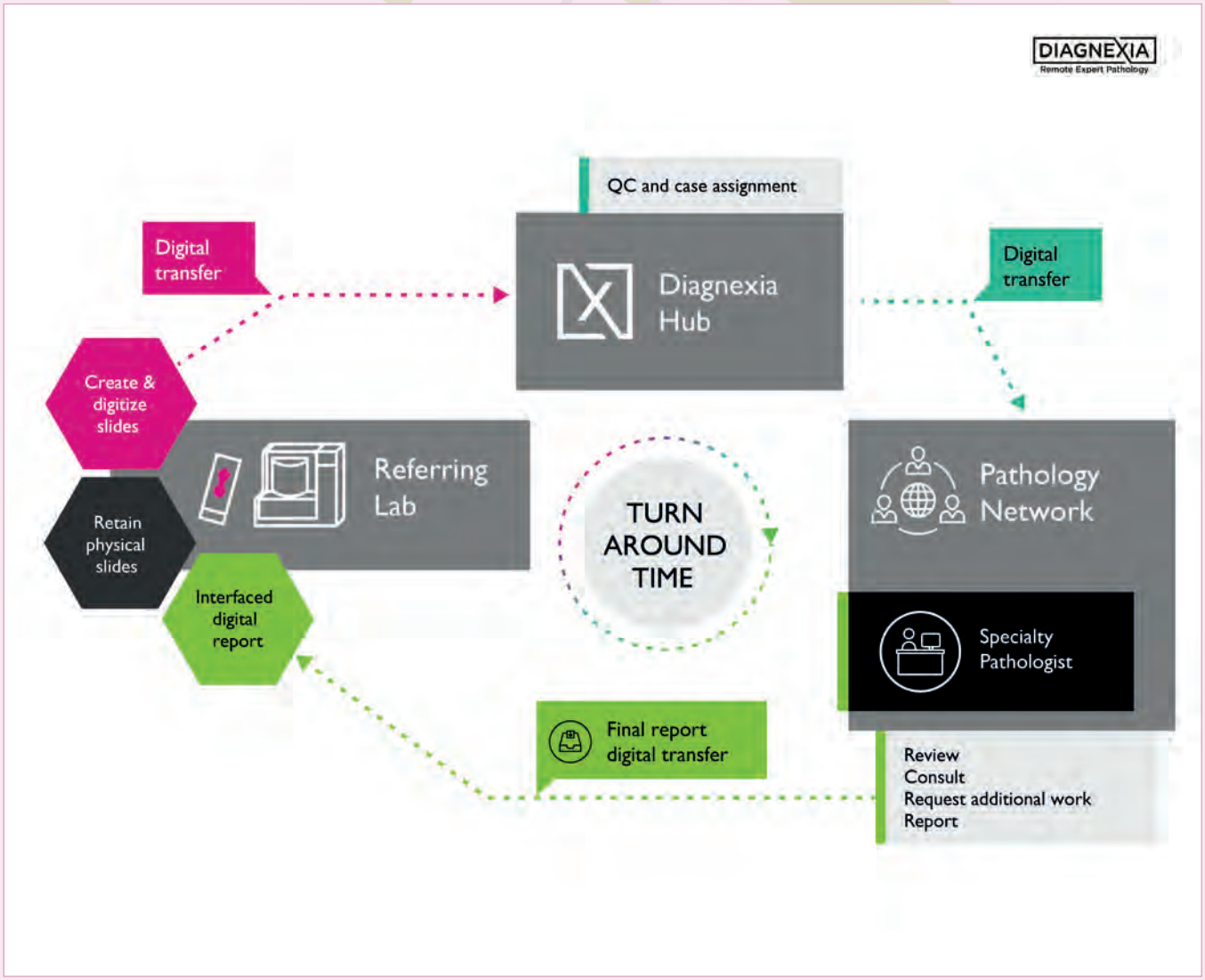
Why might pathologists use Diagnexia's services?

The most important feature is that you get an expert opinion on your case immediately. This creates time efficiencies and reduces the cost and risk of second opinions – there are no transport expenses to ship your slides from one lab to another; there's no risk of losing those slides in transit; and the scanned images are high-quality and can be reused for research and teaching purposes.

> *"If labs without slide scanning capabilities want to use our consultation service, they can send their slides to our central repository, where we can scan and upload them onto our platform."*

Of course, not every lab is fully equipped for digital pathology at the moment. If labs without slide scanning capabilities want to use our consultation service, they can send their slides to our central repository, where we can scan and upload them onto our platform. If such a lab would like to use our primary diagnostic service and the volumes are sufficient, we will install a scanner in that particular department, allowing them to interface with our reporting platform and upload their cases directly.

I think this represents an important (and interesting!) departure from our



usual mode of practice. It's exciting. It incorporates computational pathology and provides a layer of objectivity to a discipline that, by its nature, is often subjective. I think the introduction of an objective, quantitative tool will significantly benefit patient care. In no way will such tools replace pathologists – they will simply supplement our armamentarium of tools to ensure that we can not only reach the best possible diagnosis, but also help guide treatments and improve outcomes.

Are you growing your team?

We are hiring along subspecialty lines with an eye to ensuring that our clients receive the best possible diagnosis from our pathologists. At this stage, we're looking at established subspecialists in the key areas of anatomic pathology and we welcome interest from people who are interested in our approach. If you'd like to try out new techniques and apply diagnostic algorithms to facilitate and enhance your practice, Diagnexia may be the place for you!

For more information, please visit our website (diagnexia.com) or email us (info@diagnexia.com).

Find Runjan Chetty on Twitter at: @RunjanChetty
Find Diagnexia on Twitter at: @Diagnexia

Runjan Chetty is Professor Emeritus in the Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada, and Chief Medical Officer at Deciphex Ltd. and Diagnexia Ltd.

The Call of Coronal Duty

An insider's perspective on the proteomic battlegrounds of COVID-19

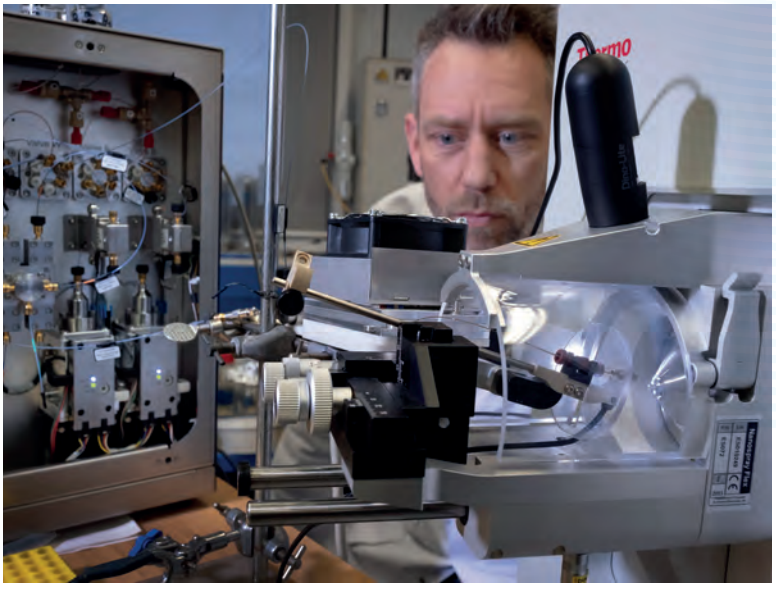
With March 11, 2021 marking one year since COVID-19 was officially classified as a pandemic by the WHO, it goes without saying that many of us – not least those on the frontline of the fight – are feeling a bit war weary. And yet, for so many in the analytical chemistry community who answered the call of duty back in 2020, the battle rages on.

With so much uncharted ground still to cover when it comes to this novel coronavirus and our response to it, there is an abundance of work ongoing across all subdisciplines of our field. For now, we decided to share some of the spoils from one battlefront in particular: proteomics. Here, experts Jeroen Demmers, Perdita Barran, and Manfred Wuhrer tell us about their work in the fight against COVID-19, and provide an insider's perspective on some of the developments we can expect to see in the coming months.

COVID-19 Detection: *Hitting the Mark*

How we successfully used targeted proteomics for the detection of SARS-CoV-2 proteins

By Jeroen Demmers, Director of the Proteomics Core Facility and Associate Professor of Proteomics, Erasmus University Medical Centre, the Netherlands



Early last year, once it became clear that COVID-19 had started to spread out across the world, there was a general sentiment in Europe that it wouldn't happen that easily here. Just like the SARS and MERS coronavirus pandemics that came before (in 2003 and 2013, respectively), many people thought that this novel virus would be kept out of the region as well. Soon enough, the pandemic hit northern Italy hard, and it wasn't long before there were messages of infected people in the southern province of Noord-Brabant in the Netherlands.

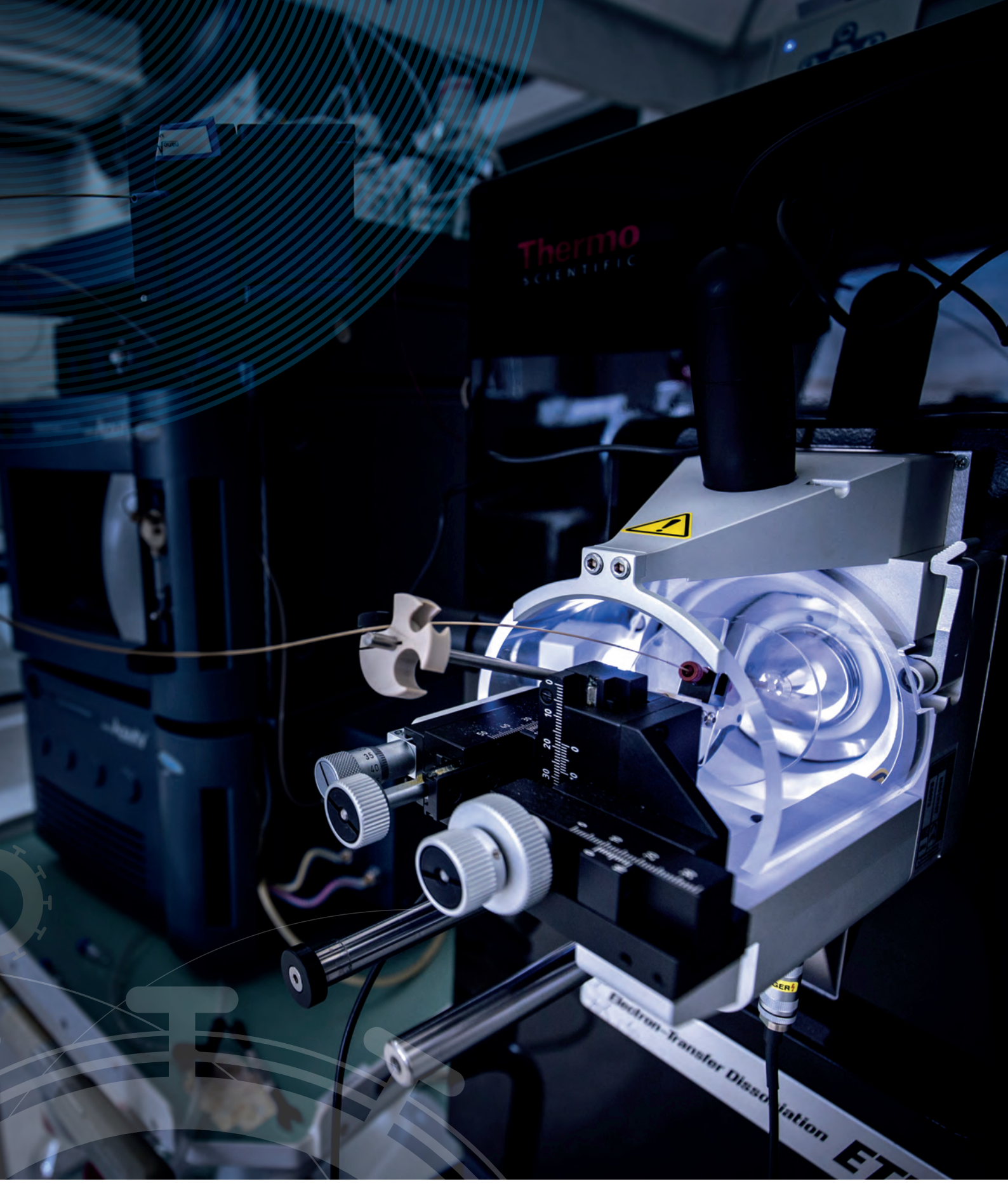
The first official outbreaks were reported in early March, and things developed quickly from there. In the second half of March our institute was shut down – like many others across Europe – and only research on COVID-19 was allowed to continue. For us, this work was happening at the Viroscience department at Erasmus MC, where several research groups had been focusing on coronaviruses for decades. My research lab and core facility had a choice: shut the lab, or grab this opportunity to contribute to SARS-CoV-2 containment by adapting our technology for use in virus detection and – if successful – diagnostics.

The journey to discovery

Thanks to our previous work during the MERS coronavirus pandemic (our lab had identified the MERS-CoV human receptor protein using MS-based proteomics; see our "Gone Fishing" sidebar on page 25 for more information), we had already established connections with the Viroscience department. I decided to contact coronavirus specialists Bart Haagmans and Mart Lamers as I knew they were working day and night on SARS-CoV-2 assays to answer questions about the mechanism of infection. For one of their assays, they were interested in analyzing the response of the host cell proteome to viral infection in a recently developed organoid-derived bronchoalveolar tissue culture. Using our technology, we were able to help them monitor up- and downregulation of large numbers of proteins upon viral infection and learn more about the intracellular pathways that are turned on or off as a result of infection. Because of this work, and our connections with the Viroscience department, we were also granted access to some of their interesting SARS-CoV-2 samples – meaning we could test whether it was possible to measure viral proteins in complex samples, such as cell lysates.

We started off with samples from an infected Vero E6 cell line derived from the African green monkey – this cell line is used to propagate viruses and serves as a rich source of viral material. A dilution series was then created to demonstrate the limit of detection of specific viral proteins. As the virus was already genotyped, the protein amino acid sequences that we needed for the analysis of proteins based on peptide fragmentation or MS/MS data were already available. Also, we were quite lucky (or unlucky?) in that just a few days before most of the institute was shut down, a brand new Orbitrap Eclipse MS was installed in the lab. The first proteins analyzed on that machine were SARS-CoV-2 proteins!

The output of a standard proteomics experiment is usually a table of identified proteins, which is generated in the final step of a database search using software tools that may take up to several hours. The progression of this process (at least in the tool that we use) is indicated by a green bar. I clearly remember the anxiety and excitement that we felt when, after the very first database search, we saw the bar hit 100 percent and the list of identified proteins popped up: the first time we identified SARS-CoV-2 proteins really felt like looking the monster directly in the eye.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How can proteomics help *in the fight against COVID-19?*

Understanding the role that proteins play in the SARS-CoV-2 infection process and disease progression is vital to the development of therapeutic and preventative strategies. In this way, proteomics has proven to be an indispensable tool in COVID-19 research, and its role will no doubt be expanded in the future.

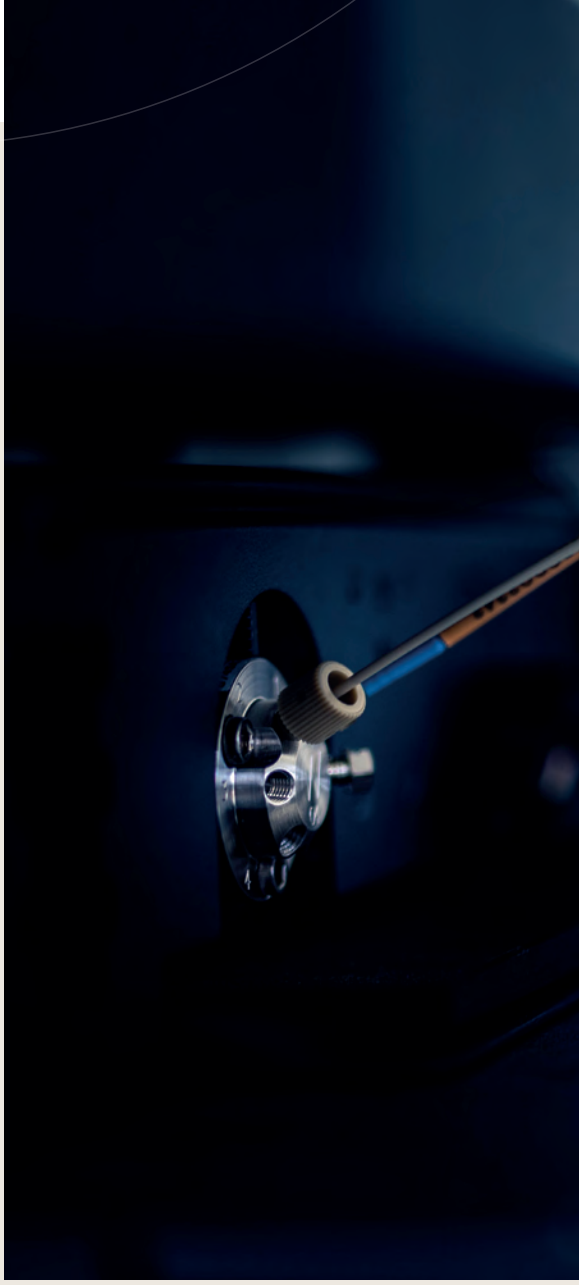
Firstly, MS-based detection of SARS-CoV-2 proteins and their proteolytic peptides offers a simple and rapid virus detection assay. Using targeted proteomics, peptides of the SARS-CoV-2 nucleocapsid and spike proteins can be detected with high sensitivity and specificity in research samples and clinical specimens. This opens up the possibility of taking this technology to clinical diagnostic labs and translating it into point-of-care devices as alternatives for nucleic acid-based methods, which could be particularly interesting from a cost-effective healthcare perspective.

Proteomics could also be used to develop approaches capable of predicting COVID-19 cases that might later progress into clinically severe disease. In fact, several studies have already identified potential protein biomarkers that are differentially expressed in COVID-19 patients and could be used to predict viral infection at early stages. (See the sidebar "Collaboration and Determination" to learn more about Perdita Barran's work around targeted proteomics and biomarkers).

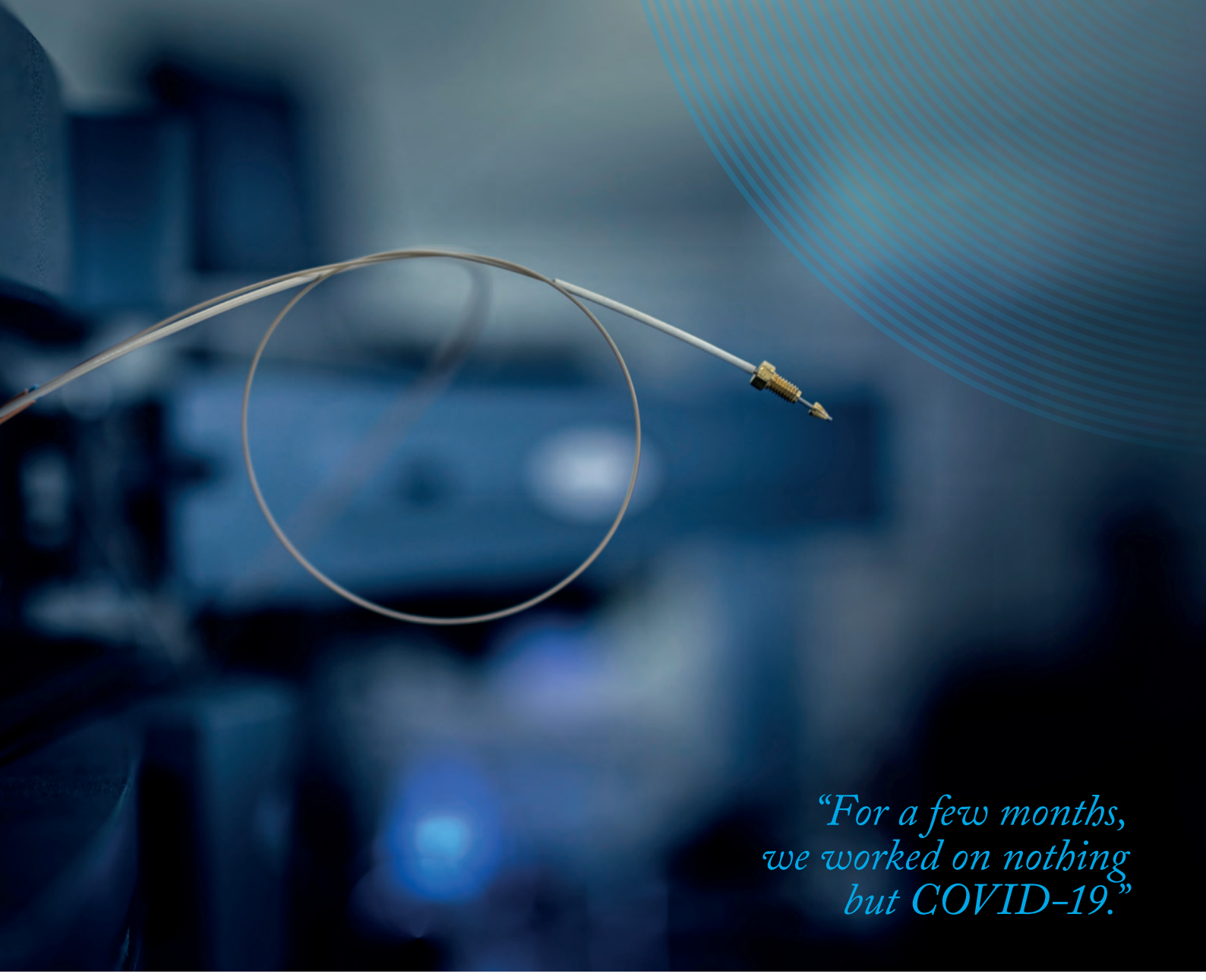
In other areas, investigation of the humoral antibody response to SARS-CoV-2 proteins has aided the development of antibody-based assays for diagnostic and therapeutic purposes. Recently, a comprehensive SARS-CoV-2 human protein–protein interaction map was generated using affinity-purification (AP) MS. Several hundreds of specific interactions between SARS-CoV-2 and host cell proteins were defined, and it was discovered that, for some of the involved human proteins, several existing FDA approved drugs were already available. Other proteomics-based research on the host cell response has shown that the complement system and metabolic pathways are severely affected in COVID-19 patients.

Unbiased, explorative proteomics has also been used to define the proteomes of autopsy samples from COVID-19 patients. For instance, it was shown that cathepsin L1, rather than ACE2, was significantly upregulated in the lungs of COVID-19 patients. In addition, systemic hyperinflammation and dysregulation of glucose and fatty acid metabolism was detected in multiple organs, which shows how the multi-organ proteomic landscape of such autopsies may help in our understanding of the biological basis of COVID-19 pathology.

Crosslinking MS has been used to study the interaction sites between antibodies and the spike protein in detail. Such studies, often combined with protein structure elucidation by tools such as cryo-EM are crucial in the development of antiviral therapeutics. In research studies, huge non-covalent assemblies of proteins – such as intact virus particles several 10s of Megadaltons in mass – can be analyzed by MS. This way, conformational dynamics of viruses and viral proteins can be uncovered and this can yield valuable information on the stability and topology of macromolecular assemblies in general and virus capsid structure in particular.



Viral proteins, in particular those in the viral envelope (such as the spike protein), are extensively decorated by protein glycosylation. To understand how this post-translational modification influences spike-ACE2 interactions with the host cell membrane, these glycan structures have been characterized in detail by (glyco) proteomics. Detailed analyses of the impact of emerging variants in spike and natural or designed-for-biologics variants of ACE2 on glycosylation and binding properties are important next steps in developing therapeutics.



The right sample

For a few months, we worked on nothing but COVID-19. Virtually all other projects were put on hold and since most meetings at work were cancelled and there were no teaching duties, it really felt like a postdoc project where the full focus is on basic science. I truly relished this lack of distraction, despite the troubling situation we – as citizens of the world – were in. On a personal level, we were building a new house and we weren't sure whether we could still sell our old house – what with the threats of a housing market collapse together with the crashing stock markets and other doomsday scenarios that circulated. It was truly both an exciting and troubling time.

We soon identified a set of proteolytic peptides that could serve as the target peptides in follow-up experiments. Also, we were able to calculate limits of detection for viral proteins in our proteomics assays. Under ideal conditions, we could go down to the mid- to low attomole range in targeted experiments, just like the numbers we had seen before in another project on non-related proteins.

While setting up these assays, I had already started asking around for patient material to see whether we could detect proteins in clinical specimens (such as nasal swabs) and to determine if it could be used as a diagnostic tool. However, getting patient samples turned out to be more difficult than I had anticipated. For conventional PCR-based testing, samples are usually stored in a " transport medium. " This medium contains a lot of protein, the signals of which dramatically mask the signals of viral proteins in our assay. Unfortunately, adaptation of standard protocols in diagnostic departments is virtually impossible, and as research scientists who are used to changing protocols if something doesn't work, this was quite frustrating.

But one day we got a message from a collaborating clinical virologist who had collected a different type of sample from a COVID-19 patient. This was a sputum sample, deposited on a little glass slide with no addition of transport medium or any other buffer solution. Upon inactivation of the virus in 80 percent acetone, we could take the sample from the BSL lab to our own lab and subject it to our standard bottom-up proteomics protocols – which basically means digesting all the proteins into peptides. This sample was in fact the first clinical specimen in which we could clearly detect SARS-CoV-2 peptides.

We used a targeted proteomics assay, which means that we set the MS in such a way that it only detects viral peptides that were selected a priori. The quadrupole in the Orbitrap hybrid MS then acts as a filter that lets only the peptides (or m/z values) of interest pass through. Upon fragmentation of the peptide to determine the amino acid sequence, the fragment ions are measured in the Orbitrap with high selectivity and sensitivity – the high mass accuracy of the Orbitrap is a clear advantage over such targeted methods in a triple quadrupole instrument here. The fragment ion fingerprint that is thus obtained is highly specific for the selected peptide. These fingerprints are then computationally compared with the specific fingerprints that were defined in the experiments on infected Vero E6 cells. Using targeted MS, the sensitivity can be increased and the limit of detection is at least 10-fold lower compared with data-dependent acquisition (untargeted) MS.

Next, we contacted clinical virologists from a hospital in the south of the Netherlands, which was located in the center of the area that was hit by the first COVID-19 wave in the spring of 2020. The clinicians there used so-called Eswabs, for which no protein rich transport medium is necessary. This results in much less background in our analyses and therefore increased sensitivity. Despite the excess of red tape, we managed to get an Eswab sample cohort to our lab. This sample set contained various swabs within a wide range of PCR Ct values and we could see a nice inverse correlation between Ct value and peak intensities of target peptides in the mass spectra, reflecting the abundance of proteins. Later, in a second sample cohort, we managed to get similar results and could detect SARS-CoV-2 peptides at fairly high Ct values (i.e., low viral counts).

Winning the war…

Where are we now? We have established the proof-of-concept and have shown that it is definitely possible to detect SARS-CoV-2 proteins using MS. The challenge now is to translate this methodology from the R&D stage to the clinical diagnostic lab. For the analyses we have performed so far, we used state-of-the-art, ultra-sensitive Orbitrap mass spectrometers – which are typically not present in clinical diagnostic labs. Still, the basic technology is comparable to triple quad MS and these are readily available in many clinical labs.

There is still a debate around the level of sensitivity we really need in COVID-19 testing. The limit of detection of PCR based methods is unsurpassed, but do we really need that sensitivity? It is unclear whether infected individuals, whose nasal swab PCR Ct values are in the high 20s or low 30s, are infectious. Although no viral proteins could be detected in most swabs with associated Ct values of >26, we have to test whether the sensitivity that can be reached by MS-based approaches is sufficient to differentiate between infectious and non-infectious people. Only then will we be able to assess the potential value of MS-based COVID-19 testing.

One clear advantage of this technology over other testing methods however, is that proteins of multiple different viruses

Gone *Fishing*

For our work on the MERS-CoV human receptor protein, we designed a relatively simple "fishing" experiment that was performed using in vitro synthesized MERS-CoV Spike 1 protein immobilized on magnetic beads. The spike protein was used as bait, and we went fishing in a pond of human proteins – or "cell lysates," prepared by crushing cells that were cultured in a petri dish in the presence of detergents. We found one human protein that showed a very specific interaction with the bait protein, suggesting this was a receptor protein present on the outside of the human host cell – for example an epithelial cell in the lung that the virus grabs and uses to enter the host cell.

The identification of the receptor protein was not only crucial in understanding how the virus infects a host cell, but also for the development of antiviral therapeutics and vaccines. For instance, by blocking a receptor using small molecule drugs or antibodies, infection of the host cell can be prevented. We identified the protein DPP4 as the receptor for MERS-CoV, and this finding was confirmed by in vitro and in vivo experiments. Since it is different from the ACE2 receptor that SARS uses to enter the host cell, this was a somewhat unexpected finding at the time.

From a proteomics point of view, this study is the ultimate example of the importance of identification of proteins in an unbiased manner – the core of MS-based proteomics technology. If more conventional experimental methods, such as screening assays, had been used at that time, the identity of the receptor is unlikely to have been found so quickly. One would have to have made a selection of possible receptors a priori, and if the protein was not included in that selection, it would not have been found.

See full article online at tp.txp.to/coronal-duty

Collaboration *and* Determination

Perdita Barran, Professor of Mass Spectrometry at the University of Manchester, UK, shares a targeted approach to SARS-CoV-2 proteomics

How can targeted proteomics help in the fight against COVID-19?

Targeted proteomics can help diagnose whether someone has the virus, but it can also help to determine the effect of the virus on a given individual by providing biomarkers that can predict the course of the disease. I am working on projects in both of these areas. Ultimately, targeted proteomics (and indeed metabolomics and lipidomics) could provide the cheapest and most robust methods to determine the course of infection – and to help doctors decide how to treat individuals.

Can you tell us a bit more about your own work?

We have found that the NCAP protein in SARS-CoV-2 is very amenable to fast digestion, and that it can be detected at 100 attomol level by UPLC-MS. This means we can determine how much viral protein is present in any individual sample. MS directly measures the viral protein, without any labeling or the need for many additional reagents.

What is interesting to me in this area at the moment is comparing the abundance of viral protein (as found by MS) to viral RNA (as detected by RT-PCR). It may be that the viral protein abundance is a better indication of infectivity, as there are lots of reports of RNA hanging around much longer than an individual is infectious. Maarten Dhaenens has been one of the pioneers in translating a method for clinical diagnosis of COVID 19 with MS.

The other role for targeted MS will be to determine the presence of mutations in the virus. This diagnostic capacity could be extremely helpful in surveillance testing, as we will need to know if the vaccine continues to provide immunity – especially as new strains emerge.

How has MS added value to the pandemic so far – and what about its future impact?

To date there are 244 published papers on PubMed that have COVID-19 and MS in the title or abstract. This is likely an under-representation of the role that MS has played in this pandemic. Increasingly, scientists are using MS to study the progression of the disease with renewed focus on understanding the effects of long COVID. I think MS will have an important role in the development of therapeutics to treat people with the virus, as well as contributing to vaccine development.

More importantly, the fact that so many scientists have been willing to work together and share knowledge has been incredible. The COVID-19 MS coalition, which I helped initiate, is a great example of this – within a few weeks we had more than 800 members. Actions like this, in the face of the threat of the virus on all of us, will lead to more collaborative science and allow us to develop public health that is less competitive.

I also hope that any new resource being purchased for coronavirus research will benefit the diagnosis and treatment of other diseases. The data being collected now all over the world will be a great future resource. The way we're accelerating rapid diagnostic tests to the point of having them validated and being used by clinicians is a real celebration.

Reference

1. *M Larsen et al., Science, eabc8378 (2020). PMID: 33361116.*

"The challenge now is to translate this methodology from the R&D stage to the clinical diagnostic lab."

can be targeted in one assay. If peptide signatures for a given virus are defined, these can be relatively easily included in the target list. This way, samples can be screened for multiple viruses simultaneously. This is not only useful now, but also in the future when differentiation between different pathogens will be needed.

One challenge to overcome will be improving the analysis time: the sample preparation for proteomics assays takes a while, mainly because of the protein digestion step. This can be dramatically reduced by microwave irradiation. Furthermore, LC gradients could be much shorter than they are now: we have managed to reduce the gradients lengths threefold and could still detect most of the SARS-CoV-2 peptides. Running clinical samples using LC gradients' of only up to a few minutes should be possible.

As a final note, I'd like to mention that part of our early work was published on biorXiv. Although manuscripts are not peer reviewed, they can be downloaded by the scientific community and the general public for free. For COVID-19 research, this has been a tremendous help in the dissemination of data, knowledge, and protocols. Even though our manuscript has not yet been published in a scientific journal, our selection of target peptides and MS data sets have been used by others and already proven useful. I believe this is a beautiful illustration of the power of open-access scientific knowledge – a trend I hope to see continue in the future.

I've demonstrated my own work using MS to detect viral proteins, but this is just one application of this versatile technology. It is clear to me that by studying proteins, both from SARS-CoV-2 and the human host cell, proteomics has profoundly changed the way we study viral infection and disease progression at the molecular level. I am excited to see the many potential novel applications that will no doubt come to fruition in this fast-moving field.

A structural and systems *biology view*

Manfred Wuhrer, Professor of Proteomics and Glycomics at Leiden University and Head of the Center for Proteomics and Metabolomics in the Netherlands, shares his view on how MS-based proteomics can contribute to COVID-19 research in the clinical lab

What's the role of MS in structural research around SARS-CoV-2?

MS largely contributes to the structure elucidation of the spike protein. Initial bottom-up proteomics studies showed that the spike protein is heavily glycosylated. This did not come as a surprise, as SARS-CoV-2 shares this feature with many other viral surface glycoproteins – it was remarkable how quickly different laboratories then performed in-depth analyses of the glycosylation of the S protein! These studies provided key insights into how glycans shape the viral surface and influence the interactions with host cell factors and the immune system.

Do you think MS could make a useful (and realistic) diagnostic tool for COVID-19?

Current COVID-19 molecular diagnostic assays focus on the detection of parts of the viral genome (PCR-test) or protein antigens ("quick" or even "self-test"). At the moment, MS does not play a role in diagnostics, but it certainly has a lot of potential in this direction. Various efforts are ongoing to establish assays for the low-resolution MS detection of viral proteins to obtain a molecular fingerprint of diagnostic value from, for example, nasal swabs or even gargle solution. These attempts build on the previous success of whole-cell MS and intact mass analysis of major microbial proteins – something that is now widely used for diagnosing bacterial and fungal infections. I am curious to see whether the MALDI-TOF-MS platforms that are widely established in medical microbiology laboratories will find their way into the diagnosis of viral infections, including SARS-CoV-2.

The bottom-up approach chosen by Jeroen Demmers likewise has good potential for translation into clinical laboratories. After transfer of these assays onto triple quadrupole LC-MS platforms, they can certainly be established in clinical chemistry laboratories, which often have the necessary hardware and increasingly also the protein expertise available.

What about the use of biomarkers in clinical diagnostics?

COVID-19 often has a huge, systemic impact on infected people, and there's a range of immunological, cell biological, and metabolic effects with biomarker potential. Due to the enormous impact of the pandemic, an array of omics technologies have been applied to COVID-19 patient materials which has led to a range of promising biomarkers. Using MS, my team has recently helped to define a specific glycosylation switch on antibodies against the viral spike protein in COVID-19 (1). This switch appears to initiate inflammation, and we are now looking at whether it can serve as an early marker predicting the development of severe COVID-19. I think a key challenge will be to integrate and scrutinize this wealth of information using a meaningful, systems biology and systems medicine approach.

Reference

1. *M Larsen et al., Science, eabc8378 (2020). PMID: 33361116.*

European Society of Pathology

33rd European Congress of Pathology

Pathology: Compass for optimal patient therapy

29 – 31 August 2021

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Understanding the catalysts behind the acceleration of digital pathology

Digitization is radically transforming pathology. This change is driven partly by force of circumstance – think pandemic – and partly by the passion of its champions. To find out more, we spoke to David Dimond (Chief Innovation Officer, Global Healthcare - Life Sciences, Dell Technologies) and Michael Valante (Chief Technology Officer, Digital Pathology, Dell Technologies)...

What triggered your interest in pathology?

MV: I have spent many years working in imaging informatics for healthcare, helping clinical departments and care providers enhance collaboration and patient care. When digital pathology began to emerge, I saw the opportunity to bring my radiology experience to the pathology lab and help pathologists with their digital transformation. Leveraging technology to enhance patient care is highly motivating!

DD: One driver was personal; I had seen family members with cancer having to cope with slow diagnostic and patient management processes. The biggest hurdle was getting all the pathology data in front of different experts at the same time. I wanted to change that. I became convinced that digital pathology was inevitable, and that Dell Technologies – given its impressive technology assets and market share – was the right place to make it happen. The main challenge was integrating different digital healthcare data systems into a unified, vendor-neutral archive, but I believed my 20-year background in electronic health records and medical image digitization would help meet that challenge.

What are the biggest opportunities in the move to digital pathology?

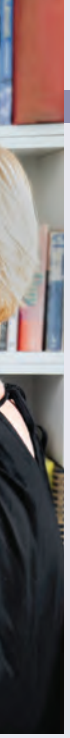
MV: I'm encouraged by the progress being made in pathology toward digital transformation; after all, other medical imaging services, such as radiology, adopted digitization years ago. Now, 15 months of lockdowns have presented a unique opportunity to transform the way pathology uses and shares images and data. COVID-19 resulted in a seismic shift to remote working, which has accelerated the opportunities for digital pathology.

DD: The pandemic has shown everybody what is possible. For example, wide availability of rapid COVID-19 diagnostics has changed expectations; patients now demand faster turnaround. Overall, people are at last beginning to recognize that digital pathology will inevitably become the standard of care and that pathology laboratories must invest in these capabilities, despite methodological limitations and inadequate standardization.

"COVID-19 has forced laboratories to operate virtually and thereby cleared a path to transformation of the pathologist's workflow."
– David Dimond, Chief Innovation Officer, Global Healthcare - Life Sciences, Dell Technologies

Why should labs make the move to digital?

MV: Removing the geographic limitations associated with traditional microscope-based workflows improves efficiency. More importantly, however, digitization allows pathologists to benefit from more



sophisticated technologies – in particular, AI tools. These advanced analytical tools allow pathologists to interrogate data more quickly and reproducibly, thereby optimizing decision-making. I have no doubt that AI will be part of digital pathology's future.

DD: Digitization releases the power of biobanking and opens up many opportunities, not least of which is in the field of long-haul COVID-19 research – which has attracted NIH funding of ~US$1 billion for projects that include the building of an imaging resource archive for pathology and other specialties. That kind of work demands that you interrogate all the data holistically, so digitization is essential. For example, a new Dell Technologies' Digital Twin initiative uses technology to leverage digital pathology imaging to identify groups of patients with similar diagnoses and genetic attributes and then applies AI to continuously search for correlations. This approach has great potential, but is not possible with legacy pathology systems.

What barriers exist to digital pathology?

MV: First, it's essential to have infrastructure capable of managing the large amounts of information and data pathology produces. This requires careful planning, especially regarding interoperability and integration. Second, the technology must be adopted in a way that is compatible with the laboratory's work processes. In addition to deciding which tools should be used, adoption plans must define initial and growth use cases that will be supported. All these factors can complicate the shift to digital pathology, but they are by no means insurmountable.

DD: The primary barriers are funding shortages and the current status quo, of which the latter can be more challenging. There is still a feeling that "real pathology" is done by humans. But younger pathologists increasingly expect a digital environment and, over time, they will drive change. Another issue might be some IT teams' reluctance to grapple with sophisticated AI tools and huge digital pathology files. These demand increasingly high-performance computer systems and can't be managed in the public cloud alone. Finally, we still need to see improvement in standards for genomic data and in the integration of images and associated data in the emerging area of personalized health called "computational pathology."

How might digital pathology evolve – and how can we future-proof it?

MV: Future challenges include accurate capture and efficient interrogation of data, as well as management of regulatory concerns regarding testing and monitoring. I am convinced that AI will provide solutions to all such issues and will, at the same time, enhance the interpretation of pathology data and its integration with diagnostic and treatment processes. More generally, we should learn from imaging fields where digitization has proceeded further than it yet has in pathology. In particular, we should embrace existing infrastructure solutions and, where possible, adapt them to support pathology's workflows. Above all, we must maximize the benefits of continuing advances in information sharing to improve clinical collaboration and its resulting improvements in care delivery.

DD: Digitization creates a store of medical information that follows each patient. The key element in this packet of patient-associated information won't be a glass slide or a tissue block; it will be an image file integrated with other data. This will radically transform our practices. Imagine accessing all available data – the entire virtual patient – via the click of a mouse! These capabilities will facilitate the adoption of AI; we will come to rely on algorithms that are continually fed with data and that not only provide augmented diagnoses, but also support development of breakthrough treatments. For example, vaccine manufacturers will be able to respond to an evolving healthcare situation by modulating their products according to information provided by digital pathology. Importantly, collaboration between different specialists will be enhanced, leading to more holistic clinical decisions. And at the same time, the AI algorithms themselves will evolve. The architectural approach is inherently future-proof!

Any final thoughts?

MV: Seek IT expertise early when planning a digital pathology strategy; otherwise, you may risk overlooking important aspects of the transformation, such as how can we best collaborate now that caregivers are not limited to relying on a physical glass slide and what we can do with the digital data to redesign the current workflows. Digital pathology requires an infrastructure capable of managing huge amounts of data in a scalable, secure, standardized, and reliable way. Such data management is a mission-critical aspect of digital pathology – so the infrastructure that supports it is vital. I make this assertion with confidence, because we at Dell Technologies have digitized data for many different healthcare services; we understand what is required in what can be a challenging endeavor.

DD: Don't underestimate the expertise required to implement an effective digital transformation in the pathology laboratory. Creating the patient data package requires attention to data validity, reproducibility, and provenance; this is a significant engineering challenge because the data is spread between multiple organizations in multiple clouds. Managing this challenge requires integration of the virtual information packets and establishment of a register of anonymized patient data. Few organizations have the requisite expertise for this; indeed, we see much confusion regarding management and reproducibility of diagnostic information. Fortunately, Dell Technologies has significant experience with this type of challenge. We maintain collaborative relationships with all the cloud providers to ensure that our customers' technical needs are fully met within the context of clinical workflows, and our skills in this area have proven valuable to many partners.

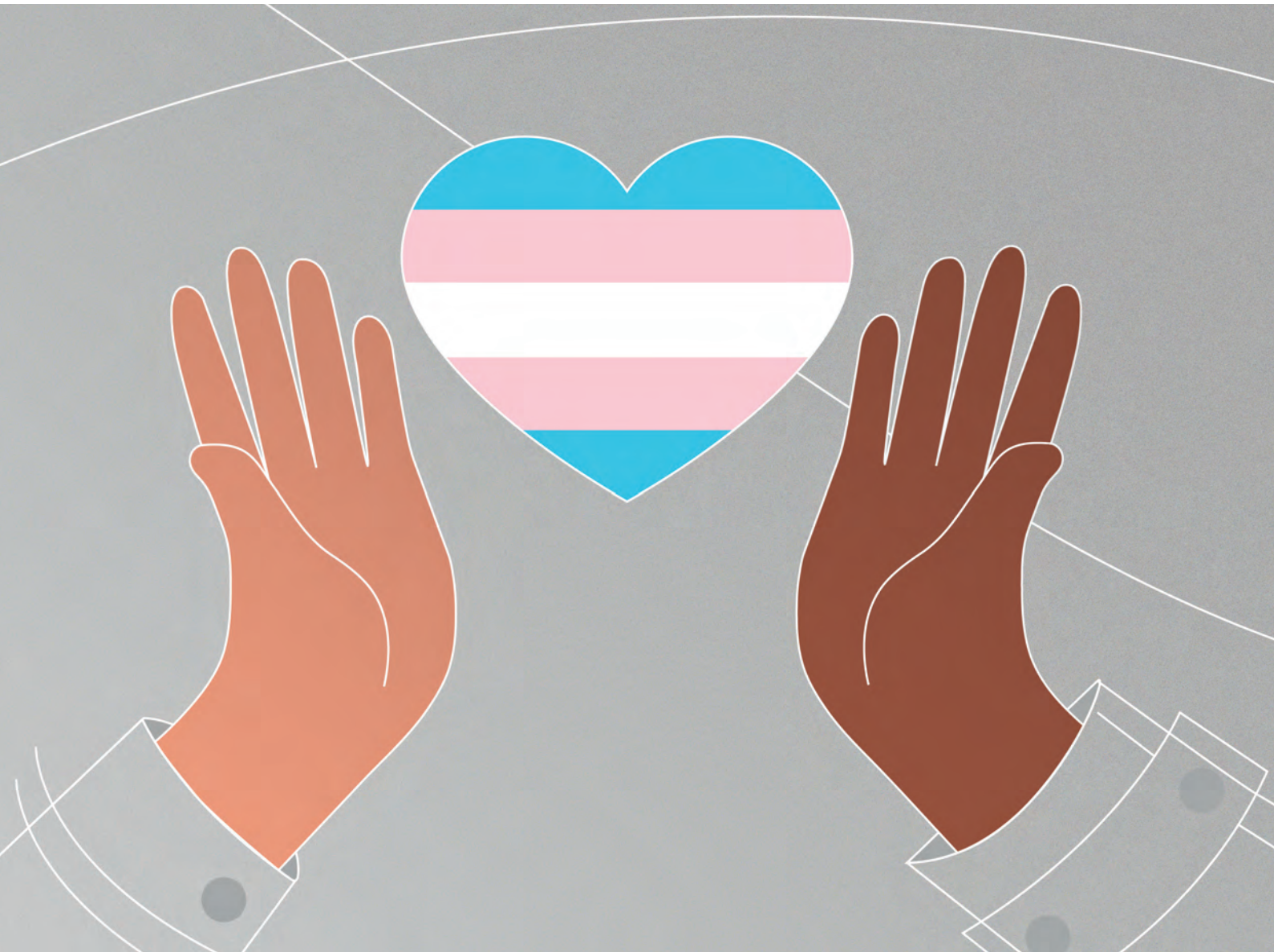
In Practice

Technologies and techniques
Quality and compliance
Workflow

Positive (Trans)Action

How can we ensure better care for transgender patients? Here, Timothy Craig Allen shares his lessons learned – and his vision for the future

Michael Schubert interviews Timothy Craig Allen



How did you enter the conversation on transgender patient care?

Like many others, I learned about transgender patients' issues and difficulties through news, social media, and conversations with friends and colleagues. My understanding has built slowly over the last few years, and I continue to seek out information and update my knowledge.

Discussions with colleagues at the University of Mississippi Medical Center (UMMC) who have specific expertise in transgender issues, as well as diversity, inclusion, and equity educational programs, have furthered my understanding of transgender difficulties. I asked myself what more I could do – and that started me thinking about how pathologists and laboratorians could engage in the discussion and be part of the solution.

What do you think are the key needs pathologists and laboratory medicine professionals must keep in mind when treating trans patients?

The goal is equitable treatment for transgender patients by all health care personnel. Stigmatizing behavior keeps these patients away and insensitive behavior adds to their discomfort. This is a responsibility borne by everyone involved in health care – including pathologists and laboratorians.

In our discipline, there are a few first steps with which we can quickly involve ourselves – specifically, the use of proper pronouns in patients' charts and medical records and the development of appropriate reference ranges for certain tests, especially hormone-related tests.

Clinical laboratory testing of hormones is the most obvious place to start, with consideration of reference ranges appropriate for our transgender patients. It's clear that tissue excisions from organs that have been affected by hormones – for instance, breast and prostate – will need special consideration related to hormonal changes and cancers that may be hormone-related.

More research is needed to assess differences between cis and trans men, and cis and trans women, in all aspects of their health, as transgender patients age and chronic diseases become more prevalent in gender-affirmed patients.

What training do pathologists and lab medicine professionals currently receive in addressing the healthcare needs of trans patients?

The issues surrounding transgender medicine are relatively new to many folks. Training and education vary; however, from my conversations with colleagues, I've found that most pathologists and laboratorians have little or no training regarding medical issues specific to the transgender population beyond what may arise in a basic institutional diversity lecture or course.

Unfortunately, I doubt that this lack of training is unique to pathology and laboratory medicine. Other health care professionals face the same barrier. There

A New Gender Gap

By Scott M. Rodgers

Regarding the unique healthcare challenges facing the transgender population, access to caring, nonjudgmental, and competent providers is perhaps the single greatest challenge. In Mississippi, only three clinical practice groups in the entire state have developed specific programming in transgender health, and two of these three are in Jackson. Many patients in the rural areas of the state have no one nearby with the capacity or willingness to provide care.

Second, inclusive health insurance benefits depend to a great extent on where a person lives in the United States. In Mississippi, insurance companies are not required to cover the costs of gender-affirming care, which means that patients are left with only self-pay options – and therefore often cannot afford treatment. To provide some contrast, Massachusetts requires all insurance companies to cover these costs, allowing patients in Massachusetts to get the care they need.

Third, transgender individuals face discrimination at higher rates in housing, schools, and the workplace, and they are more likely to be unemployed (and therefore without workplace health insurance). In states like Mississippi without expanded Medicaid to cover the uninsured, this creates massive problems for the transgender community.

Regarding recent legislation targeting this community, many of us who serve this population are very worried about the potential for real harm. By restricting and eliminating affirming healthcare for the community, we expect a resultant worsening of mental and physical health. We will likely see an increase in rates of depression, suicide, anxiety, and addictions, to name just a few potential problems. This runs entirely counter to our purpose in medicine, which involves the relief of suffering. Such legislation is regrettable, unfortunate, and unnecessary.

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"The goal is equitable treatment for transgender patients by all health care personnel."

is a great need for expanded education overall in the health care setting.

Beyond the standard diversity training pathologists and laboratorians receive, I believe we need additional focused training and education that puts transgender patients' issues in perspective, reinforces a professional culture, promotes discussion, and helps pathologists and laboratorians consider next steps in our journey to improve the lives of our transgender patients.

How prevalent are trans patients in your healthcare system?

Without knowing exact numbers, I would say that transgender patients are at least as prevalent at UMMC as at any other institution that does not specialize in transgender care. In fact, as a state medical school, it is likely that UMMC sees more transgender patients than surrounding hospitals due to the socioeconomic factors many transgender patients face. Furthermore, our institution's active focus on providing services for our transgender patients, championed by several institutional leaders, may draw additional transgender patients to UMMC.

I will share one of my own cases – a lung biopsy from a transgender patient. I paid close attention to the patient's name and made sure to use the appropriate pronouns when referring to the patient in the report's comment. This experience may sound mundane, but it highlights the fact that all pathologists and laboratorians are likely to be involved in transgender patients' care – not just those specialties that deal with gender-affirming surgery, such as breast, gynecologic, and urologic pathology. As such, we should all recognize the need to educate ourselves fully so that we can best care for our transgender patients.

Do pathologists and lab medicine professionals face any potential issues with billing and reimbursement for gender-related health care?

Billing issues may occur in cases of gender-affirming surgery; however, they

A Care Collaboration

By Jane F. Reckelhoff

Because the field of transgender studies is relatively young, the overall health of transgender individuals is not clear, especially in individuals taking gender-affirming therapies (GAT) long term. For example, most studies on cardiovascular disease risk in transgender individuals, including lipid and triglyceride levels and insulin resistance, are made in individuals who have been taking GAT for relatively short times (months to a couple of years). Thus, the long-term cardiovascular consequences of GAT are unknown.

It is well known that cisgender men have a higher incidence of cardiovascular disease and earlier incidence of first myocardial infarction than do cis women; cis women develop myocardial infarction approximately 10 years later than cis men on average, but their recovery is not as rapid – perhaps due to the increased incidence of comorbid conditions present with aging. Due to the lack of long-term studies in transgender individuals, there is little information as to whether the same gender differences in myocardial infarction that are present in cisgender patients are present in trans men and women.

The data from transgender studies are also inconsistent; some studies show that transgender men have little cardiovascular disease risk, whereas others show that they have increased incidence of myocardial infarction. In addition, there are no consistent reports on the cardiovascular health of aging transgender individuals who have been receiving GAT for many years. This is partly due to the changes in GAT guidelines over time and partly due to the relatively small populations of transgender individuals.

Given this lack of data and the need for careful testing and record-keeping, it's clear that pathologists and laboratorians can provide significant support for endocrinologists, cardiologists, and other healthcare practitioners. Together, we can define the health consequences of long-term GAT in transgender individuals with the intent to be proactive in their health care, thus improving our trans patients' long-term quality of life.

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can likely be avoided by the pathologist carefully indicating in the surgical pathology report that the surgical procedure is "gender-affirming surgery" and not merely "hysterectomy" or "breast reduction," which would perhaps cause confusion with transgender patients who are medically transitioning.

For procedures and tests that are not part of the continuum of gender-affirming surgery, appropriate use of pronouns – and diligent tactful comments – should assist the billing folks and avoid denials. The same holds true in the clinical laboratory; for instance, when performing a prostate-specific antigen test on a trans female patient.

What effects might new legislation surrounding gender-related healthcare have on trans patients?

Evolving legal, social, and cultural issues exist with regard to transgender medicine. In this situation, reasonable minds can reasonably differ and society will continue to work to balance the equities involved, including issues of patient autonomy, civil rights, and parental rights.

The best thing pathologists and laboratorians can do right now to benefit our transgender patients is to fully engage in learning – and educating others – about our transgender patients. Dispel myths. Explain terminology. Get involved in research to assess the questions that will guide public health policy.

What one key takeaway message would you like pathologists and lab medicine professionals to keep in mind when treating trans patients?

These are our patients. Even if we do not see them, we can have a remarkable impact on the quality of their medical encounters, on the quality of their medical care, and on the quality of their lives. We can reduce their anxiety, increase their feelings of acceptance, and ultimately provide them equitable health care free of gender-related stigma and discomfort. It's our job to support these patients and champion their care – and it's our job to make sure they know we're there for them.

Timothy Craig Allen is Professor and Chair of the Department of Pathology at The University of Mississippi Medical Center, Jackson, Mississippi, USA.

Realizing the Quadruple Aim

Putting pathologists at the center of digital transformation

By Monica Santamaria-Fries

The dialogue around digital transformation in pathology is growing louder – a sentiment echoed at this year's USCAP Annual Meeting (which was itself virtual). This trend is hardly surprising considering that the past year has been momentous for digital pathology. Adoption crossed an inflection point when several major organizations, including the Joint Pathology Center, Mayo Clinic, and Israel's largest health maintenance organization, announced plans to modernize – and others, such as LabPON and the UK's National Health Service, increasingly scaled their implementations. All of these developments happened in the midst of an unexpected surge in demand brought on by the COVID-19 pandemic.

Yet despite the enthusiasm around digital transformation, we often overlook a critical aspect of this journey. A recent article in the Harvard Business Review states that "digital transformation is more about talent than technology (1)." In other words, it's about empowering your team by using digital solutions to unlock new sources of value. Although much has been said about the scanners, technologies, and process changes required for digital transformation, the pathologist's experience and necessary cultural shift rarely feature in the conversation.

Humanizing digitization

Let's start by considering what matters most to pathologists. From my experience, three factors – quality of care, efficiency, and wellbeing – are at the top of the list. Pathologists are passionate about delivering high-quality care and about the impact they have on patient outcomes. They also recognize that they have limited time to read cases and feel the strain of growing workloads and increasing case complexity. A steady decline in the pathologist population combined with rising biopsy volume has led to a 43 percent increase in cancer cases per US pathologist between 2007 and 2017 (2).

This looming shrinkage of the pathologist workforce also casts a spotlight on physician wellbeing. Burnout is real. Over one-third of pathologists report feeling overworked (3); primary drivers are too many bureaucratic tasks and too many hours in the laboratory. Such a high burnout rate is unsustainable – once again calling attention to the need for efficiency – and can even lead to errors that impact quality of care.

I doubt I am the first to point out that the rationale for going digital should align with the "quadruple aim" in healthcare (4). This model starts with the well-known triple aim, which states that optimizing the healthcare system depends on improving the patient experience, lowering costs, and bettering outcomes. All of these can be achieved, in part, through efficiency and quality of care – factors that matter to pathologists and laboratory management alike. The fourth critical pillar? Improving physician satisfaction (or wellbeing), which is necessary to deliver the other three aims.

"Digital transformation is a process – and, although it heavily involves technology, it starts with people."

Improving the pathologist experience

Digital pathology can improve the pathologist experience in many ways. Take quality of care as an example – subspeciality care is easier to facilitate, collaboration is easier, and consults are more accessible, all because sharing images eliminates the complexities of mailing glass slides. Image analysis applications and, increasingly, other artificial intelligence solutions drive diagnostic accuracy and precision. And we're just beginning to scratch the surface with AI – I anticipate that, over the coming

years, we'll see a tremendous leap forward in how it supports diagnostic decision-making and workflow optimization.

Traditional analog workflows also have inherent inefficiencies related to handling glass slides. Digital pathology replaces these processes with intelligent workflows – for instance, supporting network integrations with distributed sign-out so laboratories can better manage workload-to-staffing ratios. And once again, AI delivers improvements; workflow applications are beginning to enable case sorting, triaging, and tumor detection, among other use cases.

Physician well-being comes into play when we consider the impact of these efficiency gains. They enable pathologists to work more productively, reducing burnout by helping them to cope with growing demands. More recently, we've heard a lot about digital pathology enabling remote operations and worksite flexibility during the pandemic. Even as pathologists increasingly make their way back into the laboratory, they will want to balance being physically present in their department with the benefits that they have come to appreciate from being offsite. From gaining back time typically spent commuting to the ability to read cases on demand during a free evening, digital pathology empowers pathologists to achieve the work-life balance they want.

Monica Santamaria-Fries

Hearing the pathologist's voice

We are seeing a shift in the pathology community's attitudes toward digital pathology as pathologists increasingly see firsthand how technology can help them achieve what matters most. Because pathologists are the primary users of digital pathology, accelerating this transformation requires a cultural shift centered on allowing their voices to guide future strategies and solutions.

And that begins with addressing their many valid concerns and making the pathologist a key stakeholder in change management. For example, pathologists may wonder what problem digital pathology is trying to solve. Perhaps they haven't had much exposure to digital solutions or are looking for clarity on the rationale for adoption. Often, a little education goes a long way. Socializing the change, articulating the "why," and acknowledging the cultural shift enables pathologists to provide meaningful input on goals, roadmaps, success criteria, and software selections that will ensure a successful digitization effort.

From here, the pathologist's voice should be used to design human-focused solutions that put them at the center, fitting into and enhancing their workflows. A tangible way to illustrate this is through the pathologist's workstation. The new digital office should feature an image management platform that sits at the core and integrates the entire ecosystem. This digital setup not only drives powerful efficiency and quality gains and enables worksite flexibility, but also allows easy collaboration with colleagues. Pathology is, after all, a team sport. In the end, it's not about a technology-powered workstation, but about using that technology to improve the pathologist's experience.

Digital transformation is a process – and, although it heavily involves technology, it starts with people. I truly believe now is the time to hang up your microscope and embrace the benefits that digital pathology affords – but it's important to acknowledge that even positive change involves some adjustment. By putting pathologists at the center of this change, we can help drive improvements in their experience and our practice – all in commitment to excellent patient care.

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See references online at: tp.txp.to/achieve-the-quad

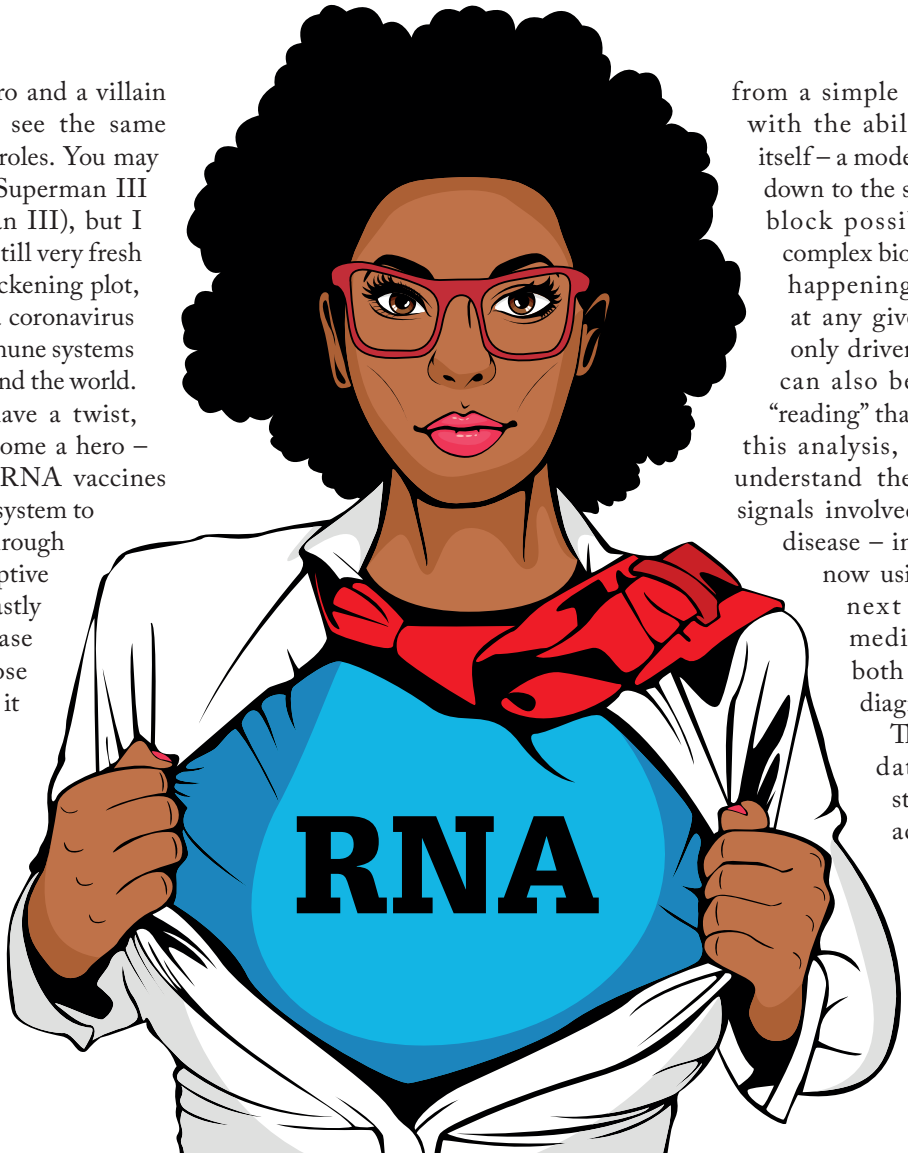
NextGen

Research advances
New technologies
Future practice

RNA: The Villain… and the Hero

How macromolecules and machine learning can work together to expand the frontiers of precision medicine

By Jarret Glasscock



Most stories have a hero and a villain – but we don't often see the same character take on both roles. You may have chosen to forget Superman III (and possibly Superman III), but I am sure COVID-19 is still very fresh in your mind. In this sickening plot, RNA plays a villain – a coronavirus that challenged our immune systems and wreaked havoc around the world. But the best stories have a twist, and RNA has also become a hero – taking the form of mRNA vaccines that allow our immune system to prepare for infection through antibodies offering adaptive immunity. These two vastly different roles showcase a macromolecule whose dynamic nature makes it biologically fascinating.

The next chapter

In fact, our bodies tell a story written by RNA. And the "RNA world" hypothesis suggests that life on earth sprang forth from a simple RNA molecule with the ability to replicate itself – a model that distills life down to the simplest building block possible. The many complex biological processes happening in our bodies at any given time are not only driven by RNA; they can also be quantified by "reading" that RNA. Through this analysis, we've begun to understand the pathways and signals involved in health and disease – information we're now using to build the next generation of medicine, including both treatments and diagnostics.

The boom of RNA data available to study is thanks to advancements in next-generation sequencing (NGS) that have exponentially decreased the

cost – and increased the output – of both DNA and RNA data. This technology yields thousands of data points for analysis – but, often, we overly distill these large datasets down to individual signals that are easier to interpret. This approach has limited our scope of understanding when it comes to the complexities of biology. We have learned in recent years that nucleic acid analysis is like watching a play – you can't focus on just one of the characters, but must observe them all to understand the story. Therefore, the next chapter of RNA – focused on enabling more precise medicine – evolves from measuring a single molecule to modeling many RNA molecules to better represent biological systems. This multidimensional approach has fueled efforts for precision medicine across a number of diseases.

Jarret Glasscock

The play's the thing

One such biological screenplay is in the field of immuno-oncology (IO). Our immune system is modulated by most oncology treatments, including both IO and standard treatments, such as chemotherapy and radiation. It has become commonplace to measure a patient's immune profile before and after therapy to understand how the immune landscape impacts tumor response. Current efforts to measure this in solid tumors begin by analyzing tumor tissue. In this application, where tissues are formalin-fixed and paraffin-embedded following biopsy, RNA technologies are arguably the best method for analysis. These tissues are unsuitable for flow cytometry and, although multiplex imaging has advanced significantly, there are still technical limitations to the number of signals we can measure. Using RNA sequencing, we can measure even highly degraded transcripts in a multiplexed fashion, allowing us to detect all of the "characters" to more fully understand what's happening at the site of the solid tumor.

Early approaches using RNA data to measure the immune components of the tumor were influenced by flow cytometry and cell surface markers. They focused on one or two transcript levels as a proxy for immune cells: high CD14 expression = monocytes, high FOXP3 expression = T regulatory cells, and so on. This is not ideal because single markers are not sufficient to uniquely define these immune cells in the tumor microenvironment. Inaccurate measurements led to inconclusive results that did not hold up when moved from the research lab to clinical practice. And immune cell composition is only one facet of an immune response; other RNA signals, including immune escape or co-inhibitory and co-stimulatory signals, also play a role. Essential to building technology that has clinical utility are more robust immune cell measurements, as well as the ability to look at every character in the play.

Comprehensive detection is only the beginning of a successful performance; interpreting the resulting data is a challenge in itself. A cast list cannot tell you what happens in the play. Thankfully, while NGS evolved to generate massive amounts of RNA data, machine-learning technologies expanded in parallel with new approaches suitable for modeling and identifying signals in the data. With these tools, we are now looking at RNA data in new ways to help us characterize and understand disease.

One of the key challenges that machine

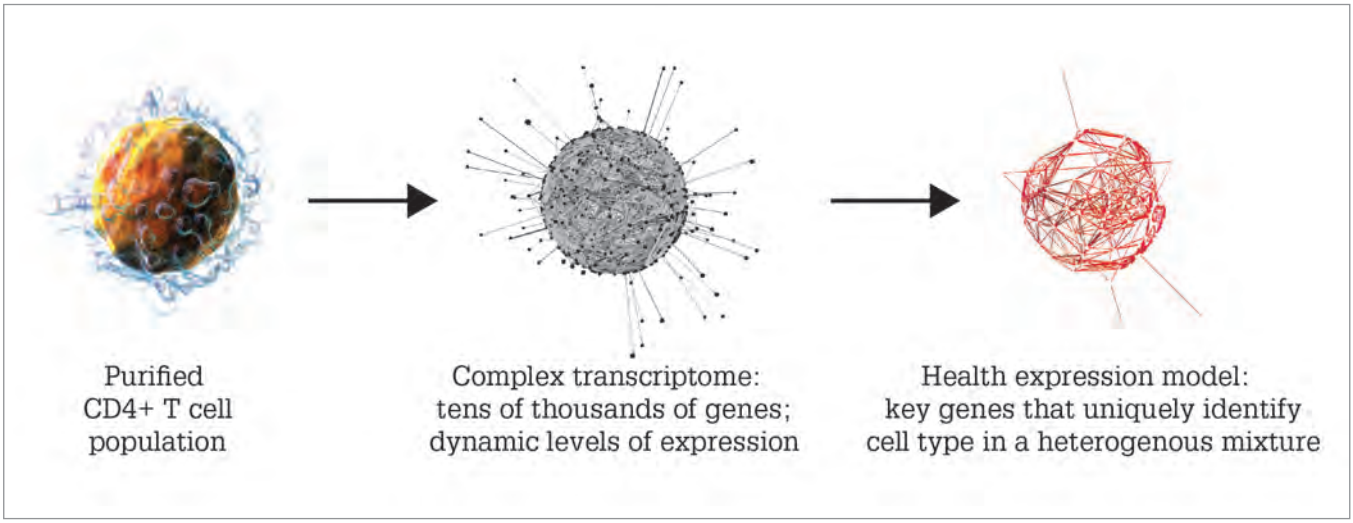


Figure 1. Machine learning is used to build iHEMs by analyzing whole transcriptome data consisting of thousands of signals to simplify into an essential-gene model. Data points in the models represent individual transcripts and their distance from the center represents expression level. T cell image from (3).

learning can address is converting RNA signals to immune cell quantification, through a process called "deconvolution." Various approaches to immune cell deconvolution have been developed and evaluated; a benchmarking study in late 2020 found that the performance relied on a number of factors including data transformation, scaling/normalization strategy, cell profiles or markers, and the deconvolution method itself (1). Not included in this study is a new method for building and deploying multidimensional RNA models that improves upon many of the performance factors described in the paper: immune Health Expression Models, or iHEMs (2). These models are built using machine learning to identify key signals from bulk RNA-seq data generated from purified immune cell populations (see Figure 1). These models enable better signal-to-noise ratios and higher specificity in quantifying immune cells in a heterogenous tumor microenvironment, leading to a more robust understanding of the immune signals modulated in response to disease, therapy, and other environmental factors. What's more, these models can even be built for nuanced cell states – historically cumbersome or even unachievable with other technologies. The combination of RNA and machine learning in these models bridges RNA sequencing data and immune cell quantification, representing a powerful new tool for IO.

A superhero origin story

However, this optimized data simplification does not accomplish the holistic characterization that would provide the most meaningful view of tumor response. To achieve this, we need to upgrade RNA from hero to superhero! We now need to use machine learning to complexify, rather than simplify, our dataset. After measuring these immune cell types, subtypes, and cell states in a clinically annotated cohort of therapy responders and non-responders, we can combine the individual signals into a multidimensional biomarker that better predicts response compared with the individual analytes alone (4,5). This process – predictive immune modeling – evaluates all possible signal combinations to build the most powerful biomarker for predicting disease response. And this heralds a shift in how clinicians use biomarker data. By combining many biological signals into easy-to-interpret prediction tools, we capitalize on our ability to measure massive amounts of dynamic RNA data in a meaningful way.

The COVID-19 pandemic put both RNA and the need for molecular diagnostics front and center in our minds. The world saw firsthand the challenges of building, validating, and deploying a clinical test. As we reflect on the incredible science and collaboration that came out of the pandemic, it's clear that new diagnostic technologies are an integral part of precision medicine. What's more, the role of RNA as a powerful tool in our precision medicine toolbox has been solidified. Although we've applied this technology to one field of medicine – oncology – its potential applications are vast. With the help of machine learning, RNA is poised to become the superhero in the story of precision medicine.

Jarret Glasscock is Co-Founder and CEO of Cofactor Genomics.

See references online at: tp.txp.to/rna-villain-hero

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1 in 2 women with HRD-positive tumors do not have a *BRCA1/2* mutation[1-4]

Homologous recombination repair deficiency (HRD) testing identifies tumor characteristics—beyond *BRCA1/2* mutation—that make it sensitive to PARP inhibition.[1,5]

Personalized medicine begins with personalized pathology. Discuss establishing a testing protocol for HRD in ovarian cancer with the multidisciplinary team at your institution.[6-8]

Learn more at **testforHRD.com**

BRCA, breast cancer susceptibility gene; PARP, poly ADP-ribose polymerase.

References: 1. Frey MK et al. *Gynecol Oncol Res Pract.* 2017;4:4. **2.** Pennington KP et al. *Clin Cancer Res.* 2013;20(3):764-775. **3.** Konstantinopoulos PA et al. *Cancer Discov.* 2015;5(11):1137-1154. **4.** Ledermann JA et al. *Eur J Cancer.* 2016;60:49-58. **5.** Watkins JA et al. *Breast Cancer Res.* 2014;16(3):211. **6.** Cheema PK et al. *J Oncol Pract.* 2017;13(2):e130-e138. **7.** Hoskins PJ et al. *CA Cancer J Clin.* 2017;67(6):493-506. **8.** Sundin T. *Med Lab Manag.* 2019;8(11):6.

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Metastatic melanoma (WV3).

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From Pot to Print

The rebirth of pathology specimens using 3D scanning and printing technologies

By Paul G. McMenamin, Sarah E. Coupland, and Justin W. Adams

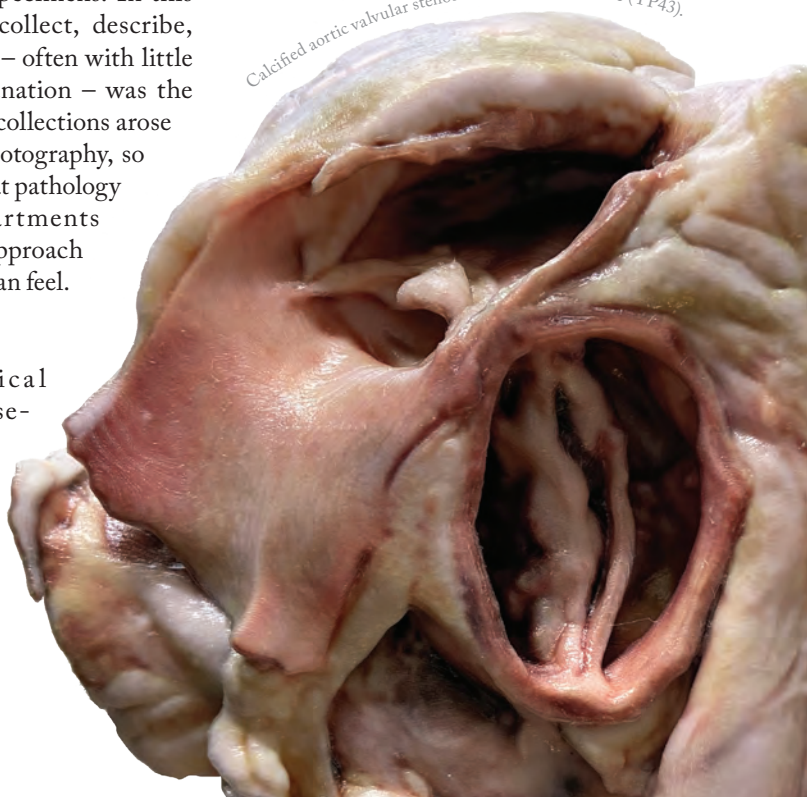
Capture a whiff of formalin scent and where does it take you? Many may recall medical school days spent in large, museum-like spaces lined with dark, wood-paneled shelves housing hundreds of jarred or bottled pathology specimens. The smell of formalin pervading the air gave those cavernous rooms a sense of historical significance, telling all who entered that they were in a "hallowed" space. The specimen "pots" themselves were likely collected from postmortems conducted over many decades or even centuries (for instance, the Berlin Museum of Medical History and the Hunterian Museums in London and Glasgow) and were a normal feature within university and hospital pathology departments (1). Medical students used the pots in tutorial sessions or self-directed learning to help them recognize gross anatomical pathology – often forming part of the practical assessments in many pathology courses.

Pathology collections were often amassed in the 18th, 19th, and early 20th centuries, when the study of zoology and other sciences relied upon museum collections that displayed cases of taxidermy specimens. In this period of history, "collect, describe, classify, and display" – often with little accompanying explanation – was the norm. Indeed, many collections arose prior to the era of photography, so it is not surprising that pathology and anatomy departments followed a similar approach and retained a Victorian feel.

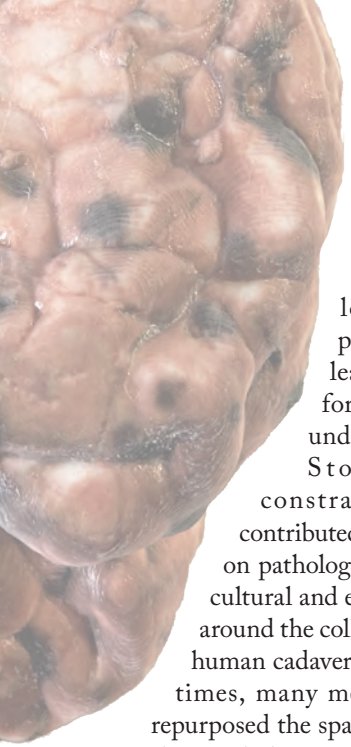
"Pathology collections [are] a constant reminder of the progress we have made in modern medicine."

Lost in the crowd

In modern medical curricula with case- or problem-based learning, pathology has become largely integrated into the broader curriculum. Pathology – despite it being a large subject incorporating several subspecialties – may not even be identifiable as an academic discipline in some medical schools, and many pathology departments have been reduced in size, combined with related disciplines, or eliminated entirely. In some institutions, pathology teaching has become integrated into clinical environments, such as in tertiary hospitals, where the heavy service



Calcified aortic valvular stenosis bicuspid aortic valve (TP43).



loads of diagnostic pathologists may leave them little time for teaching medical undergraduates.

Storage and space constraints have further contributed to reduced reliance on pathology specimens, as have cultural and ethical considerations around the collection and display of human cadaver material. In modern times, many medical schools have repurposed the spaces once occupied by their pathology specimen museums and transitioned to uploading photographic images of historical collections online as learning resources. There are some who still advocate for pathology museums (2) – arguing that the ability to identify and describe gross anatomical pathology is still a relevant and appropriate component of modern medical undergraduate training. However, we must remember that, as well as being important resources for understanding disease pathogenesis, prognosis, and clinical reasoning, pathology collections highlight diseases that have either been completely eradicated or are extremely rare – a constant reminder of the progress we have made in modern medicine.

Seeking specimen solutions

About eight years ago at Monash University, we developed expertise in producing 3D replicas of anatomical specimens using 3D surface scanning, CT scanning, MRI, digital segmentation, and 3D printing. These combined technologies allowed us to produce a collection of 3D-printed normal anatomy replicas (3,4) – including replicas of a human fetal collection (5).

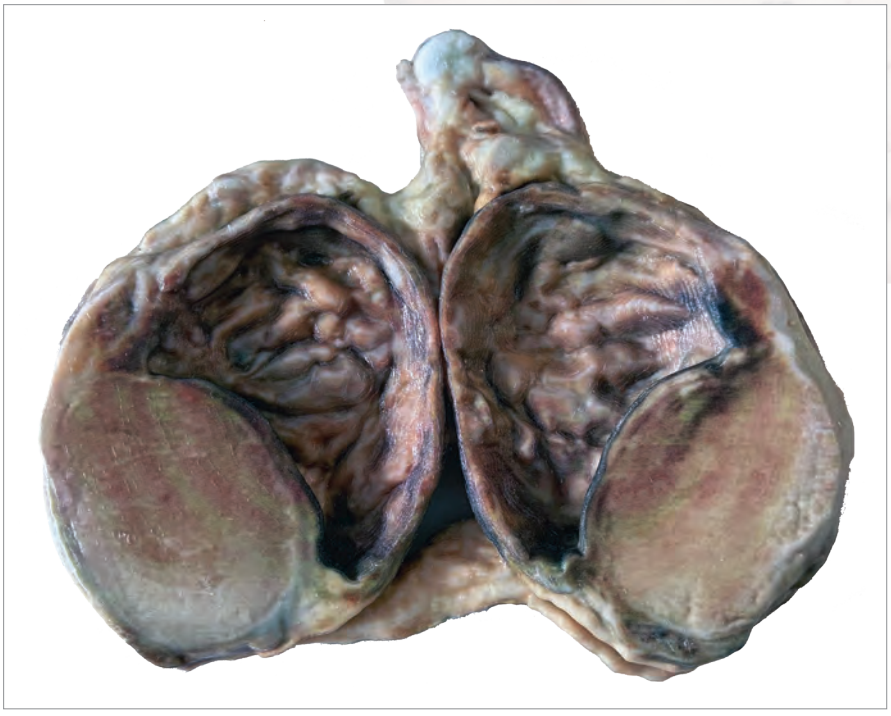
Cholelithiasis (gallstones) (AP203).

Our experience equipped us with the technical skills and resources to overcome some of the challenges of accurately recreating and replicating color, fine detail, and 3D form, which are considered essential when trying to produce 3D-printed replicas of human pathology specimens. With the lapse in time since our first study, we expected others to have applied this approach to producing human pathology specimen replicas, but – to our knowledge – only one group has since used photogrammetry and powder-based inkjet printers to create replicas of two gross specimens (6). Note that the authors did point out that these may be useful for clinicopathological correlation sessions.

At Monash University, we had a large collection of sparingly used pathology specimens in pots. But because they were collected in another era, they were considered of little value in the modern age of digital technology-based teaching. Before disposing of the specimens, we triaged them – reviewing all of the material and choosing examples of both common and rare pathologies that would be considered useful for teaching if we could replicate them at a suitable level of detail to mimic the real specimen. With full-color surface scanning and high-resolution, UV-curable 3D printers, we were able to do just that.

"There will be no fluid-filled pots to handle and no occupational health and safety issues with smelly containers and formaldehyde leakage."

By translating specimens into a digital – and then inorganic – medium, these 3D prints will allow students to physically handle pathology replicas in facilities other than licensed anatomy



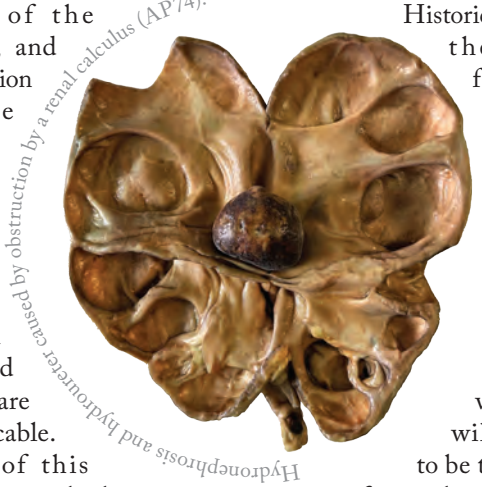
Chronic hydrocoele (R4Q2).

laboratories. There will be no fluid-filled pots to handle and no occupational health and safety issues with smelly containers and formaldehyde leakage.

Onwards and upwards

To enhance the utility of the Monash 3D Printed Pathology Collection, we created an updated synopsis for each specimen that includes the clinical history of the patient, a macroscopic description of the specimen, an overview of the disease process, and modern information about disease pathogenesis. The clinical histories written at the time of specimen collection use outdated medical terminology and descriptors that are no longer applicable. The creation of this updated collection, which is by no means exhaustive, gives students the opportunity to hold an accurate 3D replica of, for example, an infant heart exhibiting tetralogy of Fallot, with access to the associated description and individual case history.

So far, we have printed approximately 100 specimens from an original repository of around 1,800 – but, of course, there are many more interesting cases that could be preserved and archived using this approach. Historical collections around the world contain fascinating "potted" pathological specimens that only limited numbers of people will ever see. Now, the technology exists to change this situation; the only barrier is whether institutions will allow specimens to be temporarily removed from their glass containers for radiographic imaging and surface scanning.



Hydronephrosis and hydroureter caused by obstruction by a renal calculus (AP74).

We can only imagine the fascinating radiographic data that may be hidden within some of these rare and unique cases in collections across the globe – and, if there is a will for such discoveries, then we have certainly shown there is a way (7).

"We can only imagine the fascinating radiographic data that may be hidden within some of these rare and unique cases in collections across the globe."

Paul G. McMenamin is Emeritus Professor at Monash University, Clayton, Australia, and former Director of the Centre for Human Anatomy Education.

Sarah E. Coupland is Consultant Histopathologist and the George Holt Chair of Pathology at the University of Liverpool, UK.

Justin W. Adams is Senior Lecturer in the Centre for Human Anatomy Education, Monash University, Clayton, Australia.

See references online at: tp.txp.to/from-pot-to-print

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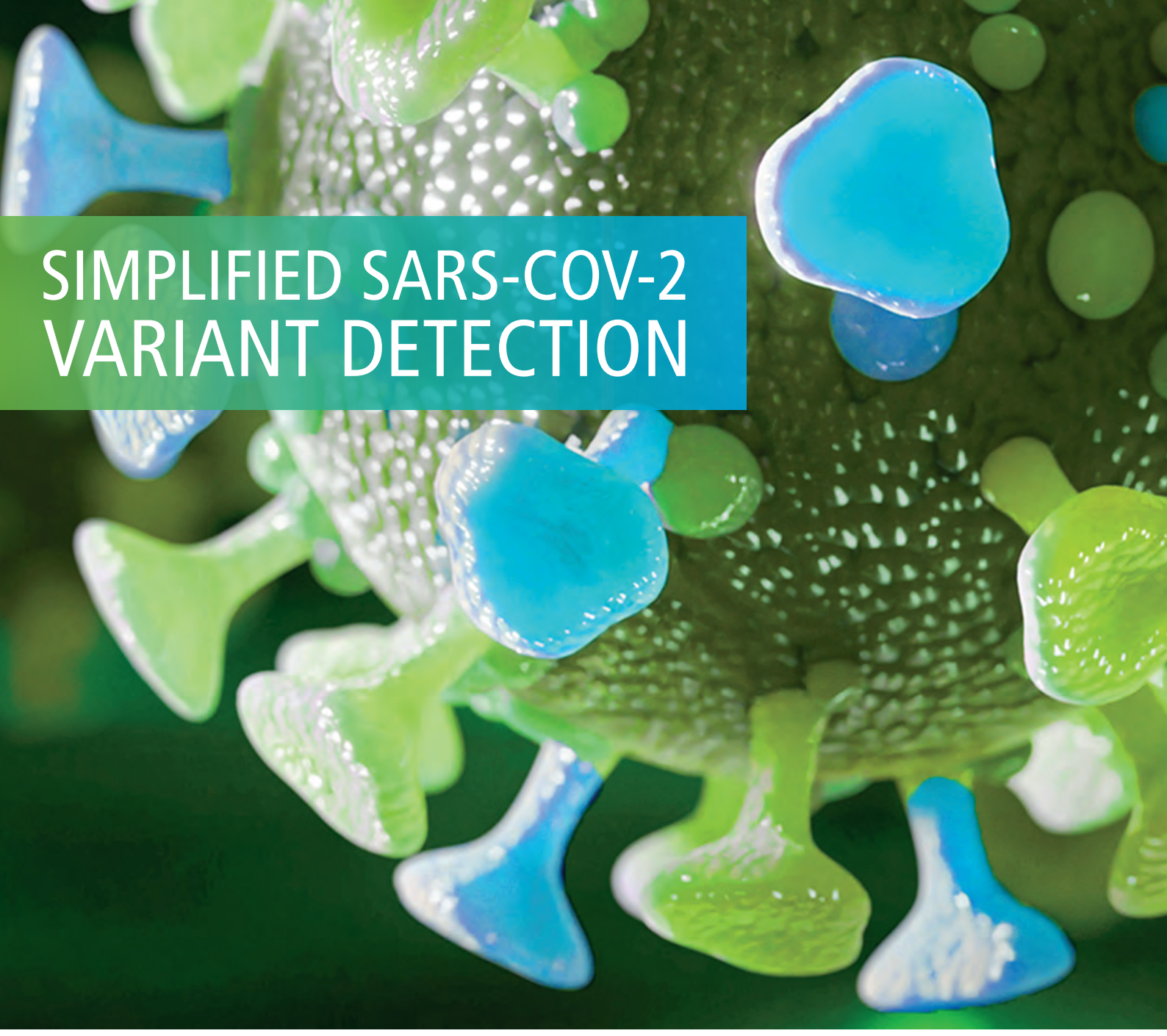

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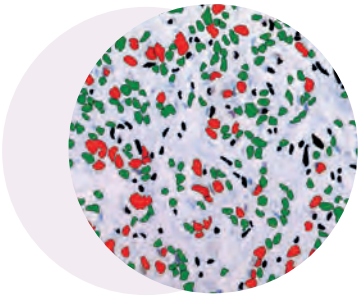

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Dancing Forward in One Health

Sitting Down With… Pikka Jokelainen, Academic Officer at the Laboratory of Parasitology, Infectious Disease Preparedness, Statens Serum Institut, Copenhagen, Denmark; Adjunct Professor of Zoonotic Parasitology at the University of Helsinki; President of the Scandinavian-Baltic Society for Parasitology; and Vice-President of the World Federation of Parasitologists

What role has pathology played in your career?

My understanding of health and disease would be much poorer without the time I spent in pathology. Although molecular methods can detect the DNA of a pathogen in a clinical sample, pathology can tell much more of the story.

I find parasites intriguing as pathogens. A good parasite does not cause its host much trouble. Host-parasite interactions are a delicate balance – and host reactions often cause more problems than the parasites themselves.

When I focused my research on zoonotic parasites, in particular *Toxoplasma gondii,* I was working as a lecturer at the University of Helsinki and participating in diagnostic work in their veterinary pathology and parasitology unit. My projects there included both epidemiological studies and investigation of fatal toxoplasmosis in different hosts. The similarities and differences between manifestations across the wide range of hosts are fascinating.

Can you tell us about your current work?

My current work focuses on One Health – the concept that human health, animal health, and the environment are closely connected. I work on a large European project, One Health EJP, which is a landmark partnership between public institutes across Europe. I am part of the One Health EJP Project Management Team, and I also lead one of the joint research projects, TOXOSOURCES, to explore the many sources of *Toxoplasma gondii* infections.

I am based at a public health institute that also has veterinary preparedness functions. Our laboratory is the national reference laboratory for parasites on both the human medical side and the veterinary side. For me this feels natural – and the synergies are obvious.

How has the pandemic changed the public's view of pathologists and epidemiologists?

The response to COVID-19 from the scientific community has shown what science can do – and I think we should not just acknowledge, but celebrate that. It's also important to note that many of the things the scientific community has been able to do in record time are based on a massive foundation of past work. That's the part that is largely invisible to the public.

The importance – and the challenges – of science communication and science-to-policy-translation have also gained visibility. We need to keep repeating the key biological aspects of pathogens and what the epidemiological expressions actually mean.

I am privileged to have been able to continue my work remotely through this pandemic. In times like these, I like to focus on the things we can do and how important it is to keep doing them. Even when COVID-19 is in the spotlight, older widespread endemic diseases such as malaria and toxoplasmosis don't disappear. The measures taken to address COVID-19 may affect these other diseases one way or another, but the old pathogens are likely to adapt and thrive. We must keep watching, studying, and addressing them – perhaps more now than ever.

COVID-19 has really shown the importance of cross-sectoral, interdisciplinary, and international collaborations and data sharing. I hope the collaborations and networks forged in this fire will be long-lived and strengthen our preparedness for future pandemics. One Health is, and will remain, more relevant than ever.

SARS-CoV-2 is mainly driven by human-to-human transmission – but how well prepared are we for a pandemic where animal hosts and animal-to-human transmission plays a key role? The necessary collaborations across sectors are easier to establish when they aren't urgently needed – and that includes building collaborations to focus on zoonotic diseases.

You hold a number of positions, including Vice-President of the World Federation of Parasitologists. How do you balance your many commitments – and a personal life?

My work gives me more energy than it takes – and I appreciate the opportunities it gives me to change things for better. One example is the International Congress of Parasitology, the World Federation of Parasitologists' main event, which takes place in Copenhagen in 2022. The pandemic inspired us to turn it into a hybrid event, making it more inclusive.

Having many commitments requires planning, organizing, and prioritizing. My to-do list runs long, but the top item is a quote – "Do what you love and do it often." I really like my work and it's important to me to actively notice the joy in it.

The other thing I love and do often is dance. I miss dancing in the studio – but, during the pandemic, I have made most of my living room into a small dance space. I start my home office days with a few pliés!

What can human and veterinary pathologists learn from one another?

I think we should collaborate more with one another. It could be interesting to share observations across our disciplines, apply comparative approaches, and harmonize our methods.

What advice do you have for others who wish to follow in your career footsteps?

I would encourage combining skills across fields and disciplines. I don't just mean academic skills – my stage experience from my dance background and my interest in learning languages have benefited my work and made it more interesting – more like me.