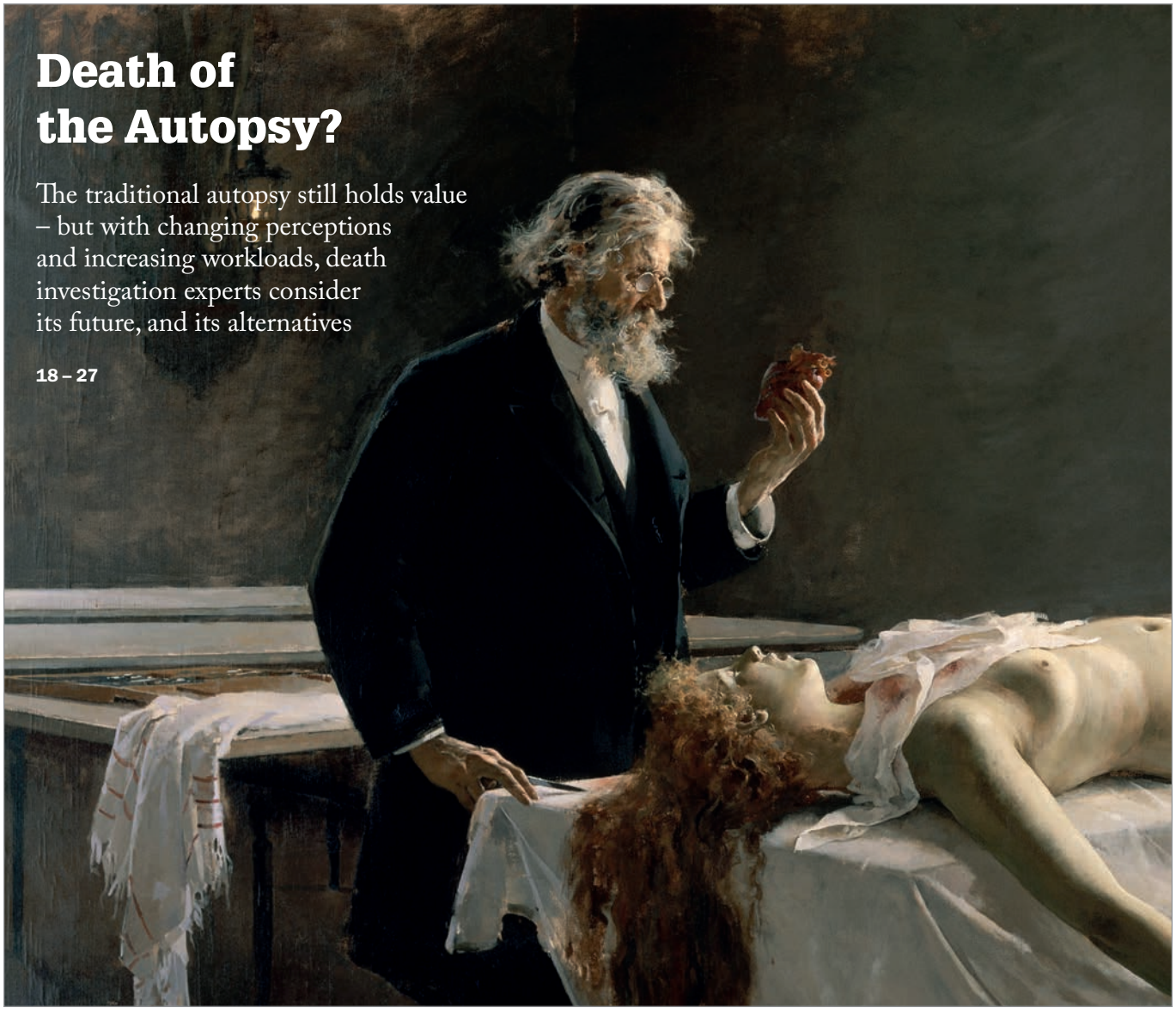
AUGUST 2017 # 33

the Pathologist



Death of the Autopsy?

The traditional autopsy still holds value – but with changing perceptions and increasing workloads, death investigation experts consider its future, and its alternatives

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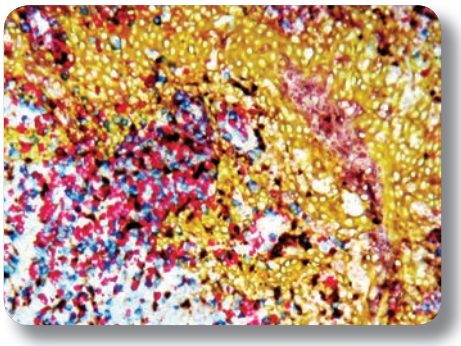

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Case of the Month

Peripheral Lung Tumor

A well-circumscribed peripheral lung tumor measuring 3 cm in diameter was discovered on a routine X-ray examination of an otherwise healthy 50-year-old man. The tumor was positive for CD34, Bcl-2, CD99, and STAT6, and showed low proliferative activity by MIB1 (Ki-67) immunostaining. Cytogenetic analysis revealed no evidence of X;18 translocation.

What is the most likely diagnosis?

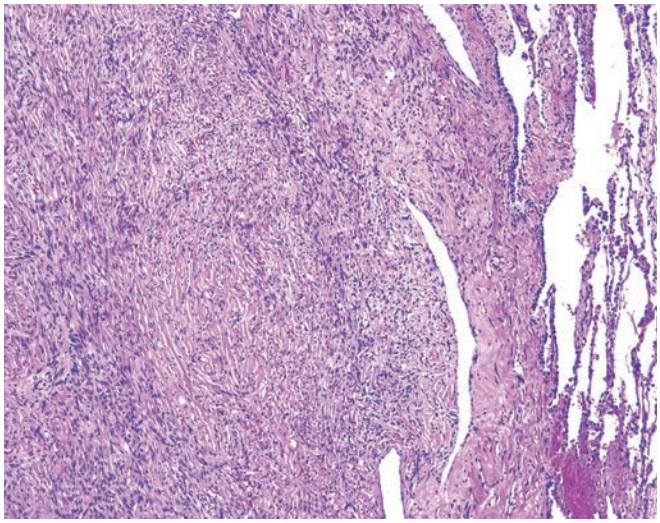
a Synovial sarcoma

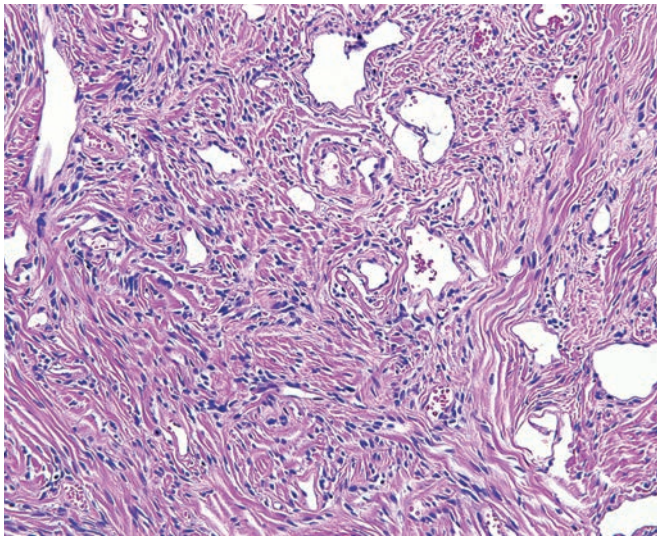
b Benign fibrous histiocytoma

c Solitary fibrous tumor

d Myopericytoma

e Malignant mesothelioma





Answer to last issue's Case of the Month…

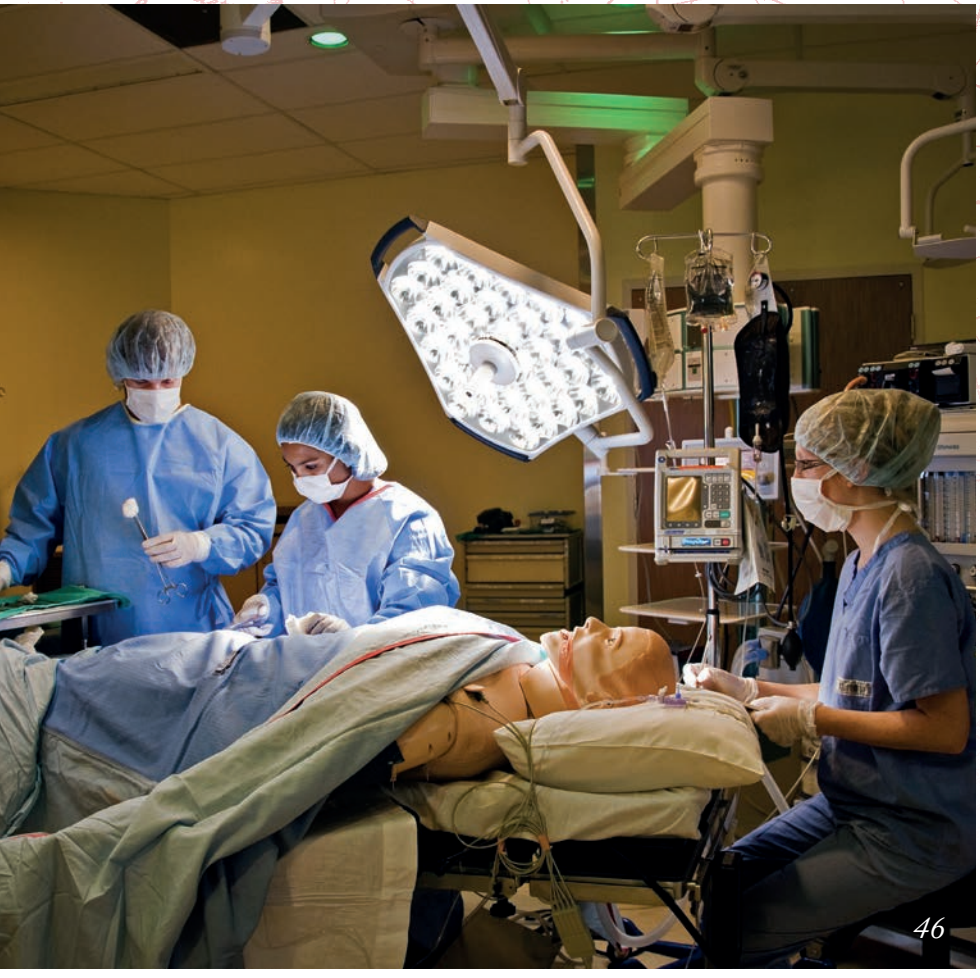
A. Systemic lupus erythematosus

The light microscopy of this periodic acid-Schiff stained slide shows marked thickening of the capillary loops, colloquially known as "wireloops." The electron microphotograph shows osmiophilic deposits on the sub-endothelial side of the capillary loop and also in the mesangial area. These findings are diagnostic of lupus nephritis, a common feature of systemic lupus erythematosus.

Submitted by Ivan Damjanov, The University of Kansas, Kansas City, USA.

To register your guess, please go to http://tp.txp.to/0817/case-of-the-month
We will reveal the answer in next month's issue!

Contents

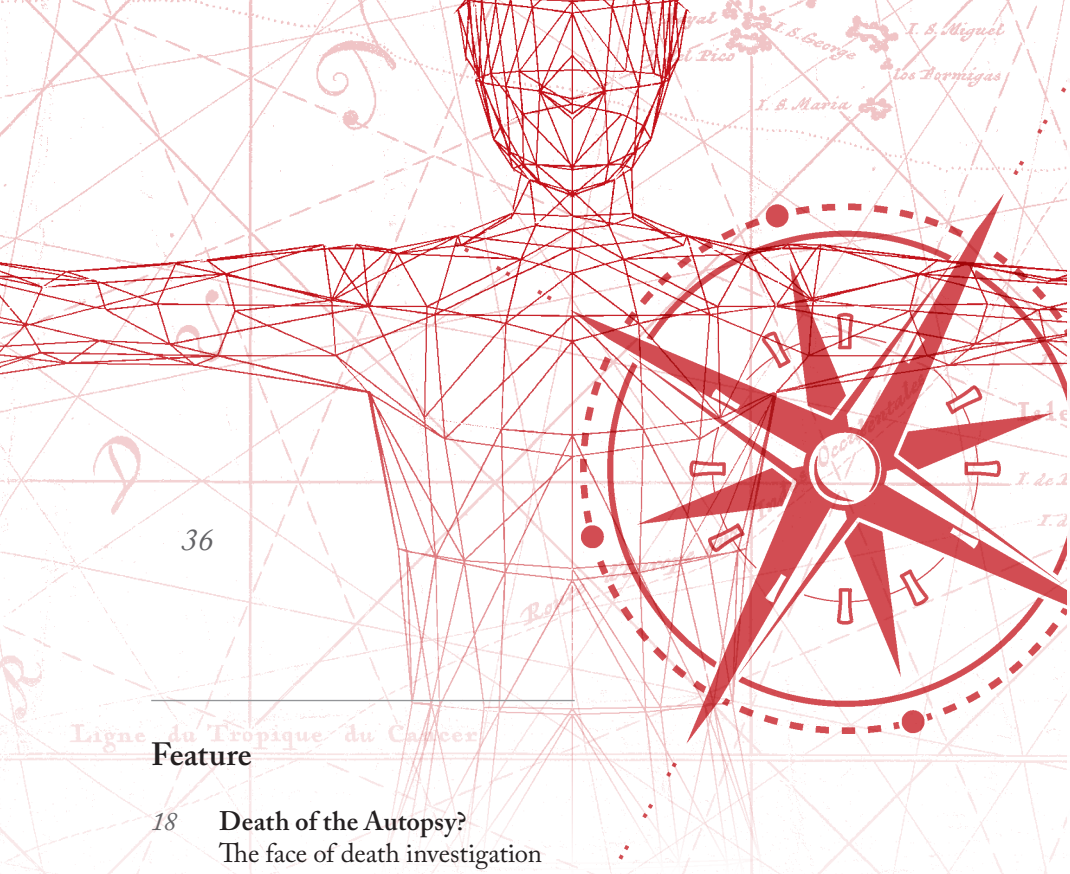


On The Cover

Enrique Simonet, "¡Y tenía corazón! (Anatomía del corazón)." English translation: "And she had a heart! (Anatomy of the heart)," oil on canvas, 1890.

Upfront

In My View



36

Feature

In Practice

NextGen

Profession

Sitting Down With

the Pathologist

ISSUE 33 – AUGUST 2017

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A Time to Reflect

Three years on, many inspirational stories told and issues addressed – and we've only just begun…

We're already racing towards the end of summer (and what a year it has been so far!), which means The Pathologist is about to reach its third anniversary. And, looking back, I never could have imagined what a personally gratifying and exciting journey it would be. We have addressed some fundamental (sometimes controversial) topics that have provoked passionate reaction and conversation among you – a group of professionals often considered by your medic counterparts to be silent and non-communicative! As editors, we have had the immense privilege of working with some truly inspiring people. We've spoken with professionals who care deeply about what they do and who are contributing to the dramatically changing face not only of diagnostic medicine, but of patient care and the overall healthcare machine. Your profession is arguably going through one of the most substantial transformations in decades, and we have been honored to tell the stories of those who are working hard to make their mark – challenging convention but also retaining the important lessons of the past.

To that end, we tackle a big issue in this month's cover feature by questioning where autopsy fits into modern diagnostic medicine, if indeed it fits at all. Run a quick search for autopsy articles online, and you will see gloomy words such as "endangered," "extinct," "miserably low," associated with the practice. It is in huge decline globally. To some, the fall is deemed acceptable: though not overly costly, autopsy is sometimes regarded as non-critical and therefore a great way to save time and money in an already overstretched pathology service. And that seems somewhat reasonable, but not when the practice still holds an immense amount of value. This month, we speak with experts who feel the same way, but recognize the need to seek new and creative ways to conduct the procedure.

As we progress through the rest of this year and beyond, we will continue to address the big topics that are shaping and challenging your profession – it is our ongoing commitment to you. One aspect that will change, however, is the face that you will see atop this page and the masthead. I am delighted to announce that Michael Schubert will be taking up the enviable position of Editor, working with me and my editorial colleagues, Rich Whitworth and Roisin McGuigan, to better serve you and to continue to grow and support The Pathologist community. It has been a real honor to serve as Editor of this publication; heartfelt thanks to you all for your continued support and for sharing your opinions, your work and your inspirational stories. I look forward to continuing this exciting journey with you.

Fedra Pavlou
Editor

Upfront

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

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

Email: edit@thepathologist.com

Finding the Freshman 15

Circulating erythritol levels may predict which young adults are more likely to gain weight during their first year at university

Do you remember the start of your higher education? For many of us, it was our first time away from home, our first time fending entirely for ourselves – and our first experience with the "freshman 15" – the weight gain (of an arbitrary 15 pounds) that affects so many university students during their first year. According to Patricia Cassano, Professor in Cornell University's College of Human Ecology, the phenomenon affects about 75 percent of new students – but why? And is there any way to identify those at risk?

Cassano and her group are trying to answer those questions as part of Cornell's EnHANCE project, an initiative that is trying to solve the medical mysteries of the post-secondary transition. The goal? To improve student health not just in their first year, but throughout their undergraduate education and even beyond. And now, the group may have cracked the code: erythritol, best known as an artificial sweetener, is strongly associated in the blood with an increase in weight and abdominal fat (see Figure 1).

"We found that students who gained weight and abdominal fat over the course of the year had 15-fold higher blood erythritol at the start of the year compared with their counterparts who were stable or lost weight and fat mass over the academic year," says Cassano. Her study used a technique developed by senior co-author Karsten Hiller, Professor of Bioinformatics and Biochemistry at Technische Universität Braunschweig, Germany, to investigate how metabolites are generated and further metabolized: they had participants drink ^{13}C-labeled

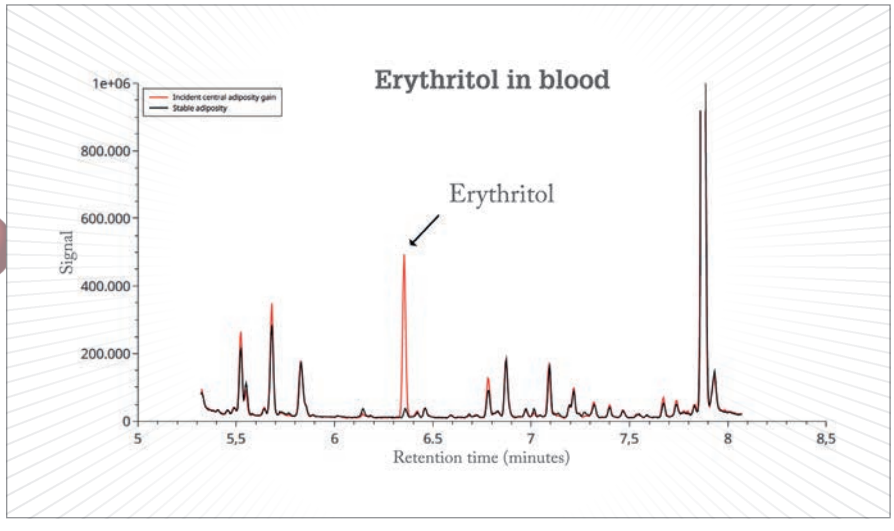

Figure 1. A chromatogram comparing signals in the pooled baseline blood samples for the incident central adiposity group (red line) and the stable adiposity group (black line). The arrow marks the signal for erythritol, and the much higher peak for this metabolite in the pool of participants with incident central adiposity gain is evident.

glucose and followed its path through the body. To their surprise, the labeled atoms began to appear in circulating erythritol. Glucose metabolism, thought to be so well known, had a trick up its sleeve – a previously unknown product of its metabolism. In direct contrast to previous assumptions, erythritol can be both absorbed from food and synthesized by the body (1).

Right now, those most impressed by the new biomarker are biochemists and molecular biologists. But one day, it may hold more significance for clinical laboratories, primary care doctors, and young adults heading off to start their university lives. "Our study raises the possibility that erythritol metabolism may play a role in weight gain and adiposity change," says Cassano. The association between the biomarker and the physical outcomes must be confirmed and replicated; the metabolic pathway itself must be better understood and characterized; and the role of exogenous erythritol has yet to be factored into the equation. The Cornell researchers are working on animal and cell-based studies to decipher the exact biochemistry of the metabolic pathway from glucose to erythritol. "Once we know the exact mechanism, we will be able to shed more light on the link between elevated blood erythritol levels and weight gain," says Cassano. *MS*

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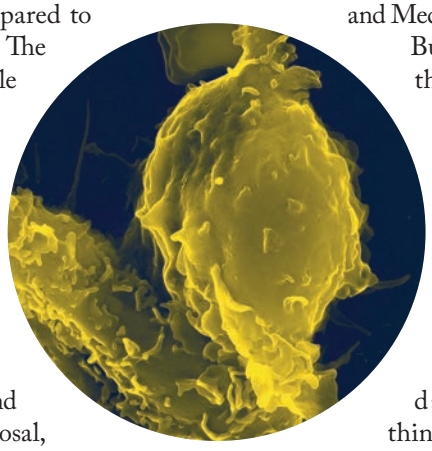
Creating an Immune Inventory

An EHR-derived phenotypic disease catalog has allowed researchers to replicate four decades of work on human leukocyte antigen

Autoimmune diseases are as varied as they are poorly understood, with the potential to affect almost all organs and tissues of the body. There are around 80 recognized autoimmune diseases, and many have been linked to human leukocyte antigen (HLA) – a gene complex that codes for major histocompatibility complex (MHC) proteins, and plays a crucial role in the immune system.

Over the years, many studies have linked HLA genes to a variety of specific diseases – but one team decided to take the wider view. To create a catalog of diseases associated with HLA, they used data from 13,835 people, screening for the presence of almost 1,400 phenotypes using patient samples and electronic health records (EHRs). The resulting phenome-wide association study (PHeWAS) essentially offers a reverse perspective to a GWAS by analyzing many phenotypes compared to a single genetic variant. The results are freely available online for researchers to access and use (1).



"Through this bioinformatics study we have essentially replicated forty years' worth of research on this topic, in one fell swoop. The strong associations we found, and the resources at our disposal, allowed us to do this both quickly and efficiently," says Jason Karnes, Assistant Professor at the University of Arizona College of Pharmacy, and co-first author of the paper.

The research team found strong associations between HLA genes and over 100 diseases, many of them autoimmune. There were also associations with other diseases, such as cervical cancer, but the researchers expected more. "We had a hypothesis that maybe we'd find that some HLA variants protected against cancers, or against infectious disease – diseases outside the autoimmune space. But we didn't find too many surprising results," adds Joshua Denny, senior author of the study and professor of Biomedical Informatics and Medicine at Vanderbilt.

But in many ways, their catalog is just the beginning. "Our hope is that other researchers will be able to use our database as a resource to aid their own research. People will download this data and do new things that we haven't even thought of, which is fantastic," says Denny. The team also have their eyes on other areas that could be studied using this approach. "It doesn't stop with HLA – there's a lot of data that we can create using a similar approach, but looking at different areas of the genome," says Karnes. *RM*

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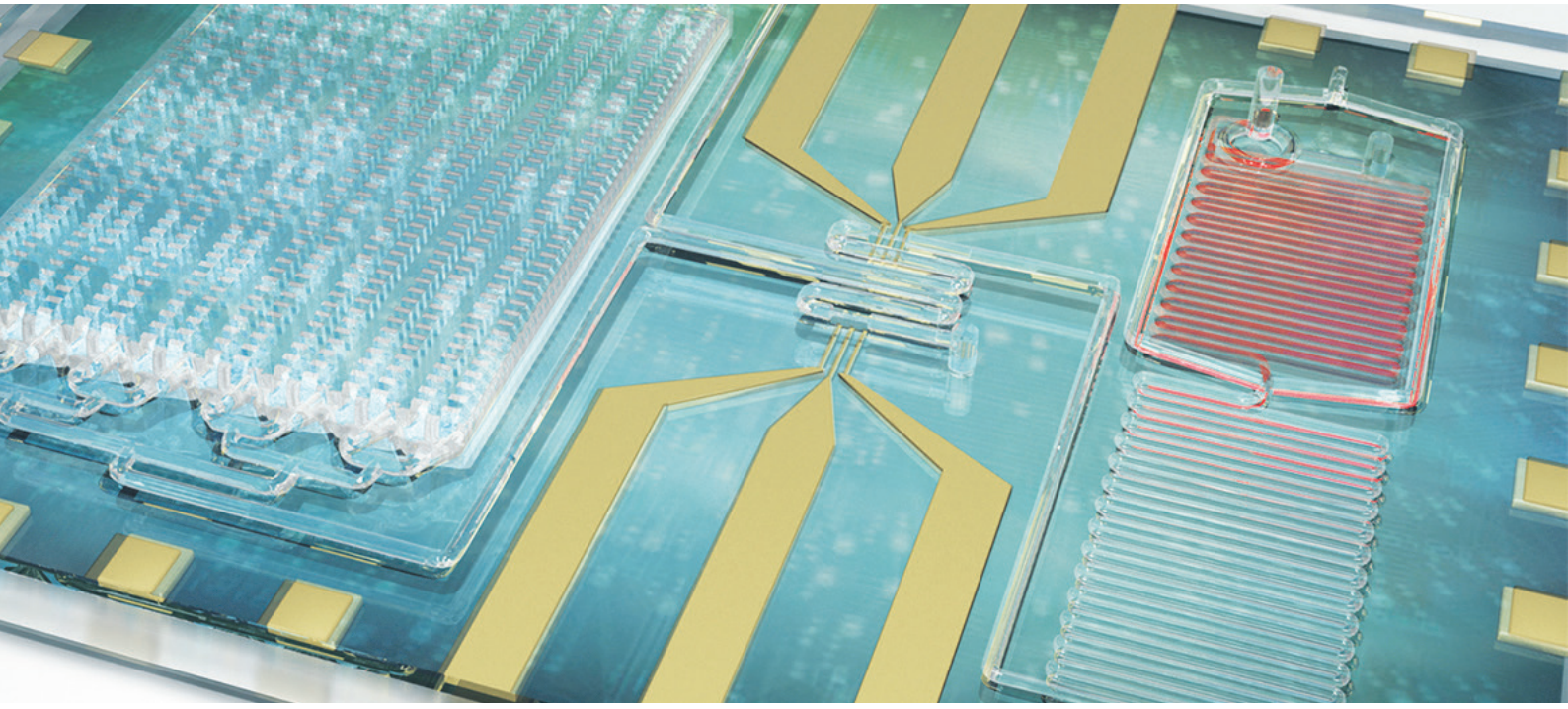

The lab-on-a-chip system that uses a patient's immune response to diagnose sepsis. *Credit: Janet Sinn-Hanlon*

When Minutes Matter

New tests can speed up the diagnosis of severe sepsis, ensuring patients get the right treatment before it's too late

Sepsis is one of the most time-critical diagnoses a hospital can make. In the most severe cases, it's estimated that patients' likelihood of survival decreases by 7.6 percent each hour that passes without effective treatment (1). But with common symptoms like fever and pain, it can be difficult to conclusively identify sepsis in a timely manner.

Fortunately, science is on the case. Two groups of researchers have recently published tests that promise rapid, reliable diagnosis of sepsis: one, a new PCR-based method, and two, a portable lab-on-a-chip device.

The first, a TaqMan-based multiplex real-time PCR detection system, probes conserved regions of the 16S rDNA gene in 10 common bacterial pathogens (2). It not only detects the organisms causing sepsis, but also positively identifies them in a matter of hours, ensuring that patients can receive appropriate antibiotic treatment as soon as possible – and freeing doctors from the need to wait a day or more for blood cultures to provide the same information.

The second test takes a unique approach – instead of looking for the cause of infection, it detects the patient's immune response (3). How? The device takes a complete white blood cell count, a neutrophil count, and measures levels of the CD64 neutrophil cell surface marker. As the immune response increases, so do these numbers, giving doctors a rapid heads-up that the patient's condition is deteriorating. In some cases, the immune response can spot sepsis even before the causative pathogen is detectable in the blood.

"We think we need both approaches," said Rashid Bashir, senior author on the latter study (4). "Detect the pathogen, but also monitor the immune response." *MS*

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CEA: Overlooked and Underused

Could an existing blood test improve treatment choices – and outcomes – for some colon cancer patients?

A Mayo Clinic study has found that a simple blood test could improve treatment for nearly one fifth of patients with stage II colon cancer; but many who could benefit from the test are not receiving it.

The test measures levels of carcinoembryonic antigen (CEA), which has an established link to patient prognosis, and can help to predict recurrence-free survival. But researchers found that it could also play an important role in selecting the most appropriate therapy for some patients (1).

Using information from the National Cancer Database, the team studied over 40,000 patients and found that the results of the CEA test could improve colon cancer staging predictions, raising the risk from average to high in 17 percent of stage II patients. The new classification could have affected potential treatment options, including the decision to use chemotherapy. The researchers also found that adjuvant chemotherapy following surgery appeared to reduce the increased mortality associated with stage II patients with an elevated CEA level.

"The decision to give a patient chemotherapy after surgery is not a light one, and physicians must weigh the risks and benefits," said Kellie Mathis (2), a Mayo Clinic colon and rectal surgeon and senior of the study. "There is no good reason for a physician to omit this blood test, and more work needs to be done to ensure that all patients receive it." *RM*

Reference

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Sampling the Spectrum

Blood biomarkers may offer reliable early diagnosis of autism spectrum disorder

Most parents of children with autism will agree that early diagnosis and intervention is best. But autism isn't always easy to diagnose – the label encompasses a broad spectrum of traits and may present differently in every patient. As a common saying in the community goes, "If you've met one person with autism… you've met one person with autism."

So how can doctors contend with this diagnostic challenge and ensure patients are treated as early as possible? New research from the University of Texas Southwestern Medical Center has shown that levels of two blood biomarkers, measured together, can accurately diagnose autism in over four-fifths of patients. The study examined a total of 82 boys (43 with autism spectrum disorders; 39 typically developing). In the former group, levels of thyroid stimulating hormone (TSH) were significantly suppressed, whereas levels of interleukin-8 were significantly elevated. Each biomarker predicted autism with approximately 75 percent accuracy, and combining them increased the overall accuracy to 82 percent.

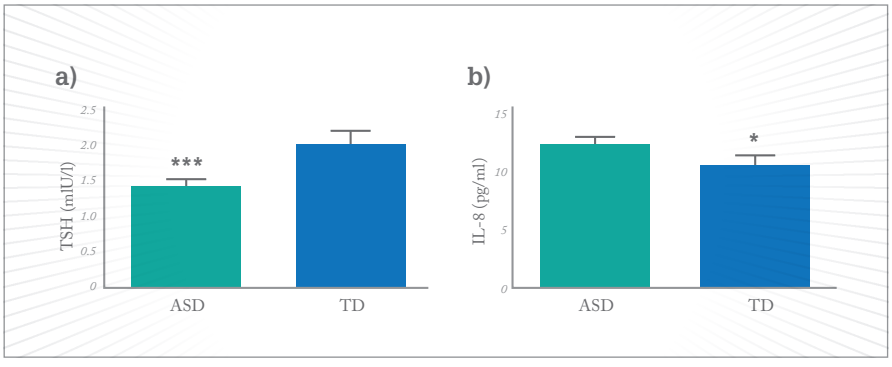

Figure 1. Serum levels of a) thyroid-stimulating hormone and b) interleukin-8 in boys involved in the study. TSH is significantly lower and IL-8 significantly higher in boys on the autism spectrum than in typically developing boys. Adapted from (1).

The study's authors suggest pursuing panels of blood proteins as a method of accurately identifying and characterizing autism spectrum disorders. Their next step: to increase their sample size, and to add two further proteins (apolipoprotein E and stem cell factor) to their panel with the hope of improving overall diagnostic accuracy. *MS*

References

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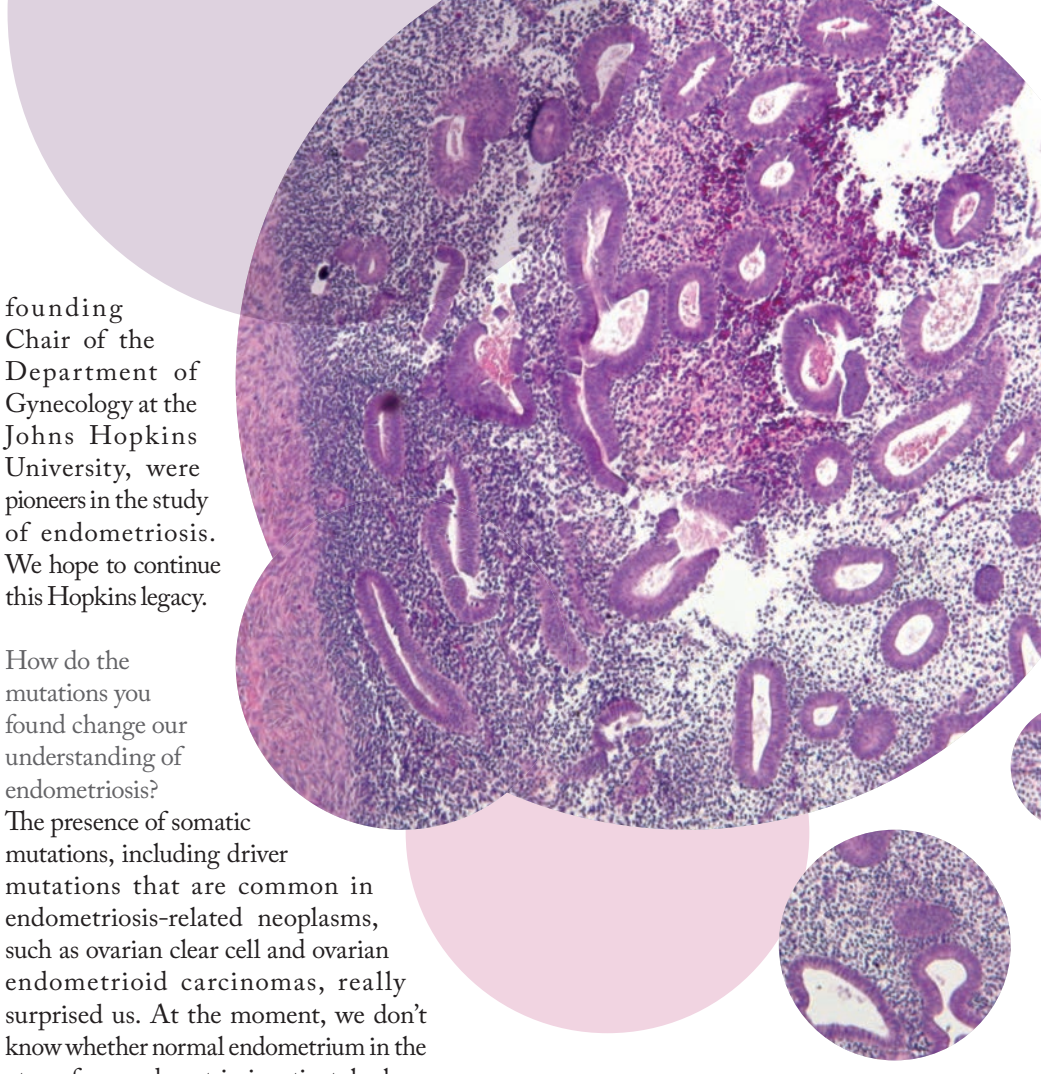
The Endometrial Exome

Sequencing of tissue in endometriosis patients turned up some puzzling results – and could eventually lead to better classification of the disease

In endometriosis, endometrial tissue grows outside of the uterus. More than half of those affected experience chronic pain, but the amount and location have little correspondence to the stage of disease. Staging is complex, and requires a survey of the lesions present. One international team of researchers decided to take a different approach, by sequencing the exomes of 24 women with benign endometriosis, with the eventual aim of developing a system for classifying endometriosis with a genetic basis. We asked Ie-Ming Shih, co-author of the associated paper (1) and Professor of Gynecology and Obstetrics at Johns Hopkins Medicine, about the implications of the findings.



What motivated this study?

Endometriosis is a highly intriguing lesion in terms of its originality and clonality, as well as its cancer-like behavior. Although it has been almost 90 years since the lesion was first described, very little is known about the etiology and molecular pathogenesis of this common condition. Interestingly, its molecular landscape has not been elucidated in the post-genomic era, even though the genomes in most human tumor types and lesions have been thoroughly sequenced. As a gynecologic pathologist and a cancer researcher, I think the time has come to apply cancer research tools to study benign diseases like endometriosis. We've also been inspired by our institution's own history – John Sampson, a former student and resident, and Richard TeLinde, the founding Chair of the Department of Gynecology at the Johns Hopkins University, were pioneers in the study of endometriosis. We hope to continue this Hopkins legacy.

How do the mutations you found change our understanding of endometriosis?

The presence of somatic mutations, including driver mutations that are common in endometriosis-related neoplasms, such as ovarian clear cell and ovarian endometrioid carcinomas, really surprised us. At the moment, we don't know whether normal endometrium in the uterus from endometriosis patients harbors any somatic mutations, and whether the mutations found in endometriosis are also present in the corresponding uterus.

In cancer, driver mutations are sufficient or even essential for tumor growth and progression – invasion, metastasis and acquisition of drug resistance, and so on. But those mutations in normal-appearing epithelium in an endometriotic lesion are puzzling. One can speculate that mutations such as *KRAS* and *ARID1A* may contribute to the survival of ectopic endometrial tissue outside the uterus. These mutations can also equip endometriosis with the invasive capacity to deeply infiltrate into peritoneal tissues (the tissues covering the organs in the abdominal cavity) and the bowel wall. But the main challenge is to develop an endometriosis animal model to start addressing these possibilities by manipulating specific gene mutations and expression in endometrial tissues.

Do you see potential in forecasting endometriosis patient outcomes?

This is what we are hoping for. The current classification and staging of endometriosis, although clinically useful to some extent, does not provide a correlation with clinical presentation or a prediction of disease behavior. Our next study will aim to answer this question.

We are going to study large cohorts to determine whether there is any association between certain mutation patterns and clinical presentation and outcome. With the advances in molecular pathology, we are in a good position to perform molecular tests for endometriosis if we are able to demonstrate a positive correlation.

Reference

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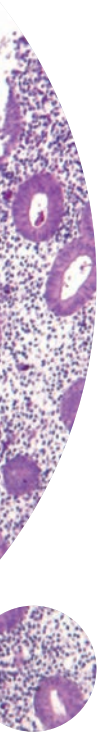

A New Gold Standard Test for PDAC?

Nanopore and plasmonic technology could speed diagnosis of pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is challenging to treat – able to survive with limited blood supply and low oxygen, and with the ability to protect itself from the immune system, the disease causes few symptoms in its early stages. As a result, PDAC is often diagnosed when the disease is more advanced – and when the prognosis for patients tends to be bleak.

Earlier diagnosis is likely to translate to improved survival, so a team of researchers, including Cesar Castro, Assistant Professor of Medicine at Harvard Medical School, have developed a new diagnostic assay that performs better than the current blood test and has the potential to diagnose PDAC earlier. "No current blood-based laboratory test exists to reliably identify early PDAC without high rates of false positives, causing undue alarm for both patients and providers," said Castro. "With reliable early detection methods, we would expect significant rather than incremental improvements for patient survival," he added.

The test uses nanotechnology to detect tumor-derived extracellular vesicles (tEVs) in plasma. These vesicles, which are structurally stable, relatively abundant, and have a biological makeup very similar to the main tumor, should be able to act as "serial peripheral windows" into PDAC tumors, without the need for tissue biopsy, according to Castro.

The tEVs are detecting using a plasmonic sensing system, in which the light emitted through gold nanopores is measured – the tEVs are bound via monoclonal antibodies immobilized on the surface of the pores, and this causes a spectral shift in the light that passes through the pores.

After initially studying around 50 proteins, the team found that individual tEV markers didn't appear to be accurate enough to be clinically useful in isolation. However, a panel of five tEVs produced an accuracy of 84 percent, a sensitivity of 86 percent and a specificity of 81 percent when differentiating PDAC from other pancreatic diseases (1). Analysis takes 10 minutes and costs US$60 per patient – $42 for the chip, and $18 for the cost of the antibodies.

"Rather than seeking one universal, yet elusive PDAC marker that likely does not exist given human biological complexity, our work identified a multi-component PDAC signature with excellent diagnostic performance," said Castro. Next, the team plans to demonstrate the real-world performance of the test, and look into its potential use for screening patients at high risk of developing PDAC.

Reference

1. *KS Yang et al., "Multiparametric plasma EV profiling facilitates diagnosis of pancreatic malignancy", Sci Transl Med, 9, eaal3226 (2017). PMID: 28539469.*

In My View

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

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

Contact the editor at fedra.pavlou@texerepublishing.com

A Failed Model

We need biobanks to operate efficiently and successfully – and that means taking a lesson from an unlikely source: the automotive industry

Dominic Allen, Chief Operating Officer of the Integrated BioBank of Luxembourg, Luxembourg

I have to agree with the point made in "Curating Pathology's Future" (1) that, right now, biobanks are not providing the service they are designed to deliver.

As a member of our Society had the courage to say, "Ours is a failed model." We are loading biobank freezers with samples that will never be used. What better illustration than the seemingly desperate offer of "samples for free" (2) from a European organization representing a list of prestigious biobanks? In the past, we griped that no one was willing to pay the true value of our samples – and now we have to give them away. What happened?

Medical research in the era of personalized medicine is based on the analysis of samples with clinical data – and, because the associations are often weak, we need these samples in large numbers. TIME'S March 2009 cover feature (3) reinforced this idea by declaring biobanks one of the "10 Ideas Changing the World Right Now." It was clear to everyone that the more samples were available, the faster research would advance and the healthier we would be. At the same time, there would be more recognition – and therefore more funding – for biobanks. We had also learnt that the value of a sample goes up with time, because we are able to look back at samples from previously healthy patients, potentially opening the door to discovering predictive biomarkers and heading diseases off at the pass. So, naturally, we launched large population cohorts to amass as many samples as possible. Now there are perhaps 100 million samples in stock in Europe and no one seems to want them – at least, not in numbers that justify the cost of collecting and storing them. Why?

It's not about the money. The prices are taxpayer-subsidized – and, as any biobanker will tell you, when an impoverished researcher can't afford to pay for samples, he gets them anyway.

The problem with biobanks is analogous to one that troubles the research world as a whole. Research output is notoriously difficult to evaluate, so funding bodies tend to use simple measures, such as numbers of publications, to evaluate research centers and allocate future funds. It's no wonder that we now have a tsunami of publications, many of piteous quality. For biobanks, one of the simplest measures of "success" is the number of samples in stock – and we have a biological sample mountain! Number in stock has become the virility symbol of the biobanking

"When an impoverished researcher can't afford to pay for samples, he gets them anyway."

world: "Mine's bigger than yours!"

But we are confusing two value models. We need to learn from now-successful industries that, 40 years ago, were where we are now.

The first model is that of vintage wine, which appreciates in value over time. Cohort samples, too, appreciate over time. But what proportion of samples and data used for research actually need to be longitudinal? Five percent? Ten? We are fusing the vintage wine model with the conviction that more is better to justify building up massive stocks of samples, most of which do not need to be aged. We mix up the need to have longitudinal samples – true for a small fraction of research – with the need to have a large stock from which to satisfy any specific demand. We delude ourselves that, if only we could have enough in store, we could supply all demand from stock. We fail to see that there are so many variables in a sample that, to have one that corresponds to a specific need, we must have hundreds or even thousands in store. And the delusion suits us, as it justifies the need for massive inventories and comforts our funding bodies on the road to approving big budgets.

That brings me to the second model: cars. Some readers will remember the good old 1960s and forecourts full of new cars for sale. You went to the garage, looked for the car you wanted, rarely found the combination of model, color, engine, and options you had hoped for, and bought a compromise. When did you last see a forecourt full of cars for sale? Every automobile manufacturer has now adopted the Toyota revolution of the 1970s and there is, to a first approximation, no more stock. Instead, you get the car you really want in a matter of weeks, made to order.

Stock is waste. It costs money to store; it degrades; it becomes obsolete; and it is discounted or discarded. It's often the result of processes with high setup costs and times. And stock in a process provides a buffer that masks operational inefficiencies and poor quality. Eliminating stock, producing to order, delivering when needed: this is basic manufacturing good practice. But what is its relation to biobanking?

"Number in stock has become the virility symbol of the biobanking world: 'Mine's bigger than yours!'"

All signs point to the same problem. Storing samples costs money; samples degrade at -80°C and FFPE tissue samples at room temperature; they certainly become obsolete (who wants old samples without pre-analytical data?); and we are already seeing discounting and discarding of collections. Setting up ethics and Data Protection Authority approvals, sponsor, PI, clinicians, contracts, CRF, collection kits, LIMS and logistics can take a year. Longitudinal cohorts will remain a "vintage wine" business, but they represent a minority of the samples actually needed for today's research. It's time to look at the two models we use for collecting non-longitudinal samples – "open collection" and "project collection" – and see how we can do better.

In the open collection model, we store samples today hoping they will correspond to tomorrow's needs. It provides the advantage of samples immediately available to researchers, but suffers from the same crippling disadvantages as the 1960s car manufacturing model and most biobanking today – uncontrolled stocks. Researchers seldom get exactly the samples they want; biobanks have high

"In our zero-growth research budget world, the choice is to collect smarter or not collect at all."

storage costs and high levels of waste. And there is a further hidden cost: the cycle from production to client feedback is long and the voice of the customer faint. If we produce stock today, it might be years before customers tell us that our quality does not correspond to their needs. If, in a few years, the focus of medical research moves to metabolomics and proteomics, we will simply throw away today's stocks as not fit for purpose.

The project collection model, in which we collect to order for specific research work, should produce the samples needed, but involves high setup costs and long lead times. Nonetheless, this is the way the whole biobanking business needs to evolve. So how can we dramatically reduce the setup times and costs of this model? And how can we collect fast enough to avoid delaying research? The answer lies in trusted collection networks and broad, ongoing ethics approval. The goal is to form small, flexible, ad hoc groups of biobanks for each sample request, based on the type of sample and the difficulty of collection. Biobanks should build up their own networks – preferably local – of others with ethics approvals, similar validated protocols, compatible quality standards and quality control, common data items, pre-agreed Material Transfer Agreement formats and pricing structures, and solid personal relations.

This is not a model suitable for centralization. Requests will continue to come to biobanks, and the responsibility to deliver will always lie with a biobanking institution. Clients want to deal with the "factory," not the intermediary, and prefer a single contact point. The model provides for this: one lead biobank (which is compensated for project management) establishes the precise need, checks the feasibility in its network, takes on the contract, establishes the ad hoc collection group, coordinates the project, and provides the point of contact. It's a self-organizing system that can start tomorrow. Each biobank establishes its own network and independently markets its own capabilities. Quality standards between biobanks will naturally converge as clients preferentially do business with those offering the price and quality they require, and eventually, international bodies will formalize these standards for the benefit of all.

The "vintage wine" collection model has a different challenge. By definition, population cohorts generate stock – but we can improve the depressingly low utilization rates by reducing the stock needed to provide a given number of samples, rather than by artificially boosting usage rates. One way to do this is to focus cohorts on specific disease domains and enrich enrolment by consciously biasing recruitment towards those with risk factors. This may be anathema to "no-hypothesis" epidemiologists, but in our zero-growth research budget world, the choice is to collect smarter or not collect at all.

To criticize the current collection model is not to question the importance of biobanks – just as criticizing car manufacturing practice is not questioning the importance of the automotive industry. Biobanks are indispensable to medical research and they need to be funded. The evaluation criteria on which to base funding remain a challenge – but at least let us replace number in stock with number distributed, or ratio of stock to distributed, and introduce a way to measure service.

Let us conclude with a thought experiment. If there are 100 million samples in stock in Europe, and medical research projects each consume 100 samples, take three man-years, produce two publications and cost €500,000, then using up the samples would require a million research projects, occupy three million man-years, generate two million publications, and cost €500 billion. Don't ask who would read – let alone peer review – those publications, but at least the funding bodies would be happy and the funds would continue to flow!

A note on biobanking and business: This "In My View" article uses business vocabulary and logic for the simple reason that we are dealing with an exchange in which some parties supply products and services others wish to use, money changes hands, and the user has choice. This is the definition of a competitive marketplace. But we must remember that the object of the exchange is personal biological material donated by altruistic individuals and covered by legal and ethical constraints. The use of business language in no way detracts from this – but for everyone's good, we need to identify and use the operating model that most efficiently matches supply with demand and reasonably covers costs. Medical research depends on it, and business language can help deliver it.

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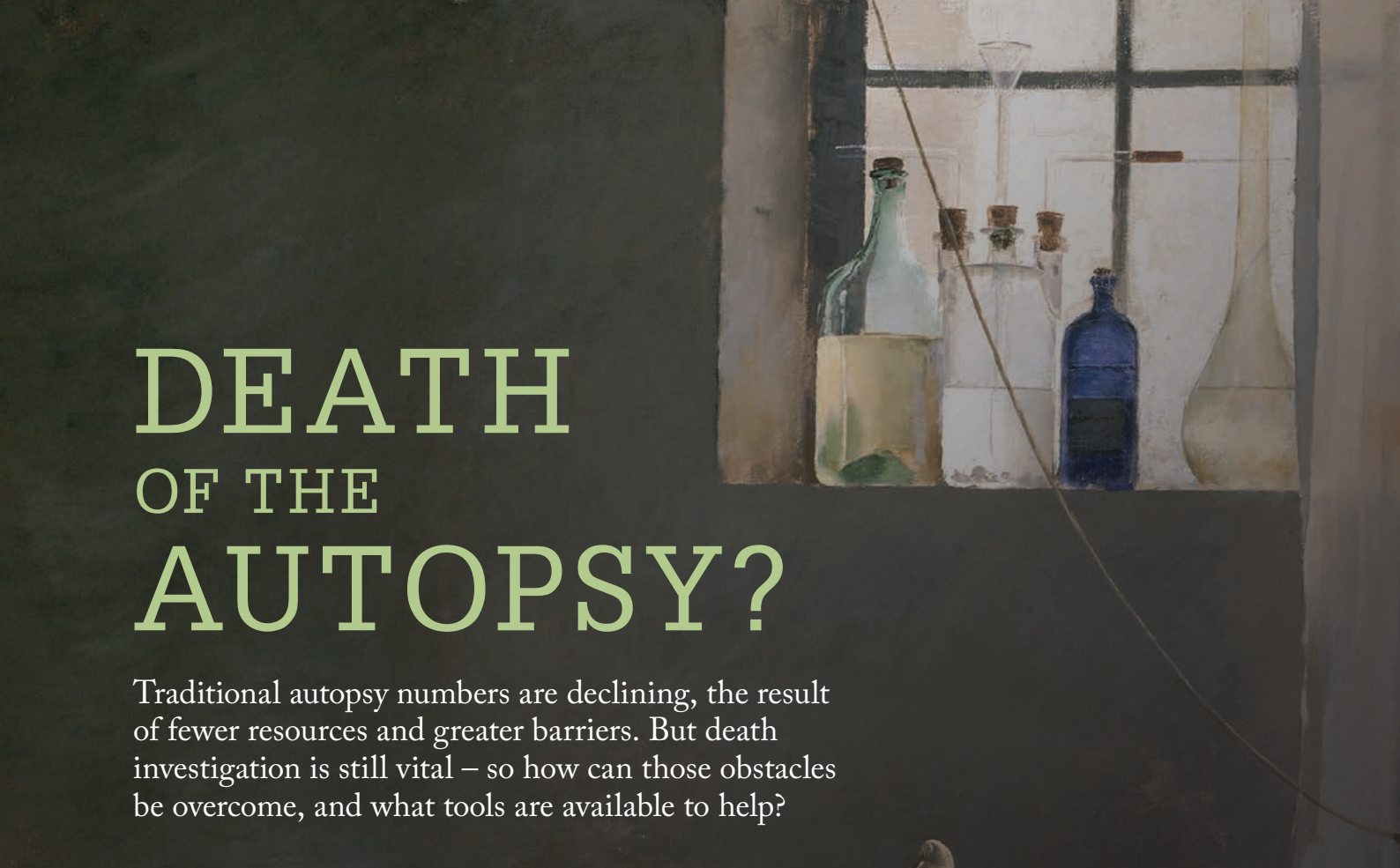

DEATH OF THE AUTOPSY?

Traditional autopsy numbers are declining, the result of fewer resources and greater barriers. But death investigation is still vital – so how can those obstacles be overcome, and what tools are available to help?

FINDING ANSWERS IN ANGIOGRAPHY

Post-mortem cross-sectional imaging, supplemented with angiography, can provide a minimally invasive alternative to traditional autopsies

By Guy Rutty

The complete diagnostic autopsy has changed little since its inception. It involves invasive surgery to examine the three body cavities – head, thorax and abdomen – and works on the assumption that it will provide enough information to identify a cause of death on a balance of probabilities.

But that examination omits many parts of the body, meaning it's possible to miss significant information – especially if the person conducting the autopsy lacks training, experience, or sufficient time for a thorough investigation. That's where post-mortem cross-sectional imaging – computed tomography (CT) in adults and magnetic resonance imaging (MRI) in children – enters the picture.

Post-mortem computed tomography (PMCT) alone can identify 60–70 percent of natural causes of death; that alone isn't sufficient, so the technique is most commonly used as an adjunct to invasive autopsy. Why does PMCT fail to provide an answer in so many cases? The principal reason is that you can't confidently diagnose coronary artery disease – a leading cause of natural death – with PMCT alone. To see that, you have to add in angiography (PMCTA), which increases the diagnostic success rate to about 92 percent of all natural causes of death, as well as 100 percent of non-suspicious trauma cases (1). So if you want to know absolutely everything about a particular case, you do both – but if you need a more volume-based system, you can save time and resources by starting with PMCTA, triaging out those cases that need to be autopsied.

How it's done

We use a triage system to determine the investigations needed for each case – the idea being that you don't go any farther through the process than you need to answer your specific question (2).

We begin with a clinical history, information on the place of death, and establishment of "the question." An external examination follows to ensure there are no suspicious aspects to the death; at that stage, we also prepare the body for PMCTA – and, if necessary, take toxicology samples.

The PMCTA procedure itself begins with the insertion of a catheter into the left common carotid artery through a small (~2 cm) incision at the base of the neck. We advance the catheter to just above the aortic valve of the heart, inflate a balloon to secure it, and at the same time insert a small tube into the airway to ventilate the lungs and enhance their imaging. Then we perform a native CT scan – that is, we scan the body from head to toe without contrast or ventilation to check the position of the catheter and, if possible, identify the main pathology.

At that stage, if there is a life-threatening pathology – for example, a ruptured abdominal aortic aneurysm – we can stop the process, because we have answered the question. If no such finding is present, we then inject a small quantity of both air (negative) and then positive contrast medium into the coronary arteries for a double-contrast examination. To distribute the contrast, we have to roll the body; we do that five times and image the heart and vessels each time. We also ventilate and image the lungs. Finally, we remove the catheters, close the incision, and place the body back into storage.

We then report those images – and if, on a balance of probabilities, there is a cause of death that is consistent with the external examination and the history, then we provide a cause of death and go no further. If not, we decide whether to proceed with a limited or a full autopsy (triage), which would hopefully yield any missing information. Laboratory tests would be next in the chain; we simply keep investigating until we answer the question.

Where it came from

About 100 years ago Upton Sinclair said, "It's difficult to get a man to understand something if his income depends on him not understanding it." I think this could be one of the reasons why PMCT has not been widely adopted to date.

The technique has been used in autopsy practice since the early 1980s, but always as an adjunct to traditional methods. In 1994, a group in Israel undertook a study of suspicious deaths and concluded that, in trauma cases, PMCT could replace autopsies. However, the outcome was not very well received by the general pathology community.

Since then, though, the field has undergone transformational change: improved technology and software – and a more open view to the concept. A number of research groups around the world – in Switzerland, Japan, and the United Kingdom – have looked at different aspects of minimally invasive autopsy. The result? In 2017, we (my co-authors and I, and undoubtedly a wider number of researchers, practitioners and authors) agree with the 1994 research that the vast majority of death investigation could be done purely with PMCT. So in essence, we've come full circle; we've established an evidence base for the early work and come up with the same answer.

Why is PMCT now at the forefront? Firstly, I think there is a perception that members of the public don't want traditional autopsies; there are cultural, religious, ethical, and even aesthetic concerns – and minimally invasive techniques are often acceptable where a complete diagnostic autopsy may not be. Secondly, we use the technique more than ever now, and I myself know that it yields equivalent – if not better – findings than actually dissecting the body. It makes me question my own practice, and that's a good thing. If I know that there's an equivalent or better system to achieve the same result, why aren't I using it?

To PMCT or not to PMCT?

We use PMCTA whenever we need to image blood vessels. Our standard technique targets the coronary arteries, but there's another method – multi-phase PMCTA – that looks at the other vessels of the body. So it comes back to asking the right question; you must decide what you want to learn from your investigation, and then you can decide which of the two techniques is better suited to your needs.

There are also situations where PMCTA is not the solution. At the moment, we can't confidently diagnose sepsis, meningitis, gastrointestinal bleeds, or pulmonary thromboemboli with these minimally invasive techniques. You might still use them, but only as an adjunct to a limited or even a full autopsy. We're trying to improve PMCTA for those scenarios where it is not yet ideal, though; at the 2017 meeting of the International Society for Forensic Radiology and Imaging, our unit presented work on a system that identifies most, though not all, pulmonary thromboemboli (publication in development). It's a system I'm optimistic will increase the diagnostic ability of PMCTA further toward the 100% goal for natural death.

At the Victorian Institute of Forensic Medicine in Australia, I understand they perform approximately 6,000 of these examinations every year. In Scandinavia, most mortuaries have their own CT scanners and use them routinely. Large areas of continental Europe have adopted PMCTA for death investigation, and it's now even making inroads into the United States. Penetration into the UK has been comparatively slow thus far, but it's gaining momentum. All of the research proving its suitability has already been successfully completed, so all that's left is to engage in the process – and, of course, to overcome issues of cost and bureaucracy. But I envisage that it will become the standard form of autopsy examination in the

not-too-distant future – and that invasive autopsy will be limited to specific cases.

Who is responsible?

Right now, there is some debate as to who should report the images when undertaking PMCTA. In most countries, the pathologist reports the images under the guidance of a radiologist – but some people argue that it should be the other way around.

My colleagues and I at Leicester run the only UK-based training programs for people who want to work on PMCTA (3). We train the mortuary staff – the APTs and the radiographers – to prepare and scan the bodies. We also help educate radiologists, because they know the anatomy and the disease processes, but need minor additional training in relation to the changes that occur after death and the medico-legal questions they haven't previously dealt with. Pathologists, too, need additional training if they plan to report scans – I, for instance, report all my own scans under the guidance of a radiologist, but I've had to learn how to read CT scans and use diagnostic software. Personally, I don't think either way – having pathologists report under radiologists' guidance or vice versa – is objectively better. All that matters is that it's done properly, and that the person who does it understands the changes that occur after death and the questions being asked.

Importantly, though, I'd like pathologists to know that they don't need to worry about this kind of change. I've heard concerns – "Does this mean we aren't going to do autopsies anymore?" "Are we no longer involved in death investigation?" – but that's not the case at all. Rather, it gives us a completely new perspective on the body. We can see and appreciate things that just can't be seen or appreciated in a traditional autopsy. Modern scanners and software generate detailed images and even allow users to virtually manipulate the body in 2D and 3D, to the point where I truly believe that CT should be the gold standard of clinical practice in the dead – just as it is in the living. So if you really want to fully understand the human body and the process of death, then my advice is: get involved. It will change your entire autopsy practice, revitalize your interest in it, and take you into a world that you just can't fully appreciate any other way.

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POST-MORTEM POTENTIAL

Changes to the UK's system of death investigation must improve the service and increase awareness of its value

By Suzy Lishman

I don't think it is overstating things to say that there is a crisis in the provision of post-mortem services in the United Kingdom. Almost all post-mortems performed are for the Coroner, with very few hospital or consented examinations taking place. Coroners are increasingly finding it difficult to get their post-mortem examinations done, as pathologists are in short supply these days and must concentrate on diagnostic work for the living.

A recent report on forensic pathology and coronial post-mortems (1) sets out the current challenges. But it's getting worse. My hope is that we can develop a new national service for the investigation of deaths, as the report recommends. Unfortunately, three different government departments – Justice, Health, and the Home Office – are involved, so coordination is complex. The Royal College of Pathologists is working with all of the agencies involved to try to find a solution.

What surprised me most when I began practicing pathology was how little understanding many of my clinical colleagues have about the work we do. It's not unusual for a consultant to fail to request a post-mortem on a patient because they truly believe there is nothing new to learn. Educating service users about the role of pathology and the value of the post-mortem examination remains a major challenge.

The changing face of death investigation

A national system of medical examiners (MEs) was proposed in the UK in 2005. MEs are experienced doctors who can scrutinize and confirm the cause of all deaths that do not fall under the coroner's jurisdiction – but they don't have to be specialist post-mortem pathologists; they can come from any specialty. Unexpectedly, it has taken – and, in fact, is still taking – much longer than anticipated to introduce MEs across the country. Although the legislation was passed in 2009, progress since then has been slow.

In 2016, the Secretary of State for Health announced that MEs

would be introduced in April of 2018. At that point, a consultation exercise was undertaken – but we are still awaiting the outcome of that exercise, and the implementation date has been further postponed to 2019. The College, which is the lead organization for the recruitment and training of MEs, has campaigned for many years for their introduction and believes that they will make significant contributions to improving the accuracy of death certification, collecting data to inform health policy, and improving the quality of care for patients.

The introduction of the Human Tissue Act in 2006 is another factor that can affect death investigation. The Act brought about much tighter regulation of the removal, use and storage of human tissue in England, Northern Ireland and Wales. The new regulations, which apply to any material from the human body that includes cells (including bodily waste), made it an offence to remove, store or use tissue without appropriate consent. Premises where human tissue is used now need a license for treatment, research, post-mortems, anatomical examination and public display. There are penalties of up to three years' imprisonment for failing to obtain appropriate consent for the use of tissue or storing tissue without a licence. Although pathologists recognized the need to update previous legislation, there were significant concerns about the bureaucratic burden and expense of implementing the act and the detrimental effect on research.

With the strengthened regulations for handling human samples, the forthcoming introduction of medical examiners, and potential future changes to forensic pathology and post-mortem examinations, we are hoping to overhaul the UK's system of death investigation for the benefit of our patients – both past and future – and their families.

Suzy Lishman is President of the Royal College of Pathologists. She is Head of the Department of Cellular Pathology at Peterborough City Hospital, Peterborough, UK.

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A SIMPLER SYSTEM

Minimally invasive autopsies can improve death investigations and health – even in settings with limited resources

By Jaume Ordi with Paola Castillo, Quique Bassat and Clara Menendez

The complete diagnostic autopsy (CDA) is often considered the gold standard of death investigation. But in many situations, such an examination is not possible – for instance, when necessary facilities are lacking or in areas with cultural or religious taboos. Unfortunately, less invasive methods are not always possible in resource-limited settings either, because many of them rely on expensive equipment and techniques, or on the availability of highly trained professionals. And yet, the need for death investigation is greater than ever in such settings – so what can be done to balance the importance of conducting such investigations against the difficulty in doing so? The answer lies in a simplified method of minimally invasive autopsy that requires less time, less training and less overall effect on the subject of the examination.

What is minimally invasive autopsy?

As the name suggests, a minimally invasive autopsy (MIA) aims to minimize the impact of the pathological examination on the body. The MIAs developed by our team are specifically designed for low- and middle-income areas with limited trained personnel and resources. In general, MIAs in high-income countries involve highly sophisticated imaging techniques, such as magnetic resonance or CT scan, combined with directed biopsies. Our simplified MIA procedure (see Figure 1) begins with a careful disinfection of the body surface (1,2), after which we collect blood and cerebrospinal fluid samples and perform blind puncture of solid organs such as the liver, lungs, heart, and central nervous system using biopsy needles. Finally, we apply histological and microbiological techniques to analyze the samples.

Compared with the CDA, the MIA is much simpler and does not require a fully trained pathologist; medical agents, nurses, or even trained technicians can perform MIAs of adequate quality, which is a major advantage in many low- and middle-income countries. Additionally, the minimally invasive approach and strict disinfection offer some advantages in the realm of infectious disease. Indeed, those features allow a good assessment of the microorganisms present on the body, something seldom possible in the CDA because of the high contamination rate that ensues from dissection. Finally, MIA is a non-disfiguring technique and more rapidly completed than CDA, which represents a significant advantage in many cultural environments. Indeed, our team's socio-behavioral studies indicate that MIA is more acceptable than CDA in a range of different geographical, cultural and religious communities.

When should it be used?

MIA has shown high accuracy for disseminated neoplasms, as well as in infectious disease diagnosis. One of its most notable advantages is that it allows the identification of the specific microorganisms

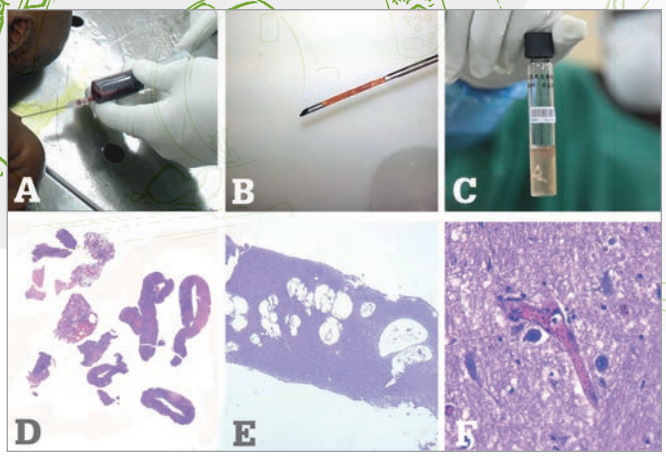

Figure 1. a) Blood sampling through supraclavicular puncture; b) tissue sampling with biopsy needle; c) formalin jar with tissue sample; d) H&E stain of MIA sample; e) MIA brain tissue showing severe cryptococcal infection; f) massive Plasmodium falciparum parasitization of erythrocytes in brain capillaries.

causing mortality – meaning that it could significantly improve our understanding of causes of death in areas and age groups where infectious diseases are common (3,4,5). On the other hand, MIA is less accurate for some non-infectious illnesses, such as cardiovascular or digestive diseases, and for identifying internal malformations in the perinatal group. Our team is now working on new procedures to increase the tool's accuracy in such cases.

MIA, as it currently stands, has one major caveat – it is still a costly procedure. Biopsy needles are very expensive, and in cases where infectious disease is the suspected cause of death, the technique requires molecular microbiological analyses to accurately identify the causative microorganism. The real obstacle here is that these tests necessitate frozen tissue, which is logistically complex in many settings. We haven't found a good way around that yet – but we are analyzing the value of each specific sample and test to optimize and reduce the protocol.

With so many obstacles, why conduct autopsies?

Autopsies have a long history of correcting, clarifying and confirming ante-mortem clinical diagnosis. They help physicians improve their medical knowledge and ultimately lead to a higher rate of accurate clinical diagnoses. Despite the many diagnostic advances of recent decades, clinico-pathological discrepancies are relatively frequent even in high-resource settings with sophisticated laboratory and imaging techniques. Such inaccuracies occur at even higher rates in low-income countries, where pre-mortem diagnostic support is often very limited (6)

However, the value of the autopsy doesn't end in the clinic; they are also an indispensable research tool. Many advances in medicine have been made possible through post-mortem examinations, including the investigation of emerging infections, genetic or metabolic diseases, or transplant-associated lesions. The routine practice of autopsies also offers an important epidemiological window to the health status of populations – vital in any country, but especially those with endemic infections and unevenly distributed access to medical care. For all of these reasons, I think increasing the number of autopsies performed in resource-limited settings can significantly impact medical practice and provide valuable research and epidemiological data to improve the health of their populations.

What's unique about the settings in which you practice?

One interesting aspect is the difference in customs and practices surrounding death. Implementation of any post-mortem procedure in areas where such examinations are uncommon requires a profound understanding of what is culturally and religiously acceptable. Data from previous studies suggests that CDA has a low acceptability in many settings. When designing the project, we hypothesized that MIA would be more readily accepted than CDA – and we were right. We recently conducted a study in five distinct settings in Africa and Asia with different cultural and religious backgrounds: Gabon, Kenya, Mali, Mozambique, and Pakistan. The study revealed that over 70 percent of the participants interviewed would want to know the cause of death, and would be willing to have MIA performed on a relative, if this were requested in a real-world situation (7). Importantly, this was also the case for individuals who had recently experienced the death of a relative. Thus, MIA seems to be acceptable in places where post-mortem procedures were previously believed to be unfeasible.

Early community engagement, transparency and accuracy in terms of the information provided to family and community members are key for enhancing community acceptability of any procedure, but MIA in particular. Guaranteeing the necessary sensitivity and human rapport from health professionals when asking for consent and performing the MIA is also crucial – and a good example of where pathologists really do need to step out of the laboratory and connect.

Things are somewhat easier medically than they are culturally. Although some training on how to handle the small samples obtained during MIA is highly desirable, the histological processing only requires basic pathology lab tools, which are available – at least in tertiary hospitals – in most low-income countries. Transportation of the histological samples is also quite simple; for the most part, no special conditions are needed. The sole notable exception to this is infectious disease diagnosis; the accurate identification of causative microorganisms requires frozen tissue, which implies serious logistical difficulties in terms of storage and transportation. It also requires a central laboratory able to conduct complex molecular microbiological analyses, facilities that are scarce in most low-income countries and therefore overwhelmed by routine clinical work.

How can other pathologists get started with MIA?

As part of our research project funded by the Bill and Melinda Gates Foundation, we have developed a training and research center on post-mortem activities (TRePMA Center) with two aims: i) to promote the use of MIA in middle and low-income countries, and ii) to help scientists and pathologists include MIA in their research projects and

activities. The center provides theoretical and practical training on how to conduct MIAs, together with guidance related to the essential pathological procedures required for preparation and interpretation of samples. The TRePMA Centre headquarters are located in Barcelona, Spain, and Maputo, Mozambique, and each conducts several courses a year on MIA procedure and sample processing. We have also put special effort into developing both technical and human resources in local pathology departments and into stimulating collaborative training and research activities. We also assess the socio-behavioral component of the implementation process; we find that it is critically important to put into place locally tailored recommendations before and during the introduction of MIA procedures.

In recent decades, we have witnessed a continuous decline in the use of CDA in most settings. Although the causes of this decline are multifactorial, one major barrier is the difficulty of obtaining consent from family members (8,9). Over the last few years, we've seen increased interest in the use of imaging-based methods, such as magnetic resonance imaging, computerized scanning, or ultrasounds, to replace the CDA; these procedures can be complemented by imaging-directed biopsies when necessary. Such methods are noninvasive, highly acceptable to the general public, and can accurately identify many morbid conditions. The impetus to adopt them seems clear – but their elevated costs and reliance on sophisticated equipment and skilled personnel are critical limitations for their widespread introduction, particularly in low- and middle-income countries.

I believe that the use of autopsy – or robust substitutes – needs to be encouraged as a mechanism for the continuous improvement of clinical diagnosis and as a complement for cause-of-death investigation and surveillance. Though the many challenges for the feasibility of conventional autopsies in low- and middle-income countries are not likely to be overcome in the short-term, methods like MIA could easily be implemented on a wider scale. Such a process, coupled with programs to build the capacity of local pathologists, can increase our understanding of the diseases causing death in areas where this information is typically very limited.

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A LIGHT IN THE DARKNESS

After the loss of an infant or child, uncovering the cause of death can be difficult – but has far-reaching implications

By Marta Cohen

No death investigation is trickier or more fraught with ethical and legal concerns than one concerning a young child. And yet, these are the investigations where providing families with a conclusive cause of death can bring the most relief – and, in some cases, make the most difference to a court case. These cases are inevitably heartbreaking, so it is a true honor to be entrusted with them and to select the most sensitive and appropriate examinations to bring closure to the question: why did this happen?

A comforting connection

When speaking with parents after a post-mortem, I seek to provide some kind of consolation to them in their grief. As I specialize in the study of sudden infant death syndrome (SIDS), the cases I investigate are always tragic losses of very young, apparently healthy infants. After every post-mortem, I make myself available to explain my findings to the parents, because I consider these conversations a very precious duty. I take great care to show the parents how much the entire hospital team cared about their baby, so I carefully check the name, sex and circumstances before I introduce myself.

"THESE CASES ARE INEVITABLY HEARTBREAKING, SO IT IS A TRUE HONOR TO BE ENTRUSTED WITH THEM"

In general, I work to give parents a positive message on which to focus. For instance, in cases where a child has died from sudden infant death syndrome (SIDS), I let families know about a parent support group called the Lullaby Trust and provide information about the group's helpline. I inform parents about the research we are actively conducting, and sometimes this discussion naturally opens up the opportunity to approach them for consent for future studies. It is a very difficult time – and above all we must be sensitive to the family's needs – but the potential for some good to come out of the tragedy is often a comfort.

When we are able to provide it, the answer to the question of what happened also helps a great deal. I understand that the answer cannot eclipse the pain, but knowing the reason behind a death seems somehow healing. In fact, I find that part of my job very rewarding, because I can see the comfort provided by the answer – or the knowledge that we're working as hard as we can to find one. I find it most important to communicate with the families at this time. When I diagnose a patient's cancer, for instance, I know that that child will be seen immediately by an oncologist and started on treatment, so there is an entire care team participating in the process. After the death of a child, the pathologist may be the last health professional the family sees.

The future of death investigation

When I began studying cases presenting as "SIDS" in 2003, we were able to identify the cause of sudden unexpected infant death in only one out of every five cases. Nowadays, that number has risen to three out of five. What has caused this reduction in the number of unexplained deaths? Better post-mortem examinations contribute significantly. In 2004, Helena Kennedy led a report on sudden unexpected death in infancy (1) that put into place the first protocol for its investigation. At the same time, I was appointed head of my department, representing my hospital at meetings with coroners, midwives, mortuary staff, police, coroners, emergency and ambulance personnel. We became one of the first NHS Trusts in the United Kingdom to fully implement the protocol, which meant we also began helping other Trusts – first by assisting with their post-mortems, and then by actually performing them ourselves.

What does a sudden infant death investigation look like at Sheffield Children's Hospital? Our protocol begins with microbiology; this allows us to investigate infections (bronchopneumonia, meningitis, hemolytic syndrome and so on), which explain about 30 percent of our sudden unexpected death population. Next, we do toxicology, which clarifies another 2 percent of deaths; then we look into things like cardiovascular conditions (10 to 15 percent of our population have a long QT mutation, 2 percent have an undiagnosed congenital heart malformation, and 3 percent have genetic conditions like cardiomyopathy). We take samples for metabolic analysis, which reveals the 3 percent of the population with undetected metabolic conditions – and then there are less common issues, such as mitochondrial diseases (approximately 2 percent of cases), abnormalities in the hippocampus or brain stem, and more. Some unusual genetic conditions are known to cause sudden infant death, but we don't yet have methods to detect them in many of our patients.

We also do imaging studies, both as a service provision and for research. The main post-mortem magnetic resonance imaging (MRI) facility is in London – but, luckily, we are well-acquainted with the doctors there, so we sat down with them in 2014 and developed a business case for something completely innovative: the fetal and perinatal post-mortem MRI, which we offer to parents who don't accept the traditional autopsy. We work in partnership with the radiology department, so after I complete my initial examination, the case goes to them for X-ray and MRI, and then comes back to us for the final report. We've done over 100 of these cases now, and we've identified a Relevant Condition at Death (ReCoDe) (2) in at least 90 percent of them. Considering that this technique is not the gold standard, that's an excellent result – and it's achieved at no additional cost and often within a 24-hour turnaround time. It's sensitive to cultural needs, too; some families need to bury their children on the same day, whereas others don't want the baby to be touched or to have any marks or scars. This method helps them, but it also helps us to improve our imaging and molecular genetics techniques, so it is a good balance.

Post-mortem MRI is diagnostically useful, of course, but with parental consent we can also keep the images and use them for future studies. We are currently collaborating on a project with the University of Sheffield's Department of Sociological Studies in which they are considering the impact of bereavement on parents who have had either a traditional post-mortem or magnetic resonance imaging (MRI), evaluating people's access to these services, and exploring how family members and professionals understand and interact with the images. With MRI, the parents can also keep the images of their child's heart or brain or face; they can have a final, lasting memory to assist them in the process of grief – so the study also examines the impact of that on the family.

I think that this is the future of post-mortem examination. Imaging, genetic and metabolic testing, microbiology and

toxicology will find a cause of death for two-thirds of patients, and only the remaining population will need a full diagnostic autopsy. This is the standard I am trying to develop and promote in my own department, although there are still obstacles to overcome. We are working with our genetics experts to develop a panel that can screen a battery of genes per case – something that I hope will become cheaper, easier and more widespread as genomics advances. We also don't have a dedicated MRI scanner in the mortuary at the moment, but we expect to in the near future. These scanners are gradually getting smaller and more affordable, so I predict that every mortuary will eventually have its own. We haven't quite achieved the "ideal" 21st-century post-mortem yet – and I believe we are still a decade or two away from it – but both the legal and the technological landscape are bringing us closer and closer.

Conflict in the courts

In the course of my work, I have seen post-mortem examinations where a test that could have been conducted was not, or where something that could have been spotted in the patient was missed. These all come as coroner post-mortems, so I take instructions directly from the coroner. In this role, I don't have contact with the clinicians at the hospital where the death occurred. I make efforts to phrase my reports in a way that does not assign blame; the point of an inquest is not to ascribe fault, but to provide the facts of the matter, so these are the details I include in my report. Occasionally, in significant cases, the coroner will telephone me before the inquest for a conversation about my findings and I try to speak as neutrally as possible – but, of course, if I think something has been missed, I must be honest about that during the inquest.

Many of the cases I have dealt with in the past have been difficult because they represented "science versus science." Shaken baby syndrome is a good example of this; in most instances, there are many signs that point to trauma – but also many that suggest other possibilities. For instance, the triad of subdural hemorrhage, encephalopathy and retinal hemorrhage could be the result of non-accidental injury, but may alternatively result from a metabolic condition or an infection. These post-mortems are carried out jointly with forensic pathologists. Some forensic pathologists treat these cases like trauma deaths in adults – but the way a child responds to trauma or disease is different to the way an adult would respond, and I feel that sometimes the subtleties and uncertainties are missed when the cases are investigated by professionals without pediatric experience. Medicine is never black and white, especially when it comes to infants and children, whose health is far less extensively studied. Unfortunately, in the Crown Court, everything has to be "black and white"; everything has to be true beyond reasonable doubt, because people's liberties are at stake. It is difficult to reconcile medicine and law, so nowadays I try to avoid such cases and focus on research that I hope will eventually yield information that gives us better insight into unexpected deaths with uncertain causes. The downside to this is that many others have made the same choice – so there's a serious lack of available medico-legal experts to address these challenging cases at the moment.

I had this same discussion recently with a prominent Queen's Counsel. He asked me to take a case and I said, "I'm no longer doing this work, and neither is the other expert who can act for the Defense in this field." He told me he would find one in the US. "They won't come here either," I replied. "I worked for the Innocence Project there, and they asked me what could be done to resolve the conflict between science and law – and I told them that the only thing I could do was to stop accepting these cases."

Doctors and scientists alone cannot change the judiciary system. But, at the same time, we can't participate in it in its current form, because it's risky. You put your profession – your whole life – on the line when you state your opinion in court, and it isn't always possible to be entirely certain. An investigation by the Karolinska Institute in Sweden commissioned a group of experts to search the literature for papers to substantiate trauma as a cause of Shaken Baby Syndrome. The group found 3,000 papers, of which only 100 met the criteria for inclusion and only two provided supportive evidence that shaking a child can cause the relevant symptoms. In my opinion, it's not safe to base an entire court case on two papers. Eventually, I suspect we'll reach a point where no one is willing to challenge such cases anymore, and then it will be a breach of human rights to try them in court – it wouldn't be a fair trial if the defendant had no medical expertise on his side. This is why I believe that, to maintain a just legal system, the establishment is going to have to change to accommodate the inherent uncertainties of science and medicine.

Death investigation is a difficult subject for many reasons, and it is still in its early evolution. As technology advances, we get closer and closer to finding a cause of death in every case – and, increasingly, we can do so without a complete diagnostic autopsy. It's my hope that, one day soon, we will be able to provide grieving families with the comfort of conclusive answers without the legal, technical and cultural obstacles we currently face.

Marta C. Cohen is a Consultant Histopathologist at Sheffield Children's Hospital, UK.

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SCIENCE HONORS SACRIFICE

A unique facility dedicated to a unique kind of death investigation where identification and re-association are the goals

By Robin Stombler

In late March 2017, United States Marine Private Harry K. Tye was laid to rest at Arlington National Cemetery – more than seven decades after he was killed in the Pacific Theater during World War II. His remains had finally been located and identified using DNA sequencing technology. The emotional story ran on the front page of the Washington Post, and the US Secretaries of Defense and Homeland Security were there to honor the private who came home.

Not even 100 miles up the road is the place where many such stories unfold.

The city of Dover, Delaware, is home to the Armed Forces Medical Examiners System (AFMES). Here, forensic science is performed with remarkable proficiency, sensitivity, and innovation. The military base is home to a laboratory that processes and tests dental, skeletal, tissue and hair specimens – the largest of its kind in the world. A database, whose ultimate purpose is to aid in the identification of missing service members from past military conflicts, collects and processes family reference specimens. DNA profiles submitted electronically for upload, storage and searching are processed and automated at such a high level that the laboratory's global throughput is unmatched.

The majority of scientific and technical personnel for this endeavor come from one company: ARP Sciences. "For 40 years, we have served this major civilian and military mission," explains Cynthia Thomas, CEO. "We are enormously proud to perform world-changing research and method development in support of identifying missing service members and medical examiners' investigations into the cause and manner of death of deceased personnel. It's a humbling experience."

And it's a unique challenge for those involved in the death investigations. The bone and tooth fragments this laboratory receives are not typical forensic samples; they may be contaminated, mixed or disassociated. The investigators have established specially designed protocols for handling these precious samples; they must employ specific DNA extraction methods to prevent contamination and customized PCR-based methods to obtain reliable results. Scientist Charla Marshall, who led a team to validate a novel next generation sequencing method for use in processing past accounting specimens, says, "We are the first forensic laboratory to utilize this new technology."

One of the challenges the laboratory faces is the degradation of DNA. Living cells maintain their DNA by enzymatic repair processes. After death, the cellular compartments that house catabolic enzymes break down and release their contents. Once the DNA molecules themselves start to degrade, whether through lysosomal nucleases, bacteria, fungi, or insects, there is no repair mechanism in place. The damage simply continues to accumulate.

Scientists from ARP Sciences may encounter cases as old as 140 years past death. When so much time has passed, samples are generally fragmented and environmentally challenged. Direct references for the cases are rarely available, so it's usually necessary to rely on mitochondrial DNA (mtDNA) analysis. mtDNA sequencing provides a high level of success compared to other testing technologies – in fact, its success rate is over 90 percent, much higher than other sequencing techniques like short tandem repeat on the Y-chromosome (Y-STR) and autosomal short tandem repeat (auSTR) testing, which have a success rate just under 50 percent. Why such a significant difference? Nuclear testing relies on the single copy of the nuclear genome, which degrades with age to the point where it can no longer deliver reliable results. Each mitochondrion in the cell has its own mtDNA, meaning an increased likelihood that sequencing will be able to extract usable information.

Mitochondria don't always hold the answers, though. Sometimes, the laboratory receives small, unidentifiable bone fragments. The first question, of course, is: are they human remains? To find out, the lab uses 12S rRNA gene testing. Since the implementation of this testing protocol, 45 percent of samples tested were found to be non-human – helping investigators work out which ones need continued investigation.

Samples at the laboratory vary from the highly challenging to the quite ordinary. Routine samples may include human remains and reference samples. The former are usually bones, teeth, tissue, hair, dentures, envelopes and clothing (with body fluid stains like saliva and blood extracted from the latter two). Reference samples include bloodstain cards and buccal swabs, from both subjects and family members.

Bloodstain cards became standard in the early 1990s, shortly after Operation Desert Storm. At that point, the US Department of Defense began collecting blood specimens from military service personnel. A quality control process for extracting DNA from the cards, which are solely used to identify remains, had to be developed and implemented. The DNA must be collected in such a way that its integrity is maintained and the test results are meaningful and legally defensible – so how do they do it? Samples must be labeled and stored separately in appropriate containers, and personnel must change their tools and personal protective equipment between each collection. Despite the rigorous protocols, though, not every forensic sample leads to a new identification; samples might have degraded, remains commingled, there might not be appropriate references, and it's possible that a submitted sample may not be of human origin.

But even with these caveats, advancements in forensic science have come so far so fast that there may never be another truly unknown soldier. In 1972, an Air Force pilot was shot down in Vietnam. The remains of this Vietnam War Unknown were laid to rest in the Tomb of the Unknown Soldier at Arlington National Cemetery. This would once have been the end of the soldier's story – but in 1998, his remains were exhumed. With mtDNA testing, AFMES was able to identify the remains of First Lieutenant Michael J. Blassie, United States Air Force. The crypt of the Vietnam Unknown is now vacant.

Robin Stombler is President of Auburn Health Strategies, Arlington, USA.

Cancer's Circulating Secrets

Liquid biopsy is a powerful research tool that allows investigators to better understand cancer's intricacies without subjecting patients to invasive procedures

Alberto Bardelli, Professor of the Department of Oncology, University of Torino, Director of the Laboratory of Molecular Oncology at the Candiolo Cancer Institute-IRCCS, Torino, Italy, and President Elect of the European Association for Cancer Research.

Liquid biopsy is an ever more vital component of cancer research. Circulating tumor DNA (ctDNA) offers a window into the characteristics, the changes, and the vulnerabilities of tumors, and as a result, its adoption in research laboratories is becoming more widespread. Alberto Bardelli, an expert in cancer genetics and personalized medicine, explains how liquid biopsy has benefited his work – and why he thinks more researchers should take advantage of it.

How did you begin using liquid biopsy in your research?

I first became interested in the technique because of my work on cancer clonal evolution and drug resistance. A number of years ago, my colleagues and I reported that colorectal cancer patients who respond to EGFR blockade therapy do so for a limited period of time, then relapse (1). We needed a way to identify the mechanisms of secondary drug resistance and patients who were likely to relapse – and the best way of doing so at the time was to take tissue biopsies. It was difficult to engage patients in the protocol, though, because it was invasive and didn't provide them with an immediate, tangible benefit. We found ourselves running in circles with no way to identify the cancer's mechanism of resistance to this targeted therapy.

Then I thought, "Perhaps we can use blood as a surrogate for tissue." There was already evidence that ctDNA was a potential source of information for this type of research (2) – and so, starting in 2009, we began to build our ctDNA collection. Ever since, in every clinical trial we have designed, we have collected blood and isolated plasma and peripheral blood mononuclear cells (PBMCs) from our patients. Thanks to those ctDNA samples, we were able to uncover the mechanism of EGFR blockade resistance in colorectal cancer patients (1). And that made us realize that liquid biopsy could be an incredible source of information on how tumors evolve during therapy.

A few years later, we asked the question, "When treatment stops, will resistant clones in the blood increase, decrease or remain constant?" To our surprise, we found that, when therapy was suspended, the resistance mutations we saw in ctDNA would drop almost immediately (3). We're now beginning a clinical trial called CHRONOS – in which, for the first time in colorectal cancers, clinicians will make treatment decisions based on liquid biopsy. It will be a large trial (>250 patients), and our plan is to track levels of *KRAS* mutation in the blood during anti EGFR therapy and use those to determine when to restart the therapy.

> *"For the first time in colorectal cancers, clinicians will make treatment decisions based on liquid biopsy."*

How can liquid biopsy benefit cancer research?

It can answer key research questions. For instance, which cells release ctDNA most effectively? If you have a patient with metastatic colorectal cancer, will the ctDNA recapitulate the disease progression? Will it accurately represent all the lesions present in that patient?

One way to address this is by coupling liquid biopsy to "warm autopsy" – the practice of procuring samples soon after death. We have designed a rapid donation trial (DONUM) to collect tissue and blood from patients with multi-organ disease to

understand how each tumor contributes to the total ctDNA pool. Eventually, we hope to understand enough about individual tumor contributions to study patients with metastatic disease primarily through their ctDNA. Ultimately, I hope the knowledge we gain will lead to better disease tracking over time, better monitoring of minimal residual disease, and – one day – large-scale screening for early cancer detection.

Five years ago, you would go to a meeting and find perhaps one or two presentations on ctDNA studies. Now, entire sessions are focused on liquid biopsy; there's been incredible interest. We now need to define clinical settings in which liquid biopsy can inform treatment approaches. We have already shown it's a valuable approach to track tumor dynamics and identify mechanisms of drug resistance, in the future it might be used to prescribe drugs, determine the need and timing of CT scans and other tests, assess minimal residual disease, decide when further chemotherapy is warranted, and when it is not…

How does research benefit from NGS and biomarker analysis?

Next generation sequencing (NGS) and multi-biomarker panels are crucial to understanding and improving therapies that specifically target tumor cells. In colorectal cancers, we discovered the importance of *KRAS*, then *BRAF*, then *NRAS*… Now we're looking at alterations like *HER2* and *MET* amplifications and MAP kinase mutations. The list is constantly growing – who knows what we may need next? Cancer is in essence a genetic disease, so next-generation sequencing of DNA and RNA will be even more relevant in the future than it already is.

The more we know about cancer, the more we realize that it continually changes – and especially so during treatment. Both kinase-targeted therapy and immuno-oncology will benefit tremendously from NGS approaches – and, in those fields, the research of today will yield the diagnostics of tomorrow. In the future, we will want to predict how many and which neoantigens are present in a tumor at a given moment, we already need to quantify the number of mutations per megabase in individual tumors. This information comes from NGS analyses, so this technology is poised to make a huge impact. We particularly need two things: platforms that simplify the process so that users don't need a dedicated bioinformatics team to understand their results, and standardization so that laboratories can work with one another's data.

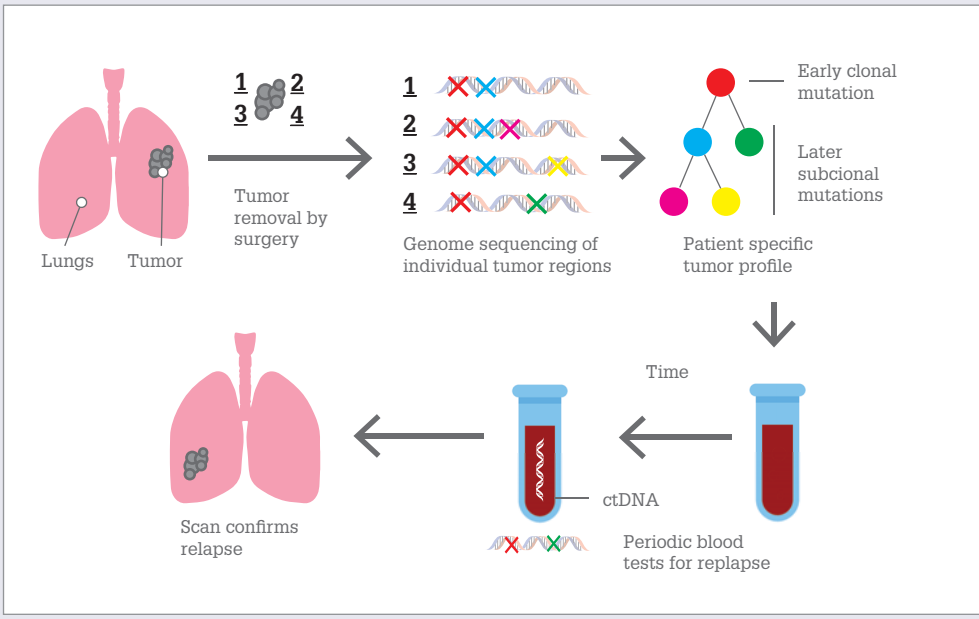

Figure 1. The use of liquid biopsy to monitor for cancer relapse after surgical excision. Adapted from (5).

What do you expect from the field in the near future?

I can think of a few advances that would be especially useful. Panels that are capable of tracking tumor evolution, for instance, can help determine when a patient may need to change treatments and what the next option should be. NGS approaches to identify neoantigens will be valuable. So too will be knowledge of T cell receptor rearrangements to study the immune system's reaction to a tumor. Analyses like these are expensive, but within reach – recently, for the first time, a personalized approach was prospectively used to track individual lesions that evolved in lung cancer patients (4). Perhaps in the future, every patient will be followed with an individualized multi-biomarker panel. And eventually, as the cost of sequencing decreases and the sensitivity of liquid biopsy improves, perhaps tumors will be interrogated longitudinally in routine clinical practice – using ctDNA as a source of information.

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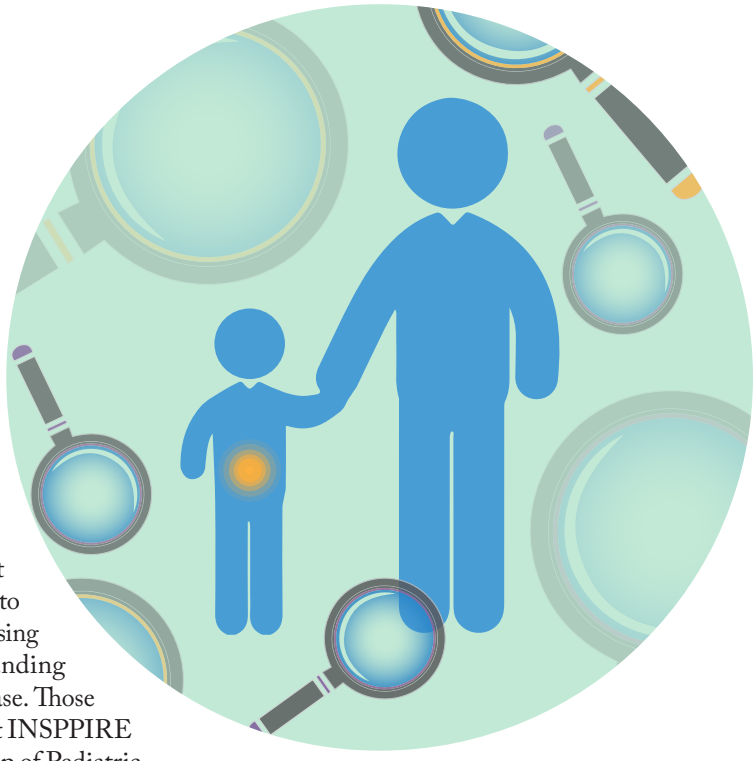
Personalizing Pediatric Pancreatitis

New gene mutations associated with recurrent and chronic pancreatitis in children may help with prediction, diagnosis and treatment planning

By Matthew Giefer

A child is taken to her primary care doctor with nausea, vomiting, and abdominal pain. What's the doctor's first instinct? It's unlikely to be pancreatitis, even after several episodes of symptoms. This is because recurrent and chronic pancreatic inflammation are uncommon conditions in young children, and many doctors – even those who are knowledgeable about the disease – may not think it occurs often enough to be considered in the differential diagnosis. Unfortunately, this bias can delay diagnosis, resulting in more suffering for the patient and potentially a less tractable disease when the correct conclusion is finally reached.



What can we do to address this issue? The first and most critical step is to make sure doctors think of pancreatitis when encountering its common symptoms in children. But beyond that, it's important to have reliable ways of diagnosing pancreatitis and understanding the mechanisms of the disease. Those were the goals of our recent INSPPIRE (INternational Study Group of Pediatric Pancreatitis: In Search for a CuRE) study (1), in which we examined clinical and genetic data belonging to 342 pediatric pancreatitis patients. We found a number of interesting associations – but the most fascinating were the mutations correlated with early-onset (prior to age six) acute recurrent or chronic pancreatitis, especially alterations in the cationic trypsinogen (*PRSS1*) and chymotrypsin C (*CTRC*) genes.

At a Glance

- *Even when children present with common pancreatitis symptoms, doctors don't necessarily consider the disease when making a diagnosis*
- *Delayed, questioned or missed diagnoses can result in prolonged suffering and increased disease severity for pediatric pancreatitis patients*
- *A new study identifies several new mutations strongly associated with early-onset acute recurrent and chronic pancreatitis*
- *The mutations may help doctors more accurately predict and diagnose early-onset pancreatitis and even personalize therapy*

Spotting the symptoms

There are three factors involved in diagnosing acute pancreatitis: abdominal pain consistent with pancreatic inflammation, amylase or lipase elevations over three times the upper limit of normal, and imaging that shows pancreatic inflammation. A patient needs to exhibit at least two of these elements to be classified as having pancreatitis. Our study focused on acute recurrent pancreatitis (ARP) and chronic pancreatitis (CP) in pediatric patients. ARP refers to multiple discrete episodes of pancreatitis with a complete return to normal in between episodes; CP, in contrast, is a permanent scarring of the gland and is accompanied by chronic pain and either endocrine dysfunction (diabetes), exocrine dysfunction, or both.

One major difference between these two conditions and acute pancreatitis is that we're much better able to treat the acute form of the disease now than we were in the past. Patients may need lots of intravenous fluids and aggressive pain control, and research has shown that restarting feeds shortly after diagnosis leads to the best outcomes. Unfortunately, we still don't know how to best treat ARP and CP – and a large part of the reason why is that, until recently, we didn't know why children developed those conditions. Now, we have shown that children who develop this condition at a young age often do so because of genetic abnormalities that affect the pancreas.

The meaning of the mutations

We have found that mutations in the *PRSS1* and *CTRC* genes have the strongest association with ongoing pancreatitis. *PRSS1* mutations affect trypsin, one of the major digestive enzymes created by the pancreas. When this gene is mutated, the result is a dysfunctional trypsin enzyme that can be activated more easily, and is not responsive to the body's normal

Study locations around the world involved in the INSPPIRE pediatric pancreatitis project.

deactivation pathways. *CTRC* mutations also seem to affect trypsin activity because this gene plays a role in trypsin degradation. Our data therefore suggest that dysregulation of trypsin homeostasis is an important mechanism for early-onset ARP and CP.

Even among patients with the same or similar mutations, the course of the disease can be quite variable. Some may have a few episodes of mild pancreatitis, whereas others may suffer from severe chronic pain and pancreatic failure. Our hypothesis is that other factors may play a role in determining the clinical severity of the gene mutations – and our task at the moment is to identify those factors.

The road to diagnosis

Knowing that early-onset ARP and CP are predominantly genetic conditions is very important, because it leads us to better screening and diagnostic tools. But even before that, physicians need to be trained to think about the possibility of acute pancreatitis in pediatric patients. If the diagnosis isn't considered, the proper tests won't be requested and the patient's symptoms will remain unexplained. Hopefully, especially for children who experience multiple episodes or develop chronic pancreatitis, our data on the genetic causes will both encourage physicians to look for the cause and provide them with the tools to do so. Considering pancreatitis in the differential diagnosis is a critical first step; once that hurdle is cleared, we hope our studies will help take care of the rest.

Patients who have had multiple unexplained episodes of acute pancreatitis should be considered good candidates for genetic testing. The younger the patient is, the more likely his or her episodes of inflammation will have a genetic cause. That said, I think it's important to point out that, even in the oldest age group in this study, over half (54 percent) of patients had a genetic abnormality. I would therefore consider genetic testing to be important in any pediatric patient with potential ARP or CP.

Our study focused on just four genes – *PRSS1, CTRC, CFTR,* and *SPINK1* – but there have been reports of other genetic causes of pediatric pancreatitis that still need further examination. It is also very likely that additional factors remain to be discovered and described. Ultimately, we hope to use any genetic information we can glean to determine what treatments work best for ARP and CP, to see whether patients' responses to these treatments can be predicted based on the underlying genetic drivers of their disease. The ability to one day apply precision medicine to pediatric pancreatitis is one of the most exciting aspects of our research.

Matthew Giefer is Director of Gastrointestinal Endoscopy and of the Gastroenterology and Hepatology Fellowship Program at Seattle Children's Hospital, Seattle, USA.

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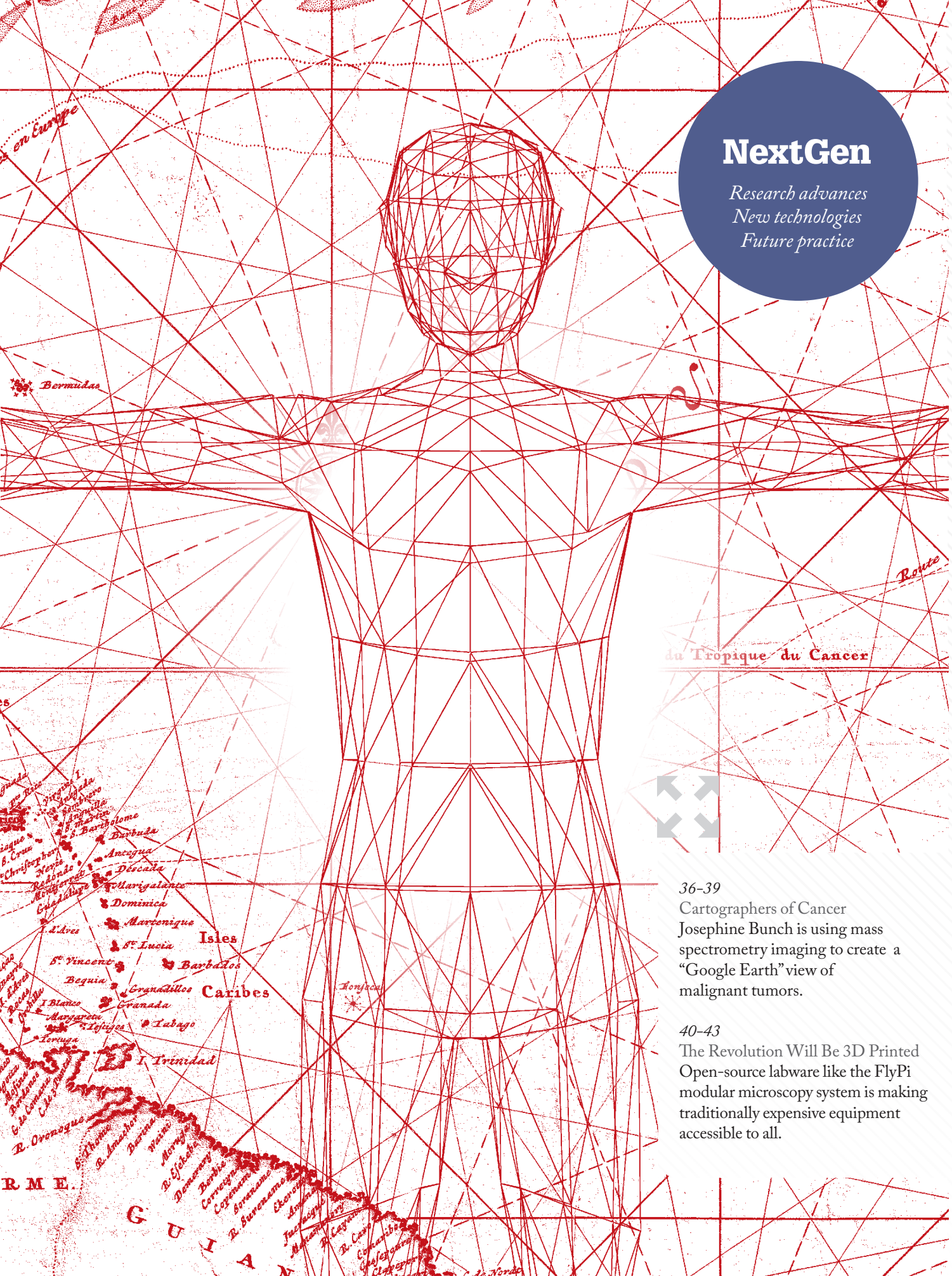


Cartographers of Cancer

Meet the researchers using mass spectrometry imaging to plot a molecular map of malignancy§

By Josephine Bunch

From Ptolemy to Google Maps, humans have been driven to record the landscape around them. By committing our world to paper, we can understand it, order it, and maybe even control it. A blank on a map is intriguing, but unnerving; medieval mapmakers, faced with unknown territories, filled them with ferocious monsters and deadly storms. These mythical beasts were vanquished as intrepid explorers charted the wilderness, filling in the gaps in our knowledge.

Can a new kind of cartography help us face down another terror? Cancer is much better understood than it was 50 – or even five – years ago but for the millions of people diagnosed with the disease every year – and the doctors who treat them – there are still many troubling uncertainties. Have we caught it in time? Will it spread? What is the best course of treatment?

An ambitious five-year project led by the United Kingdom's National Physical Laboratory (NPL) will record the most detailed map yet of the molecular landscape of a tumor. By combining new and existing mass spectrometry imaging techniques, the multidisciplinary team will create a "Google Earth view of cancer" – from whole-tumor down to subcellular level – with the hope of charting a course towards new options for prevention, diagnosis and treatment.

A Google Earth View of Cancer

In 2015, I heard a program on BBC Radio 4 about the Cancer Research UK (CRUK) Grand Challenge – a series of five-year, £20 million awards for multidisciplinary teams willing to take on some of the toughest challenges in cancer research. One of the problems they described was mapping tumors at a molecular and cellular level – creating a Google Earth view of tumors. Traditionally, mapping of tumors was performed using standard histology and histopathology tools, such as microscopy of stained tissues. The problem is that to stain for a molecule, you have to know what it is. To create a comprehensive map, we have to be able to find all molecules, including the unexpected. As an analytical scientist, I saw that what CRUK was describing was fundamentally a measurement challenge, and felt immediately that the way forward

At a Glance

- *The difficulty with molecular tumor mapping using standard histology tools is that, to stain for a molecule, you must know in advance what it is*
- *An ambitious new project sets out to image all the molecules associated with cancer using mass spectrometry imaging (MSI) instead*
- *The researchers are using a range of different MSI techniques to increase performance – but this also increases the challenges involved*
- *Ultimately, the biologists hope to learn more about how cancer grows and spreads, improving diagnosis and treatment of the disease*

would be through mass spectrometry imaging (MSI).

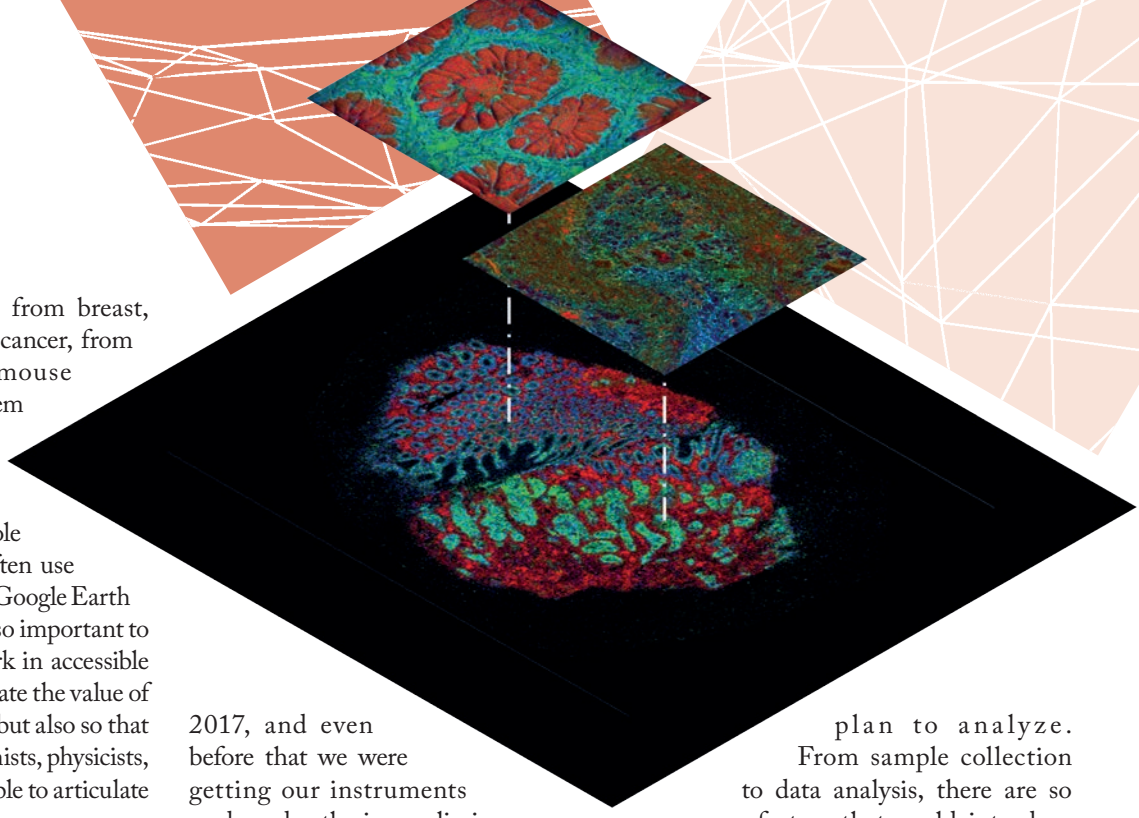
Admittedly, I may have been somewhat biased. From the moment I first encountered mass spectrometry, I was hooked. I love the sheer variety of instruments available; the many ways we can create and transmit ions gives us a huge number of different combinations. Then there's the breadth of applications – mass spectrometry measurements are being recorded everywhere from oceans to operating theaters to missions on Mars. At the time, I had been Co-Director of the National Physical Laboratory (NPL)'s National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI) for three years, leading our efforts in ambient MSI and matrix assisted laser desorption/ionization (MALDI).

"To create a comprehensive map, we have to be able to find all molecules, including the unexpected".

Friends and colleagues encouraged me to contact CRUK to see if they would consider a MSI-based project. CRUK confirmed that there were no preconceived ideas about how the challenge should be solved, and I decided to go for it.

Google Translate

First, I put together a consortium of researchers, some of whom I already knew or had heard of, and others who were recommended to me. The team consists of experts in the relevant cancers, world-leaders in developing genetic models, inventors and innovators of techniques, and specialists in various aspects of tumor biology and metabolism.



We will take samples from breast, pancreatic and colorectal cancer, from patient biopsies and mouse models, and we will use them to build chemical images – molecular maps – at a range of different scales, from single cells up to whole tumors. The reason we often use CRUK's clever analogy of "Google Earth for tumors" is because it is so important to be able to explain our work in accessible terms; partly to communicate the value of our research to the public, but also so that our team of analytical chemists, physicists, biologists and medics are able to articulate shared goals.

We all want to achieve the same thing but we don't always speak the same language. For example, if you ask a biologist about the biggest challenge in mapping a tumor, they are likely to mention the difficulties of obtaining samples for molecular or metabolic studies, and interpreting the information. A mass spectrometrist may have a completely different answer, focusing on the huge sample numbers involved or the problem of building instruments with the resolution required. The Google Earth analogy has guided us in designing our pipeline and bringing the right investigators and techniques on board. I believe that the better you can break the project down into accessible descriptions across disciplines, the better the science.

A grand measurement challenge

Getting the call to say we'd been successful in our bid was incredibly exciting. NPL is leading the consortium and will be analyzing samples with MALDI, desorption electrospray ionization (DESI), secondary ion MS (SIMS), Nano-SIMS and OrbiSIMS. We will also be coordinating imaging performed at other institutions, managing the data generated and disseminating the resulting protocols and instrumentation.

We launched the project officially in May 2017, and even before that we were getting our instruments ready and gathering preliminary data. Our results so far have shown the extraordinary amount of data possible when we combine different MSI techniques. However, there is also an abundance of challenges, from ensuring that we have sensitivity at the highest resolutions for key metabolites to maintaining the quality of each and every measurement. But mining the enormous data sets we collect is perhaps the biggest challenge of all.

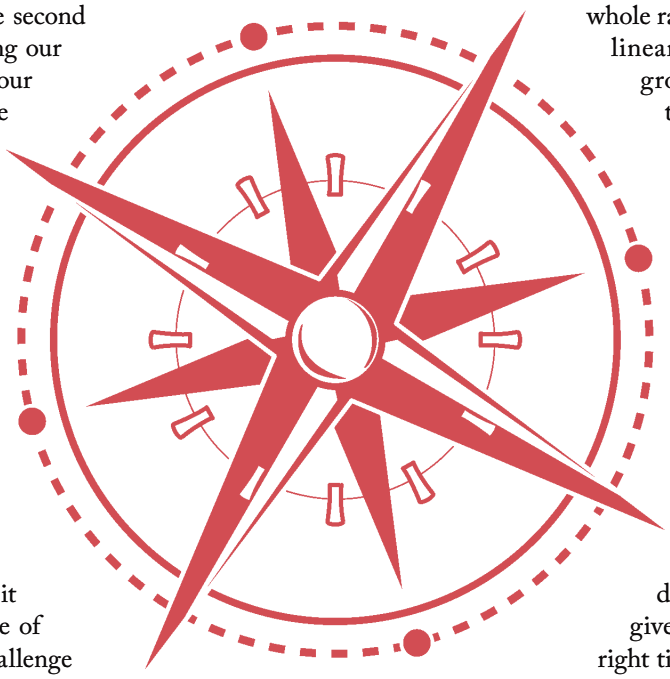
Our priority for now is to build a framework to ensure quality measurements across the huge number of samples we plan to analyze. From sample collection to data analysis, there are so many factors that could introduce variation, especially when working with multiple techniques. NPL and the Grand Challenge consortium are extremely passionate about generating reproducible data and repeatable measurements. We don't want to produce beautiful pictures that cannot be reproduced or don't accurately represent the underlying cancer biology.

"Over the five years, we hope to share results so exciting that other labs are inspired to use our techniques."

We have designed pilot studies assessing the performance of the plethora of different MSI instruments we're using – and we've made some measurements at several different sites to understand how much it affects the results. This work represents an important foundation in being able to quote the performance of the different techniques in combination. We have also been assessing the various parts of our pipeline to ensure absolute consistency. It is perhaps not the most glamorous work, but it's vital that samples from different sites are being collected, stored, transported and analyzed in the same way, and that we have robust pipelines in place for handling our data all the way from raw files to the curated data that will be available to the public.

Right now, I'm most excited about making measurements that no-one has ever made before whilst working with an extraordinary consortium of researchers

– all of whom are there to ensure that we are making measurements that matter. I'm also looking forward to the second phase of the project: interpreting our data, sharing it and broadening our network. Over the five years, we hope to share results so exciting that other labs are inspired to use our techniques. And we will be ready to help them acquire quality data as quickly as possible.



Next-level MSI

The past few years have seen fantastic work from around the world using MSI as a powerful method for mapping multiple molecules in the same tissue. It might still be an emerging technique, but it already has a fantastic pedigree of excellent results. The Grand Challenge project will build on that success by using several MSI techniques in combination to widen the range of molecules examined, and so gain an in-depth understanding of tumor metabolism – linking genes, proteins, peptides, lipids and metabolites.

We will be making use of significant recent advances in technology. An example is the 3D OrbiSIMS instrument, which combines SIMS with an Orbitrap mass analyzer. As Ian Gilmore describes in "The Super-Resolution Revolution," the hybrid instrument allows very high resolution. Going back to the Google Earth analogy, OrbiSIMS is like peering through the window of a house in Street View to see where the sofa is...

At that level of detail you can't get through enormous numbers of samples – just as you wouldn't want to record the position of every sofa in the world. Instead, we will use other techniques to identify cells and regions within the tumor that we want to pay special attention to. MALDI and DESI give a street and city view – they can get down to pixel sizes small enough for a street-level look but can also rapidly create a basic "city map" of a tumor section.

Other techniques will help us localize our search to specific "cities". In addition to MSI methods, such as SIMS, MALDI and DESI, we are also using techniques for in vivo analysis and imaging of metabolites – such as the iKnife (REIMS) and MRI.

A common aspect of all the techniques is that they will produce a series of mass spectra acquired at discrete locations across the tissue.

We are developing methods to handle this enormous hyperspectral data set, from raw files to basic pre-processing, such as peak alignment and peak picking, to reduce the volume of data. We may also need strategies for normalization so that signals collected on different days can be meaningfully compared. The real challenge comes when we want to mine those data; we will need to use a whole range of machine-learning tools, linear and non-linear methods to group similar samples together, to segment areas of relevance, and to try to understand associations between the molecules detected.

Measuring success

Our ultimate goal is to gain new insights into tumor progression that might help diagnose and treat cancer. If we can help biologists understand exactly how tumors grow and spread, that knowledge can be translated to make sure that patients are diagnosed earlier, and can be given the right treatments at the right time. Of course, it will take time to translate our findings into the clinic. Concentrating on the next five years, I will be satisfied if:

- The tumor biologists on the team have gained fresh understanding.
- We have significantly improved the performance of the techniques we're using.
- Our measurements are being adopted as standard in research labs.
- Our data have helped to produce new in vitro models that are more representative of real human tumors.

Like the cartographers of cities, countries and continents, we want to fill in the blanks on our map of cancer, and unlock the secrets of tumor metabolism.

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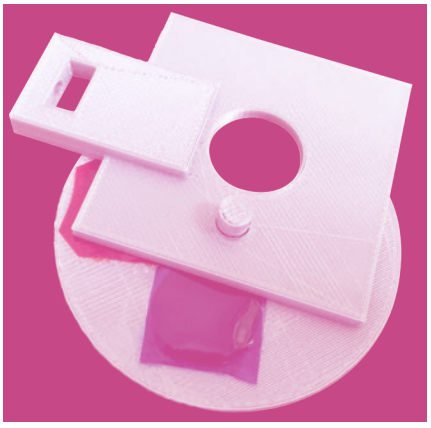
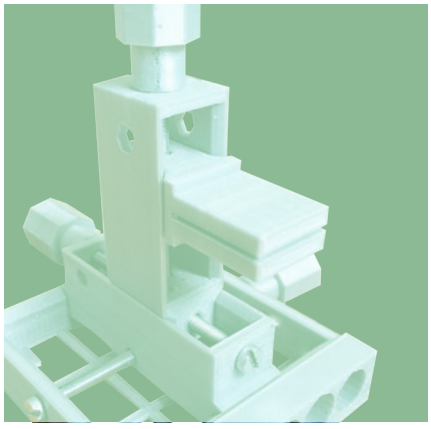
The Revolution Will Be 3D Printed

Printable, modifiable and shareable open-source labware is offering users a new way to acquire and customize traditionally expensive laboratory equipment

By Tom Baden





The single greatest limiting factor for a new, underfunded, or resource-limited laboratory is equipment acquisition. Most laboratory technology is expensive, and some pieces can be difficult to locate or transport to more remote areas. So how can a lab get itself up-and-running, or upgrade its equipment, without sufficient money or access? That's where the open labware revolution comes into play.

Open-source lab equipment is freely available – not just for 3D printing by potential users, but also for further development. Pathologists can download and print the designs they need, creating modular systems to suit their specific needs – and if the system lacks a particular function, they're welcome to design it themselves, print it, and share it online to help others with the same needs. A significant part of the laboratory at the University of Sussex runs on technologies we have designed, printed and assembled ourselves. It's my hope that by sharing our experiences with the FlyPi modular microscopy system, as well as our other tools, we'll be able to encourage others to join us on our quest for affordable, accessible, editable equipment.

Building a better microscope

FlyPi is not our only creation; we have actually built several pieces of equipment – pipettes, micromanipulators, and even a pico-injector. But some form of microscope is at the core of most biomedical labs and, as such, it's an obvious starting point. Nowadays, imaging equipment like a powerful charge-coupled device (CCD) chip is routinely incorporated into everyday items such as mobile phones, webcams, and all manner of surveillance and home automation hardware. So if you have a suitable set of lenses to hand, a makeshift digital microscope is never far out of reach. For example, if you break the lens out of a cheap laser pointer and tape it over a mobile phone camera, you already have a pretty powerful microscope in your pocket.

As it turned out, one popular adjustable-focus camera module for the Raspberry Pi platform is already a microscope, though it was not originally

At a Glance

- *Open-source labware offers equipment solutions for labs that can't afford – or whose needs are not met by – standard systems*
- *FlyPi is one such tool, a modular microscopy system that can be customized to fit the requirements of its users*
- *Not only are users invited to download and print their own FlyPi systems, but they're welcome to create and share their own unique modifications as well*
- *The open labware revolution is on the rise – all that remains is to get as many people as possible involved*

intended as one. Simply screw the objective lens all the way out, and the optics align to bring tiny objects just in front of the lens into focus on the CCD chip. We stumbled across this when using the camera system in an attempt to film some fruit flies in the lab, so we just integrated the feature into our microscope design.

Why were we filming fruit flies? We originally intended FlyPi to be a behavioral monitoring system for small, genetically tractable model organisms – including Drosophila, nematode worms and zebrafish. In these species, it is fairly easy to selectively express specific proteins in specific neurons that can then be controlled with light (optogenetics) or heat (thermogenetics). This is a core technique in modern neuroscience; it lets you study the functions of neurons and circuits in a noninvasive manner. It's also a fairly user-friendly technique in that you don't need a huge amount of light or heat to trigger these proteins. A suitable LED or hot plate will do the trick. So we built a simple camera system to which we added some basic controls for controlling LEDs and a Peltier (heating/cooling) element. Of course, the fact that our system can now also work at high zoom opens up a number of additional possibilities; for instance, with some low-cost sheets of colored plastic like those used in theater lighting, we can shape the spectra of lights reaching a sample or the camera path to enable fluorescence imaging.

Why FlyPi?

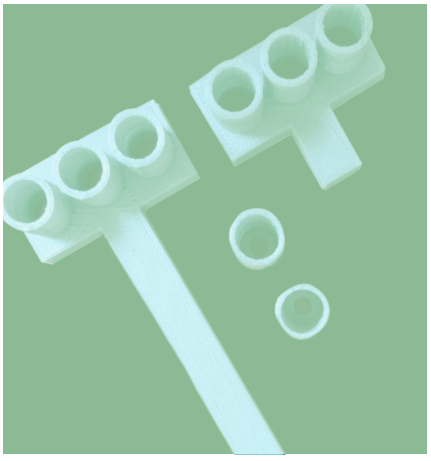
We think the power of FlyPi's design comes from its modular nature and broad range of possible use cases. Some people may use it just to magnify samples; others may appreciate the fluorescence imaging option; still others may wish to use it as a behavioral chamber for neurogenetics experiments. If you only want one or two of these functions, you only need to assemble one or two of the underlying modules – and if you change your mind later on, you can simply swap them out or add more. At its core, FlyPi is just a little camera with lights that can be flexibly arranged to suit the user's needs.

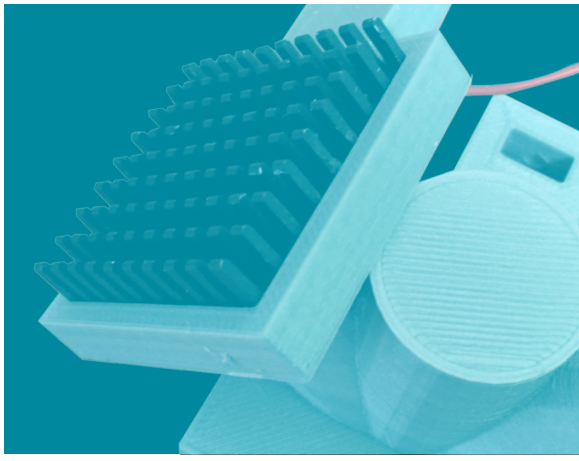
"We usually build 'new' things if we identify an obvious need, but cannot find an existing open-source solution."

In terms of peak resolution, the system will certainly be superseded by most commercial solutions, and even a number of alternative open microscope designs. For example, the 3D printed WaterScope, in combination with a commercial objective, provides much crisper images, albeit at a somewhat higher price. Where FlyPi outperforms its rivals is in flexibility. If you want to, you can point the camera at a tree, zoom out, and do a time-lapse over several days. Or you can zoom all the way in, point it at a blood smear, and count macrophages. Ironically, colleagues often come to use our system to take pictures for which their commercial microscopes are too good – like snapshots of a whole mouse brain at high resolution. A mouse brain is too big for a 4X objective combined with 10X oculars (the lowest typically available on a compound microscope), so if you use commercial equipment, you actually end up having to take multiple pictures and stitch them together with specialized software.

That's not to say that you can't take a high-resolution image with FlyPi, though. If you want better resolution without changing systems, you can simply take a commercial objective of any power and mount FlyPi's CCD chip above it to capitalize on the better optics. After all, there is nothing special about commercial solutions. You can even stick FlyPi down the ocular of a commercial system to take some snapshots if you don't have a microscope camera to hand. In our laboratory, we sometimes do this when demonstrating dissections so that students can follow along on a screen. We have even used it as an improvised gel imager by placing the ultraviolet LED beneath the stage and the camera above – works like a charm! In time, we hope that users will come up with all kinds of interesting applications that we have not thought of – and, most importantly, improve the designs to their own requirements and then re-publish them.

Think of the system as a pretty flexible digital camera system with additional control circuits and filters for light and heat. What could that be used for if you really exploit your scientific creativity? To keep an experimental animal at a suitable temperature? As a visual stimulator for vision experiments? To monitor and manipulate how colonies of organisms behave over long periods of time? Perhaps embarrassingly, I sometimes just use it as a remote webcam to monitor my 3D printer! I guess the only limit to FlyPi's applications is the limit of its users' imaginations. If you have a FlyPi sitting idle in the lab, you will soon find its use, and rarely for its intended purpose! We tend to have a few spare ones around when we run neuroscience training courses in Africa (1) and it never takes long before students and instructors ask if there are any more, because they are all in use!





Getting Involved in Open Labware

Tom Baden and André Maia Chagas

Why is open labware important?

TB: Affordability and universal access to laboratories and institutes of learning around the world are key considerations in open labware. The community effort matters, too. If you build something useful and put it online – free to use and modify – then others will soon pick it up, improve it, and re-share it. It's a very satisfying process that results in greater benefit for everyone.

AMC: Open labware fits quite nicely with all the other efforts to make academic science more reliable, robust and accessible. As more and more initiatives push for open-access publications, open sharing of study designs, and preregistration of protocols, soon the only barrier stopping anyone from being an active part of science will be the lack of appropriate tools. That's where open-source hardware comes in. It allows more people to actively participate in science by coming up with testable ideas and developing experiments to prove or disprove them. Not only that, but by being open, the communities involved also become collaborators. We can build on one another's progress, so everyone wins!

Take the FlyPi as an example. Anyone can use it as a starting point for a better microscope design. They "win" by not starting from scratch, and we "win" by having more people working to improve our original design.

A final point to consider: many new tools for science are developed inside institutions funded by public investment, so it is only fair and ethical that they are released under open-source licenses. That way, the people who paid for the technologies can start to reap their benefits faster.

How can others get involved in the open labware movement?

TB: Pick your favorite design and build it! You will soon realize how easy it is not to just follow other people's instructions, but to modify them to better suit your needs. Most people get hooked at first contact. For example, I ask every new student in my lab to build something, and so far everyone has latched onto it with ease. The result? Most things in the lab that can be printed (rather than bought) are – which saves a huge amount of money.

AMC: I completely agree; building things that are already available is a great way to start! It might seem a bit intimidating in the beginning, because we are used to being passive consumers of technology rather than active makers. The designs can seem complex, with a lot of parts users have never seen before, using concepts that most people last saw in high school. But there are a lot of tutorials online for small projects that introduce newcomers gently, as well as a very active community of people who excel at replying to questions and assuaging doubts. Additionally, once you start learning basic circuitry, you'll realize that there is a lot of repetition in laboratory equipment. The electronic circuits necessary to build an orbital shaker, a centrifuge, or the heating and cooling element of a thermocycler are all pretty much the same! Once you learn how to build one of those things, the next one is exponentially easier.

Your very own FlyPi

All of our designs are available on GitHub, and we also put the best ones on Thingiverse and Open-Labware.net so that they are freely accessible to anyone who wishes to use them. If people wish to share their own modifications to the system, the simplest way is to generate a fork to the existing GitHub repository (github.com/amchagas/FlyPi) and upload them. If the changes mostly concern 3D printed parts, it may also be worth putting them on Thingiverse and tagging the original design (thingiverse.com/thing:905172) so that it's easy for others to find the modifications. Of course, if users are interested, we are certainly happy to include substantive improvements directly to the core repositories as appropriate. Just write us an email!

Is FlyPi right for everyone? I think for diagnostic purposes, it's all about the size of the thing that needs to be resolved. Using the cheap plastic objective lens that comes with the system, we have a point-spread function of about 10 µm. Resolving objects substantially smaller than this is likely to be difficult – if not impossible – unless you use a higher-quality objective, which is a fairly easy modification. What does that mean in the clinic? Counting red blood cells or identifying types of white blood cells is fairly easy with the basic system. Identifying malaria parasites within red blood cells, on the other hand, is just beyond the limit of its abilities – you'll need to add a better lens for that.

The rest of the lab

FlyPi is a great – and very low-cost – addition to most laboratories, but you don't have to stop at just one piece of "homebrew" technology. Many standard pieces of lab equipment can already be home-built. Centrifuges, shakers, incubators and even thermocyclers have been on the makeshift market for a long time. In our own lab, we usually build "new" things if we identify an obvious need, but cannot find an existing open-source solution. If you run into the same problem, it is always worth checking a few standard sites: Thingiverse (2), Instructables (3), Hackaday (4), and the National Institutes of Health 3D Print Exchange (5). To help new users along a bit, we also curate a collection of designs that are well-documented and seem to work (6). Take a look at what's available and consider how it might fit into your current laboratory setup. We look forward to welcoming you to the open labware revolution!

Tom Baden is Principal Investigator and Senior Lecturer in Neuroscience at the University of Sussex, UK.

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Simulated Scenarios: Real-Life Benefits

Simulation has an important place in medical education – and now, in the early stages of its evolution, is the time to get on board

By Viren N. Naik

Medical education has undergone fundamental changes over the past few years, and I expect it will continue to do so long into the future. We've shifted from a didactic, lecture-based system of training to a competency-based one that focuses on creating capable doctors instead of living medical textbooks. We're moving from volume-based healthcare to a value-based model in which it's as important for doctors to know how to interact with their patients as it is to know how to treat them. And, of course, technology is insistently pressing into our classrooms and continuing professional development. So how can we best take advantage of these changes? Simulation can have a significant impact on medical education – but what exactly is it, and how can it benefit pathologists?

At a Glance

- *Simulation encompasses a wide range of activities, including standardized patients, hands-on training and virtual reality*
- *Simulation activities can help medical educators in every field maintain and improve their professional and interpersonal skills*
- *The uptake of simulation in educational scenarios has been slow, partly because users are unaware of its benefits and partly because it needs to target distinct clinical needs*
- *It's important for pathologists to get comfortable with simulation while it's still in its beginnings to reap the greatest benefits*

What is simulation?

Simulation is an umbrella term for a broad spectrum of activities. Essentially, any time you recreate the clinical experience – whether for education or to optimize clinical processes – you're working with simulation. The key is that the entire patient journey can be simulated, from admission to discharge, which means you can use it at any stage of the process to improve your understanding or ability.

The spectrum of simulation also encompasses a wide range of techniques, from something as low-tech as standardized patients all the way up to full mannequin simulators and virtual reality. It's the latter modalities that most people think of when they hear the word "simulation" – things that recreate a patient (vital signs, pathophysiology, reactions to treatment) – but, as vital as that aspect is, there are others just as important to replicate. For instance, environmental fidelity (faithfully recreating the clinical setting) or psychological fidelity (creating a scenario that "feels real").

It's important to be inclusive in our definitions of simulation; if not, we risk treating it as a standalone educational activity instead of an integrated part of the medical curriculum that touches every aspect of teaching – and that's how it should be.

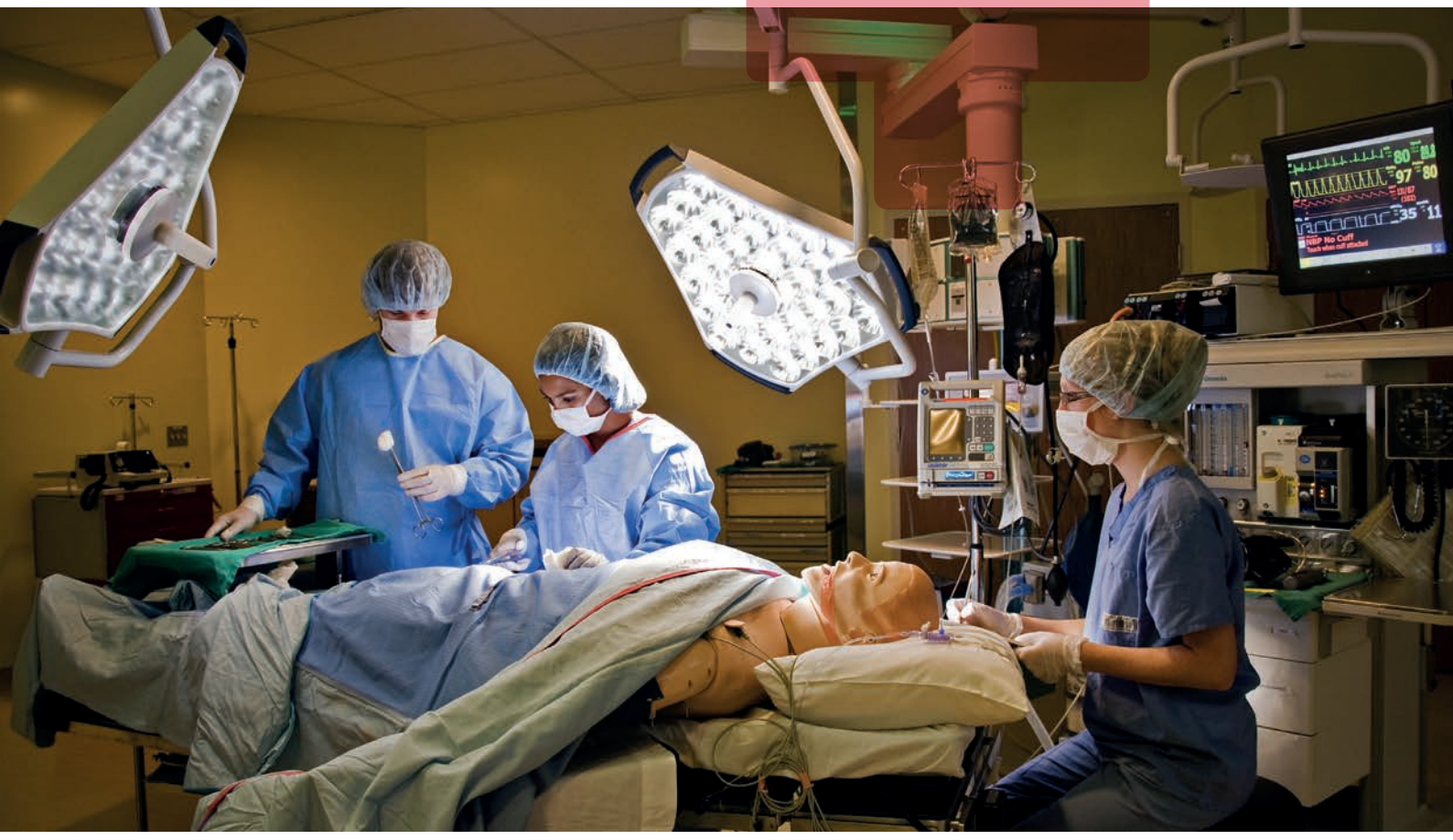
Where does it fit?

The main focus of education used to be on medical knowledge – but we now realize that doctors need to be proficient in many different competencies. Here in Canada, we call them intrinsic roles: communicator, collaborator, leader, health advocate, scholar and professional, all of which are centered on the medical expert. We've also moved from the "apprenticeship model" to more team-based care; we interact with far more colleagues than we did a century ago. Doctors don't pass on their training one-to-one anymore; instead, you develop a new doctor under multiple supervisors.

I also think that doctors spend less time in the clinical setting than they used to because of work-hour restrictions and a better understanding of wellness issues. Years ago, trainees would work extremely long hours to experience as many different cases and procedures as possible – but, since then, there has been an exponential increase in our medical knowledge, so there's no way to see and do everything you may need in the future, no matter how much time you spend in the clinic.

> *"If you rely only on what comes through the door during medical training, you'll miss out on a great deal of learning."*

So how does simulation fit into that picture? Consider a learning curve with a mastery asymptote. Simulation can accelerate a person through the steep part of that curve. If they can learn the basic skills or core competencies in a simulated environment, then when they enter the clinic, they are refining those skills as opposed to learning them for the first time on a living subject. And for some essential competencies, the right patient simply doesn't walk into the clinic often enough; if you rely only on what comes through the



door during medical training, you'll miss out on a great deal of learning. Simulation fills those gaps, and will continue to be valuable long after medical school – after all, refreshing your knowledge of core competencies is an important part of continuing professional development.

The challenge is that today's medical educators didn't train with simulation, and most of them have little exposure to it even now. Educators typically train others in the paradigms they are most used to, which may be why simulation has been slow to gain a strong foothold in medical education. But it is growing. We'll know we've been successful advocates when educators begin delivering the bulk of their curricula through simulation. It's a resource-intensive method, of course, and less cost-effective than delivering a lecture to 500 people – but does the lecture deliver effective learning? Simulation, on the other hand, is a powerful and effective form of learning – and that's where its differentiating teaching value lies.

What difference does it make?

There are different levels of evidence for the impact of simulation in education:

- T0 research measures learner satisfaction. Do they like being in the simulation environment? Do they think it is a valuable experience? The downside is that it's not valuable research because the answers are obvious. Who wouldn't like being in a simulator with a 1:5 teacher:student ratio instead of sitting in a boring lecture?
- T1 research assesses whether learners' knowledge, skills or attitudes have actually changed, and involves retesting students to see whether or not their performance has improved after learning in a simulated environment.
- T2 research assesses whether or how newly acquired knowledge, skills and attitudes affect the learner's practice when caring for patients. If a student improves their ability to perform a physical exam by practicing on – for example – a standardized patient, you'll be able to see that improvement when they begin working with actual patients.
- T3 research asks a big question: are

"I think pathologists can use simulation to learn how to better communicate with patients, and how to collaborate with other medical specialists."

learners changing patient outcomes? Emerging research suggests that doctors trained in a simulated environment are better at placing central lines – and, because of that, the infection rate after placement decreases. Others might see reduced morbidity and mortality in a cardiac resuscitation. This is all new research, but I expect to see more of it in the near future, because improved patient care is the true objective of simulation-based training.

Increasing impact

I think doctors are unaccustomed to active assessment. After our certification examinations, we go on to practice for several decades, and most of our continuing professional development is passive: lectures, conferences, reading… Of course, it's human nature not to enjoy exposing oneself to criticism – and that's why we have to change the model so it's about coaching, not criticizing. Simulation helps by letting instructors coach in replicas of real-world situations, so that learners can see the direct impact on their actions and on patient outcomes. I hope that seeing such effects will encourage people to engage with simulation even more.

Once they've tried it, people tend to think: "Hey, that wasn't so bad!" They recognize the power of the simulation; they appreciate the coaching; they learn. In this instance, the challenge is to lead the horses to the water in the first place, not to make them drink. We've tried things like incentivizing simulation training and tying it to accreditation, but we still haven't seen widespread uptake. In my opinion, if we really want to see simulation take off, we need to remove barriers. We must lower the cost and make it easier to access simulation centers. And if that doesn't work, we might need a small top-down push. I think we have to say to ourselves, "If we truly believe simulation is such a powerful form of learning, then at what point do we consider mandating it for training and professional development?" Some core competencies – for instance, resuscitation techniques – are impossible to test in any other way.

Meeting needs

We still need to improve our equipment – that's currently the least realistic aspect of simulation! One day we may get Star Trek-style holodecks for medical education – but if we want them to be functional, we have to make sure they're actually meeting clinical needs. Even the best simulator will go unused if there's no need for it – either because it teaches something that no one uses, or because it teaches something so common that there's no need to invest in an expensive, high-tech simulator to demonstrate it. For example, a frozen section

simulator for trainee pathologists would be pretty pointless – they're going to do so many throughout the course of their career that there's no need for a simulator to teach that skill. We need to figure out where the educational needs lie and then develop simulation technologies that address them.

Pathologists may wonder, "Where does simulation fit into our education? We don't do resuscitations; we don't do complex surgeries; what gaps does simulation fill for us?" In pathology, the benefit of simulation – as in any other specialty – is that it offers us the chance to improve the patient experience. However, it's not about equipment fidelity; it's about environmental and psychological fidelity. There are, of course, some procedural skills – specimen preparation and so on – that may be best attempted in a simulated fashion at first (rather than in a high-stakes patient diagnosis), but that's not where I see the highest return on investment for pathology. I think pathologists can use simulation to learn how to better communicate with patients, and how to collaborate with other medical specialists. We can all benefit from better communication skills, especially for patient interactions.

Simulation isn't the answer to every question in medical education, but I think we're on the cusp of a revolution. If we can get both teachers and trainees comfortable with using simulation techniques, and make sure that those techniques target clinical and educational needs, then we'll be heading in the right direction – and we'll also be well-prepared for the holodecks of the future!

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First in Class

Sitting Down With… Kona Williams, Junior Forensic Pathologist and First Nations Liaison at the Ontario Forensic Pathology Service, Toronto, Canada

What inspired you to study forensic pathology?

Medicine was a good choice for me because I always loved science, my grades were good, and I was always happy to help someone in need. I had some phenomenal mentors to help me along the way, too. One of the first physicians I met in medical school was a pathologist. She was (and still is!) an amazing teacher, and she inspired me to take up pathology as a career. From the first moment, it was fascinating – I got to see so much that my colleagues only read about in textbooks. Luckily, just as I was starting my anatomical pathology residency in Ottawa, a new forensic unit opened up there. Forensic pathology quickly captured my attention, and my mentors encouraged me to pursue it. I ended up doing a fellowship in Toronto, in a brand-new, state-of-the-art facility. And the rest is history…

Can you describe your typical workday?

Work life is very busy, but each day is different. I spend a lot of my time in the morgue performing autopsies, which really depend on who has died overnight. Sometimes there are criminally suspicious cases – but, regardless of the cause of death, I learn something new each time.

As with any medical profession, there's a lot of paperwork. I also spend time teaching residents, medical students and other health care professionals; I liaise with other experts in forensic science; I conduct research; and I regularly testify as an expert witness in court.

What is it like to be Canada's first (and currently only) First Nations forensic pathologist?

There aren't many indigenous people in medicine or the sciences. It was hard to be the only one for so long, until I met some of my medical school classmates. That was a revelation – and we worked hard to help develop the indigenous program at the University of Ottawa. My background didn't really factor into the equation until late in my forensic pathology fellowship. I didn't realize initially that I was the only one who was First Nations. It didn't seem like a big deal at the time, but now I am the First Nations Liaison for the entire province at the Ontario Forensic Pathology Service. Because it's a completely new initiative, it has been a rollercoaster of evolution – there is so much that can be done, and so many issues to be addressed. I'm only one person, but I'm dedicated to helping improve the death investigation system for indigenous people in Ontario.

Working closely with the cultural customs that surround unexplained death can be tough. It's important to remember that each community is different – and even within the same community, different families may have different beliefs. Understanding and respecting this is crucial.

Canada's size and diversity creates some unique challenges as well. Often, a body is transported from a remote northern community to undergo autopsy, which can mean days of travel. This completely disrupts the grieving process and many traditional practices that surround the death of a community member. If the death is that of a child or youth, the situation becomes even more complex and challenging. It takes a good understanding of all the subtleties to make the right decisions, and we must always make sure the lines of communication are wide open.

What advice do you have for trainees just starting out in pathology?

Keep your eyes and mind open. Find what you enjoy, and get good at doing that. The best feeling in professional life is to excel at what you do and enjoy it at the same time. And whatever obstacles you face, don't let anyone tell you that you can't do something you love. Never give up!

What would you like other pathologists to know about forensic pathology?

It's probably the one specialty where you can gather all the pieces of the puzzle, from what you can see macroscopically on the body to microscopic findings, radiographic imaging, microbiology, toxicology, biochemistry, and now the molecular autopsy… You need to be able to assemble all those pieces of the puzzle to determine how someone died. This information is so important to the family, and can have real implications for surviving family members, the criminal justice system, and the public. It's a huge responsibility and a great honor.

"I'm only one person, but I'm dedicated to helping improve the death investigation system for indigenous people."

Sometimes the only way to determine how someone died is by autopsy, and I like finding the answer. But the best part of my job? Being able to give families those answers. There's a myth that pathologists aren't good at speaking to families, but I really enjoy it – and it can be very beneficial for them to hear the results directly from me.

I would absolutely love to see forensic pathology become as well-known as other specialties in medicine. Most people have no idea what I do. It's not like CSI on TV – it's better!

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