OCTOBER 2020 # 69

the Pathologist®

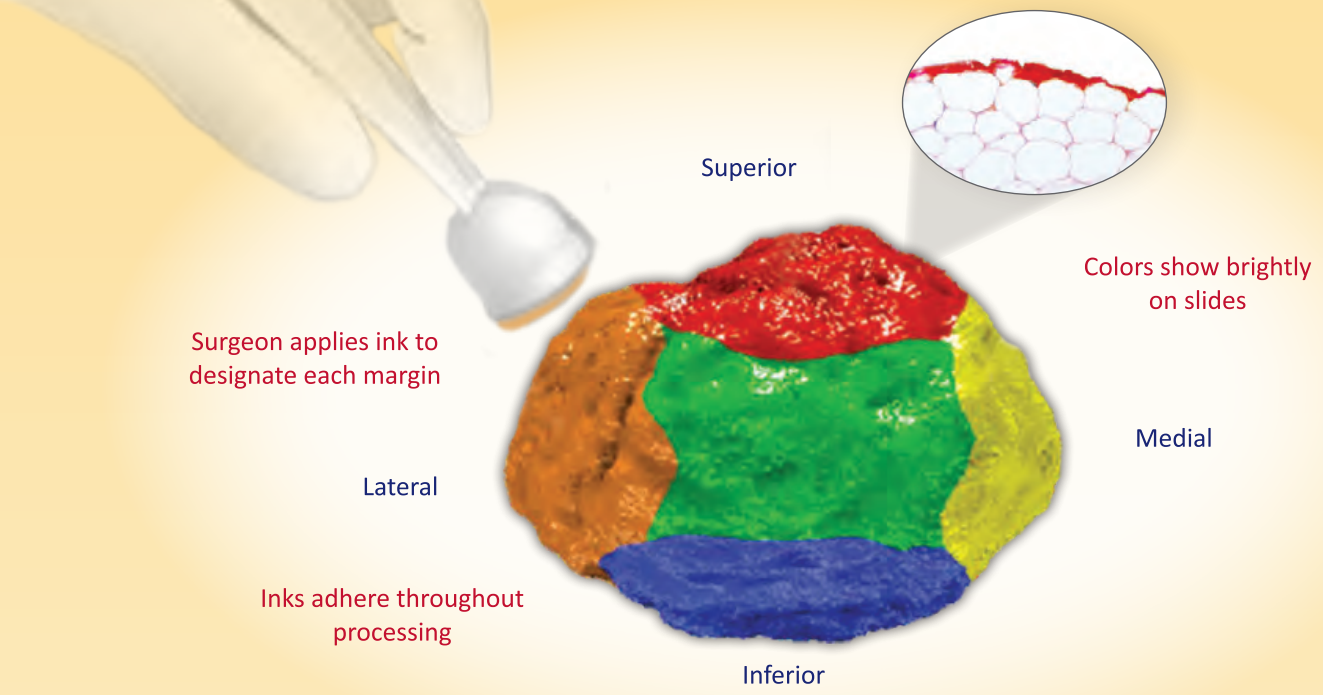
Official society partner of The Pathologist

The Promise of Precision Profiling

Three experts tackle a tough subject: the future of molecular oncology

MarginMarker™

Sterile Ink Kit for Intraoperative Use



Pathology Receives Specimens With Margins Clearly Marked by the Surgeon

In cancer surgery, the single most important predictor of local recurrence is the tissue margins.[1] Research shows discordance rates as high as 52% in the identification of specimen margins.[2,3] Re-excision rates exceed 20% in breast surgery.[4] Use of Vector Surgical's MarginMarker can result in:

- 50% fewer unnecessary re-excisions[1,5,6]
- Fewer delays of critical adjuvant therapy[7]
- More accurate re-excisions[5,8]
- Lower cancer recurrence[9,10]
- Improved cosmesis[6,8]

View Pathology Slides and Video Demonstrations | Request a Sample | VectorSurgical.com | +1 (262) 798-7970

VECTOR™ SURGICAL

Our grateful thanks to those who selflessly provide health care and support during these challenging times. Your service and dedication are sincerely appreciated.

 References: (1) Dooley, W.C. and Parker, J. "Understanding the Mechanisms Creating False Positive Lumpectomy Margins." *American Journal of Surgery* 190 (2005): 606-608. (2) Britton, P.D.; Sonoda, L.I.; Yamamoto, A.K.; Koo, B.; Soh, E.; and Goud, A. "Breast Surgical Specimen Radiographs: How Reliable Are They?" *European Journal of Radiology* 79 (2011): 245-249. (3) Molina, M.A.; Snell, S.; Franceschi, D.; Jorda, M.; Gomez, C.; Moffat, F.L.; Powell, J.; and Avisar, E. "Breast Specimen Orientation." *Annals of Surgical Oncology* 16 (2009): 285-288. (4) McCahill, L.E.; Single, R.M.; Aiello Bowles, E.J.; Feigelson, H.S.; James, T.A.; Barney, T.; Engel, J.M.; and Onitilo, A.A. "Variability in Reexcision Following Breast Conservation Surgery." *Journal of the American Medical Association* 307.5 (2012): 467-475. (5) Singh, M.; Singh, G.; Hogan, K.T.; Atkins, K.A.; and Schroen, A.T. "The Effect of Intraoperative Specimen Inking on Lumpectomy Re-excision Rates." *World Journal of Surgical Oncology* 8.4 (2010). (6) Landercasper, J.; Attai, D.; Atisha, D.; Beitsch, P., Bosserman, L.; Boughey, J.; Carter, J.; Edge, S.; Feldman, S.; Froman, J.; Greenberg, C.; Kaufman, C.; Morrow, M.; Pockaj, B.; Silverstein, M.; Solin, L.; Staley, A.; Vicini, F.; Wilke, L.; Yang, W.; & Cody III, H. "Toolbox to Reduce Lumpectomy Reoperations and Improve Cosmetic Outcome in Breast Cancer Patients: The American Society of Breast Surgeons Consensus Conference." *Annals of Surgical Oncology* 22.10 (2015): 3174-3183. (7) Lovrics, P.J.; Cornacchi, S. D.; Farrokhyar, F.; Garnett, A.; Chen, V.; Franic, S.; and Simunovic, M. "The Relationship Between Surgical Factors and Margin Status After Breast-Conservation Surgery for Early Stage Breast Cancer." *The American Journal of Surgery* 197.6 (2009): 740-746. (8) Gibson, G. R.; Lesnikoski, B.A.; Yoo, J.; Mott, L.A.; Cady, B.; Barth, R.J. Jr. "A Comparison of Ink-Directed and Traditional Whole-Cavity Re-Excision for Breast Lumpectomy Specimens with Positive Margins." *Annals of Surgical Oncology* 8.9 (2001): 693-704. (9) Gage, I.; Schnitt, S.J.; Nixon, A.J.; Silver, B.; Recht, A.; Troyan, S.L.; Eberlein, T.; Love, S.M.; Gelman, R.; Harris, J.R.; and Connolly, J.L. "Pathologic Margin Involvement and the Risk of Recurrence in Patients Treated with Breast-Conserving Therapy." *Cancer* 78.9 (1996): 1921-1928. (10) Menes, T.S.; Tartter, P.I.; Bleiweiss, I.; Godbold, J.H.; Estabrook, A.; and Smith, S.R. "The Consequence of Multiple Re-excisions to Obtain Clear Lumpectomy Margins in Breast Cancer Patients." *Annals of Surgical Oncology* 12.11 (2005): 881-885.

~~False~~ Fake Negatives

Beneath the surface of COVID-19 testing lurks a monster: the fake test

Direct-to-consumer testing has always been a contested topic – not just in pathology, but in medicine as a whole. The question of whether or not patients should have unlimited access to their own results is controversial on its own, but giving them the ability to choose and order their own tests is another matter entirely. It's an issue that first reared its head as the cost of gene sequencing decreased; but now, perhaps unsurprisingly, another test is gaining traction: the over-the-counter COVID-19 test.

Around the world, health authorities are crying out for more testing. Surely increasing access can only be a good thing. Unfortunately, many direct-to-consumer tests have poor accuracy – and some are outright fakes. Every day, my spam filter catches at least one email offering to sell me a personal testing setup for the office. Several people in the UK have been arrested for selling fake COVID-19 test kits, including one that provided users with unlabeled hydrogen peroxide and potassium thiocyanate as mouth rinses. Worse yet, a "black market" has sprung up around fake COVID-19 test results, allowing people to evade quarantines and restrictions by providing a negative test result – without ever having taken a test at all.

The risks are uncountable. At best, consumers might not swab themselves correctly, potentially leading to false-negative results. At worst, they could be ingesting harmful chemicals, misusing resources intended for hospitals and legitimate laboratories, or even infecting dozens of contacts.

It's possible that the ship has sailed on direct-to-consumer genetic testing. But is the same true of COVID-19? And – if not – what can we do to prevent its departure? The word of an expert carries a lot of weight. How can pathologists and laboratory medicine professionals contribute to the conversation and educate the general public on the dangers of illicit tests – and the value of legitimate ones?

Michael Schubert
Editor

www.purigenbio.com/**ffpe**

IONIC®
PURIFICATION SYSTEM

Get 3.5x More DNA and 2x More RNA from FFPE Samples

Automated Nucleic Acid Purification – Pure and Simple

The **IONIC® Purification System** uses isotachophoresis to extract, purify, and concentrate nucleic acid from biological samples without binding, washing, or stripping from fixed surfaces. Since nucleic acids remain in their native form, not denatured or dehydrated, the Ionic system produces more nucleic acid with higher quality – an ideal solution for low-quality samples or samples with limited starting material.

- **Simple Workflow**
  Extract and purify 8 samples at a time with less than 5 minutes of hands-on time per sample
- **Purify *both* mRNA and miRNA**
  Co-purify both mRNA and miRNA with higher yields than column-based kits
- **Simplify Lysis**
  De-paraffinize, lyse, and de-crosslink in a single reaction without using harsh chemicals
- **Improve Reliability**
  Minimize user-to-user variability, cross-contamination, and sample loss

For more information, contact **info@purigenbio.com**.

NUCLEIC ACID PURIFICATION
PURE AND SIMPLE™

PURIGEN™
BIOSYSTEMS

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Contents

the Pathologist

ISSUE 69 - OCTOBER 2020

Change of address info@thepathologist.com
Hayley Atiz, The Pathologist, Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

General enquiries
www.texerepublishing.com | info@thepathologist.com
+44 (0) 1565 745 200 | sales@texerepublishing.com

Distribution: The Pathologist (ISSN 2055-8228), is published monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK. Single copy sales $15 (plus postage, cost available on request info@thepathologist.com). Non-qualified annual subscription cost is available on request.

SPECIAL SERIES
Molecular Pathology

Upfront

Research
Innovation
Trends

Prognostic Tumor Gene Test for Ovarian Cancer

New tumor gene test reliably predicts survival chances in ovarian cancer patients

Survival rates in patients with epithelial ovarian cancer are poor, with high-grade serous ovarian cancer (HGSOC) accounting for around two-thirds of cases and most deaths. To offer patients a better chance of survival, we need biomarkers that provide insight into prognosis and personalized treatment options. Could gene expression biomarkers be the answer?

Until recently, gene expression findings in HGSOC were inconsistent and presented logistical barriers when using fresh frozen tissue, limiting real-world application. Now, researchers from UNSW Medicine have developed a robust and clinically relevant measure to identify ovarian cancer patients with poor prognosis using formalin-fixed, paraffin-embedded (FFPE) tumor tissue (1).

By analyzing 3,769 ovarian cancer tumor samples from the Ovarian Tumour Tissue Analysis (OTTA) consortium, they found that gene expression could reliably predict a patient's chance of surviving five years after diagnosis, with a difference of seven years' survival between poor and good prognosis groups. The signature was an even better predictor than age or stage of disease alone. "We were surprised at how well the assays worked on RNA from old FFPE tumor tissue blocks," says Susan Ramus, co-founder of the OTTA consortium and lead researcher on the study.

In the clinic, the new test would enable more informed, personalized treatment decisions. Ramus says, "As patients are diagnosed with ovarian cancer, their tumors could be tested for this signature, and those predicted to have poor survival with current treatments could rapidly be included in clinical trials of alternative treatments."

TIMELINE

A Molecular Journey

Key dates along the path to precision molecular diagnostics

1977
Frederick Sanger and colleagues develop Sanger DNA sequencing

1990
The Human Genome Project is launched

First linkage map of human genome: 1994

First draft sequence of human genome: 2001

Successful completion: 2003

1994
The Association for Molecular Pathology is founded

RESEARCH REVIEW

A rapid roundup of pathology and lab medicine news

CRISPR for COVID-19

The demand for rapid COVID-19 screening is ravenous – especially in settings without the necessary equipment and resources to conduct metagenomic next-generation sequencing or RT-PCR. To address this gap, researchers have developed a new test that uses CRISPR technology to provide rapid, sensitive COVID-19 diagnosis (1).

Antibody Answers

New research reveals distinct differences in the anti-gluten antibodies of patients with celiac disease and those with non-celiac gluten sensitivity (2). Whereas the former experience a strong, sustained primary B cell response, the latter evidence similar antibody levels, but less inflammation.

A Boon for Blood

After donation, blood may be stored for weeks prior to transfusion. During that time, it undergoes continuous degradation. Laboratorians are responsible for the difficult and time-consuming task of assessing viability – but now, a deep learning network that can recognize red blood cell degradation can help (3).

Radiotherapy Revealed

Radiotherapy is not typically the province of the pathologist – but, with the discovery of genes that may predict treatment success, that may change. Mutations in the ERCC6L2 gene correlate with better survival in radiotherapy-treated patients (4), suggesting its potential use as a biomarker for the likelihood of response.

A Look at Interleukin

Triple-negative breast cancer (TNBC) – a diagnosis known for its poor prognosis and lack of response to standard treatments. Now, though, new research has revealed a new prognostic biomarker and potential therapeutic target in one: interleukin-34 (5). The cytokine is highly expressed in TNBC and correlates with lower survival rates.

References

1. *T Hou et al., PLoS Pathog, 16, e1008705 (2020). PMID: 32853291.*
2. *M Uhde et al., Gastroenterology, [Online ahead of print] (2020). PMID: 32702369.*
3. *M Doan et al., Proc Natl Acad Sci USA, [Online ahead of print] (2020). PMID: 32839303.*
4. *P Francica et al., Cell Rep, 32, 108068 (2020). PMID: 32846126.*
5. *N Kajihara et al., Breast Cancer, [Online ahead of print] (2020). PMID: 32578004.*

Why Didn't They Teach This in Med School?

A series on new (and not-so-new) medical terms and diagnoses that most of us (probably) missed in training

Reading about COVID-19, you may have encountered the four parameters below. Which one indicates the risk unit defined as one-in-a-million chance of death?

a) Micromort
b) Microprobability
c) R_0
d) R_t

Answer: a) Micromort

Micromorts are used to measure the risk inherent in various daily activities. A microprobability is a one-in-a million chance of any given event; a micromort is the microprobability of death.

For an average American, the risk of dying of unnatural causes is one in a million per day, or one minimort. The risk of dying under general anesthesia is five per million, or five minimorts. For COVID-19, the exact micromort cannot be calculated yet – and it depends on many variables.

1996
The first disease-associated genes are located

SNCA: associated with Parkinson's disease

HPC1: predisposes to prostate cancer

2006
The Cancer Genome Atlas is launched

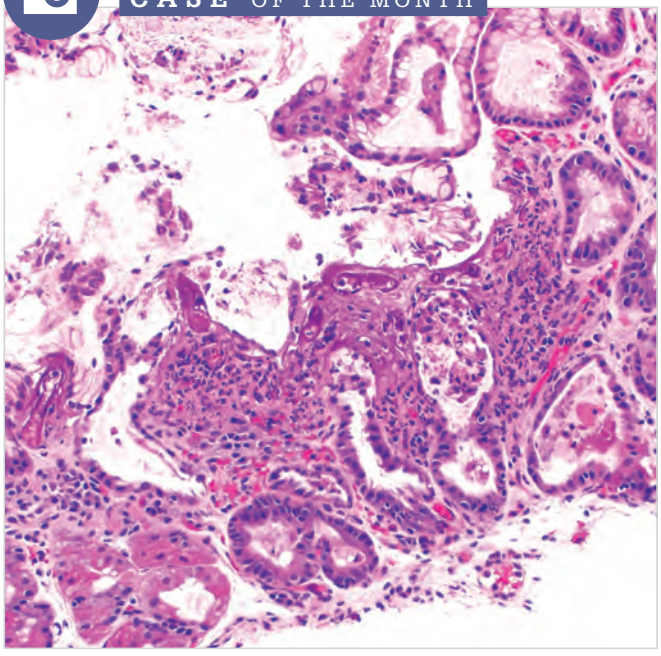
2014
The 100,000 Genomes Project is launched

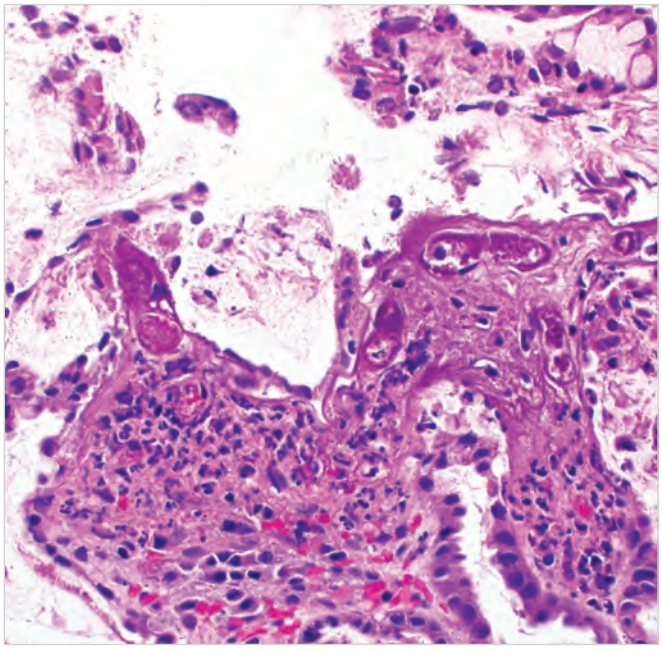
First adult diagnoses: 2015

First pediatric diagnoses: 2016

Successful completion: 2018

CASE OF THE MONTH





A 70-year-old woman with multiple medical problems presented with abdominal pain. Upper endoscopy was performed and gastric biopsies showed histologic abnormalities as displayed in the representative images above.

Which of the following diagnoses is most likely?

a) Mucosal calcinosis
b) Doxycycline-induced gastric injury
c) Strongyloides *infection*
d) Gastric antral vascular ectasia

Answer to last issue's Case of the Month…

b) Polyoma BK virus

The diagnosis is supported by nuclear immunostaining with antibody to simian virus 40 (SV40), used for demonstrating BK virus (BKV) because the two viruses share 70 percent homology. Histologically, BKV cytopathic effects are usually seen in renal tubular epithelial cells as 40–45 nm intranuclear inclusion bodies. They may also be observed in Bowman's capsule parietal epithelial cells. Associated lymphoplasmacytic tubulointerstitial inflammation may be present in the biopsy. Prolonged infection progresses to interstitial fibrosis. Routine BKV load assessment and examination during surveillance biopsy is important for early detection. Immunohistochemical staining with SV40 can be used to aid detection, especially in cases with lymphoplasmacytic inflammation, possible nuclear inclusions, and/or high clinical suspicion.

Submitted by Amanda Kitson, House Officer IV, University of Michigan, Ann Arbor, Michigan, USA.

Further Reading

1. *D Sawinski, J Trofe-Clark, Clin J Am Soc Nephrol, 13, 1893 (2018). PMID: 30242026.*
2. *P Acott, H Hirsch, Pediatr Nephrol, 22, 1243 (2007). PMID: 17377822.*
3. *B Nankivell et al., Am J Transplant, 17, 2065 (2017). PMID: 28371308.*
4. *A Zarauza Santoveña et al., Transplant Proc, 47, 62 (2015). PMID: 25645771.*

To register your guess, please go to http://tp.txp.to/1020/case-of-the-month
We will reveal the answer in next month's issue!

In advanced ovarian cancer,

If you're not testing for HRD, you're not seeing the whole picture

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

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

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

Learn more at **testforHRD.com**

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

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

Precision Medicine & Nurse Education

AstraZeneca

SPECIAL SERIES
Molecular Pathology

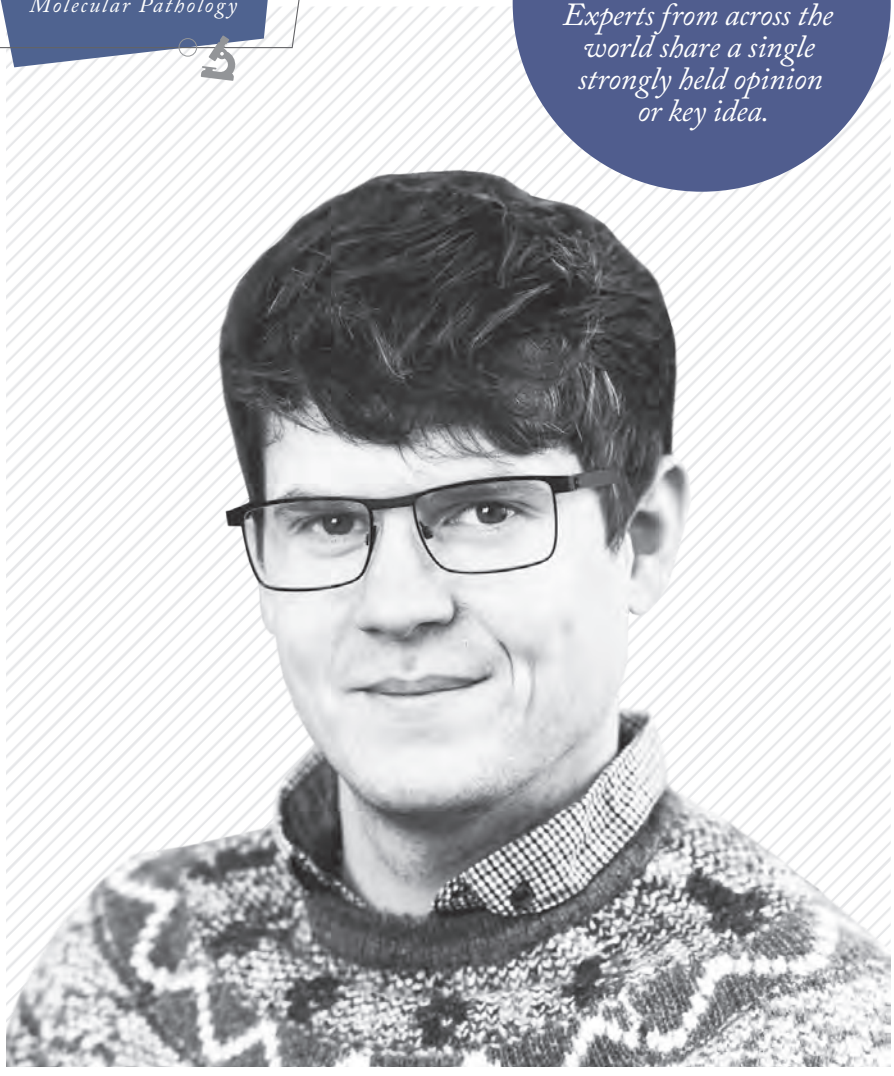
In My View

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

Breathe In – Breathe Out

Breath testing to advance early disease detection and diagnosis

By Jonathan Lawson, Head of Content at Owlstone Medical, Cambridge, UK



Invasive, labor-intensive, and potentially risky – a list of characteristics that don't sound desirable in a standard diagnostic tool. Yet tissue biopsy, with all of these characteristics, is the global gold standard in disease detection and diagnosis – and other well-established biopsy methods have their own limitations. It's clear that we have an urgent need for new approaches that are affordable, accessible, reliable, and safe to enable effective early detection of disease and advance precision medicine. Breath biopsy – the detection, identification, and precise quantification of chemicals in breath – has the potential to transform clinical pathology.

Exhaled breath is a valuable source of prospective disease biomarkers, containing over 1,000 volatile organic compounds (VOCs) in addition to respiratory droplets, which carry non-volatile compounds, proteins, lipids, nucleotides, bacteria, and viral particles. Together, these provide a rich source of information on metabolism, environmental factors, and disease processes. Breath collection is completely noninvasive and increasing sampling time allows detection of VOCs that may be present at very low levels in the earliest stages of disease. Such capabilities make breath a unique sampling option.

VOCs in breath can arise from external sources or from within the body itself. As such, they can reflect biochemical and metabolic activity, diet, prescription drugs, and environment. Many VOCs from all parts of the body are readily transported to the lungs via the blood, making breath samples compatible with whole-body disease sampling. Furthermore, because endogenous VOCs link directly to metabolic activity in the body, changes in their levels can be characteristic of specific disease processes from the earliest stages.

Respiratory droplets generated in the deep airways of the lungs have been the center of attention recently because of their role in transmitting respiratory infections. With the right collection approaches, biomarkers relevant to various diseases can be captured from respiratory droplets and analyzed using well-established

"We have an urgent need for new approaches that are affordable, accessible, reliable, and safe."

techniques, such as ELISA and PCR.

Discovering VOC biomarkers in a complex sample like breath requires both highly reproducible tools for collection and advanced chemical analysis to resolve and identify compounds. In the past, technical limitations and a lack of standardized analytical techniques have hindered the development of clinically relevant breath tests. In recent years, though, experts in the field have developed advanced collection technologies that offer consistent breath sampling. At the same time, though many techniques have been applied to breath analysis, gas chromatography-mass spectrometry (GC-MS) has emerged as the gold standard for VOC biomarker discovery. The latest high-resolution GC-MS platforms excel in the identification and quantification of biomarkers within the complexity of a breath sample, providing vital biological insight across the full range of exhaled VOCs. Together, these advances enable the development of novel breath tests in areas of high clinical need.

The range of potential applications for breath biomarkers in both research and clinical settings is expansive. Test development programs are under way in areas as diverse as respiratory disease, liver disease, cancer, and environmental exposure.

The early detection of cancer, particularly lung cancer, is a key area of interest in breath research. Despite being the most common cancer worldwide, lung cancer has one of the lowest five-year survival rates. Why? Because early diagnosis is costly and inefficient. Multiple studies have suggested that lung cancer could be diagnosed by the presence of certain carbonyls in a patient's breath (1). The benefits of a low-cost, noninvasive test that can be deployed in screening programs are clear.

Liver disease is rapidly growing as a cause of global morbidity and mortality.

"The range of potential applications for breath biomarkers in both research and clinical settings is expansive. Test development programs are underway in areas as diverse as respiratory disease, liver disease, cancer, and environmental exposure."

Existing liver function tests largely assess liver damage rather than current function and struggle to determine the stage of liver disease. Limonene, however, shows excellent potential as a biomarker of both cirrhosis and broader liver health (2,3). Originating from diet and detected noninvasively on breath, limonene abundance increases due to metabolic shifts linked to cirrhosis. Work is now ongoing to understand whether limonene and other breath VOCs can also be used to monitor liver disease, which is increasingly widespread due to high-fat diets.

Over half a billion people worldwide suffer from chronic inflammatory airway diseases including asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis. A lack of reliable diagnostic tools means that treatment often depends on trial and error – increasing costs, prolonging periods of poor disease control, and raising the risk of exacerbations. Breath analysis could provide rapid, noninvasive patient stratification to identify steroid responders and enable targeted therapies. Recent research is already exploring the identification and validation of breath biomarkers with the potential to differentiate inflammatory phenotypes in asthma (4), and there is hope for tools that could predict oncoming exacerbations.

Taken together, these three examples highlight the huge untapped potential for breath biomarkers to revolutionize early disease detection and precision medicine. And with the unprecedented attention COVID-19 has brought to breath research, the next few years promise to be an incredibly exciting time of development for the field, with the possibility of disruptive new healthcare technologies just around the corner.

References

1. *Y Saalberg, M Wolff, "VOC breath biomarkers in lung cancer," Clin Chim Acta, 459, 5 (2016). PMID: 27221203.*
2. *R Fernández del Río et al., "Volatile biomarkers in breath associated with liver cirrhosis – comparisons of pre- and post-liver transplant breath samples," EBioMedicine, 2, 1243 (2015). PMID: 26501124.*
3. *ME O'Hara et al., "Limonene in exhaled breath is elevated in hepatic encephalopathy," J Breath Res, 10, 046010 (2016). PMID: 27869108.*
4. *FN Schleich et al., "Exhaled volatile organic compounds are able to discriminate between neutrophilic and eosinophilic asthma," Am J Respir Crit Care Med, 200, 444 (2019). PMID: 30973757.*

SPECIAL SERIES
Molecular Pathology

Multiplexing the Tumor Microenvironment

Spatial biomarkers are needed to improve immunotherapy outcomes

By Cliff Hoyt, Vice President of Translational and Scientific Affairs, Akoya Biosciences, Marlborough, Massachusetts, USA

Delivering on the promise of precision medicine in oncology depends on the predictive performance of biomarker tests. Immuno-oncology treatments can be lifesaving – but only for a fraction of patients. Put simply, the predictive biomarkers available today don't always accurately identify the patients who will respond, leading to higher incidences of adverse events and treatment resistance. Conventional immunohistochemistry (IHC) techniques assessing PD-L1 expression and, to a lesser degree, microsatellite instability status are currently the only clinically validated predictive biomarkers for checkpoint inhibitors. However, most experts agree that neither assay is sufficient; some lead to false positives or false negatives, whereas others address only a small fraction of the patient population. There is much room for improvement.

It is evident that our current repertoire of immuno-oncology biomarkers does not adequately capture the root-cause biological mechanisms that correlate with response. New evidence suggests that a critical source of information is revealed in the cell-to-cell biology of the tumor microenvironment (TME). But most efforts to identify clinically useful biomarkers do not preserve spatial information related to the arrangement of cell types in TME and are thus blind to cellular and tissue cartographic coordinates. When it comes to cellular interactions and spatial clustering, many of which are critical for cancer development and progression, we are left in the dark.

A recent study underscores the need for spatial information to accompany immunotherapy-related biomarkers (1). This meta-analysis of more than 50 studies – spanning more than 10 solid-tumor cancers and over 8,000 patients – found that spatial characterization significantly improved the predictive power of biomarker assays. The authors reviewed traditional biomarker assays – including gene expression profiling, tumor mutational burden assessment, and immunohistochemistry – as well as two new types of assays that incorporate spatial data: multiplex IHC (mIHC) and multiplex immunofluorescence (mIF). The latter is a new type of biomarker assay that makes it possible to quantify multiple immune and tumor markers in a tissue section while preserving and imaging the spatial architecture of the tumor microenvironment.

The authors found that traditional biomarker types performed comparably in distinguishing between responders and non-responders to anti-PD-1/PD-L1 immunotherapy – but the spatially resolved biomarker assay types performed significantly better. These assays had fewer false positives; that is, they were less likely to predict positive response for patients who would not benefit from checkpoint inhibitor immunotherapy. Moreover, the results from this study showed that combining traditional biomarker assays improved predictive performance, but even this additive effect paled in comparison to the singular performance of mIF and mIHC assays.

> *"When it comes to cellular interactions and spatial clustering […] we are left in the dark."*

mIF and mIHC assays represent robust and standardized tools that can generate spatially resolved molecular maps of biopsy tissue sections, allowing us to see the cell-to-cell interactions and spatial biology occurring in the TME. Multidimensional examination of the TME should play an integral role in immuno-oncology research and in the search for better predictive biomarkers. From predicting immunotherapy treatment response to characterizing tumor heterogeneity, we now have access to a new dimension of information to benefit patients and help us expedite the battle against cancer.

Reference

1. *Steve Lu et al., "Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade", JAMA Oncol, [Online ahead of print] (2019). PMID: 31318407.*

A Precise Roadmap to Recovery

Precision medicine is critical to patient care

By E. Blair Holladay

If ever there was a misconception about the laboratory, it's that our job ends when a test result goes out the door. That couldn't be further from the truth; in fact, the moment a test result leaves the laboratory is the moment our job as pathologists and medical laboratory scientists truly begins.

As healthcare has moved from volume- to value-based care over the past decade, it has become increasingly vital that our profession step out from within the four walls of the laboratory and take on a more involved role in patient care. This evolution of healthcare has also driven the rise of precision medicine, which provides the perfect opportunity for pathologists and medical laboratory scientists to step into a more visible role. And, as precision medicine continues to expand, the role of the laboratory will become ever more vital to patient care.

At a high level, precision medicine is challenging the idea that patient care is one-size-fits-all. Just as no two patients are alike, neither should two treatment plans be the same. On a more in-depth level, precision medicine is taking the knowledge, skill, and expertise that pathologists and medical laboratory scientists employ on a daily basis to detail what a diagnosis truly means for a given patient and how it will impact their treatment. That knowledge is exactly what clinicians need to tailor a care plan for each one of their patients. Without that input from the laboratory, clinicians can only make educated guesses at what might be best for patients.

The American Society for Clinical Pathology has patients at the heart of its mission. We continually evaluate what more we can do for patients, how our programs benefit patients, or how a new initiative will improve patient care. And isn't that the ultimate driving force behind the rise of precision medicine – to improve patient care? We know that the laboratory's role is not over when a test result goes out the door. We understand that we are a part of a much bigger journey. A test result is not just words and numbers on a chart; it is the starting point on a person's roadmap to recovery.

Keeping the patient at the forefront of our work in the laboratory is vital. It is what will push precision medicine forward and improve outcomes for patients overall. As pathologists and medical laboratory scientists, we are the beginning and the end of a patient's healthcare journey. Understanding and embodying that truth is what will strengthen the laboratory's role in healthcare and allow precision medicine to reach its full potential.

"At a high level, precision medicine is challenging the idea that patient care is one-size-fits-all."

The PROMISE *of* PRECISION PROFILING

A roundtable with Marc Peeters, Özlem Er, and Philip Beer

Precision medicine is an increasingly common buzzword in healthcare – but what does it mean to pathologists and laboratory medicine professionals? How does it affect the patients who seek out molecular profiling – whether for cancer treatment, pharmacogenomics, or other reasons? And what is its outlook in the near future and beyond? Three experts gather to discuss molecular pathology, precision oncology, and the future of diagnostic and prognostic medicine.

MEET THE EXPERTS

MARC PEETERS

Professor and Head of Oncology at Antwerp University Hospital, Coordinator of the Multidisciplinary Oncology Center Antwerp, and Chairman of Oncology at the University of Antwerp, Belgium.

ÖZLEM ER

Professor and Head of the Departmen tof Medical Oncology at Maslak-Acıbadem University Hospital in Istanbul, Turkey.

PHILIP BEER

Specialist in precision oncology; leadership roles at various public and private centers in the United Kingdom.

WHAT IS YOUR BACKGROUND IN PRECISION MEDICINE – AND HOW DO YOU USE IT IN YOUR WORK?

Marc Peeters: Over the years, my profession as an oncologist has changed dramatically. At the beginning of my career, we were happy to have any drugs at all to treat our patients. Nowadays, it's a question of selecting which of a wide array of drugs will be best for each individual patient.

Certain types of tumors have a limited number of biomarkers that we routinely test for treatment selection. In other cases, we're searching for a solution for patients who have become resistant to standard lines of treatment. We need biomarker information to locate clinical trials or explore drugs that, although not indicated in the patient's tumor type, might yield a response in that specific patient based on their biomarker profile. At the moment, we don't fully profile every patient at my institution (beyond the routine testing); it's reserved for those who have exhausted the standard options. Smaller sets of biomarkers are tested if they are clearly linked to a registered (and thus reimbursed) drug.

Özlem Er: I am a clinician trained as an internal medicine and medical oncology specialist treating solid tumors. As a medical oncologist whose goal is to improve the therapeutic options of cancer patients, I use several tests to detect actionable targets in tumors. These tests can be performed on either blood or tissue samples. It's an approach that I hope will expand to many areas of oncology in coming years until it's common in the clinic. The more reliable biomarkers we have to help select appropriate candidates and match treatments to their molecular profiles, the more this kind of testing will improve the lives of people with cancer.

Philip Beer: I'm a clinician. My initial training was in hematology and I'm dual-accredited in internal medicine and pathology, which means I have both diagnosed and treated patients with leukemia.

Hematology was ahead of the game in using genetics to guide therapies – after all, the first really famous targeted therapy was imatinib for chronic myeloid leukemia. In hematology, clinical trial enrollment rates are 20–40 percent for adult leukemia (versus 5–10 percent for common solid tumors). That said, the solid tumor space is catching up; the genomics are a bit more complex, but the diseases are far more common, so there's a lot to be gained from better biomarkers and corresponding treatments. And that's the space I've been working in almost exclusively since 2014. I have a deep interest in using genomics and other

complex biomarkers to accelerate cancer drug discovery – and I'm pleased to be at the interface between healthcare delivery and drug discovery.

There are historical barriers between academia and the commercial sector – and, indeed, between healthcare delivery and commercial diagnostics and drug discovery. I hope stories like mine, where people overcome those barriers, become more common. In fact, I would like to see those barriers come down altogether. People should be free to move between the different sectors. We have a lot to learn from one another!

WHAT IS THE CURRENT STATE OF PRECISION MEDICINE IN EUROPE – AND HOW DOES IT COMPARE TO THE REST OF THE WORLD?

MP: I think precision medicine, for the moment, is still a work in progress. Each country has its own management approach; some have a central organization, whereas in others adoption depends on individual institutions with the resources to integrate precision medicine into daily practice.

The desire to take up precision medicine is there, but we must work to translate that clearly into clinical practice. Although some big companies are working to make information available to physicians for treatment decision-making, we have to be careful about how we use the data. First of all, the information that we get from genomic analysis cannot always be linked to specific tumors or therapies. And, second, it's possible to rely too much on these analyses to guide our decisions, especially when they are new and unfamiliar. In some cases, it's better to stick to the standards and integrate new information judiciously.

That said, it's a fast-moving field. If we talked about this again in three to five years, it would be a totally different conversation!

ÖE: Precision medicine in oncology is relatively new worldwide – and becoming more important every day. By definition, precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person (1)." This approach allows us to decide more accurately which treatment strategy will work for a specific patient by taking into consideration the genomic differences between tumors.

Precision medicine in oncology is more common in North America and Europe than in other parts of the world. As populations age, cancer incidence increases – and so, in turn, does the need for cancer treatment. In the long run, genomic testing will increase demand for tailored cancer therapeutics.

PB: I think we are at an interesting point in Europe. We've made some early gains – but I think the low-hanging fruit has been plucked. We have had successes with therapies like EGFR inhibitors for lung cancer, which have made a big impact. The recent success with TRK inhibitors may well be the last time that we have really powerful results, though. Now, we need to move beyond the single-gene/single-drug model. It's time for comprehensive genomic profiling to inform targeted therapies.

We've had a number of fairly high-profile failures in the targeted therapy realm, too. A recent example is IDO inhibitors, which failed in a fairly spectacular fashion in late-phase clinical development. Although the biological rationale for IDO inhibitors is strong, clinical trials were not backed up by comprehensive biomarker studies to understand how these drugs work in humans or if their activity is limited to a subset of patients. And that's why I feel we need to change the infrastructure of precision medicine to get to the next level; we need to bring in complex genomic profiling and use the data to make better use of therapies.

In terms of the rest of the world, America generally leads the way when it comes to medical progress – mainly through sheer scale of numbers and clinical trial infrastructure. In the large hospitals, where they pull in enormous numbers of patients, they can run complex clinical trials more easily. But there are disadvantages to taking up genomics through centralized providers. Not all patients have equal access, which means that the studies don't necessarily include all relevant populations. Europe has the potential to "get ahead" by embedding complex genetic profiling within the publicly delivered healthcare system so that it's available to everybody. We are still a little bit behind in Europe at the moment – and funding is always an issue – but I think we have a unique opportunity to overcome the infrastructure barriers other systems may face.

I think that the key to unlocking precision oncology lies in better profiling of patients. Genomics is a big part of that and immunotherapy will also bring in other profiling techniques. What we need, though, is to routinely perform these techniques on all cancer patients referred into hospital, rather than waiting until they're in a clinical trial. The other important part is that we collect outcome data. We need to match complex biomarker profiles not only to the treatments our patients receive, but also to what actually happens as a result. It may be easier to do that in a socialized healthcare system, such as we have in Europe, than to get the big institutions to harmonize and share data in the context of the American model. By ensuring equitable access to therapy and collecting outcome data, we can work out the true significance of complex biomarkers.

HOW WILL PRECISION MEDICINE CHANGE IN THE NEAR FUTURE?

MP: Genetic profiling is rapidly moving forward and will be a significant part of our daily routine in the next few years.

Bioinformatics is also becoming much stronger. The rest is still a work in progress. We need to work on integrating all of the available data into a clinical interpretation. We have yet to fully optimize our methodologies, and there's still a lot we don't know.

If we talk about the microbiome, for instance, it's clear that we're just at the beginning of our scientific exploration. What information do we get out of the microbiome? Does it help us develop or select treatments? Can we modify the microbiome in therapeutic ways? These types of questions remind me of where we were only a few years ago with genetic profiling – but now, tumor genetic profiling based on tissue or even liquid biopsy is becoming routine. The outlook is promising.

At the moment, we focus our profiling efforts mainly on treatment response and efficacy – but we sometimes forget to consider toxicity. Can we identify patients who should receive modified doses because they are at greater risk of toxicity? I think so – and that's an area in which we'll make a lot of progress in the future.

PB: An interesting thing that's going on in a few different European countries is a restructuring of genomic services. If that's done properly, it will probably take the better part of five years to get useful systems up and running, let alone big data collection – but at least people are beginning to think about the practicalities of making it happen.

The process of precision oncology is happening hand in hand with an interest in real-world data. There are companies in America set up solely to aggregate real-world patient record data – and, by doing it at scale, they've defined patterns of physician behavior, tracked prescription habits, and identified some interesting therapeutic connections. It will take a while for us to learn how to use real-world data effectively, but – if things go well – we could be in a great place in five years to accelerate the process of drug discovery.

We live in uncertain times for a number of reasons, so it can be difficult to look much farther ahead than that. But look at immunotherapy; it hasn't been long since people working in that field were considered charlatans and outcasts by mainstream scientists. And now – almost out of nowhere – immunotherapy has completely changed the face of cancer treatment. I don't think anyone, perhaps even the experts, expected quite such a sea change. So I hope that, in the next decade or so, we might be in a similar situation. I'd like to see individualized treatment based on patients' biomarker information become standard practice.

With the advent of cheaper, better sequencing, our understanding of inherited diseases is improving by leaps and bounds. England is now considering a move to whole genome sequencing as a standard test for suspected inherited disorders. On a wider scale, pharmacogenomics is an interesting space to watch. We're aware of a few markers with strong predictive effects right now but, when the effects become weaker, we need to use "polygenic risk scores" that combine many genomic features to predict a patient's risk or response. It all comes down to data generation – and we don't yet have the enormous amounts of data we need. Nevertheless, I do think how patients' genomes impact their drug responses – and how we can use that information to target treatment – will be a big story over the next 10 years.

It seems clear to me that bioinformatics will be the skill of the future – and not just pure bioinformatics, but everything that goes along with it: data architecture, natural language processing, and a variety of computational skills.

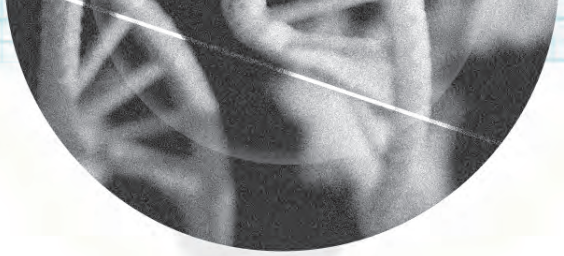
WHAT ARE PRECISION MEDICINE'S GREATEST STRENGTHS... AND WEAKNESSES?

MP: Its greatest strength at the moment lies in situations where patients have driver mutations with clear links to known therapies. Identifying a patient's individual mutations can create new treatment options – some that offer dramatic improvements.

Its weakness lies in the challenge of integrating information and increasing access in routine clinical care – not just in big academic institutions, but also in smaller centers that handle more routine cases. I think an important task for key opinion leaders is to ensure that all clinicians have access to this information and all patients receive optimal treatment. Another weakness is the current strong focus on genetics and genomics; to see the full benefit of precision medicine, we need to take a multidisciplinary approach that integrates proteomics, the microbiome, and more. Precision medicine made its first step in cancer, but the future is much more complex.

ÖE: Precision medicine's greatest strength is increased cancer treatment efficacy accompanied by less toxicity than

> *"The information that we oncologists get from pathologists is becoming much more important. It's no longer used only for diagnosis – we need [it] to guide treatment decisions."*



conventional chemotherapy. It also creates new treatment options for chemorefractory patients. However, because genomic tests are not reimbursed by insurance companies, we need financial solutions to enable routine testing.

PB: I think the greatest strength is that it has the potential to completely change the face of cancer therapy. We hear about amazing breakthroughs all the time in the papers – but the actual path will be slower and more laborious, of course, and it will have ups and downs. Despite all that, the breakthroughs we've made so far in immunotherapy are extraordinary. For instance, nearly half of all patients with metastatic melanoma are now being cured by immunotherapy. TRK inhibitors are now also approved to treat cancers in many different tissue types. We've begun to realize the long-held thought that cancer is a molecular disease, rather than a tissue disease.

Unfortunately, we're still stuck in old paradigms; patients don't get comprehensive biomarker profiling and, when they do, the data aren't necessarily good quality. As a result, I still have a slight concern that precision oncology will be written off before we can really ask the important questions. What is the role of genomics? What is the role of complex biomarkers? How can we find the right drug for each patient? We're beginning to ask these questions, but there's still a long way to go. For me, the big downside at the moment is that people may become disillusioned with precision medicine – before we've understood the full potential.

CAN YOU SHARE ANY ANECDOTES FROM YOUR EXPERIENCE WITH PRECISION MEDICINE?

MP: The information we got from tissue 20 years ago was relatively limited. If you consider the information available to us today – not only from tissue, but also from new techniques like liquid biopsy – we've made dramatic progress. Along with that progress have come changes in mindset; now, the information that we oncologists get from pathologists is becoming much more important. It's no longer used only for diagnosis – we need the information to guide treatment decisions in certain patients. A few years ago, we couldn't adequately treat many of our patients with kidney cancer; today, we can link a specific marker to a specific immunotherapy treatment – a sea change in which we now see dramatic responses and achieve disease control we never previously imagined.

We see the same situation with *NTRK* fusions and the drugs targeting the TRK pathway. These patients, who were previously poor candidates for systemic treatment, now show responses of 60 to 70 percent in some cases. It's clear that the current diagnostic and prognostic landscape is very different to what it was just a short time ago.

ÖE: Metastatic non-small cell lung cancer (NSCLC) has a poor prognosis; statistics show a median survival of 12 to 18 months. But, thanks to precision medicine, one of my patients with metastatic NSCLC survived for over five years after diagnosis. His tumor was EGFR-positive, confirmed with genomic testing, so he was treated with an EGFR tyrosine kinase inhibitor for almost two years before the disease progressed. While on this oral medication, he was asymptomatic, performance status ECOG 0, and lived life as if he were disease-free. Upon progression, we did another genomic test, which revealed a resistance mutation – *EGFR* T790M – whose activity can be inhibited by osimertinib, another oral tyrosine kinase inhibitor. The patient was able to continue treatment with oral medication and manageable side effects that didn't affect his daily life. It wasn't until his fourth year of metastatic disease that he progressed to the point where chemotherapy was needed. Not only does a survival of greater than five years show a clear advantage to this treatment approach but, for much of that time, the patient and his family were able to enjoy an excellent quality of life.

PB: I'm involved in the molecular profiling of patients for several projects. The more you provide patients with complex molecular genetic profiling as a matter of routine, the more you see things you might not otherwise have noticed. You might identify therapeutic pathways that wouldn't otherwise have been considered. Recently, we picked up an inherited predisposition with consequences to the patient and their family – but not one that was typically associated with the patient's (rare) cancer type. You might also unlock access to clinical trials – a frequent occurrence. In perhaps one in four patients who come to us for genetic profiling, we find something interesting that can potentially take them down a different avenue for therapy.

The barriers lie in changing the attitudes of both physicians and patients. It can be difficult to encourage people to consider clinical trials when making decisions about their treatment. In particular, we should be considering them earlier in the therapeutic pathway; clinical trials are still seen by the general public as a bit of a "last-ditch attempt" at treatment. There's a misconception that the patient is not going to benefit from the trial and that it's only for the benefit of future patients. But, more often than not, that's untrue, and the persistence of that false belief means that the number of patients registering for clinical trials is quite small – which, in turn, slows down progress in drug development.

But it's not only patients who view clinical trials as "the end of the road." Physicians, especially those who don't work in large academic centers, often seem to have a similar view. I've noticed that the majority of oncologists – certainly in the UK and the US – still very much stick to chemotherapy-led pathways. Immunotherapy is making inroads and, in specific areas, targeted therapies are beginning to expand – but I think we still have a little way to go to persuade oncologists that clinical trials should be thought of early in the treatment selection process. Molecular profiling can be expensive, so patients tend not to be profiled until they've run out of traditional treatment options and

are in poor health. As you can imagine, this affects their options in terms of selecting effective treatments. I think it would be beneficial to get patients access to genomic profiling earlier, find out what clinical trials are available, and consider them as part of the standard of care for cancer therapy. We shouldn't wait to explore potentially promising options until there's nowhere else to go.

IF YOU COULD MAKE ONE BIG CHANGE (IN YOUR FIELD) TO ADVANCE PRECISION MEDICINE, WHAT WOULD IT BE?

MP: I would integrate multidisciplinary decision-making into a model of pathology that goes beyond just precision medicine; that's where we have to go in routine clinical practice, but we're not there yet. At the moment, the majority of centers have molecular tumor boards, which are mostly separate from the multidisciplinary tumor boards in which we discuss patients. In the future, we need to bring those two entities together – at least when we're discussing tumor types that benefit significantly from molecular profiling.

PB: For precision oncology, I would prioritize genomic profiling of all cancer patients (or at least all patients with advanced metastatic disease) at diagnosis. And, following closely on the heels of that, I would begin collecting outcome data at a national level. It's a lot of work but, without it, I think precision oncology will stall. Despite our early successes, we'll have an increasing number of clinical trials that have failed because we didn't select the right patients. And that's why I think widespread genomic profiling and data collection must be the next challenge we tackle.

WHAT ADVICE DO YOU HAVE FOR PATHOLOGISTS AND LABORATORY MEDICINE PROFESSIONALS WITH AN INTEREST IN PRECISION MEDICINE?

MP: Networking is important. This type of technology is expensive and not everyone has equal access to it. Additionally, knowledge is becoming much more specialized than before, so it's no longer possible for one person to have all the answers – having contacts with different areas of expertise is vital. Finally, investment is key, especially if you want to do routine molecular pathology. You need technology robust enough to give you the information your patients need. For those working in academic centers, it's important to focus on new predictive and prognostic markers, and to consider markers for toxicity as well as for efficacy.

Funding is often a sticking point, but the cost of gene profiling has dropped dramatically over the last five to 10 years because we're using it so much more. I expect that pattern will continue. Most of the major pharmaceutical companies are also investing a lot in companion diagnostics, which should further our goal of getting the right drug to the right patient at the right time and with minimal toxicity.

ÖE: Pathologists and laboratory medicine professionals are important members of the multidisciplinary team in the management of cancer patients – and their importance will only increase as precision medicine evolves. As a clinician, I recommend that they take an active part in clinical tumor boards – especially molecular boards – to help appropriately direct each patient's treatment options.

Remember that we are not just doctors, but also potential patients. Cancer is a major global health problem – and almost half of all cases can be prevented by good lifestyle choices: not smoking, moderating alcohol consumption, exercising, maintaining a healthy weight, eating a balanced diet, and protecting ourselves from the sun. That said, it's also important to be aware of family history and take preventative measures against known genetic risks – and, of course, to follow the relevant cancer screening recommendations.

PB: For these efforts to be truly successful, they need to happen at a national level. We are beginning to build those infrastructures, but it's a little fragmented because bringing everybody together is difficult. As a pathologist, I can say that colleagues of mine have seen this as a threat. They feel that a lot of pathology skills could become less relevant and practitioners will increasingly rely only on molecular profiling.

I disagree, though. I think history has shown us more than once that people don't just suddenly find themselves out of a job. Things don't change that quickly. But you do need to "go with the flow" – and right now, to me, that means encouraging pathologists to become more genetically aware. We need to understand these novel technologies and embrace them as a part of our future routine work. As medicine evolves, to protect the future of pathology, we need to evolve along with it.

WHAT'S YOUR KEY TAKE-HOME MESSAGE ABOUT PRECISION MEDICINE?

MP: It's clear that the profile of the pathologist has changed dramatically over the last five to 10 years.

ÖE: Precision medicine is very important for the management of oncology patients, and its impact will only increase in the near future.

PB: Genomics is here to stay. It is going to touch on many aspects of cancer – and beyond, for instance in pharmacogenomics. So what I want to get across is that it is not something to hide from, but something to understand and embrace. Get involved!

Reference

1. *P Garrido et al., "Proposal for the creation of a national strategy for precision medicine in cancer: a position statement of SEOM, SEAP, and SEFH", Clin Transl Oncol, 20, 443 (2018). PMID: 28861725.*

Pathology's Best-Kept Secret

Clinical trials offer pathologists the opportunity to work at the forefront of diagnostic discovery

What role do pathologists play in clinical trials?

Pathologists have a variety of responsibilities, including identifying suitable patients and evaluating biomarkers expressed in tissue biopsies. From a diagnostic and prognostic viewpoint, we function similarly to community pathologists. At Q² Solutions, we focus 60 percent of our work on oncology – but we also regularly deal with other therapeutic areas.

Clinical trial pathologists are as essential to the development of novel therapies as frontline oncologists – and, together, we help bring cutting-edge drugs to market. A striking example of this is the significant contribution clinical trial pathologists made with PD-L1 expression case review in several tumor types, which led to the approval of both immune-checkpoint inhibitors and companion diagnostics that later demonstrated major clinical success.

What are the differences between practicing pathologists and those in clinical trials?

Our work is a lot more quantitative than qualitative, especially when it comes to diagnostic immunohistochemistry. Our pathologists are trained on many different numerical cutpoints for immunohistochemical and FISH markers, with their skills tested by the assay manufacturers. To determine whether a patient can be enrolled in a trial, the pathologist identifies positive cases around a cutoff value predetermined by the pharma partner. Working in clinical trials also offers a unique opportunity for exposure to what would otherwise be considered rare cases. For instance, we often work with relatively rare tumors over a short time – one of my colleagues once reviewed 1,800 cases of glioblastoma multiforme over a six-month period. That exposure is equivalent to 10 years of neuropathology fellowship training.

How do regulations differ between the two?

All of our labs are fully licensed and accredited, just like any other clinical diagnostic lab – but we also have to submit detailed data to the FDA and other international bodies. The various regulatory agencies for clinical trials ensure that patient safety is paramount and provide confidence in clinical trials to encourage patient participation.

Are there any differences in the client base?

Pathologists in private practice communicate with clinicians about one patient at a time. In contrast, clinical trial pathologists communicate with entire biopharmaceutical companies, including their clinicians, oncologists, radiologists, gastroenterologists, translational scientists, and manufacturers of assay kits and instruments. Pathologists working in clinical trials can't hide behind the paraffin veil. Instead, we must liaise regularly with clients who want to monitor the progress of their studies. For that reason, one criterion for a pathologist to work in clinical trials is excellent communication skills.

What technologies are pathologists working in clinical trials exposed to?

One of the things that excites me most about working with Q² Solutions is the ability to evaluate cutting-edge technologies, sometimes years before they're introduced to the clinical diagnostic commercial market. We were among the first labs globally to employ PD-L1 as a diagnostic assay – and we're currently examining image analysis and artificial intelligence algorithms to help diagnose cancers. Earlier this year, one of our labs purchased a digital spatial profiling platform. Although in its infancy for clinical trials, this technology holds huge promise and is probably the highest order of multiplex immunohistochemistry available by leveraging anatomic pathology and genomic technologies.

How has COVID-19 affected clinical trials?

It's clear that the pandemic has adversely affected everyone's lives and many businesses, including clinical trials. The whole industry has seen COVID-19's impact on cancer trials, with many patients' visits canceled. It was clear from the beginning that fighting COVID-19 would require new therapies and vaccines – and their discovery and development are areas in which Q² Solutions and its pharma partners excel. Many of our scientific divisions adjusted operations to support our partners in their unprecedented efforts to identify effective drugs and vaccines. To this end, we deployed SARS-CoV-2 qualitative and quantitative, sequencing and serology (total Ig, IgG, and IgM) assays. At our peak, we performed approximately 3,000 SARS-CoV-2 PCR tests each day.

How do career opportunities compare?

When I was training, my professors rarely mentioned opportunities in biopharma and clinical trials. Typically, they discussed the three major practice settings: academia, community pathology, and commercial reference labs. It wasn't until I had been practicing for five years that I became aware of the real need for pathologists in clinical trials. For me, it's one of the best-kept secrets in our specialty – and I want to spread the word that there is another avenue through which pathologists can contribute to medicine. One of the best things about working in clinical trials is that our work impacts the lives of thousands or millions of patients.

John Cochran is the Chief Pathologist at Q² Solutions.

clonoSEQ®

NGS MRD MONITORING

SEQ TO STANDARDIZE YOUR MRD RESULTS

Leverage the power of the 1st and only FDA-cleared and Medicare covered assay for the detection and monitoring of minimal residual disease (MRD) in chronic lymphocytic leukemia, multiple myeloma, or B-cell acute lymphoblastic leukemia patients.

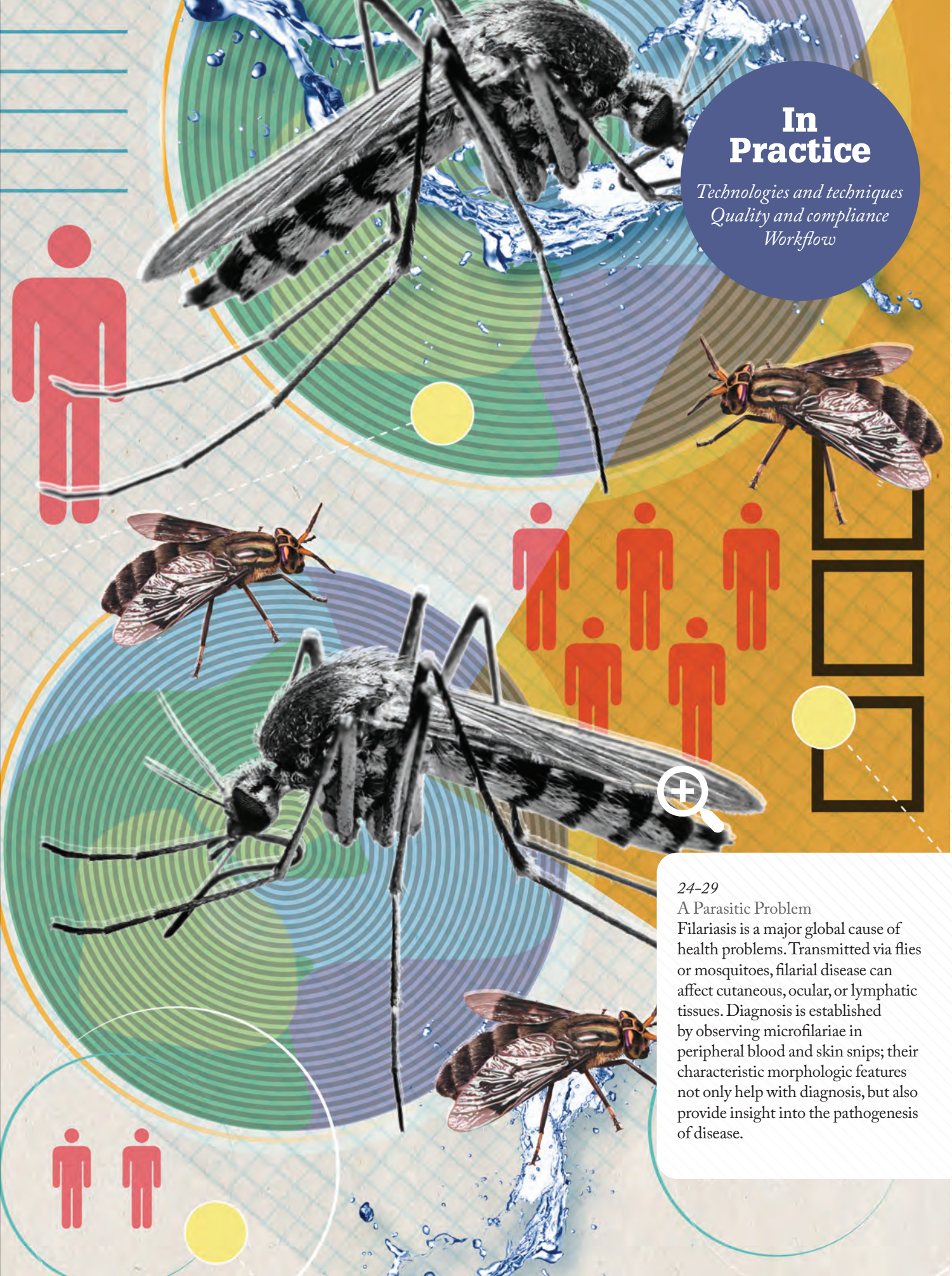
Testing service available nationwide.
Learn more at clonoSEQ.com.

clonoSEQ® is available as an FDA-cleared *in vitro* diagnostic (IVD) test service provided by Adaptive Biotechnologies to detect measurable residual disease (MRD) in bone marrow from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (B-ALL) and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). clonoSEQ is also available for use in other lymphoid cancers as a CLIA-validated laboratory developed test (LDT) service. For important information about the FDA-cleared uses of clonoSEQ including test limitations, please visit clonoSEQ.com/technical-summary.

clonoSEQ®. [technical summary]. Seattle, WA: Adaptive Biotechnologies; 2020.
Ching T, Duncan ME, et al. BMC Cancer. 2020; 20: 612.

clonoSEQ.com

Adaptive biotechnologies®



In Practice

Technologies and techniques
Quality and compliance
Workflow

A Parasitic Problem

A systematic review of filarial disease

By Harsh Mohan and Poonam Bhaker

The term "filariasis" may seem remote to some – but for others in the medical field, it is far too close to home. Filariasis refers to a group of neglected tropical diseases caused by nematodes of the superfamily Filarioidea, transmitted through arthropod vectors. These diseases are classified as lymphatic or cutaneous/ocular filariasis based on which tissues are the primary home of adult worms (1). It is estimated that over 120 million people are infected worldwide, 40 million of whom are disfigured or incapacitated by the disease. The social, psychological, and economic burden of filariasis – amounting to a loss of 2.8 million Disability Adjusted Life Years annually (2) – is clear. The World Health Organization has committed to eliminating lymphatic filariasis as a public health problem by 2020 – and river blindness, a cutaneous/ocular form of the disease by 2025 – by i) mass drug administration in endemic regions and ii) targeting the vectors to halt transmission (3,4). However, even regions that have eliminated filariasis may see its re-emergence due to travel in and out of the area (5).

How and where

Parasitic nematodes responsible for human lymphatic filariasis include *Wuchereria bancrofti* (which causes over 90 percent of lymphatic filariasis), *Brugia malayi*, and *Brugia timori*. Nematodes causing cutaneous and ocular filariasis include *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *Mansonella ozzardi*, and *Mansonella streptocerca*. Transmission of lymphatic filariasis occurs through the Culex (in urban and suburban areas), *Anopheles* (in rural areas), and *Aedes* (Pacific islands) mosquitoes. Black flies are vectors for *M. ozzardi* and *O. volvulus*, and deer flies for *L. loa*. Biting midges transmit *M. ozzardi*, *M. perstans*, and *M. streptocerca* (1,2,6).

To make an accurate diagnosis, the geographic distributions of these parasites are key.

- *W. bancrofti* has widespread distribution, mainly in the tropics and subtropics including Asia, Pacific islands, Africa, South America, and the Caribbean basin.
- *B. malayi* is concentrated in southeast Asia, including India, China, Philippines, Malaysia, Indonesia, Korea, and Vietnam.
- *B. timori* is limited to some islands of eastern Indonesia including Timor (2).
- *O. volvulus* is endemic to west and central Africa, as well as parts of eastern Africa and Yemen.
- *L. loa* is found only in the tropical regions of west-central Africa; exercise caution in diagnosing loiasis outside Africa. Dirofilaria causes ocular infections clinically similar to loiasis in Europe and Asia (7,8).
- *M. perstans* is distinctive to Africa south of the Sahara, Central and South America, and some Caribbean islands.
- *M. ozzardi* is distributed from Central to South America and in the Caribbean.
- *M. streptocerca* is native to tropical areas of western and central Africa (9).

The circle of life

Filarial nematodes need two hosts to complete their life cycle: mosquitoes or flies as intermediate hosts for development and maturation, followed by humans as definitive hosts for reproduction (10,11). Humans have adult worms localized to lymphatics (*W. bancrofti*, *B. malayi*, *B. timori*), subcutaneous tissues or eyes (*L. loa*, *O. volvulus*, *M. streptocerca*, *M. ozzardi*),and body cavities (*M. perstans*). Adult worms can survive for years in human hosts. When they undergo sexual reproduction, female worms release millions of larvae (microfilariae) into the skin or blood. These motile microfilariae stay in pulmonary vessels during the day and migrate to peripheral blood at night, reaching peak peripheral blood concentration – and thus ideal sample collection time – around midnight in parasites with nocturnal periodicity (*W. bancrofti* and *Brugia* spp.).

How does it work? A vector picks up microfilariae circulating in blood or cutaneous tissue during a feed. The development stages, which occur in the vector and take 10–14 days, are L1 (inactive), L2 (pre-infective), and L3 (infective). Finally, the vector bites a human host and releases L3 larvae that migrate to their respective localization site.

Studies have demonstrated the presence of *Wolbachia* bacteria as endosymbionts in some filarial nematodes. This association is implicated in disease pathogenesis, and host immune response, and adult worm viability and development. However, when the nematode dies, these bacteria are released and can initiate an immunologic reaction (12) – which is why it may be effective to accompany antifilarial treatment with antibiotics targeting *Wolbachia*.

Clinical manifestations

The spectrum of disease differs between patients living in endemic regions and those who have traveled or recently migrated to those regions. In general, filarial disease has a severe, acute presentation in recent travelers or newly exposed individuals, whereas people native to endemic areas experience a more chronic and debilitating disease course.

Lymphatic filariasis

In lymphatic filariasis, asymptomatic or subclinical microfilaremia is the most common presentation. In endemic areas, this begins in early childhood (13). Patients may exhibit lymphangiectasis or dilated lymphatics (including scrotal lymphatics), microscopic hematuria, and proteinuria (1).

In acute filarial lymphangitis, patients present with high-grade fever, retrograde lymphatic inflammation, lymph node enlargement, and transient edema. Genital lymphatic involvement in *W. bancrofti* filariasis leads to funiculitis, epididymitis, and scrotal pain. Acute dermatolymphangioadenitis is characterized by fever, chills, myalgia, red tender edematous inflammatory plaques, and vesicles or ulcers. Ongoing lymphatic inflammation and subsequent obstruction leads to elephantiasis: brawny edema, thickening of subcutaneous tissue, and hyperkeratosis. Hydrocele, scrotal lymphedema and chyluria may be noted, and obstructive lymphedema may involve other organs. Recently exposed individuals present with short-lived lymphadenitis, followed by retrograde lymphangitis (1,5,14).

Tropical pulmonary eosinophilia is an occult filariasis characterized by marked eosinophilia, paroxysmal cough and wheeze (mostly at night), low-grade fever, and weight loss. Microfilariae are not usually identified in peripheral blood, but may be rarely observed in lung biopsies. A rapid initial response to diethylcarbamazine is hallmark of this disease (15).

Onchocerciasis

Cutaneous manifestations include intense pruritus, generalized papular eruptions, eczematous dermatitis, lichenification, and hypo- or hyperpigmentation ("leopard skin"). Longstanding cases may exhibit lax skin with atrophy. Onchocercomata – firm, non-tender, mobile subcutaneous nodules composed of adult worms – are present over bony prominences. Ocular symptoms may include conjunctivitis, keratitis, corneal opacities, uveitis, and optical atrophy. Patients also frequently experience hanging groin with enlarged inguinal and femoral lymph nodes covered by atrophied loose skin (14,16,17).

Streptocerciasis

The clinical features of this disease resemble those of onchocerciasis.

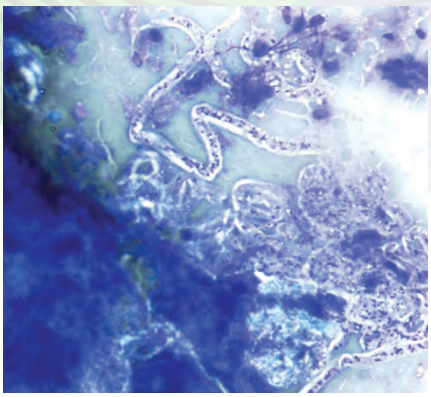


Figure 1. Fine needle aspirate from a breast lump showing the presence of numerous coiled microfilaria (MGG stain).

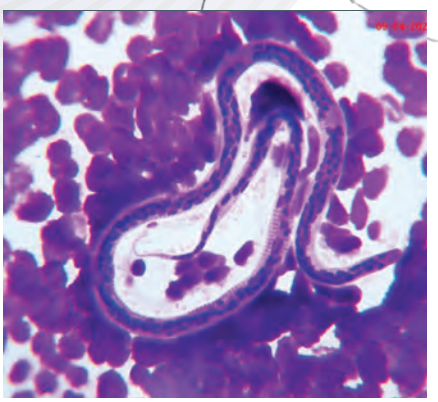


Figure 2. Microfilaria in thick peripheral blood smear (Leishman stain).

They include pruritus, skin rash, and lymphadenopathy, but lack the subcutaneous nodules (16).

Loiasis

Loiasis is mostly asymptomatic. Episodic Calabar swellings (transient, localized subcutaneous angioedema) over extremities may be observed, as may subconjunctival presence of microfilariae. These are transitory and resolve with complete recovery (1,16). Atypical manifestations include arthralgia or arthritis, heart failure due to endomyocardial fibrosis, respiratory symptoms due to pleural effusion or pulmonary fibrosis, and rarely gastrointestinal and renal symptoms. Encephalopathy, either spontaneous or more often after initiation of ivermectin, may occur in patients with high parasite load (>30,000 microfilaria/mL). Some patients experience partial or complete vision loss.

Mansonellosis

Most patients infected with *M. perstans* are asymptomatic – but some may present with transient subcutaneous swellings, rash, pruritus, effusions, or systemic symptoms. Individuals with *M. streptocerca* infection present with papular dermatitis and pruritus, most commonly on the upper parts of the body (18).

Making the diagnosis

Diagnosis of filariasis can be made by direct visualization of adult worms in biopsy; by observing microfilariae in peripheral blood, aspirate smears, and skin snips; and by serologic and molecular testing.

Histopathology

The lymphatics of the lymph nodes, breast, epididymis, and legs are commonly affected. In subclinical lymphangiectasia, intact adult worms are found lying freely in dilated lymphatics lined by thickened endothelium. The worms are 30–75 µm wide and have a thin cuticle with fine transverse striations (19). There is paucity of inflammatory response. Fibrosis alternates with atrophied or hypertrophied smooth muscle. Nonspecific reactive lymphoid hyperplasia occurs in the lymph node nearest to the dilated lymphatics harboring the parasite (20).

Host inflammatory response occurs in response to the death of an adult worm and intensifies as the filarial worm degenerates. The dead worm lies within fibrinous material adherent to the vessel wall. Eosinophil-rich inflammation, initially mild, becomes florid with time, obliterating the lumen. The degenerated worms and fragmented cuticles form their own inflammatory centers, consisting of lymphocytes, plasma cells, histiocytes, and eosinophils; granulomatous reactions (20,21) and central neutrophilic micro-abscess formations may be present. Finally, the dead parasite calcifies, surrounded by fibrous healing. Granulation tissue forms in the surrounding zone and obliterated vessels may undergo recanalization (20). Differential diagnosis includes:

- cat scratch disease-related lymphadenitis, which shows similar central necrosis surrounded by micro-abscesses and granulomas
- other granulomatous lymphadenitis
- Hodgkin lymphoma (19)

The subcutaneous nodules of onchocerciasis reveal coiled adult worms surrounded by a fibrous capsule (22). Skin biopsy shows the presence of microfilariae in all layers of the dermis, along with chronic inflammation comprising a variable number of lymphocytes, plasma cells, and histiocytes, with a few eosinophils. After treatment initiation, plenty of eosinophils are seen around degenerating microfilariae. Granulomas may be present in some cases. All patients show dermal scarring with loss of elastic fibers and fibrinoid necrosis of collagen. The cornea in punctate keratitis reveals edema and lymphocyte and eosinophil infiltration. Inflammation is extensive in heavy parasitemia, and the accompanying neovascularization and fibrous scarring leads to sclerosing keratitis (22,23).

Lymph nodes from patients with onchocerciasis show thickening and fibrosis of the capsule, obliteration of the subcapsular sinuses dilated in the early phase, and replacement fibrosis of lymphoid tissue. The characteristic perivascular symmetrical fibrosis with fibrinoid material deposition is probably due to immune complex deposition. The medulla displays plenty of eosinophils, plasma cells, and histiocytes.

	Sheath	Size	Morphology	Nuclear pattern
W. bancrofti	Yes	244–296 µm long 7.5–10 µm wide	Short head space Tapered tail	Loosely placed within nuclear column; do not reach up to the tail
B. malayi	Yes Stains pink on Giemsa stain	177–230 µm long 5–6 µm wide	Long head space Compact nuclear column	Terminal and subterminal nuclei in tapered tail
B. timori	Yes	310 µm long 6–7 µm wide	Long head space Compact nuclear column	Terminal and subterminal nuclei in tapered tail
L. loa	Yes	231–250 µm long 6-8 µm wide	Short head space Tapered tail	Non-uniform distribution of nuclei
M. perstans	No	190–200 µm long 4.5 µm wide	Blunt tail Compact nuclear column	Nuclei reach to tip of tail
M. ozzardi	No	220 µm long 3-4 µm wide	Tapered tail	Anucleate
M. streptocerca	No	180-240 µm long 3-5 µm wide	Hook-tipped tail	Nuclei reach to tip of tail
O. volvulus	No	220-360 µm long 5-9 µm wide	Tapered tail	Anucleate

Table 1. Differentiating between species causing filarial disease.

Microfilariae can be identified in fibrous capsule, interstitial tissue, and rarely in the medulla (24).

Skin biopsies from cutaneous loiasis lesions reveal adult worms with randomly arranged cuticular nodules. *Dirofilaria* is an important differential; these species have taller, more numerous muscle cells and possess longitudinal cuticular ridges on skin biopsy (7). Loiasis lymphadenitis shows unique features: atrophied lymphoid follicles and sinusoidal dilatation containing histiocytes and eosinophils. Other features include capsular and trabecular fibrosis and dilatation of capsule and medullary lymphatics (25).

Streptocercal lymphadenitis reveals depletion of lymphoid tissue due to inhibitory factors released by microfilariae (24).

Cytologic findings

Parasites may be identified in cytological specimens, such as fine needle aspirates, effusion cytology, and gynecologic smears. Several case reports have been published on the cytological diagnosis of filarial disease from various sites. Aspirate or fluid may appear milky, turbid, or hemorrhagic. Microfilariae are detected on a background of inflammatory cells rich in eosinophils (see Figure 1). At times, giant cells and epithelioid cell granulomas are part of the inflammatory reaction. Rarely, cytology reveals adult worms or fragments (26, 27).

Erroneous diagnoses can present a challenge; extraneous material like cotton fibers, vegetable matter, or airborne contamination with fungal conidia may be mistaken for microfilariae (28). Accurate diagnosis relies on recognizing the characteristic morphologic pattern of the microfilariae.

Peripheral blood smear

Adult worms residing in lymphatics are usually inaccessible; the traditional method of diagnosing lymphatic filariasis is to detect microfilariae in the peripheral blood, particularly thick smears (see Figure 2). Circulating microfilariae can be identified in peripheral blood smears and occasionally bone marrow aspirate (29). Associated eosinophilia is a frequent hematologic finding in filariasis; in particular, counts are strikingly high in tropical pulmonary eosinophilia, a complication of lymphatic filariasis.

The absence of microfilariae in the peripheral blood does not exclude a diagnosis of filariasis. Even in patients with overt disease, microfilaria may be absent; adult worms may still be sexually immature, have passed reproductive age, or died (20). The time of sample collection should be appropriate for expected peak

concentration; blood samples should be collected at midnight for filarial forms with nocturnal periodicity and midday for diurnal periodicity. Microfilariae survive in venous EDTA blood for 48 hours at room temperature. Often surrounded by empty space, they are found at the edges and tail of smears – so it's vital to screen the entire smear at 4X magnification, or else microfilariae may be missed. Wet-mount preparation may reveal motile microfilariae.

When microfilariae cannot be detected in smears, concentration techniques are indicated. The filtration method is the most sensitive, but risks potential infection. The recommended approach is the lysed capillary blood method, which can reveal moving microfilariae through a partially closed condenser iris or dark-field microscopy. Field's stain A or 1% methylene blue can facilitate species identification. Where the lysed capillary blood cannot be applied, buffy coat preparation also concentrates microfilariae (30).

Species identification is done on stained smears by morphologic assessment of sheath (present or absent), distribution pattern of nuclei, and size of microfilaria. Sheath identification is an important feature, but interpretation should be in context of size. *W. bancrofti*, *B. malayi*, and *L. loa* are sheathed filariae; *Mansonella* spp. are unsheathed. However, some features can complicate sheath identification:

- Sheathed microfilariae may lose their sheaths if there is a delay in sample processing.
- The sheath may be retracted.
- Sheath color is dependent on pH and type of stain.
- The sheath cannot be identified in skin snip preparations.

For size context, microfilaria can be compared with red blood cells. *W. bancrofti* and *Brugia* spp. are equal to or larger than red blood cells, whereas *Mansonella* spp. are half the size.

Nuclear pattern is the most important diagnostic parameter (see Table 1).

Skin snips

Diagnosis of *O. volvulus* and *M. streptocerca* microfilariae in skin snips – a specialized bloodless biopsy – is standard. Skin snips are collected from multiple sites. The skin tissue is immersed in isotonic saline, covered with a coverslip, and the slide is examined for motile microfilariae after 15 to 20 minutes. Prolonged incubation is required in low-density infections.

Serologic and molecular testing

Serologic testing and detection of antigen or antibody are convenient diagnostic modalities compared with demonstration of microfilaria in smears. These testing methods are unaffected by periodicity and thus do not require nocturnal sampling. Sensitivity is high, meaning that these assays can identify the patients with low-density microfilariae as well as amicrofilaremic patients.

- *W. bancrofti* filariasis can be diagnosed by detecting circulating filarial antigen by enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic card test. These are extremely sensitive and specific methods. Filarial antigen tests can yield positive results for at least three years following mass treatment in endemic regions and are thus not suitable for determining the efficacy of control programs. Filariasis test strips are more sensitive at low antigen levels and have longer shelf life (5,31,32).
- *Brugia* can be detected by a rapid test for IgG4 antibodies with high sensitivity and specificity. Rapid antibody-based assays are available to diagnose onchocerciasis. The main drawback of serologic testing is cross-reactivity with *W. bancrofti* and other protozoal parasites, making it insufficient for establishing the diagnosis independently (32,33).
- Polymerase chain reaction (PCR)-based assays are available for diagnosing *W. bancrofti*, *Brugian* spp., *L. loa*, and *O. volvulus* (13,33).
- Finally, hypergammaglobulinemia may be present and serum IgE levels are high in all filarial diseases.

In essence, a combination of characteristic geographic or travel history, clinical presentation, blood eosinophilia, demonstration of microfilariae, and/or serologic testing allows a correct diagnosis of filarial nematodes. No single feature is enough for diagnosis – and this is a particular problem in resource-limited regions where diagnosticians may lack the resources for a multimodal approach and patients may lack the ability to reach a clinic once, let alone attend repeatedly for diagnosis and treatment. The reasons behind regions with endemic filariasis are manifold and complex, but for those of us tasked with identifying and treating the disease, this combination of methods may allow us to modify our approach to suit our resources and our patients' needs.

Harsh Mohan is former Professor and Head of the Pathology Department, Government Medical College and Hospital, Chandigarh. He currently serves as Senior Consultant Pathologist at Oncquest Laboratories, Paras Hospitals, Panchkula, Haryana, India.

Poonam Bhaker is a Consultant Pathologist at Oncquest Laboratories, Paras Hospitals, Panchkula, Haryana, India.

Please see references online at: tp.txp.to/filariasis

Asuragen®

Not all BCR-ABL tests are created equal. Choosing the right test is essential.

Targeted therapies have dramatically improved outcomes for patients with CML, who now experience a near-normal life expectancy. Optimizing these outcomes is dependent on routine, sensitive molecular monitoring.

For clinicians and their patients, the best molecular results are delivered by tests with a demonstrated track record of performance. The QuantideX® qPCR BCR-ABL IS Kit enables highly-sensitive (MR4.7, 0.002%IS) detection and quantification of Major BCR-ABL1 mRNA transcripts, producing results directly traceable to the WHO International Scale via an in-run calibrator system.

CML Patients Deserve Better Answers

To learn more, visit: **asuragen.com/bcr-abl**

QuantideX® qPCR BCR-ABL IS Kit

Asuragen, Inc.| 2150 Woodward St | Austin TX 78744 |asuragen.com

Leading From the Lab

Minimal residual disease testing and next-generation sequencing in lymphoid and plasma cell malignancies

An interview with Mohammad Hussaini

Minimal (or measurable) residual disease (MRD) refers to the small number of cancer cells that remain in a patient after treatment, potentially spurring a resurgence of the disease. Recent advances in MRD diagnostic testing, including the advent of next-generation sequencing (NGS) techniques, have improved our ability to detect these cancer cells and make more accurate prognoses. To explore this developing field's impact from a laboratory perspective, we spoke with Mohammad Hussaini, a pathologist at the Moffitt Cancer Center in Florida. Hussaini's expertise is in hematologic cancers and his research background includes using molecular techniques, such as NGS, to enhance the personalized treatment of patients.

Tell us about your background in MRD for hematologic malignancies…

Early in my education, MRD wasn't well-understood in molecular pathology circles. I never expected to become an MRD researcher, but I happened to train with some of the big players in NGS – Elaine Mardis and Timothy Ley – and they inspired me to become one of the first molecular genetic pathology fellows. I was just in time to see NGS mature as a technology and as a clinical test.

My involvement in hematologic MRD followed a similar path. In 2013, I was helping to implement NGS, which was novel at the time. I was asked to review a compelling new technology from Adaptive Biotechnologies called clonoSEQ® – a way to assess and monitor MRD in trials and in practice. That was the beginning of my journey into the MRD space, and I feel fortunate to have been an early adopter and to have worked extensively with Adaptive on one of the first high-level MRD testing centers.

Later, I gained more practical experience running MRD advisory boards and developing a more mature grip on MRD testing. At the moment, I am spearheading multiple outputs comparing molecular MRD platforms, including things like single-cell genome and bulk NGS versus the standard, flow cytometry.

How has MRD testing evolved over the course of your career?

Awareness of MRD testing has been growing all along, but the really big shift has been the advent of NGS for this purpose. When I started, pathologists used labor-intensive tools such as multiparameter flow cytometry and allele-specific PCR for MRD testing. When NGS first came into common use, no one considered its potential for MRD detection because background noise made it difficult to detect very low levels of disease. But, over recent years, smart, focused people have produced a number of advances. For instance, molecular barcodes can help eliminate PCR duplicates, produce cleaner sequencing, and lower the technology's limit of detection. Bioinformatics software is more accessible, more refined, and better able to discern true MRD from artifact. And new technologies, such as single-cell genomics, are helping us break that detection barrier as well. Although NGS testing may still miss MRD in some instances, it happens much less often than with other methods.

In recent years, conventional MRD testing has become more widespread – and I think NGS MRD testing will follow. Unfortunately, a lack of awareness makes implementation a challenge. It takes time for things to trickle down from the literature to the guidelines to actual deployment in clinical laboratories. Fortunately, a lot of literature supports the use of NGS MRD – particularly in the lymphoid system, where I believe its accuracy and quality have established it as the new gold standard test. Myeloid disease is trickier; testing approaches are more diverse and less standardized, so it's harder to compare the performance of NGS with that of existing methods. And that's one of the challenges my colleagues and I are working on right now.

Adaptive Biotechnologies

Where are things headed in the near future?

I think the future is molecular. Diagnosis is often subjective and there is an art to interpretation – but objective, fact-based assays like NGS remove a lot of uncertainty. The initial sequence shows exactly how many copies exist. Better yet, it's a portable result; if you go elsewhere for treatment, you can take your MRD results with you without worrying about differences in interpretation or risking interruptions to care.

Standardizing use of NGS, particularly for diseases like lymphoblastic leukemia and multiple myeloma, is what will make it the go-to approach – but full adoption will take time. People trained decades ago may not have encountered NGS or may be more comfortable using traditional approaches. Newer pathologists learn about NGS in training and are much more likely to embrace it going forward. Even as technology marches on, though, there will always be a niche for flow cytometry and other non-NGS technologies.

Can you describe an ideal patient scenario for MRD testing?

An ideal situation for MRD testing would be a B-cell acute lymphoblastic leukemia patient who has completed therapy and is preparing for a bone marrow transplant. Here, MRD status can have a significant impact on outcomes – not only MRD positivity, but also how positive the patient is. The presence of MRD will influence the course of treatment and may delay a transplant. Such patients might receive additional treatment to achieve MRD-negative status. In my opinion, patients should be reassessed for MRD several times: after induction, just prior to transplant, and about 30 days post-transplant.

What should labs consider when implementing MRD testing?

Many hands make light work when it comes to NGS. You can benefit from expertise in molecular pathology, bioinformatics, oncology, and more – including a wide range of trained support staff. It's also vital to understand the pros and cons of each MRD testing method. They complement one another, so having just one test might not solve all of your problems. And, for each one, you must ask yourself: How many of these tests will we need to run? Do we have the in-house expertise to interpret the results? Do we have the hardware and implementation support necessary to do it properly? If not, do we have the resources needed to send it out? How will we handle billing and payment?

Work closely with clinicians to determine your testing algorithm. Consensus is critical for success. It helps to have well-developed guidelines and to consult colleagues at other centers while you're moving things along. It also helps to have support staff who can track and coordinate the testing, send out appropriate samples, and ensure a manual review. Costs will always be a consideration, so they must be monitored and controlled.

For pathologists, MRD testing can be a big help. Although it can be difficult to get off the ground, it ultimately reveals how effective treatment has been, enabling pathologists to provide robust, clear, and definitive answers about whether – and to what extent – disease remains. Laboratorians and pathologists need to take ownership of this area, so our expertise can drive the conversation to improve patient outcomes.

My advice? Stay up to date with the literature. Study review articles and meta-analyses on different diseases and the role of MRD testing and ensure that you understand the guidelines. Your initial investment – of both time and money – might be substantial, but it's worth tackling the inevitable sooner rather than later. We're becoming much better at treating diseases, but we need to identify the patients most prone to relapse to provide more effective treatment. This prognostic realm is where MRD testing really comes to the fore – so the sooner we adopt it, the better for our labs and, most importantly, our patients.

SHIMADZU
Excellence in Science

Quadrupole Time-of-Flight
Liquid Chromatograph Mass Spectrometer

LCMS-9030

Effortless performance

The LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer integrates the world's fastest and most sensitive quadrupole technology with TOF architecture. It delivers high resolution accurate-mass detection with incredibly fast data acquisition for routine applications.

Greater accuracy and higher sensitivity
based on patented Ultra-Fast technologies

Identify and quantify more compounds with greater confidence
in food applications, forensics, drug identification, proteomics and metabolomics

Effortless performance
with less recalibration and easy switching between ionization units

Small footprint
due to a simple and compact floor-standing design

DESIGN AWARD 2019

www.shimadzu.eu/effortless-performance

NextGen

Research advances
New technologies
Future practice

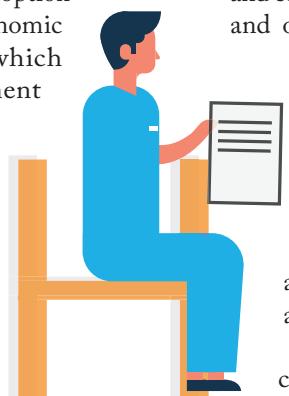
At a Tipping Point

Comprehensive genomic profiling is essential to advancing cancer care – and here's what lies ahead in the precision medicine journey

By Cindy Perettie

Oncology has reached a tipping point and its name is "precision medicine." No doubt you will have heard of it; in fact, it may well form a substantial part of your daily work. Over the last few years, we have seen tremendous advances in research and in the clinic, thanks, at least in part, to the adoption of comprehensive genomic profiling (CGP), which helps guide treatment decisions based on the molecular drivers of disease. Precision medicine is changing the outlook for many people living with cancer – especially those facing advanced disease.

In breast cancer specifically, we've seen an avalanche of research and subsequent progress that spans multiple decades and shapes modern oncology. The progress we're making for breast cancer patients every day is exciting and provides a glimpse into the potential of CGP and precision medicine not just for breast cancer, but for oncology as a whole.

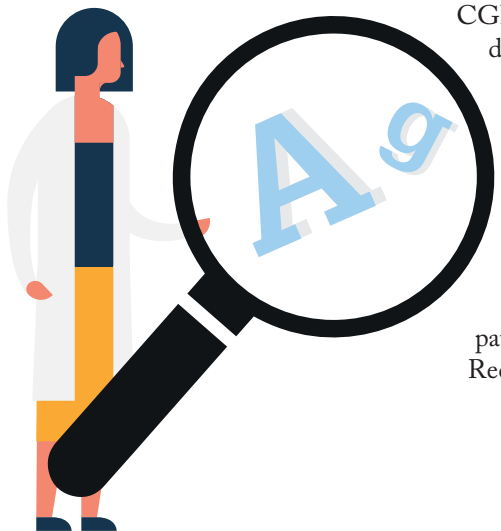


An age-old decision

True personalization of cancer treatment goes beyond tumor type or stage. Consider, for example, the influence of age in advanced breast cancer – an aggressive disease with a higher mortality rate for younger patients than for older patients (1). For younger women, the need to balance treatment with reproductive health concerns adds a layer of complexity. The rate of breast cancer in younger women has almost doubled in the last 40 years (2), so it's vital that we better understand the specific drivers of breast cancer in this population. CGP is helping us gain that understanding.

A recent study used CGP to identify and compare genomic drivers in younger and older women and found specific age-related differences that were consistent across histological and molecular breast cancer subtypes (2). For example, the study found markedly lower frequencies of *PIK3CA* alterations – but higher frequencies of *BRCA1/2* alterations – in women under the age of 45 than in those over 45.

In the clinic, this knowledge could help guide treatment decisions or match breast cancer patients with clinical trials. But these results also pose a question: how many other cancers exhibit age-dependent differences in genomic drivers? How many patients are missing testing that could influence treatment decisions and, ultimately, outcomes?



"The rate of breast cancer in younger women has almost doubled in the last 40 years, so it's vital that we better understand the specific drivers of breast cancer in this population."

This study strongly reinforces the pivotal role CGP can play in truly personalizing the patient experience. However, we can only harness that potential through widespread and routine access for all patients.

Beyond diagnostics

Emerging research has also shown that CGP may have a role in monitoring disease and predicting recurrence in earlier-stage cancers. This incredibly exciting glimpse into the future shows how the clinical utility of CGP could evolve – from a tool matching advanced cancer patients with appropriate therapy to a technology informing cancer treatment paths throughout the patient's journey.

Recently, investigators studied triple

Q² Solutions®

Turning Hope into Help

Requires expert diagnosis

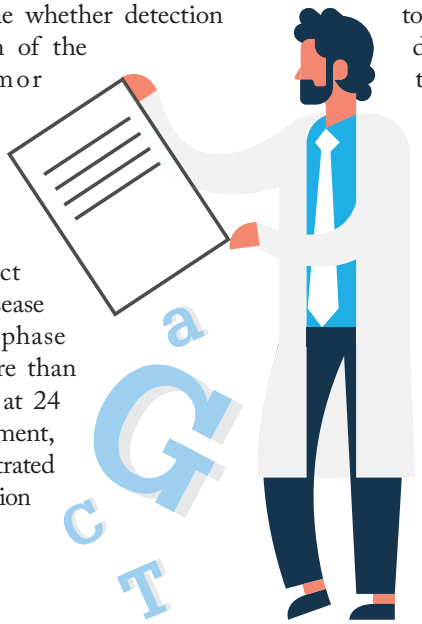
At Q² Solutions, we continue to invest in state-of-the-art diagnostic solutions for clinical trials, as well as in pathology experts. Visit **www.q2labsolutions.com/careers** to learn about career opportunities for pathologists and anatomic pathology professionals.

Together, we can turn hope into help.

"The ability to better predict outcomes and more accurately stratify risk at the start of a patient's TNBC treatment could have big implications for success."

negative breast cancer (TNBC), another aggressive cancer with high recurrence rates and fewer treatment options than other subtypes (3). Neoadjuvant chemotherapy has become standard treatment in early-stage TNBC, but disease recurrence is still high. Today, decisions around adjuvant treatment after surgery and radiotherapy are based on clinical factors and there is no consistent approach to risk stratification.

In their study, the researchers used CGP to determine whether detection and quantification of the circulating tumor DNA (ctDNA) that remains in patients' blood after neoadjuvant chemotherapy could help predict the risk of later disease recurrence. The phase II trial tested more than 150 patients and, at 24 months post-treatment, the results demonstrated a significant association between disease recurrence and the detection of ctDNA. Furthermore, the higher the quantity of ctDNA detected, the higher the risk of recurrence.



The ability to better predict outcomes and more accurately stratify risk at the start of a patient's TNBC treatment could have big implications for success. And that's welcome news for such a difficult-to-treat cancer. Using CGP in this setting means that the specific driver mutations can be identified early on, allowing the optimization of adjuvant treatment approaches for those with a high risk of recurrence. We are among the researchers now looking at potential designs for new basket trials that would do just that and generate further evidence for this approach.

Moving forward with CGP

Although CGP is currently used to help match patients with advanced disease to targeted treatment and clinical trial options, the results highlight the promise of CGP from a much wider perspective at every stage of cancer treatment. To move toward a truly personalized approach, we need to look beyond tumor type or stage to understand the genomic drivers that could inform treatment decisions and patient outcomes.

The ability to accurately predict recurrence risk alongside identification of specific mutational drivers for a patient's cancer also gives us a much more complete picture of a patient's likely cancer experience. It means we can strategically plan our approach to treatment, assessing different combinations and strategies based on our knowledge of how the cancer is likely to respond, and choose the best possible treatment pathway.

Using new technologies to develop monitoring tools means we also have great potential to evolve and adjust treatment strategies in real-time.

I'm proud to play a role in the tremendous advancements in cancer care, but I also recognize that there is still a lot of work to do to make precision medicine a reality for everyone. To progress, the entire precision medicine community will need to focus on the same objective: enabling better outcomes for all patients living with cancer. And by sharing insights, our expanding community can deliver more impactful advancements. This collaborative community includes researchers, physicians, payers, advocates, patients, and the investment of many innovative diagnostic and biopharmaceutical organizations. Together, we can help advance and enable personalized medicine for more patients.

Cindy Perettie is Chief Executive Officer of Foundation Medicine, Cambridge, Massachusetts, USA.

References

1. *AH Partridge et al., "Subtype-dependent relationship between young age at diagnosis and breast cancer survival", J Clin Oncol, 34, 3308 (2016). PMID: 27480155.*
2. *ES Sokol, "Advanced breast cancer (ABC) in young women (YW): a comprehensive genomic profiling (CGP) study" (2019). Available at: https://bit.ly/35ccgER.*
3. *M Radovich et al., "Detection of circulating tumor DNA (ctDNA) after neoadjuvant chemotherapy is significantly associated with disease recurrence in early-stage triple-negative breast cancer (TNBC): Preplanned correlative results from clinical trial BRE12-158" (2020). Available at: https://bit.ly/3eWVhLb.*

Learning and Growing with In-House Testing

Keeping molecular testing in-house offers benefits for pathologists, oncologists, and patients

An interview with Wei Song

How – and why – does your institution conduct precision oncology testing?

Precision medicine is a newly evolving discipline and, to fully realize its potential, all adequately sized institutes should be able to provide in-house genomic profiling for tumors from both tissue and plasma samples. I always think as though I'm running a startup company, so my number one consideration is the customer – who are they and what do they need? Our customers are oncologists and they need genomic profiling tests to enable their patients to benefit from novel precision oncology treatments.

Also, I believe molecular diagnostics is the future of pathology. So while centralization plays an important role currently, if we don't practice in-house testing, we won't be able to develop alongside the science and provide the best possible standard of care. That's why we do as much as possible in-house – to support our clinicians and patients and to keep learning and developing.

Have you participated in a move toward test centralization?

The opposite, in fact. In our institution, all testing was initially sent to a central laboratory – but, ever since we established molecular profiling in our lab, it has come to us instead. Bringing our testing in-house was a real game-changer for the oncologists, for the institution, and for the entire community.

What do you think about test centralization and its promotion by large commercial laboratories?

First, it's important to look at the central laboratories' testing success rates. Some of them are unable to test as much as 30 percent of patient samples due to limited size and tumor content. Because we can test even very small samples in-house, our success rate is 98 percent. The second thing to consider is turnaround time. We provide results in three to four days. Most of the central labs can't compete with that; it takes them one to two weeks – a huge difference. Third, pathologists and oncologists must work together to examine each patient case individually. Sometimes, we need to drill down to the detailed results and assess everything in clinical context. It can be extremely difficult to get detailed data from a commercial lab – and it might take up to a month. In-house, we're ready 24/7. Any time a clinician calls us, we can jump on the computer and review the data with them virtually. This is hugely important for them – and it's no less important for our development and for the research that drives modern medicine.

Precision medicine is in its infancy, so continual development is key and every case deserves a thorough investigation. If you look at the molecular testing report without also having the opportunity to examine the raw data and interpret the results in context, you won't be providing the best possible service – and you'll be depriving yourself of the chance to learn and progress.

How does in-house testing contribute to better patient care?

First, we facilitate and improve patient care by providing results much faster – and for many more patients – than a central lab. We also participate in tumor boards and, as I mentioned, we have regular telephone conversations with clinicians. This level of interaction is not possible when sending out tests to a central lab. I have had experience communicating with central labs; usually, the people I spoke to were not trained pathologists and lacked the expertise to address my questions.

The difference in turnaround times is also hugely important. We are talking about patients for whom even a single day – let alone a week or two – can make a lifetime of difference. Often, oncologists ask for urgent results because their patients are deteriorating. Cancer doesn't take weekends off. Whereas a central lab might take two weeks to provide those crucial results, we can be flexible and expedite delivery.

There's also the question of potential sample loss. Sending FFPE tissue blocks to a central laboratory means taking on the risk of losing them. That can lead to tragedy because, often, one result is not enough; we want to confirm that result via another method. If we don't have the block, we can't do that – and, in the future, we can't use those samples for further testing or for clinical research (another way in which we support our oncologist colleagues). That's a loss for the patient, the pathology department, and the institution.

What would you most like to emphasize?

The number of targeted therapies is growing and, one day, the standard of care will include a genomic profile for every tumor sample to help to guide treatment decisions. If we want to train the next generation of pathologists to understand molecular pathology and cope with these novel demands, they must be exposed to the entire testing process to give them the necessary education and experience.

Wei Song is Director of the Clinical Genomic Laboratory at the Englander Institute for Precision Medicine, Assistant Professor of Pathology and Laboratory Medicine at Weill Cornell Medical College, and Assistant Attending Pathologist at the New York-Presbyterian Hospital, New York, USA.

SOLVING PROBLEMS SAVING LIVES TOGETHER

2021 ASCP MEMBERSHIP

ASCP remains committed to serving you and helping you achieve your professional goals, while you continue to lead from the laboratory to solve critical problems and save countless lives. The laboratory is the cornerstone of healthcare, and pathologists and medical laboratory professionals are critical to successful patient care.

As we move forward together, we can accomplish so many great achievements, even in extraordinary times. ASCP is dedicated to you. We're solving problems. We're saving lives. And we're doing it together.

Visit **www.ascp.org/renew** to renew your membership with ASCP today.

If you have experienced a hardship due to the current COVID-19 pandemic that prohibits you from paying for or renewing your membership, you may be eligible to receive a hardship membership discount. Visit www.ascp.org/membership for more information.

Profession

Your career
Your business
Your life

Peer-to-Peer, with Vijay Joshi

Insights from a leader in pediatric pathology and international cooperation

Megha Joshi interviews Vijay Joshi

Pediatric pathology is among the most challenging fields of pathology and laboratory medicine. Although it spans every facet of pathology from genetics to hematology, its patients exhibit very different features and very different "normal" values to those of adults – and yet, this vital discipline is often overlooked. Megha Joshi interviewed Vijay Joshi, recipient of many awards for his achievements in pediatric pathology and in teaching, to find out what inspired his interest in diseases of childhood and to learn more about his decades-long journey to draw others into the field.

What led you to a career in pediatric pathology?

I became interested in the subject through interaction with one of my mentors, Daria Haust, the "Grand Lady of Pediatric Pathology" in Canada. She recommended me for a faculty position at Montreal Children's Hospital in 1970. Since then, I have worked as a pediatric pathologist in nine different institutions – including children's hospitals and teaching hospitals affiliated with medical schools. After working at the Medical College of Virginia from 1972 to 1975, I went back to India to work at the Postgraduate Institute of Medical Education and Research in Chandigarh and then at G.S. Medical College in Mumbai. It was 1980 when I returned to the USA – and I have been here ever since.

I had always wanted to further the development of pediatric pathology in India. After five years' experience in diagnostic pediatric pathology and clinicopathologic research in North America, I thought that I had gained sufficient expertise to do that effectively – but the environment in India was somewhat different. The institutions I worked in did not require a full-time pediatric pathologist, so I also had to sign out general surgical pathology cases and there were few opportunities to do clinicopathologic research on a large scale. My desire to do more pediatric pathology and academic research prompted my return to the USA – and I truly feel that I have been able to do more for pediatric pathology in India as a result.

What goals did you set for yourself?

First, I wanted to develop expertise as a diagnostician. Pediatric pathology is vast; it encompasses the study and diagnosis of diseases of all types – congenital anomalies, inborn errors of metabolism, infections, immune and autoimmune disorders, neoplasia and iatrogenic lesions – involving all organ systems in relatively divergent groups of pediatric age. Many of these disease processes are unique or more prevalent in pediatric age groups ranging from fetus to adolescent. To gain focused expertise, I chose to concentrate on specific areas and conduct clinicopathologic research driven by my own interests and the cases that landed on my desk.

Second, I wanted to expand my scope as a pathologist, which I accomplished through several means:

- I gave my work a clinical orientation by collaborating with a variety of clinical subspecialists.
- I took on the challenge of learning electron microscopy, which I used to help examine the morphologic features of certain childhood tumors and other lesions.
- Identify an underlying systematization to explain morphologic subtypes of peripheral neuroblastic tumors.
- In teaching, which I love, I aimed to provide a personal perspective based on my experience and my work – looking for clues to etiology, pathogenesis, and natural history in the diagnostic and morphologic features of a disease process.

Third, I wanted to make the most of insufficiently characterized or misinterpreted rare lesions. For example, I saw two cases each of glomerulocystic kidney disease, cystic partially differentiated nephroblastoma (CPDN), and atypical mesoblastic nephroma. Based on detailed study of both my own cases and the literature, I wrote articles to definitively characterize these three lesions.

Last, but not least, I wanted to assist the spread and development of pediatric pathology in India. I viewed it as an attempt to "give back" to my teachers and to the underprivileged patients being treated in inadequately equipped 1950s-era public sector hospitals. I also wished to spread the word of pediatric pathology in the USA (and other countries) because, despite the establishment of the Society of Pediatric Pathology and a certification in pediatric pathology, a number of major teaching hospitals did not have a bona fide pediatric pathologist on staff and – considering that about 70 percent of pediatric hospitalizations occur at general hospitals – pathology trainees were getting insufficient guidance in pediatric pathology.

Luckily, I happened to be in the right place at the right time – not once, but four times in my career.

In the early 1970s, I worked with Mary Ellen Avery and Leo Stern – internationally recognized pioneers of neonatology – at Montreal Children's Hospital. Mortality and autopsy rates in premature neonates were relatively high at that time, so I got to see and study, in neonatal autopsies and surgical pathology, lesions that were previously unknown or inadequately documented. Because of that

Collaborative study group (1982–88) of faculty members, trainees, nurses, and social workers in the Department of Pediatrics, Children's Hospital of New Jersey.

opportunity, I published a classification of primary causes of perinatal mortality and a large series of neonatal cases of iatrogenic lesions. Montreal also provided opportunities for me to become an ice hockey fan by watching live Montreal Canadiens games, and for my introduction to Western classical music by attending a Montreal Symphony Orchestra concert conducted by Zubin Mehta.

In the early 1980s, when the AIDS epidemic broke out in adults, I worked with Jim Oleske, Director of the Division of Infectious Diseases at the Children's Hospital of New Jersey. There, on the basis of morphologic studies of autopsy and biopsy material, we recognized and documented the occurrence of AIDS in children before its etiology was known. In fact, the fresh autopsy and biopsy samples of thymus and lymph nodes we sent to the National Institutes of Health (NIH) grew HIV at a later date. I went on to publish original articles on the effects of HIV in virtually every organ system in children and eventually edited monographs on the pathology of HIV infection and on common problems in pediatric pathology. I also continued working with clinicians as an active member of the Pediatric AIDS Lymphoma Network. In 1989, the NIH designed and later carried out a prospective study of pulmonary and cardiac complications of AIDS in children based on our original articles.

Before starting his talk on Pathology of Wilms' Tumor at St. John's Medical College, Bengaluru, India, Joshi greets a boy who recently underwent successful surgery for the tumor.

In 1988, I was asked to sign out all placentas (about 1,500 each year) received in the general surgical pathology section. I studied placental pathology intensely to take on this challenge and was rewarded with the chance to turn an assignment into an opportunity. A few years later, I published a Handbook of Placental Pathology which, with the co-authorship of Ona Faye-Petersen and Debra Heller, is now in its second edition.

Founding President Usha Kini and Secretary Nandita Kakkar (second and fifth from left) of the APPI with the Joshis at the announcement of the APPI's formal registration in Chennai, India, on February 5, 2020.

In the early 1990s, I was appointed Chairman of the erstwhile Pediatric Oncology Group's Neuroblastoma Pathology Committee because of my previous work on neuroblastoma. I inherited untapped archives of histology slides and clinical data on about 800 cases of peripheral neuroblastic tumors (pNTs). After intensive study of cases with adequate pathology material for a systematic review, I came up with a new, prognostically significant classification of pNTs and clarified their terminology. Later, Hiro Shimada and I put together the International Neuroblastoma Pathology Committee.

How did you promote pediatric pathology in India while working in another country?

After returning to the USA, I began to give lectures and organize workshops both within the country and abroad. I also started visiting India once or twice a year as a volunteer teacher in its medical centers. Eventually, I parlayed that experience into giving "mini-CME courses" in different regions of India so that larger numbers of pathologists could learn about pediatric pathology and serve India's 400 million children.

Sadashivayya Jambayya Nagalotimath and I co-founded an annual international CME course in general surgical pathology and cytology, jointly sponsored by the Association of Indian Pathologists from North America (AIPNA, founded by Megha Joshi) and the Indian College of Pathologists. I made it a point to include pediatric pathology topics in that course every year from 1996 until 2007, when other AIPNA members took over.

In 1990, I collaborated with Anand Pandit, a researcher in pediatrics, and Avinash Pradhan, a pediatric pathologist in my hometown of Pune, on two research projects in neonatology and Indian childhood cirrhosis. As an extension of that collaboration, Ashok Patwardhan (the founder of Foundation for Understanding and Enhancement) and I obtained a US$1.5 million grant from the United States Agency for International Development to upgrade the Departments of Pediatrics, Pediatric Pathology, Obstetrics, and Radiology at Pune's KEM Research Institute.

In my 25 years of academic volunteering, I became well-acquainted with pediatric pathologists from across India. Eventually, with a generous travel grant from AIPNA and the assistance of colleagues at Children's Hospital in Pittsburgh and Los Angeles, I arranged mini-fellowships for two senior Indian pediatric pathologists, Usha Kini and Nandita Kakkar, and got funding from AIPNA to start a pediatric pathology fellowship program at St. John's Medical Center in Bengaluru, India. Through the combined efforts of Usha Kini, Nandita Kakkar, and other pediatric pathologists from India – along with Sarangarajan Ranganathan and Anita Gupta from Children's Hospitals of Pittsburgh and Cincinnati respectively – India's first International CME Course in Pediatric Pathology, sponsored by AIPNA, took place in 2016. Three more have happened since; four more are planned from 2021 onward under the leadership of Sarangarajan Ranganathan, currently of Cincinnati Children's Hospital.

My dream has come true; pediatric pathology is now a recognized subspecialty with its own courses. In 2020, the discipline even started its

own organization, the Association of Pediatric Pathologists in India. The USA's conducive conditions for international academic work were vital to this – but the dream would not have been realized without support of AIPNA and the active participation of my colleagues in both India and the USA, so I owe all of them my thanks.

You have written both pathology texts (in English) and liberal arts books (in Marathi). How did that come about?

As a young man in India, I believed that science was the reason behind the West's ascendancy in the world. However, after spending time in North America, I realized that a liberal arts education is just as important. This initial impression was reinforced by regular study of secular ideas of Western civilization: rationalism, humanism, liberalism, critical thinking, disciplined curiosity, the spirit of giving to society, and more. I wanted to familiarize the 110 million Marathi-speaking people in India with these secular ideas, so my wife and I wrote four books on Ideas of Modernity, as well as articles in Marathi on the importance of including classical Sanskrit and Marathi literature in the high school curriculum. Royalties from these books go to the Indian charity involved in primary education there.

You were born during the Independence movement in India. Did you meet any of the leaders of that movement – particularly Mahatma Gandhi?

Although I was born in British India during its struggle for freedom, I regret never meeting the greatest leader of that time – Mahatma Gandhi. I did, however, attend one of his regular evening prayer meetings when I was 10 years old. Even at that tender age, the sanctity of the prayer meeting had a deep impact on me and, as an adult, I read his autobiography and biographies avidly. His message of peace is still relevant today. I also read Gitanjali, the Nobel Prize-winning collection of poems by Rabindranath Tagore, and carry a copy in my briefcase to this day.

Vijay Joshi honored in the traditional Indian manner with a shawl at the APPI meeting in Chennai, India.

Have any professional moments made a similarly deep impression?

In 1970, in the very first week of my pediatric pathology career, I remember rushing to the pediatric surgery floor of Montreal Children's Hospital to give a diagnosis of measles because I had spotted Warthin-Finkeldey giant cells in the resected appendix of a child. The diagnosis was confirmed when we demonstrated Kolpik spots on the buccal mucosa!

I also recall a four-year-old girl in whom I diagnosed neuroblastoma. She and her parents later established the Alex's Lemonade Stand Foundation for Childhood Cancer, which gave a grant to one of our International Neuroblastoma Pathology Committee meetings.

My final memory is of taking the wrong set of Kodachrome slides to my workshop at an American Society for Clinical Pathology annual meeting. Thankfully, such a situation could never occur in today's digital era!

You've remained healthy and active well into your 80s. What's your secret?

I'm certainly still active… I recently gave a pediatric pathology mini-CME course in Muscat for the Oman Association of Pathologists and I intend to continue teaching pediatric pathology as widely as I can in 2020 and beyond.

How? When I look through my "retrospectroscope," I feel that regular brisk walks, optimal amounts of food, regular reading, and "meditation" by listening to Indian classical music might have helped me in that regard!

How do you achieve a balance between professional and personal life?

I must admit that, on occasion, I have not given my family the time they deserved. I am deeply grateful to my wife and our two sons for being gracious and cooperative in enabling me to pursue my career so intensely over so many years.

Megha Joshi is Staff Pathologist at Winchester Hospital and Medical Director of two outpatient laboratories affiliated with Winchester Hospital, Massachusetts, USA.

Vijay Joshi is Affiliate Clinical Professor of Pathology at Medical College of Virginia, and Consultant in Pediatric Pathology at Hartford Hospital, Connecticut, USA.

When Coffee Is More Than Just Coffee

Two years in an Albanian pathology lab

By Susan Oupadia

My work as a pathologist was taking its toll. The daily grind, challenging group dynamics, demanding clinicians, difficult cases that kept me up all night… After 20 years in practice, I needed a break. It's a feeling many pathologists experience. But, after endless discussion and soul-searching, my cardiologist husband and I decided to break the mold by pursuing our lifelong dream of joining the Peace Corps.

To begin with, we were not at all sure where we would be placed or what we would be doing – but we finally got word that we would serve in the post-communist Baltic country of Albania. The health sector staff at Peace Corps Albania found what was probably the one placement in the whole world that was perfect for me: to establish an anatomic pathology laboratory in the second-largest city in the country.

After three months of pre-service training – living in a village with a host family, attempting to learn the language, sitting through endless required Peace Corps training sessions, and experiencing every possible positive and negative emotion – we were shipped off to our permanent site of Durrës on the Adriatic coast. Little did we know that, when we got there, we would find not only all of the same professional challenges we faced at home, but also a host of brand-new ones.

Starting from scratch

Nothing could have prepared me for my first day in the "lab." I discovered two almost empty rooms and a non-English-speaking histotechnologist who had spent over a decade doing nothing more than producing distilled water for the hospital. Since the fall of the Communist regime, Albania had experienced a severe decline in public hospital services, along with a general collapse of government and societal structure as a whole. The lab had slowly declined to nothingness. There was no visible tissue processing equipment, only one elderly microtome, and an equally antiquated microscope. There was no pathologist in sight.

A few days later, my husband, who worked in the emergency department, was introduced to a forensic pathologist. My spirits shot up; I honestly believed things were going to be okay. Several sets of slides had been donated to the lab by pathologist friends in the US and I was excited to review them with my new colleague – but, after one afternoon together (with me communicating in my rudimentary Albanian), it became apparent that his fund of knowledge was unfortunately quite poor.

I would show him a slide and ask for his interpretation. Time after time, the diagnoses were completely off-base – an invasive squamous cell carcinoma of the skin was "normal," a well-differentiated hepatocellular carcinoma was "colon cancer," and so on. We would check the dictionary each time to make sure there was no miscommunication. I began to wonder just what kind of training existed in Albania. Did all pathologists lack the basics – and, if so, why?

The basic building blocks

Of course, before this service could be realized, I had to find some equipment. Mysteriously, a second histotechnologist showed up one day and opened a dusty closet containing a tissue processor, an embedding station, and a slide stainer – all still in boxes. These instruments had been there for at least two years, purchased from an Italian company one fine day when the hospital administration had seen fit to do so. I was ecstatic to see these devices, because the Peace Corps did not provide funds for projects and I had already been told that there were no hospital funds available for equipment purchase.

My ecstasy was short-lived. We set up all the equipment, only to discover that the processor did not function. Apparently, although funds were allocated for new instrumentation, part of the cash had somehow disappeared and so used equipment had been purchased instead. It took about a month and the assistance of two technicians in the capital city, Tirana, to finally locate the problem: a broken motherboard in the processor's computer. It took another two months to beg the company in Italy to send me a new one for free. After titling my email "Urgent Request from American Doctor" and explaining the situation, I think the Italian sales representative just felt sorry for me. He knew the many obstacles faced by his neighboring country and probably anticipated the imminent failure of my project – but he helped me, and so I am forever grateful to Giorgio.

A brief history of Albania

By now, I was getting the idea of how things worked (or didn't) in Albania.

I probably need to explain a bit about the culture and the unique character of interpersonal interactions. Don't get me wrong; the Albanian people are some of the most loving, loyal, and generous I have ever met (and, luckily, they love Americans). However, it seemed as though they didn't like each other much at all.

After a 500-year occupation by the Ottoman Empire and almost 50 years of Communist rule and extreme isolation from the rest of the world, Albania was a land that knew nothing but domination. Emerging from the Communist regime of Enver Hoxha – which practiced religious persecution, forced labor and military service, near starvation, mass torture, imprisonment, and execution – Albanians developed an understandable mistrust of humanity. During this time, people lived in constant fear of being reported to the police for some real or imagined infraction of the Communist manifesto.

(Interesting fact – over 173,000 concrete bunkers were erected across the tiny country during Hoxha's Communist rule. These were built to protect against American aircraft bombers, who had no interest whatsoever in the country…)

In the 1990s, as a new "democratic" administration gained political power, rampant corruption, misuse of public funds, and questionable financial and business practices emerged. In 1997, a catastrophic government-led Ponzi scheme was uncovered in which as much as a third of the population lost their life savings. Albanians were in shock.

The resulting mistrust of everyone and everything somehow seemed to have contributed, over the decades, to the people's deeply rooted custom of "going to coffee." Literal hours are spent each day in the innumerable coffee lounges across the country. Only through these lengthy interactions with friends, colleagues, family members, and especially new acquaintances or old competitors do Albanians establish a kind of tenuous trust – one that finally allows them to live and work together. It was entirely routine for doctors to invite each other (and me) to "coffee" for sessions of up to two hours during the workday. Multiple coffee sessions with different people were not at all unusual. It is considered an integral part of the workplace and is certainly the one thing that holds society together. And believe me, it is strong coffee.

One may ask then, how does anyone get any work done? The pace of clinical work is unbelievably slow and, frankly, the patients seem like an afterthought. In the doctors' and nurses' defense, however, there really wasn't much diagnostic or treatment capacity in our hospital anyway. The drug cabinets were bare, the X-ray machines were usually broken, and the ambulances rarely had enough gasoline.

The grand opening

After the first year of work, we were ready to host our anatomic pathology lab's grand opening. (To me, it is still incredible that it took only one year to get the service functioning!) Attended by the Albanian Minister of Health, various other dignitaries of medicine and government, all of the Peace Corps administration, and a host of doctors and medical staff, we had a marvelous photo op and speeches galore. Albanians like nothing more than photo ops. The content of the event doesn't matter – the importance of the attendees and whether or not one gets a photo with them is the main attraction.

Soon thereafter, specimens began to trickle in. It became apparent that only about one half of the operating room specimens reached our lab. I was not surprised to learn that significant financial gain was to be had when surgeons continued to submit their cases to the private labs in the capital city. It was going to take a lot of coffee to change that behavior. Over time, however, things did improve – and I was particularly happy to hear that it was no longer necessary for patients to carry their formalin-filled containers on the public bus to the outside reference labs (the only way the tissue was going to get there).

Turnaround times in the country were abysmal, with many patients dying of their already advanced disease before a diagnosis was rendered. We tried to win the hearts of the public by achieving and advertising a three-day turnaround time by word of mouth. Many patients brought their specimens to the lab just because they had heard there was an American doctor there. I was just happy that we were beginning to see – and help – more patients.

Continuing challenges

After many coffees, my relationships with my clinical colleagues slowly improved. However, I still remember the first time I called a gynecologist with questions about a hysterectomy. His immediate and terse response was, "Why are you calling me? I have never had a call from a pathologist in 30 years!" Unbelievably, it turned out that the clinicians generally did not talk to each

other. Radiologists did not communicate with surgeons, who did not communicate with oncologists, who did not see the need to talk to pathologists (me, initially). The prevailing attitude was that if you didn't know the answer to a question, then you shouldn't be a doctor. The combination of ignorance and arrogance was especially hard to understand and deal with.

I eventually made friends with a hematologist and a surgeon. They kept me sane and tried to negotiate difficult circumstances with (and for) me. I'll never forget, though, when the surgeon asked me – during an especially long coffee break – if I could change a diagnosis on a thyroid case. It was a benign multinodular goiter that he wanted to diagnose as cancer. "Can't you just make a little cancer in there?" he asked. "What would it hurt?" You can imagine my horror. He explained, "The patient is very poor and, if she gets this diagnosis, she will get money from the government."

I'll never be sure if this was a true story or if there was financial gain or fear of litigation on his end. Fortunately, things ended well; my answer, which included an explanation of medical ethics, cemented our relationship forever. I think he respected me for demanding the truth.

Contrary to most American pathologists' experiences, patients were often in my office. They came with their wrinkled paper pathology reports, family members, gifts, worries, and feelings of mistrust and frustration with the system. "Unofficial payments" (that is, money given to ensure good care) are a fact of life in the Albanian healthcare system. The expectation is that money will change hands from the patient to the doorman, the receptionist, the technician, the nurse, the surgeon or other clinician, and the billing person, right up to the time of discharge. Only then can one be sure of being attended to at the hospital. Several times, I had to remove currency, hastily shoved in my lab coat pockets by patients or family members, explaining that American doctors did not participate in this system. One wealthy patient, whose thyroid slides I sent to Juan Rosai (who consulted pro bono) in Italy, drew me aside in the coffee lounge and whispered, "If you need anything, anything at all, while you are Albania, here is my phone number." I felt like I was in a Godfather movie!

A training adventure

Things weren't always difficult. In fact, for 18 months of my time in Albania, I was fortunate to teach two female residents in their last year of training. I sent out numerous emails to microscope companies in the US asking for donations and hoping for a miracle. Lo and behold, John Hubacz of JH Technologies in California sent us a refurbished multi-head scope! This was a joyous thing. I was ready to start sitting with the residents.

Resident training in Albania takes place in the university hospital in Tirana. In the bleak classroom, there was one dusty old multi-head microscope, disheveled stacks of old reports, no books, and no Internet. Unfortunately, with few shining exceptions, attending pathologists have little interest in spending time with residents. They are told on day one to "go to your room and figure it out" and are basically left to their own devices thereafter. The senior doctors' dismissive attitude was accompanied by the expectation of "paying respects" and, frankly, well-founded fear on the residents' part.

Shocking displays of anger and verbal abuse are not uncommon in Albania – not only in the hospital, but at schools, businesses, and other public places. In fact, early on (after being called by the hospital director to train another resident at the Obstetrics and Gynecology Hospital in the capital), I was summarily dismissed by her attending with a barrage of loud, angry words. The resident was sobbing, the lab staff was cowering behind the door, and – luckily for me – my command of the language was not good enough to understand what he was saying. I later learned that the attending believed I had been called there to prove his incompetence and get him fired.

Because of the inequality in payment between public and private hospitals, most attendings work at private labs in addition to the public ones, with attention very much centered on the more lucrative sector – a great disservice to the residents who train in public hospitals. These same pathologists trained in the same system, however, so the only pathologists who seemed to be trained to an acceptable capacity were the ones who had spent time learning their discipline outside Albania. And, of course, these were the ones who had the funds to support this training.

But my two residents, despite the gaping holes in their understanding of pathology, were smart and motivated. Their hunger for knowledge at the grossing table and the microscope drove them to learn as much as they could in the months we spent together. Both rode the public bus for over an hour and a half each way, every day, just to sit with me and learn the basics. My pathologist colleagues elsewhere sent a great library of texts. I also obtained several digital short and long course donations from the United States and Canadian Academy of Pathology, which I presented to pathologists from around the country during a three-day workshop. It was exhausting, but definitely worth the time and effort – to hear the questions and see the glimmers of understanding of things they had probably never heard or understood before. It was a highlight of my Peace Corps service.

The transition home

As our last weeks in Albania approached, I finally saw that my time there – although full of challenges and frustrations – would be something truly unique and unforgettable in my life. I am forever in debt to the Peace Corps, who had the foresight to give me the hardest, but most fulfilling, assignment I could imagine. The small changes I saw in the medical culture – doctors actually talking to each other about cases, asking each other questions, coming to me with questions – never ceased to surprise and amaze me. My husband had similar experiences. Of course, we weren't going to change this broken society in a major way in just two short years – but the examples we set as American clinicians had made, and continue to make, a difference.

The lab is still operational, despite some temporary closures due to a lack of reagents. One of the residents has become one of my best friends; she's like a little sister to me. She has immigrated to the US with her family and hopes to take the USMLE and eventually become a pathology resident here. I know she can do it. She has the brain power – but her confidence sometimes lags behind her abilities, thanks to all those years of dismissal and mistreatment; I encourage her every chance I get.

Returning home was wonderful and difficult at the same time. It has been a rough transition professionally. I love pathology and continue to pursue international opportunities. I read slides digitally from Uganda through the American Society for Clinical Pathology's Partners in Cancer Diagnosis and Treatment in Africa initiative. I may return to overseas projects from time to time, just to get the feeling of fulfillment I attained in Peace Corps. I have tremendous respect for that organization – they understand international aid and development like many others do not.

The daily grind, challenging group dynamics, demanding clinicians, and difficult cases led me away from my US practice. I found both similar and different challenges in Albania. Even in my late 50s, I discovered I still had much to learn about myself and about humanity and its strengths and weaknesses. I would do it all again in a heartbeat… but only if I can go to more of those coffees.

Susan Oupadia is a retired anatomic and clinical pathologist and currently reads slides digitally from Uganda via the ASCP Partners in Cancer Diagnosis and Treatment in Africa Initiative.

The opinions expressed herein are those of the author and do not necessarily reflect those of the US Peace Corps.

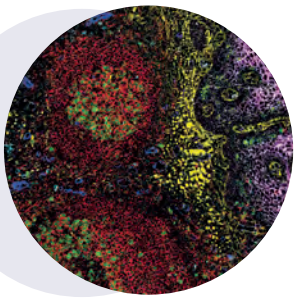
Spotlight on... Technology

Cancer Diagnostics: Want to Provide Your Patients with Answers Faster?

Designed for managing whole patient cases, Dako Omnis enables continuous delivery of complete patient cases to pathologists throughout the day. Patient case management supports your lab in completing more patient cases the same day they are requested – and in delivering fast results to patients.
Visit the web page below to learn more: https://thepathologist.com/inside-the-lab/continuous-delivery-of-complete-patient-cases

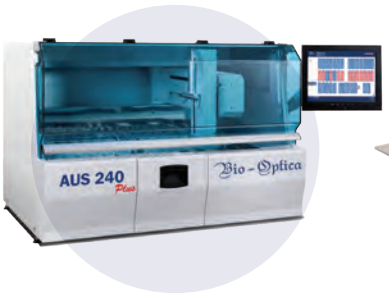
Ergonomic, Mobile Grossing Workcenter with Digital Documentation

eGROSS: fully equipped, all-in-one system with integrated digital documentation system for capturing and storing macro images of specimens. Key benefits include adjustable height of working area, ease of mobility as the unit is on wheels, and guaranteed high safety standards due to a built-in, high-power exhaust system with backdraft and downdraft.
https://www.milestonemedsrl.com/product/egross/



Get High-Dimensional Spatial Single Cell Data

Fluidigm Therapeutic Insights Services team can help you gain deep insights by leveraging Imaging Mass Cytometry™ services for your translational or clinical research. Our expert scientists can work with you to tailor and execute a sample-to-answer project to generate high-multiplex single-cell imaging data from your heterogenous tissue samples.
fluidigm.com/tis



Automatic Stainer AUS240 Plus – Stronger Than Before!

AUS240 Plus can work in stand-alone mode or in combination with CVR909 Plus as a workstation. Now available with the new Reagent Management System, priority staining protocols, user-friendly interface, and possibility to substitute the reagents in continuous. Discover all the features of Bio-Optica's automatic stainer.
Contact us at: info-export@bio-optica.it
https://www.bio-optica.it/en

RedRick Technologies Ergonomic Workstations Alleviate the Risk of Repetitive Stress Injury

The shift to digital pathology will require pathology departments to create flexible and stable ergonomic workspaces that accommodate both a digital pathology viewer and a microscope. As other digital clinical departments have discovered, a well-designed workspace also facilitates collaboration and teaching and maximizes the use of space.
https://bit.ly/2GXBUBT

The Laboratory Leader

Sitting Down With… Gary Procop, Director of Molecular Microbiology, Virology, Mycology and Parasitology, and Vice Chair of Pathology Education at Cleveland Clinic, Cleveland, Ohio, USA

Why did you pursue a career in laboratory medicine?

The story goes all the way back to high school, when I used to see old black-and-white films of healthcare workers delivering vaccines and other treatments to people in the heart of Africa. I became extremely interested in infectious diseases and pursued microbiology at undergraduate and Master's level. Through the guidance of colleagues, I discovered pathology as a route to involvement in microbiology – and I've enjoyed a wonderfully rewarding career ever since.

You are the former Co-Chair of the Enterprise Laboratory Stewardship Committee – what did this aim to achieve?

Pathologists have traditionally focused primarily on the analytic phase of testing, which is obviously crucial when providing useful and accurate information to the clinician. Laboratorians today are extremely proficient in that regard – our tests are highly reliable, reproducible, and demonstrate all the quality data required. More recently, our work has expanded to pre- and post-analytics to encompass specimen handling and the meaningful reporting of results. The next step – which some people have termed pre-pre-analytics and post-post-analytics – is to provide guidance around why a certain test is required and exert some influence over how the test results are used. This is all part of our duty in the US under Clinical Laboratory Improvement Amendments regulations – and it encompasses the real nuts and bolts of test utilization. As laboratory medicine professionals, we must be able to participate in conversations with our clinical colleagues who order the tests to ensure that all decisions are evidence-based.

Our aim is to create a consensus around evidence-based, best practices for test use to ensure consistency between patients. I'm most proud of the way we have achieved this collaboratively; during my nine years at the helm of the committee, laboratorians and clinicians have worked together to agree on best practice. Moving forward, we must train residents and fellows to understand that their job is not just performing tests, but actively coordinating with the entire system. That's why good interpersonal communication and systems-based practice skills are increasingly important for the laboratory workers of tomorrow.

What role have medical laboratories played during the COVID-19 pandemic?

There is one inescapable word that every radio and TV station has been repeating throughout the pandemic – testing. William Osler – widely regarded as the father of American medicine – once said that there are three stages to treatment: diagnosis, diagnosis, diagnosis. Our response to COVID-19 hinges on accurate and timely testing and diagnosis, meaning that those infected can self-quarantine and not transmit the disease. As the driving force behind those tests, the medical laboratory plays a crucial role – and I just hope that the public acknowledge laboratory medicine as a pivotal point of healthcare and that it drives the best and brightest of the next generation to consider it as a career.

How can we encourage people to pursue careers in laboratory medicine?

I'm disappointed by the amount of exposure pathology currently receives in medical schools. I think many medical students don't even know what a pathologist is or what the medical laboratory does when they choose their preferred subspecialties. I'd like to see greater exposure early on for students; for example, I visited my old high school this year to present pathology and laboratory medicine as an attractive career path and encourage bright young minds to consider a future in the laboratory. We also need much greater exposure in undergraduate communities and within medical schools to introduce our field as an exciting and rewarding opportunity.

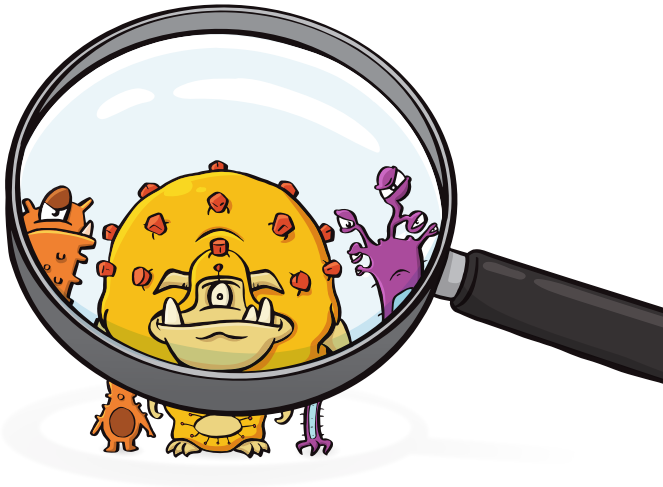
How do you find time for your personal interests among all your professional endeavors?

I love to sail and own my own sailboat; that's probably my biggest hobby and a great way to relax. We also have a cabin in northern Michigan that we often escape to and do things totally unrelated to work, such as chopping wood and taking long walks in the woods. I love getting out into the open air in general; unfortunately, I definitely don't get enough of those things. I have to squeeze them in around the edges whenever I can! I probably over-commit myself sometimes – but I think it's important to have scheduled activities away from work because it always helps to re-energize and come back refreshed.

What advice would you give to those at the start of their careers?

You have to follow the passion in your heart. My primary interest was always in infectious disease pathology and microbiology – but temptations have arisen along the way. For example, I was offered a staff position in urologic pathology early in my career at an institution where I really wanted to stay. I chose to turn it down because I decided to pursue the subspecialties that really interested me and – even though I had the option to stay at an renowned institution – saying no was the best decision I ever made. When you do things that you really love, your passion will be sustained throughout your career and you'll continue to love it many years later!

Detect SARS-CoV-2 and 21 other pathogens.



The new BioFire® Respiratory 2.1 (RP2.1) Panel* covers COVID-19 detection.

Right now, SARS-CoV-2 is everyone's top suspect, but respiratory bugs can cause similar, overlapping symptoms. Now you can test for 22 common respiratory pathogens, including SARS-CoV-2, with the BioFire® Respiratory 2.1 (RP2.1) Panel—now available under an FDA Emergency Use Authorization.*

Syndromic testing means all it takes is one test and just 45 minutes to round up SARS-CoV-2—and all the other usual respiratory suspects. Rapid answers on a broad range of pathogens can inform patient management and alleviate patients and staff alike. What's your frontline solution into respiratory season and beyond?

To learn more, visit **biofiredx.com/Covid-19**

BIOFIRE®

BY BIOMÉRIEUX

Syndromic Testing: The Right Test, The First Time.

*This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for the detection and differentiation of nucleic acid of SARS-CoV-2 from multiple respiratory viral and bacterial organisms. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

BFR0001-0005-01