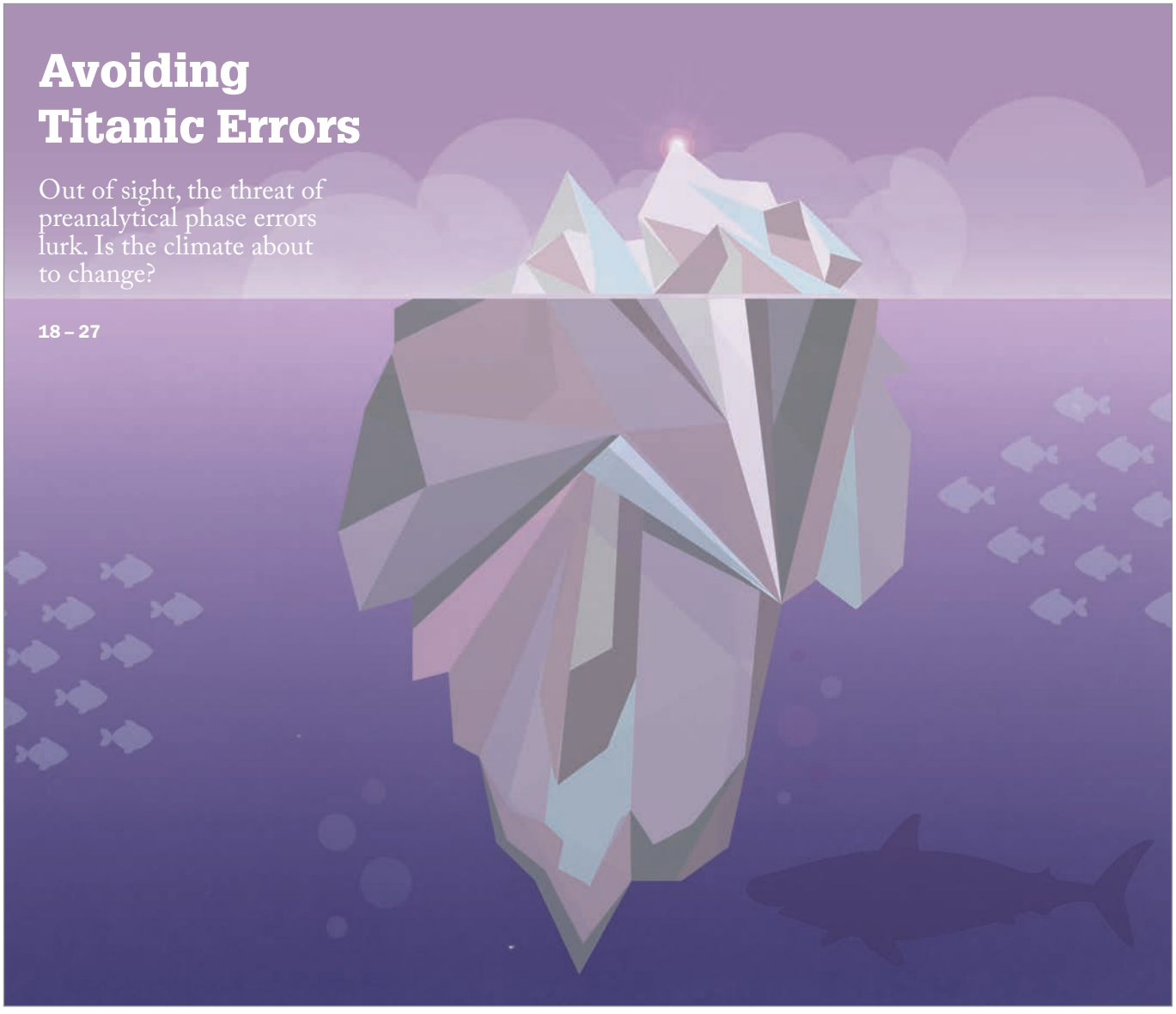
MAY 2015 # 08

the Pathologist



Avoiding Titanic Errors

Out of sight, the threat of preanalytical phase errors lurk. Is the climate about to change?

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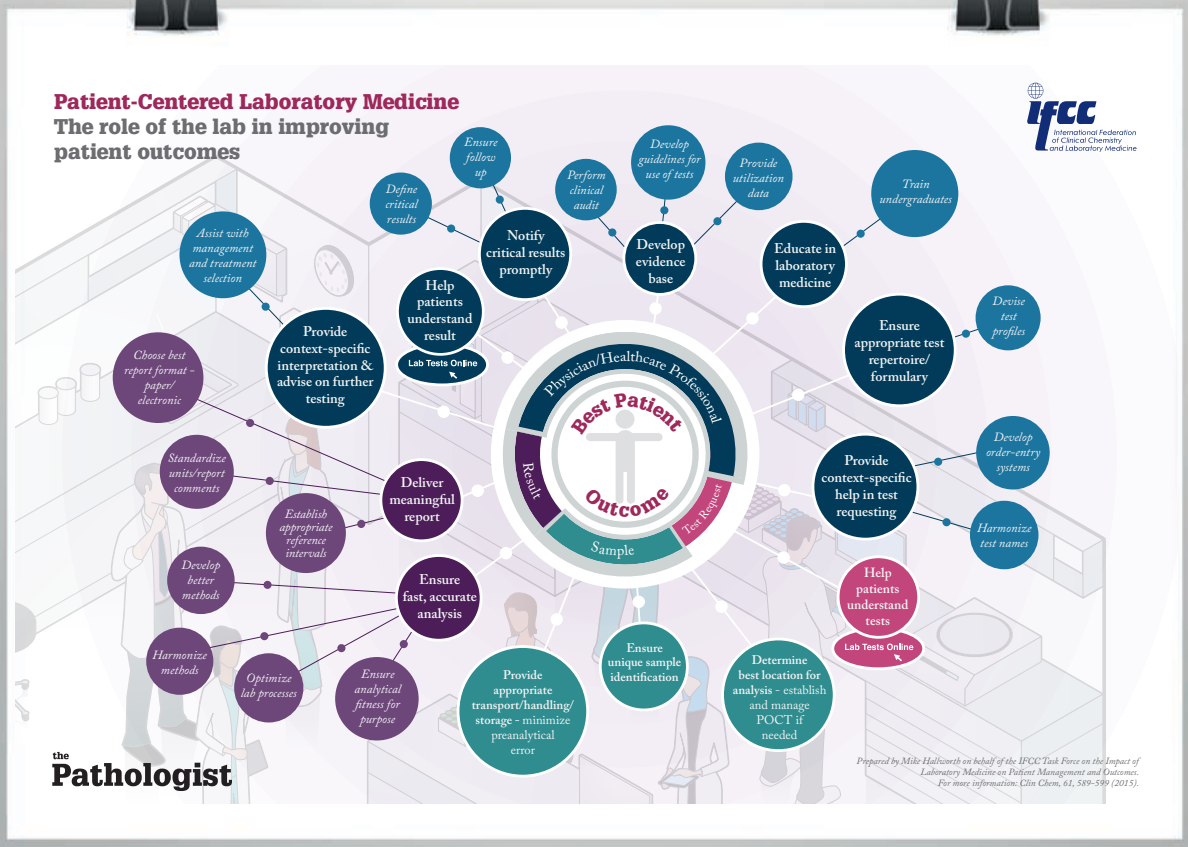
"Patient Centered Laboratory Medicine – The Role of the Lab in Improving Patient Outcomes"



at IFCC Booth # B028, Euromedlab Paris 2015, 21–25 June, Palais des Congrès de Paris, or email tracey.nicholls@texerepublishing.com

Poster prepared by a collaboration of The Pathologist and Mike Hallworth on behalf of the IFCC Task Force on the Impact of Laboratory Medicine on Patient Management and Outcomes.

Online this Month

"I believe it is time for an educational revolution, to give power to all and make education possible everywhere."

We sit down with George Kontogeorgos, President Elect of the International Academy of Pathology on page 50. He discusses the wide disparities in education levels in pathology he has observed among countries, and the crucial issues which must be addressed in order to assist pathologists in underserved countries. He also explains that the Internet and digital pathology have a key role to play.

Read our in-depth interview online now: thepathologist.com/issues/0515/701

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A Rather Unconventional Career:
http://bit.ly/1xxU318
"Interesting story. In fact, that's almost what happened with me. Went through the 3-year residency in anatomical and surgical Pathology in Brazil (my home country), then 4 years of PhD course in Japan, with emphasis in endocrine Pathology and cancer. After all that, just decided to work in the industry, and that's where I am now." – *Saulo Felizola, Japan*

Collaboration Critical:
http://bit.ly/1FmIo8W
"The resolution of the monitor and the speed of the processors is critical. While "pathologist neck" (an ailment largely of the old monocular 'scopes) may become a thing of the past, the headache from pixellation and re-pixellation is a very real hazard and the discomfort dramatically impacts efficiency." - *LuAnn McKinney, USA*

Capsule Capture:
http://bit.ly/1BQVwLw
"Just as primary screening of pap tests is done by cytotechs, in any other screening setting it is impossible for pathologists to do it all, and not appropriate, since they would lose their skills. So a new type of technician would have to do primary screening, or perhaps secondary, after the computer did the first level."– *Jiom, Canada*

Last Month's Top Tweets @pathologistmag

Doctors urged to stop 'over-diagnosing' and 'over-treating'
http://bbc.in/1Hd1L1E @BBCNews
8:45 AM - 13 May 2015

RC of Pathologists @RCPath
New - ISO 15189:2012– An approach to the assessment of uncertainty of measurement for cellular pathology laboratories
http://bit.ly/1HerDx7
12:39 PM - 12 May 2015

Einstein Pathology @EinsteinPath
Thanks @pathologistmag for featuring Dr. Prystowsky and how he is inspiring the next generation of #pathologists !
http://bit.ly/1S5buxg
4:08 PM - 11 May 2015

Congratulations to Simon Heales who won our competition for an iPad after completing our clinical diagnostics survey!
http://bitly.com/1IGUhra
1:38 PM - 11 May 2015

Michael Misialek @DrMisialek
Pathologists climb Capitol Hill today to shine light on our value and crucial role in healthcare @Pathologists @pathologistmag #cappolicy15
http://bitly.com/1PQC6OB
3:40 PM - 6 May 2015

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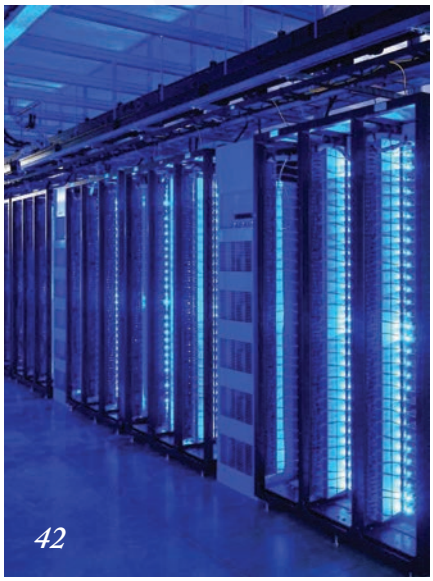
On The Cover

Beneath the analytical phase of lab testing, the more substantial, menacing preanalytical phase of the iceberg lurks.

Upfront

Feature



In Practice

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Sitting Down With

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Best Choose Wisely

Countries should be uniting against "wasteful medical practices" and "harmful tests". But how realistic is change?

"Over diagnosis and overtreatment are the products of a broken system," – strong words by Aseem Malhotra in an interview with the BBC (1). Malhotra is lead author of a recent report by the Academy of Medical Colleges (AoMRC) (2), and it seems as though the UK-based group is on a mission: to "reduce the harms of too much medicine". They argue that doctors have an ethical responsibility to cut down on "wasted use of clinical resource because, in a healthcare system with finite resources, one doctor's waste is another patient's delay." And it's looking to its American colleagues for inspiration.

The Choosing Wisely initiative in the US and Canada was set up to stop doctors using interventions that are not supported by evidence, are unnecessary or are duplicated. With the help of medical organizations, the campaign has compiled top five lists of interventions for each specialty that should not be used routinely, if at all. So far, 60 specialist societies in the US have joined Choosing Wisely since its introduction in 2012. Has it been effective? The jury's still out; a recent telephone survey of 600 US doctors found that only 21 percent had heard of Choosing Wisely, despite the publicity around the campaign (3). It has gained international following though; to date it's been adopted in Australia, Germany, Italy, Japan, the Netherlands, and Switzerland.

Now the UK wants a bit of the action. AoMRC is spearheading its own Choosing Wisely campaign and hopes to have its top five specialty lists later this year. It acknowledges that it will likely not be successful unless it has the backing of patients, but with some patient groups and charities fearing that people could miss out on a crucial, early diagnosis, how realistic is this? And how confident are doctors in their own knowledge of benefits versus risks?

With doctors being caught between the demanding patient, pricing and workload pressures, and a plethora of often confusing statistics, it's no wonder that cynicism of change remains. Tim Allen (@TimAllenMDJD) recently highlighted the closing sentence of the recent BBC news report on the initiative via Twitter: "there is no guarantee that this approach will necessarily reduce the use of unwarranted and sometimes harmful tests." One thing that is guaranteed though, is pathologists' vital role in supporting improved test utilization and in securing the best overall outcome for the patient. How? Take a look at Mike Hallworth's poster that accompanies this month's issue, where he clearly highlights all of the ways that laboratories can deliver exceptional patient care. We hope you display it on the walls of your lab with pride.

Fedra Pavlou
Editor

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Mauro Panteghini

Mauro is Professor of Clinical Biochemistry and Clinical Molecular Biology at the University of Milan Medical School, Italy. President of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), he has published over 460 manuscripts, and given over 110 invited lectures, both nationally and internationally. Speaking of the work of the EFLM preanalytical phase working group, he says, "Today [it] is an internationally-recognized driving force in this field, leading the way towards global harmonization."

We interview Mauro on page 27 on the work of the EFLM working group for preanalytical phase and how the society aims to improve lab medicine on an international scale.

Ana-Maria Šimundić

Ana-Maria is President of the Croatian Society of Medical Biochemistry and Laboratory Medicine, and serves as EFLM Executive Board Secretary and Chair of their working group for Preanalytical Phase (WG-PA). She has amassed numerous awards since 2000, including Best Young Scientist and Best Research by the Croatian Society of Medical Biochemistry and Laboratory Medicine, as well as the Per Hyltoft Petersen award by the Slovak Society of Laboratory Medicine.

On page 19, Ana-Maria discusses the substantial contribution preanalytical lab errors make to the overall risk of preventable medical errors, and what can be done to address them.

Mike Hallworth

A former President of the European Communities Confederation of Clinical Chemistry and Laboratory Medicine (EFCC) and past Chairman of the UK Association of Clinical Biochemistry, Mike is Chair of the IFCC task force on the impact of laboratory medicine on clinical management and outcomes. He was awarded 2008 UK Healthcare Scientist of the Year, and was the 2011 winner of the EFCC-Roche European Scientific Award for Laboratory Medicine.

On page 34, Mike discusses the main outcomes of the IFCC task force's evaluation of the evidence supporting the impact of lab medicine on patient outcomes, and suggests where improvements can be made.

Simon Patton

Simon is Director of the European Molecular Genetics Quality Network (EMQN) – a provider of External Quality Assessment (EQA) schemes to diagnostic laboratories in the fields of genetics and pathology. Based at the Central Manchester Hospital NHS Foundation Trust, Manchester, UK, his work has focused on improving the quality of lab testing, including lecturing, course development and consultancy.

Simon weighs in on the importance of quality assurance in the era of personalized medicine, with a focus on tumor testing, on page 30.

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Measles-Induced Immune Amnesia

The measles virus may erase immune memory, leaving patients vulnerable to other childhood infections for up to three years

The measles vaccine has been around, in one form or another, for over 50 years. Ever since its introduction, we've seen a striking reduction in childhood morbidity and mortality – by as much as 90 percent in impoverished populations (1), but the revolutionary effects aren't limited to developing countries. Even in the United States, it's estimated that the first 20 years of measles vaccination prevented 52 million cases and over 5,000 deaths, saving the healthcare system about US$5 billion (2). But the benefits of the vaccine aren't fully explained by its prevention of measles infections – so what accounts for the profound effect of measles vaccination on children's health?

The nonspecific effects of childhood vaccinations have been noted since the late 1980s, but the reasons behind them have remained a mystery – researchers have proposed various beneficial immunological mechanisms, but none can be confirmed as the cause of the reduction in disease mortality. Recent work, however, suggests a different method: that measles infection may erase a patient's immune memory, replacing lymphocytes designed to defend against non-measles pathogens with those specific to the measles virus (3). Because the previous memory cells are replaced with measles-specific ones, the overall blood counts return to their original levels within a few weeks, masking the patient's "immune amnesia" to other infections.

One group of researchers hypothesized that this effect, if indeed present, should be easy to spot by tracing the relationship between measles cases and deaths from other infectious diseases during times when measles was common. Not only would this kind of analysis confirm the existence of the effect, but also its duration – by asking how long the correlation between measles and deaths from other infections lasts, the researchers would also find out how long thew immunosuppression might last. And it worked; the mathematical analysis of data from England, Wales, Denmark and the United States consistently revealed a correlation that lasts for two to three years (4). That may be the amount of time needed for a measles-depleted immune system to rebuild.

Of course, the idea has yet to be mechanistically proven – there's no guarantee that the children who contract measles are the same ones who later die of other childhood infections, nor have there yet been any human studies of immune memory after measles infection. But the evidence from the mathematical study is compelling and, if nothing else, should provide one more reason for doctors to encourage childhood vaccination. *MS*

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Reflex Testing a No-No for CKD?

Current tests for nephropathy may not be good enough; but research could yield new options for effective diagnosis

Chronic kidney disease (CKD) is both a relatively common condition and associated with significant morbidity, mortality, and expense. A recent study of US patients has shown, however, that many of the laboratory and imaging tests conducted on patients with CKD may have little or no effect on its diagnosis and management (1).

The researchers examined how often lab and imaging tests were ordered, and what effect those tests had in 1,487 patients referred for initial evaluation of the condition to two medical centers in Boston, US, over a three-year period. "Our main finding was that a number of tests, including renal ultrasound, paraprotein testing, and serologic testing, were commonly ordered despite low diagnostic and management yield," says lead author Mallika Mendu, of Brigham and Women's Hospital (BHW), Renal Medicine Division, Boston, "conversely, urine quantification and hemoglobin A1c testing had the highest diagnostic and management impact."

Mendu and his colleagues suggest that reflexively ordering many tests to evaluate CKD is likely not the best approach, and that an evidence-based, targeted approach will result in more efficient and cost-effective diagnosis and management.

It's obvious that more is needed to improve the situation; an improved knowledge of the intricacies of the disease pathways, and the discovery of more accurate diagnostic markers would be steps in the right direction. A University of Manchester, UK, team has begun taking those steps, with some promising results. "In humans, kidney disease is more common in males, and certain racial groups," explains lead author Rachel Lennon, "but we don't fully understand why. We hypothesized that the matrix scaffolds which support the glomerular cells of the kidney filters would be different, and could account for susceptibility to glomerular disease (the most common type of kidney disease). To study this we used mice, who, similar to humans, may be susceptible or resistant to the condition."

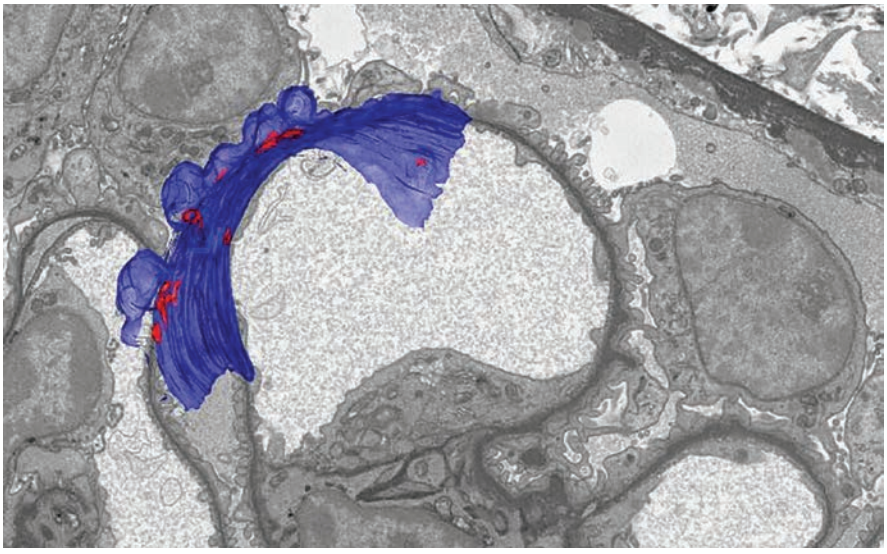

Figure 1. The structural defects in the glomerular basement membranes observed using serial block face scanning electron microcopy. A glomerular capillary lumen is shown, and the basement membrane modeled in blue, with membrane splits shown in red.

Lennon and her colleagues found that protein expression in the glomerular extracellular matrix (ECM) revealed unique signatures which could be correlated to a predisposition to nephropathy (2). Examination with electron microscopy also revealed structural differences in the glomeruli, with abnormal structures seen in susceptible mice (Figure 1).

"We have found a number of changes in the proteins of the kidney filters and they occur early in the process. These proteins could prove to be useful early biomarkers of kidney disease," says Lennon. "Our ultimate goal is to understand more about the mechanisms of glomerular disease, and design new therapeutic strategies to prevent progression or even reverse the disease process," she adds.

It is clear that tests for kidney disease, both old and new, must be used carefully in order to ensure the best care for patients. Though the goal of eliminating unnecessary testing and developing new, more effective diagnostics may not be a reality in the short-term, certainly making wiser and evidence-based choices around kidney testing is something that can be addressed now and will yield immediate improvements in diagnostic accuracy for the patient, and time and cost efficiencies for laboratory services. *RM*

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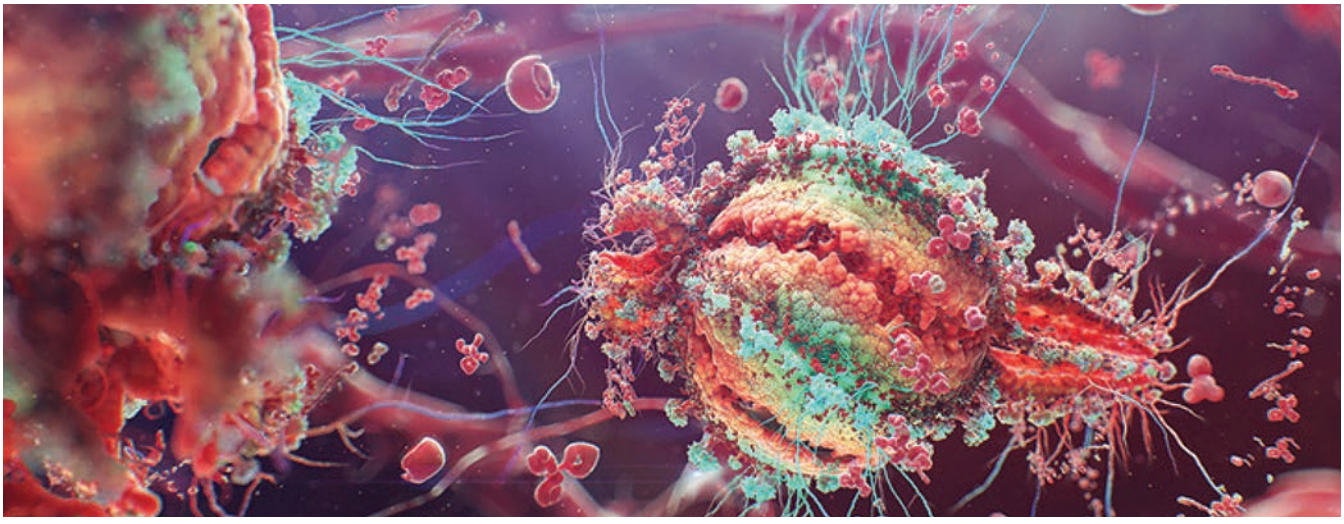

First HIV Home Test Kit Hits Europe

The first HIV home testing kit to receive a CE mark may help reduce the number of patients diagnosed late or not at all

Another kit has joined the ever-growing ranks of medical testing people can perform in their own homes – this time, for HIV. But unlike many home testing kits that require samples to be sent away and outcomes interpreted by an expert, the HIV test manufactured by BioSure (Nazeing, UK) provides a clear, easy-to-understand result in about 15 minutes. Not only is it the first home test kit to do this, but it's also the first of its kind to gain CE mark approval, permitting EU marketing.

The test itself is simple to use – patients pierce one finger with a lancet, touch the tip of the testing device to the drop of blood, then leave the device in an included pot of buffer for 15 minutes. The readout closely resembles those of commonly used pregnancy tests; a single purple control line confirms the test was correctly performed, while a positive result yields a second line below the first. Although the test is highly accurate (negative results are correct 99.9 percent of the time, while positive results are correct 99.7 percent of the time), the manufacturer recommends that all positive home test results be confirmed by a healthcare professional (1). It's important to note that the test isn't flawless – it takes up to three months for HIV antibodies to appear in the blood in sufficient quantities for testing, so people who have been exposed more recently won't be able to get an accurate result until that time period has passed. Another notable concern is that, unlike clinic testing, the home kits cost money – in the case of the BioSure kit, £29.95 (~€40).

Despite their cost, tools like these spare people not only the time, inconvenience and discomfort of needing to go to a clinic for HIV evaluation, but also the embarrassment of requesting the test. They ease the pressure on clinics, too; Gary Carpenter, clinical products director at BioSure, says, "We don't see self-testing as a replacement for testing in clinical settings […] We think that self-testing will provide wider access, getting people to test that would otherwise not have done so."

An estimated quarter of people living with HIV in the UK are unaware of their infection – and even among those who are aware, 42 percent are considered to have been diagnosed late (2). It's hoped that a rapid, discreet home testing kit will relieve overburdened clinics and encourage reluctant people to be tested for HIV, especially as a late start to treatment can result in as much as 15 years' loss of life (3). HIV is still considered a major public health challenge, despite the widespread availability of free or low-cost testing, and innovations like easy home test kits provide a welcome new addition to the tools available for fighting the virus. *MS*

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Ocular Ebola Threat

Health worker's ocular fluid tests positive for Ebola months after virus became undetectable in blood

A doctor working in West Africa was found to have the Ebola virus in the aqueous humor of his left eye 10 weeks after it was no longer detectable in his blood, and developed severe uveitis which threatened his vision – a finding which could have far-reaching implications for survivors of the recent Ebola outbreak.

Ian Crozier, an infectious disease specialist, contracted Ebola while helping to fight the epidemic in Sierra Leone in August 2014, and within a few weeks had contracted the virus and was evacuated to Emory University Hospital in Atlanta, USA. After spending some time in a critical condition he began to recover and was discharged. However, he then began to develop symptoms of ocular disease, including pain and intolerance to light, which progressed to blurred and decreasing vision. His aqueous humor tested positive for the Ebola virus using quantitative real-time PCR, despite the surface of the eye, the tear film and peripheral blood samples remaining negative for the virus.

The resulting case study (1) reports that the pathology of ocular Ebola infection is unknown, but the researchers involved theorize that the severe uveitis observed was a direct cytopathic effect of the virus. "The presence of viable Ebola virus in the eye could mean that other Ebola survivors may also be at risk for the development of uveitis," says Steven Yeh of the Emory Eye Center. "The thousands of Ebola survivors in West Africa and healthcare workers in their home countries will need to be monitored for eye disease in the post-Ebola period." *RM*

Reference

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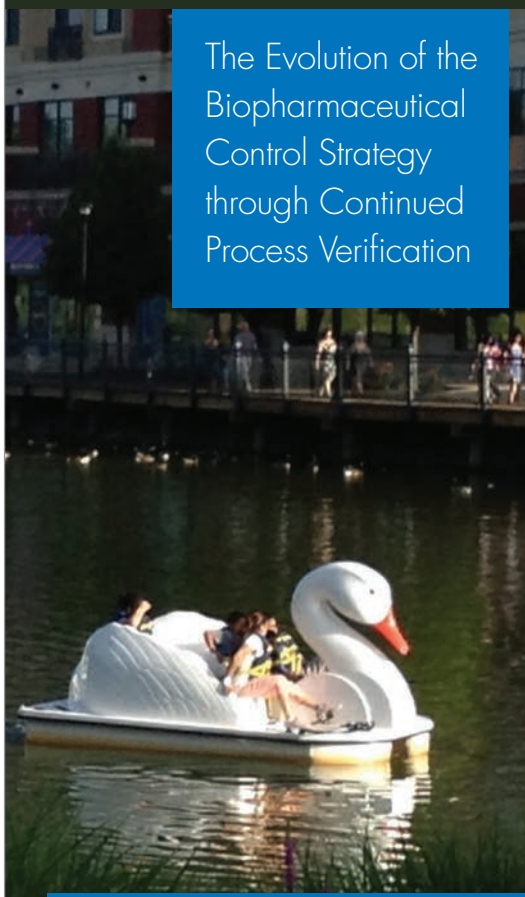

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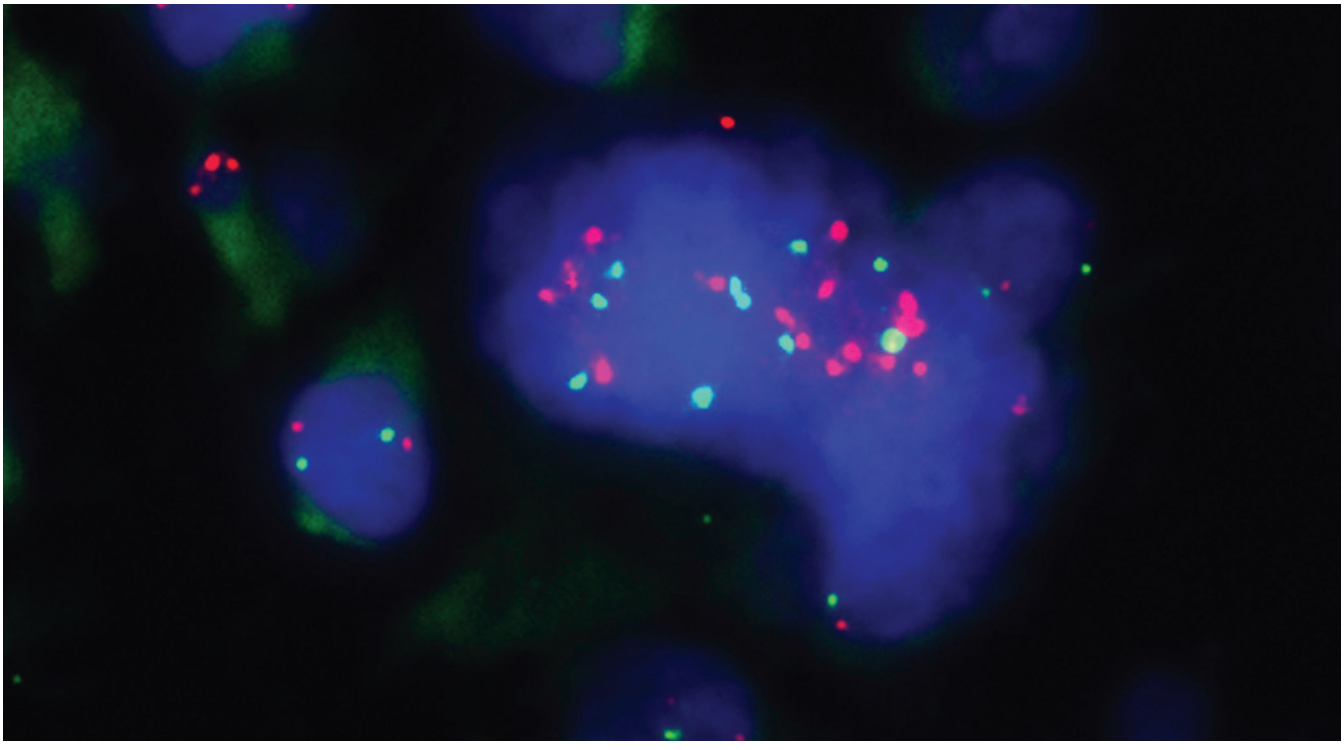

Multicolor FISH for *BPTF* locus (red), centromere of chromosome 17 (green) and DAPI (blue) as nuclear counterstaining, showing elevated *BPTF* copy number in patient progressing on *BRAF*-targeted therapy.

Molecular Clues to Skin Cancer

A better understanding of melanoma could lead to new strategies for tackling therapy resistance

In recent years, targeted therapy using *BRAF* inhibitors has substantially improved survival rates for patients with advanced melanoma. However, the majority of patients eventually become resistant to therapy and the result: treatment cessation. Now, researchers from California, USA, have shed light on a gene that could potentially provide a solution to the problem of *BRAF* inhibitor resistance, and play an important role in melanoma progression.

"Our broad-based goal is to understand factors that mediate melanoma progression, in the hope of developing markers that predict melanoma metastasis, or that may serve as targets for therapy of metastatic melanoma," says co-author of the study (1), Mohammed Kashani-Sabet. "We found that the bromodomain PHD finger transcription factor (*BPTF*) gene plays an important role in melanoma progression, and that higher levels of *BPTF* expression in melanoma cells promoted resistance to *BRAF*-targeted therapies," he explains.

Kashani and his team are hopeful their discovery will impact the diagnosis and treatment of melanoma, as *BPTF* may prove to be an important molecular marker for diagnosis and prognosis of cancer. Further, they have shown it to play a role in activation of the MAP kinase pathway, which is key for melanoma proliferation, and an important therapeutic target along with *BRAF* inhibition. A future strategy for preventing resistance to treatment could involve teaming *BRAF* inhibitors with a therapy that targets the resistance mechanisms, suggests Kashani.

"This discovery may give pathologists an additional tool to assess melanoma diagnosis and prognosis, and a marker to allow identification of patients in whom treatment with *BRAF* inhibitors can be continued", says Kashani. "In the future, we aim to gain further understanding of the role of *BPTF* in tumor progression, and develop it as a target for cancer therapy," he concludes. *RM*

Reference

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Big Push for Big Data

Bioinformatics gets a boost from educational and training initiatives

Advances in disciplines such as genomics and proteomics have caused the profile of big data in the life sciences to rise dramatically – but graduates may not be getting the training they need to analyze it. This has inspired an international group of bioinformatics educators and trainers to form GOBLET – the global organization for bioinformatics learning, education and training, with the aim of creating a training portal to support the next generation of bioinformaticians (1).

"Traditional life science degree programs have tended not to focus on the development of bioinformatics skills, so that graduates and postgraduates are often ill-equipped to understand either the data or the data quality in open databases," says Vicky Schneider, a GOBLET executive, speaking on behalf of the organization and colleagues, including Teresa Atwood, Michelle Brazas and Fran Lewitter from the UK, Canada and the US. "The advent of high-throughput technologies has exacerbated the problem, bringing not only "traditional bioinformatics" (e.g. sequence searching, multiple alignment, variant detection), but also data-driven science sharply into the spotlight in terms of the ongoing, and now urgent need to provide training in data-analysis and data-interpretation," adds Schneider.

GOBLET aims to help bridge the bioinformatics skills gap by providing a support network, and developing standards and guidelines for education and training. "We have grown to around 30 organization members including leading societies, networks and institutes, and we have launched an open training portal. Increasingly we are being invited to collaborate and share efforts," says Schneider.

In other bioinformatics news, several organizations including the European Bioinformatics Institute (EMBL-EBI) and The Genome Analysis Centre (TGAC) have received funding from the Biotechnology and Biological Sciences Research Council (BBSRC) to support scientists from the UK and China in managing and sharing their metabolomics data; skills the BBSRC believe are essential for furthering the impact of scientific research. Christoph Steinbeck of EMBL-EBI commented, "There is already a lot of commitment in the metabolomics research community to data sharing and reuse – our main challenge is simply in training people how best to incorporate this into their regular working practices. The BBSRC has recognized that this area of molecular biology is growing more quickly than any other, and that we need to do everything we can to train and support scientists in sharing data. That will lead to better quality data, more efficient research and shorter time to discovery."

It is clear that, as the sheer volume of data being obtained from processes such as next generation sequencing continue to increase in leaps and bounds, further training and education is needed to ensure the new generation of researchers are prepared to deal with the new generation of big data. The advice from GOBLET? "Get involved! Join GOBLET, and shape and influence the future of training courses by sharing your training needs." *RM*

Reference

1. *TL Atwood, "GOBLET: the global organization for bioinformatics learning, education and training" PLoS Comput Biol, 11, e1004143 (2015). PMID: 25856076.*

New Guidelines on the Block

Preanalytical accuracy in surgical pathology targeted by new recommendations

The College of American Pathologists (CAP) and the National Society for Histotechnology (NSH) have released guidelines on the uniform labeling of paraffin blocks and slides in surgical pathology (1). Their hope is that the recommendations will address current inadequacies, such as variations in practice in different laboratories.

"Careful and consistent labeling of paraffin blocks and microscopic glass slides is essential in the practice of surgical pathology to ensure patient safety and to reduce the potential risk of preanalytic error," says Richard W. Brown, guideline co-chair. "We encourage pathologists and histology laboratory professionals to implement the new guideline in their individual practice settings as an additional quality assurance measure," he adds.

The guidelines addressed 12 key recommendations (see Box), and CAP plans to update the guidelines as new evidence becomes available, with the aim of reducing error and improving patient care. *RM*

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1. *RW Brown, et al., "Uniform labeling of blocks and slides in surgical pathology: guideline from the College of American Pathologists pathology and laboratory quality center and the National Society for Histotechnology", Arch Pathol Lab Med, [Epub ahead of print] (2015). PMID: 25897820.*

To read about what is being done in Europe to address the issue of preanalytical errors, please read our cover feature on page 18.

Summary of the 12 Key CAP/NSH Recommendations
All blocks and slides should be unambiguously labeled with two patient identifiers
The accession designation on the pathology report, and all blocks slides from that accession, should include the case type, the year, and a unique accession number
If the patient's name is used as a patient identifier, labs should make sure the name format will link the blocks and slides to the correct patient
When an accession number hasn't been assigned yet, blocks and slides should be labeled with at least two patient identifiers, one of which is the patient name
Each specimen container should be labeled with a unique alpha-numeric designation that incorporates the accession designation
Each block obtained from a single specimen should be labeled sequentially with a unique alpha-numeric designation that can be unambiguously linked to a gross description within the pathology report
When multiple slides are cut from one block, label each slide sequentially in order of cutting
Slides should be labeled with the histochemical, IHC and/or special procedure, (e.g. FS for frozen section, AFB for acid fast bacteria). The technique or specific antibody used should be included if it may affect interpretation
No recommendation regarding standardization of abbreviations and conventions is made
On paraffin blocks, the accession designation should be the most prominent printed element (e.g. larger font, bolded)
On microscopic slides, the accession designation should be the most prominent printed element
Blocks and slides received in consultation should be labeled with the recipient institute's accession designation, and original labeling should not be obscured by relabeling

Screening Hopes for Autism

Will gene expression be the key to mass pediatric screening?

Diagnosing ASD (autistic spectrum disorder) is often a challenge, especially in very young children, but early identification of those at risk could lead to better monitoring and earlier detection. Now, a team of international researchers are working on a blood test that they believe has the potential to be an autism screening tool. We spoke with Eric Courchesne, principle investigator and co-director of the Autism Center of Excellence at the University of California, San Diego, and co-author of the associated paper (1).

What motivated you to develop this test?

ASD is a highly heterogeneous disorder. Many genomic analyses have been conducted, which have led to the discovery of dozens of mutations. However, each mutation can only explain a small fraction of the cases, and the pathways involved in its development are unclear. This genetic complexity makes it difficult to conclusively diagnose the disorder before a child's fourth year of life. In fact, the median age of diagnosis in the United States is 4 and a half years. This late identification is a serious concern – research shows early identification and treatment by age 2 or 3 years leads to much better clinical outcomes. At present, there is no successful early biomarker, leaving affected infants undetected and untreated.

How does it work?

We used weighted gene expression values to classify ASD vs typical and non-ASD developmentally delayed toddlers. The test uses over 700 genes in specific gene networks that we found to be abnormally expressed in blood leukocytes of 1- to 3-year-old toddlers with ASD, as compared with non-ASD toddlers. The expression of each gene is given a weight based on the gene's aberrance in ASD and its contribution to dysregulation of the network.

So far, our test has 83 percent accuracy, 80 percent specificity and 85 percent sensitivity. This substantially outperforms all known biomarkers at very young ages in ASD, including genetic markers. A large number of genetic defects have been identified as possible risk factors in ASD, but each individual defect typically occurs in less than 1 percent of all patients with ASD. By some estimates, when combined these gene defects account for only 2–3 percent of variance. In my opinion this shows genetic markers are currently unsuitable for general pediatric screening. We are aiming to develop an accurate, cost-effective first or second tier screening test.

Practically, our test is simple: blood is drawn, then quickly processed so that leukocyte mRNA can be extracted and stabilized. We then use the Illumina HT-12 to obtain gene expression data and expression values for the genes are weighted, and the computed values are used to classify infants as either ASD or non-ASD.

Could this test be used clinically?

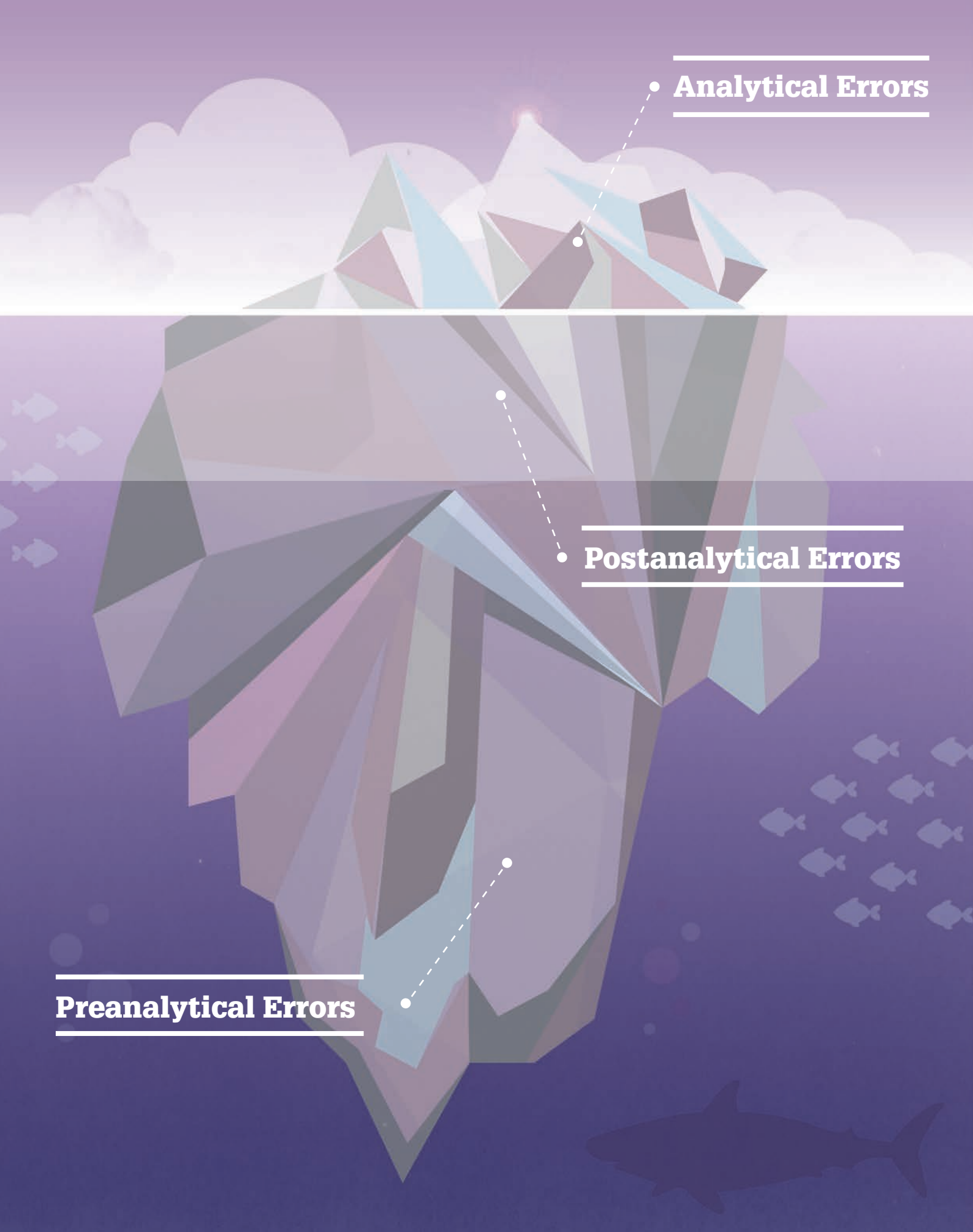
Our current test was a proof of concept. We are in the process of further developing and validating our classifier approach, and in the distant future, hopefully some clinical trials will be conducted. I believe that if a validated and robust test emerges, it could be used clinically. Many steps stand between successful proof of concept and actual clinical use – but a truly successful screening method for high risk of autism is very important to achieve, and someday someone will do it.

What are your next steps?

We are using RNA sequencing and new systems biology methods to develop even more accurate, specific and sensitive functional genomic biomarkers of early risk for ASD in infants and toddlers. We have quadrupled our sample size, and aim to double that again.

Reference

1. *T Pramparo et al., "Prediction of autism by translation and immune/inflammation coexpressed genes in toddlers from pediatric community practices", JAMA Psychiatry, 72, 386–394 (2015). PMID: 25739104.*


Analytical Errors
Postanalytical Errors
Preanalytical Errors

Avoiding Titanic Errors

The preanalytical phase is subject to more error than any other part of the testing cycle – what can we do to improve it?

By Ana-Maria Šimundić

Preventable medical errors are not something that people working in healthcare really want to think about, but they are a reality and the numbers aren't small. A recent report estimates mortality resulting from medical error in the US alone to be more than 400,000 deaths per year (1). According to WHO, one in 10 patients suffer from some kind of error during hospitalization in developed countries (2) and 8–12 percent of patients in EU countries are thought to experience preventable adverse events (3); unsurprisingly a huge spotlight has been cast over patient safety, placing it as an issue of concern by the European Commission.

Given the importance of laboratory tests on the overall medical decision-making process, laboratory errors make a key contribution to the overall risk of error in healthcare. In a separate study that looked at the frequency of diagnostic errors, the testing phase (failure to order, report, and follow-up laboratory results) were found to contribute to the majority of diagnostic errors (44 percent) (4). This number is unacceptably high. Like any clinical or diagnostic field, laboratory medicine is subject to error. While this may not come as a surprise to anyone, it's less well known that most of this error occurs during the preanalytical phase (5), which includes the collection and handling of diagnostic specimens. Because most of these problems are preventable through effective quality control, the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) has set up a working group to focus exclusively on preanalytical phase quality (WG-PA) (see "For the Greater Good").

All errors are not equal

Why is it so important to ensure quality and harmony during sample collection and handling? Numerous studies (6,7) suggest that nearly two-thirds of all laboratory error occurs in the preanalytical phase (see Figure "Laboratory Testing Error Sources"). Why? First of all, there are not many standards for safe, patient-centered and evidence-based preanalytical practices – and those that do exist are often not evidence-based. Second, many steps in that phase (for example, test selection, patient preparation, patient identification, sample collection and delivery to the lab) are performed manually by non-laboratory staff, outside the direct supervision of laboratory professionals. And finally, stakeholders involved in this part of the testing cycle often lack not only the necessary training to conduct the tests, but also an understanding of the procedures they are performing and how their actions affect sample quality and patient results. In one recent study (8), we showed that blood sampling across Europe is done by members of different professions with different education, background, competence, and skill levels. In some countries, even administrative staff are involved in venous blood sampling! All of these factors contribute to the high potential for error in the preanalytical phase.

Not all errors are created equal, though. Some are made more frequently than others, and some carry a greater risk of harm to the patient. When thinking about ways to reduce errors, your first step should be a careful risk assessment; problems that are more common or carry high risk to the patient call for immediate corrective action. One common example of this kind of error is

the use of unsuitable specimens, such as blood samples that are hemolyzed, clotted, of insufficient volume, or feature an inadequate ratio of blood to additive. Analyzing samples like these leads to unreliable test results. Another issue, less frequent but with higher risk to the patient, is identification error. Its rate of incidence is currently at less than one percent, but with the possibility of harmful consequences it carries, laboratories worldwide should adopt a zero tolerance attitude toward this kind of error.

The search for answers

There is ample evidence (9) of the impact that preanalytical phase errors have on patient safety. In fact, laboratory and radiologic issues have been found to account for almost half of all diagnostic errors (4). Though the impact to the patient can vary widely – from physical discomfort to potentially permanent disability or fatality – these problems can be minimized by the implementation of standardized, patient-centered, evidence-based policies and procedures. But this is no easy task – other studies (10) have shown that even if standard procedures are in place, adequate compliance is extremely difficult to ensure. Education improves compliance numbers, but even that only works in the short term; for full effectiveness, compliance education needs to be continuously offered and updated.

The problem with making evidence-based safety decisions is that it's almost impossible for a single laboratory to collect the necessary evidence for every procedure. Preanalytical phase quality is a "hot topic" in laboratory medicine at the moment; the number of published studies has dramatically increased over the last few years. This means that many of the questions labs are asking have been answered in the literature already – but researchers must not only search for that evidence (which is not always easy to find), but also critically assess its suitability to their testing environment and specific clinical context. And for those questions that lack an evidence base, labs must research and prepare their own – because without it, making responsible decisions about routine work becomes an impossible challenge. Without research into the effect of hemolysis, for example, how can we know whether or not a hemolyzed sample is acceptable for analysis? Without evidence of the effect of delayed transport, how can we know whether a delayed sample is acceptable? It's obvious that evidence-based decisions are needed to ensure that patients get appropriate, timely and reliable tests.

Lack of compliance and clarity

Existing guidelines aren't always appropriately followed in the laboratory. There are several reasons behind this: if procedures have standards or recommendations at all, they're often outdated, not universally applicable, not evidence-based, or lacking in vital details. For example, the Clinical Laboratory Standards Institute (CLSI) guidelines for venous blood collection state that laboratory staff should ensure that patients have been properly prepared for testing, but don't explain what this preparation should be. As this is a key step in the blood collection procedure, proper patient preparation is necessary for reliable and accurate test results (see "No Blood Sample is Better than a Bad Blood Sample"). CLSI standards aren't free, either, and many labs in developing countries aren't able to purchase them.

Guidelines represent the best possible practice at the time of their creation, and unless they are freely accessible, they won't see widespread adoption. This problem is only emphasized when these practices are difficult to implement, because humans are often resistant to change. We don't always like having to replace our well-known laboratory routines with new ones; changes of that magnitude require a lot of continuous effort, education, assessment, and monitoring. The only way to make real headway is to get all of the stakeholders involved. International professional

For the Greater Good

Who? Working Group for Preanalytical Phase (WG-PA) created by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

When? Formed in 2012

Who? WG-PA has three full members, one young member, several corresponding members from different European countries, and two expert consultants from industry

Why? All members share the goal of harmonizing and improving quality standards for the preanalytical phase. It's a goal that breaks down into several parts:

- to promote the importance of preanalytical phase quality across Europe
- to assess current preanalytical phase practices and policies in European laboratories, identifying the most critical problems and issues for improvement
- to produce recommendations and provide guidance for implementation across Europe
- to educate laboratory professionals by providing educational materials, organizing conferences, courses, webinars, and more
- to encourage harmonization of preanalytical practices in all European countries

associations can take the lead in defining best practices, whereas national ones can act as a link between overseers and individual members of their societies. National organizations can also promote the importance of standards and recommendations and encourage their implementation – in fact, a great way to begin is by establishing a national working group for preanalytical phase, which can raise awareness, promote research, and provide education. Of course, individual laboratories also carry responsibility, in their case for adopting recommendations, implementing them in routine practice, and assuring compliance. At all levels, though, education and continual improvement are the keys to success.

Small steps towards the dream

The WG-PA is quite a young group, but we've already had some encouraging results (see "What We've Found So Far"). We've organized three international meetings where we hosted world experts, presented new research results, and offered practical tips for dealing with preanalytical phase issues – including analyzing workflows and bottlenecks, assessing impact of biological variability, managing sample hemolysis and lipemia, dealing with patient identification errors, and tips for successful phlebotomy. A third conference has just taken place where 19 national societies in Europe were invited to present their activities to almost 600 attendees. Forums like these give organizations a voice for interactive discussion so that different views and experiences can be shared.

We've also conducted two large surveys – one to assess the level of training provided to personnel performing venous blood sampling, and another to evaluate the level of compliance with CLSI phlebotomy standards. We opted to look at blood sampling because it's available worldwide, it's the most common invasive procedure in healthcare, and it's also the most common source of preanalytical errors – which often go unrecognized. These errors can include things like unnecessary delays, incorrect test results, or even harm to the patient or phlebotomist. We found that only a quarter of European countries have national guidelines for phlebotomy, and that it's performed by both medical and non-medical personnel with a wide range of background education – meaning that not all patients receive the same level of care (8), especially when overall guideline compliance levels are low and critical steps like patient identification are often omitted (10).

As a result of our work, we've published several recommendations, including one outlining the requirements for fasting bloodwork (11) and another calling for harmonization of color coding systems for blood collection tube closures (12) (see "No Blood Sample is Better than a Bad Blood Sample"). But this is only the beginning. In the future, we hope to provide many more guidance documents to assist our colleagues in their efforts to standardize and harmonize preanalytical phase policies and practices. Awareness of preanalytical issues is rising, and so is the interest and commitment of laboratory professionals to upholding international standards in their work. It's not something we can achieve overnight, of course, but even small steps forward indicate that preanalytical quality management is not a dream, but a reality for the very near future.

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12. *AM Simundic et al., "Colour coding for blood collection tube closures – a call for harmonization," Clin Chem Lab Med, 53, 371–376 (2014). PMID: 25324449.*

No Blood Sample is Better than a Bad Blood Sample

According to the WG-PA of the EFLM, existing guidelines for phlebotomy need revision (11). Standardization would allow harmonized reporting of scientific data in the field of laboratory diagnostics and revised guidelines should include:

1. The exact definition of requirements for patient preparation for laboratory testing. Blood for all blood tests should be drawn preferably in the morning from 7 to 9 am. Fasting should last for 12 hours during which water consumption is permitted. Alcohol should be avoided for 24 hours before blood sampling. In the morning before blood sampling, patients should refrain from cigarette smoking and caffeine-containing drinks.
2. Professional associations (IFCC, EFLM and others) should support harmonization efforts by disseminating standardized recommendations for fasting.
3. Laboratories worldwide should implement standardized procedures for blood sampling and patient preparation.
4. Laboratories should have policies for sample acceptance criteria related to fasting samples. Blood samples for routine testing should not be taken if a patient has not been appropriately prepared for sample collection. 'No sample is better than a bad sample' should always be the leading principle.
5. Laboratory professionals are responsible for disseminating information about fasting requirements to patients as well as to clinicians and general practitioners who are the preferred source of patient information.

Recent work by the group (12) has also recommended the standardization of color coding for blood collection tube closures and labels to reduce the risk of preanalytical errors. They propose the following roadmap:

- All stakeholders, including all manufacturers working in the field, should be invited to join a dialog to establish a universally acceptable color coding standard for blood collection tube closures;
- Standard writing bodies (ISO, CLSI) should add the color coding standard agreed onto the existing recommendations;
- Manufacturers should implement the agreed color coding standard.

Members' Musings

The views of the members of the EFLM Working Group for Preanalytical Phase

Stephen Church (SC)
is associate director medical affairs at BD Diagnostics – Preanalytical Systems, Oxford, UK.

Michael Cornes (MC)
is principal clinical biochemist at The Royal Wolverhampton NHS Trust in Wolverhampton, UK.

Pinar Eker (PE)
is a biochemistry and clinical chemistry specialist at Umraniye Research and Training Hospital, Istanbul, Turkey.

Why is the preanalytical phase the biggest source of lab errors?

Giuseppe Lippi: There are several reasons, including the fact that: (a) it's often overlooked as a cause of errors (all lab errors are still too often associated with analytical errors); (b) it's poorly standardized (too many national and international guidelines exist about best practice in this phase); (c) there is poor training of doctors and nurses on how to collect a quality specimen; (d) no internal or external quality control systems have been established so far.

Edmée van Dongen-Lases: It relies on humans, and therefore it's prone to human error.

Kjell Grankvist: Most laboratories still focus solely on analytical quality. Laboratories need to take more responsibility to try to minimize errors regardless of which phase of the total testing process they occur.

Michael Cornes: There are multiple reasons: i) it's often outside the direct control of the laboratory; ii) it's poorly standardized both nationally and internationally and guidelines are often inadequately followed; iii) there is a lack of understanding of the

Kjell Grankvist (KG) is a professor and senior consultant of the Department of Medical Biosciences, Clinical Chemistry, Umeå University, Sweden.

Mercè Ibarz (MI) is head of the Clinical Biochemistry Unit and the laboratory quality assurance manager at the University Hospital Arnau de Vilanova, ICS Leida, Spain.

Gunn Berit Berge Kristensen (GBBK) is head of the Norwegian EQA organization for medical laboratories in Norway.

Edmée van Dongen-Lases (EvDL) is a clinical chemist and staff member at the Department of Clinical Chemistry of the Academic Medical Center in Amsterdam, The Netherlands.

Giuseppe Lippi (GL) is director of the clinical chemistry and haematology laboratory of the University Hospital of Parma, Italy.

Luděk Šprongl (LS) is head of the Central Laboratory and manager for Complement, Nemocnice Šumperk, Czech Republic.

consequences of errors as there is a disconnect between where the error occurs and where its impact is seen; iv) staff are under a lot of pressure because of decreasing staff numbers, decreasing funding and increasing workload, which leads to increasing human errors; v) there is insufficient funding for technological solutions leaving healthcare years behind other industries (i.e. private sector). The technology is there but we are unable to use it.

Stephen Church: I agree, the source of these errors are often outside of the direct control of laboratories, and the staff who collect samples are not aware of the impact that, what seem small errors in their practices, have on sample quality and identification and therefore a lab's ability to provide accurate results. I call it the domino effect: if something goes wrong at the beginning, the further the erroneous sample advances through the analytical process, the greater the impact on laboratory efficiency, laboratory cost and ultimately patient care.

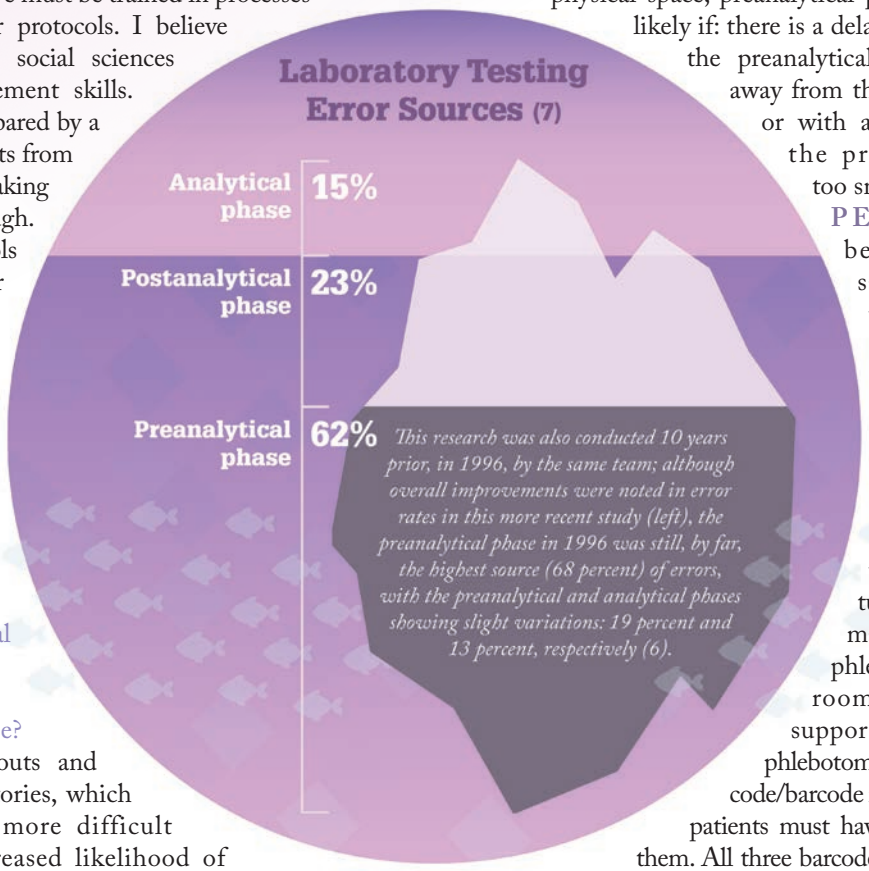
Pinar Eker: Preanalytical actions are outside the walls of the lab; it is always easier to manage what we can see. For many years, laboratory professionals have been too busy in their labs dealing with analytical procedures – we liked playing with numbers, which is always easier than managing people. The preanalytical phase is the part of our work that is mainly governed by "human factors"; as long as the challenge of managing "human factors" exists, so too will our preanalytical challenges. Besides, clinicians generally do not know much about the preanalytical phase and the impact it has on the total test process. They think the analytical phase is the most error prone stage, and this is a really big issue. All health professionals must know more about what the preanalytical risks are and their effects on test results. We must change the way of thinking; this phase is not only the responsibility of lab professional and this means we will need much more training. Patients also need to be trained.

What are common mistakes laboratories make in protocol design that may lead to an increased likelihood of preanalytical error?

Gunn Berit Berge Kristensen: Guidelines and protocols are often too comprehensive and too long. They should focus on important issues and be as short as possible. They stand a better chance of being used if they are simple, logical and perceived as useful.

Luděk Šprongl: Intelligible and clear instructions often don't exist for those who prepare the patient for phlebotomy, and this causes problems. Common errors are also made in the transportation of phlebotomy samples, so it's important that labs are made aware of the optimum time and transport conditions.

PE: As laboratorians we must be trained in processes before we design our protocols. I believe we need some basic social sciences training, like management skills. Protocols must be prepared by a team that has specialists from different disciplines Making protocols is not enough. We have some protocols for every phase in our quality management systems, but we must follow the indicators and analyze the results and replan according to the outcomes of this process.



What are common sources of preanalytical error relating to laboratory setup, equipment setup or use?

EvDL: Designs, layouts and placements of laboratories, which make sample flow more difficult may lead to an increased likelihood of preanalytical phase error.

GBBK: When you get new equipment in the laboratory it is often randomly placed where there is space. One should think LEAN (manufacturing) – a way of thinking and acting for an entire organization. LEAN represents a culture of continuous improvement that depends on the alignment between purpose, process and people. This way of thinking and acting should be used when organizing a laboratory in general, in blood sampling and how external/internal samples are sent to the central laboratory.

LS: The most common mistakes are during the centrifugation. There are different conditions (temperature, time, speed) for different analytes. However, some technicians don't follow the rules because they want to simplify the process or reduce time. I also believe that lack of optimum storage conditions can sometimes pose a problem – time, temperature, repeated thawing.

Mercè Ibarz: Common errors in equipment setup, in my view, mainly include: incorrect sample centrifugation, belief in the value of serum indices without validating the method and lack of consideration of possible interferences. With regards to the physical space, preanalytical phase errors are more likely if: there is a delay in sample transport, the preanalytical area is located far away from the laboratory entrance or with a difficult access, and the preanalytical area is too small.

PE: The distance between the central sampling point and the laboratory is important. Laboratory specialists must be able to control every sampling step easily, whenever they want. The system mustn't allow patients access to their sample tubes; they should be managed only by the phlebotomist. Sampling rooms need more IT supporting solutions. Every phlebotomist must have a special code/barcode reader and recorder, and patients must have codes/barcodes with them. All three barcodes – that of the patient, tube and phlebotomist – must be combined at the point of sampling. Another issue is hemolysis and laboratory analyzers must have a serum indices program with the ability to measure the HIL parameters quantitatively.

From your own experience, what are the most common sources of preanalytical error?

GL: Hemolyzed specimens due to poor collection practice or catheter blood drawing.

KG: Without doubt, hemolysis of the venous blood specimen.

MI: Blood samples, specifically: the sample not being received, hemolyzed samples, clotted samples and insufficient samples. Urine samples not received is also a common error.

MC: In our lab the most commonly seen errors are tests not being initially requested, hemolysis of samples, booking in

errors, and samples not being received.

SC: The collection of blood samples from catheterized patients is a common source of error based on my experience, and this is because nursing staff do not want to subject the patient to a further needle stick. However, there are no recognized standardized processes in order ensure that a sample of the highest quality is collected. Common errors include hemolysis and contaminated samples. These can be avoided by implementing good practices, such as not drawing the blood sample immediately after catheter insertion, and never collecting from an infusing line.

What are the easiest preanalytical phase errors to avoid and how?

GBBK: I think implementing electronic requisitioning of laboratory samples will reduce patient identification and tube labeling errors. Continuous training and education is also very important.

LS: The majority of preanalytical errors are avoidable in my opinion. The best way is to provide clear instruction, regular education, and also to regularly control all parts of the preanalytical phase.

What We've Found So Far

Report Title	*Available Online*	*Key Findings*
Compliance of blood sampling procedures with the CLSI H3-A6 guidelines: An observational study by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for the preanalytical phase (WG-PRE)	http://bit.ly/1Ba3sdu	• Overall compliance with CLSI guidelines is unacceptably low • Issues with a high combination of probability and potential risk of harm include patient identification and test tube labeling • Administrative staff often fail to adhere to patient identification procedures • Physicians often fail to adhere to test tube labeling policy
Survey of national guidelines, education and training on phlebotomy in 28 European countries: an original report by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for the preanalytical phase (WG-PA)	http://bit.ly/1Nk2GRL	• The quality, compliance and critical steps in current phlebotomy need to be assessed • Existing CLSI guidelines should be adapted and used locally in areas that do not have their own guidelines • National EFLM societies must develop basic training programs and continuously educate phlebotomy staff
Preanalytical quality improvement. In pursuit of harmony, on behalf of European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working group for Preanalytical Phase (WG-PRE)	http://bit.ly/1Gn13AF	• The vast majority of laboratory errors occur in the preanalytical phase • Matters of concern include unnecessary testing, prevention of needlestick injuries, harmonization of phlebotomy practices, and quality assurance standards
Preanalytical quality improvement: in quality we trust	http://bit.ly/1w6yV1f	• A summary of the potential for error in laboratory diagnostics • Matters of concern include quality indicators for the preanalytical phase, phlebotomy practices (especially in pediatric samples or for blood gas analysis), urinalysis practices, and auditing the preanalytical phase
Preanalytical quality improvement: from dream to reality	http://bit.ly/1EiB6Sb	• There is an inherent possibility of error in the "brain to brain cycle" of lab testing • Preanalytical errors account for 60 to 70 percent of all such problems • Though most are intercepted, nearly one-fifth of those cases result in inappropriate clinical decisions and unjustifiable increases in cost • Standardization and monitoring of preanalytical variables is vital
Colour coding for blood collection tube closures – a call for harmonisation	http://bit.ly/1KqQTlM	• Blood collection tubes are identified both by label and by closure color • Tube closure colors have not been standardized between manufacturers, so labs risk error when switching brands • To reduce the risk of error and improve patient safety, tube closure and label colors should be harmonized worldwide
Standardization of collection requirements for fasting samples: for the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)	http://bit.ly/1M8Fr9H	• Standardized protocols for patient preparation for laboratory testing are currently lacking • Great heterogeneity exists in the definitions of "fasting" currently being used among healthcare workers and in the literature – and different types of fasting result in metabolic and hormonal differences in blood samples • Patient preparation for fasting tests must be standardized to avoid variation in results

MC: The easiest errors to avoid are as follows: sample contamination by following the correct order of draw; sample identification errors by education and automation – using machines that automatically provide the correct tubes and label them is a great help; booking in errors by increasing education, increasing staff numbers and/or automation; hemolysis can be decreased by using dedicated trained phlebotomists.

Overall the key steps to improve the preanalytical phase are standardization, education and automation, all of which require continuous funding. Education alone is not enough unless it is continually monitored. You cannot improve what you do not measure!

What is your top piece of advice for laboratories looking to reduce preanalytical phase error?

GL: Strengthen the education of doctors, nurses and technicians about preanalytical quality, and establish a comprehensive system of quality in the preanalytical phase that entails systematic monitoring of non-conformance.

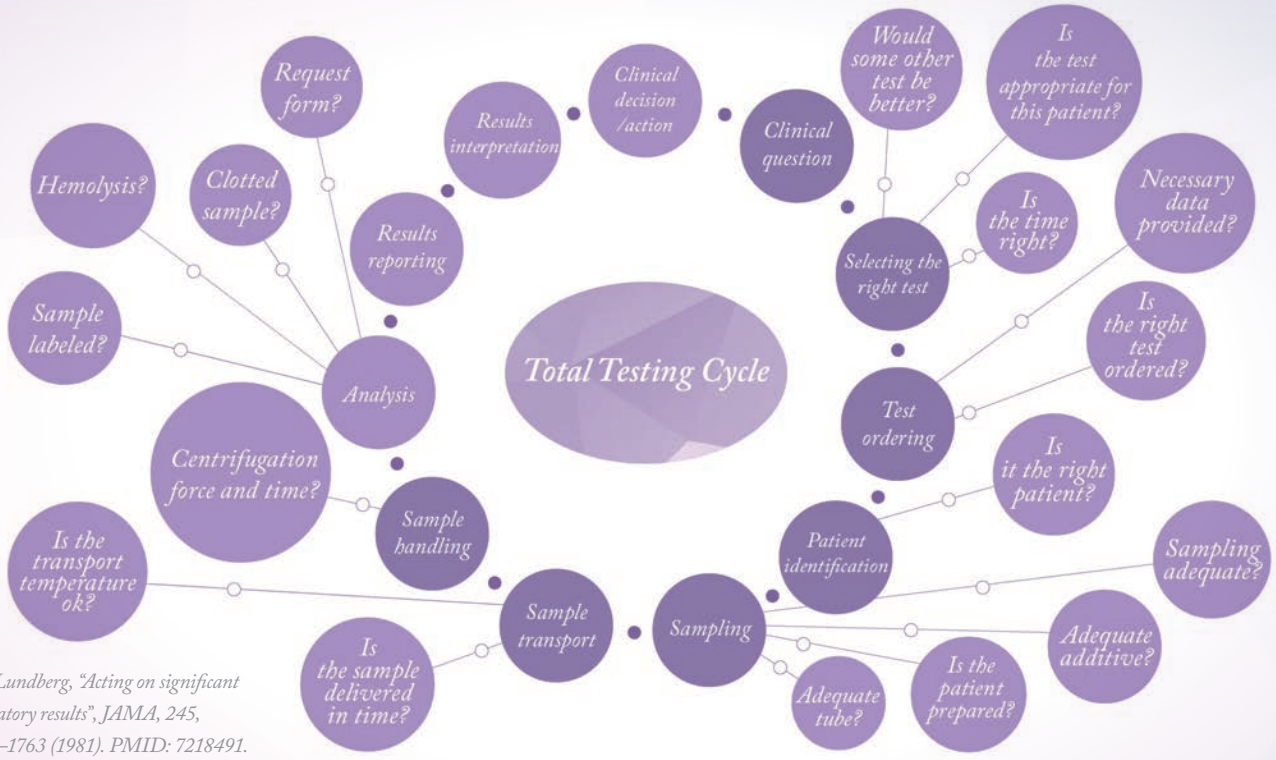
EvDL: Automation. In my experience, automating functions has led to the biggest reduction in preanalytical phase errors.

KG: Repeated local observational studies with error frequency assessment and risk analysis of preanalytical practice errors, combined with direct feedback, discussions and reflection amongst involved personnel, seems to be the most efficient strategy for sustained good preanalytical practices.

MI: I would say the following are needed: continuous training, well-defined processes that are written and accessible in the workplace, clear definition of responsibilities and fluid communication with phlebotomists.

SC: The best piece of advice I can offer is to leave the laboratory and go and observe the processes outside of your laboratory that influence the sample quality. Look at how the patients are identified, how the samples are collected, understand the challenges that the phlebotomist has in collecting a sample from various patients, and how the samples are transported to the laboratory. Conduct systematic reviews of these processes in order to understand the challenges, create partnerships and help educate those that are collecting samples that the laboratory is so reliant on.

"Brain to Brain Cycle" of Lab Testing



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EFLM President Mauro Panteghini Speaks Out

What is your view on the achievements of the WG-PA versus the overall objectives set out by EFLM?

EFLM WG on Preanalytical Phase (WG-PA) is doing an excellent job. At the time when this WG was established (in 2012), there was no other formalized activity by international bodies relating to the preanalytical phase. The objective of the EFLM executive board and the science committee was to increase the level of awareness about the importance of the preanalytical phase among healthcare professionals. Today, this working group is an internationally-recognized driving force in this field, leading the way towards the global harmonization of this very important part of laboratory medicine.

What, in your opinion, are the most important initiatives that the group is involved in?

WG-PA is very active in several specific areas; all of them are equally important. They have set up a series of highly successful biannual conferences on the preanalytical phase, starting from the first one held in Parma (Italy) back in 2011. These conferences get an increasing number of participants globally; the most recent was held in Portugal in March 2015 and was attended by almost 600 participants. Furthermore, the WG has published a number of papers that report the results of their surveys, recommendations, opinions, etc. Finally, this WG has recently initiated an important project on the standardization of the colors of blood tube closures (12). Besides professionals in laboratory medicine, important stakeholders in this project include manufacturers of blood drawing systems. This is a good sign and reflects the appreciation of our IVD partners of the work of this group.

In your view, how much of an issue is preanalytical phase error; what parts of the testing cycle are most prone?

The preanalytical phase has been demonstrated to make the largest contribution to the overall error rate in the total examination process (TEP). Every step of TEP is prone to errors. However, errors cannot have the same effect on patients. Some errors may just cause patients discomfort, some may lead to delays in treatment and some even produce a fatal outcome. To minimize the risk, one needs to know all sources of errors and their consequences. Education and standardization of preanalytical phase steps is the key to success. This is exactly what the EFLM WG-PA is working on.

What, in your opinion, have been some of the key successes as a result of the work of this group? And why would you consider those successes to be so important?

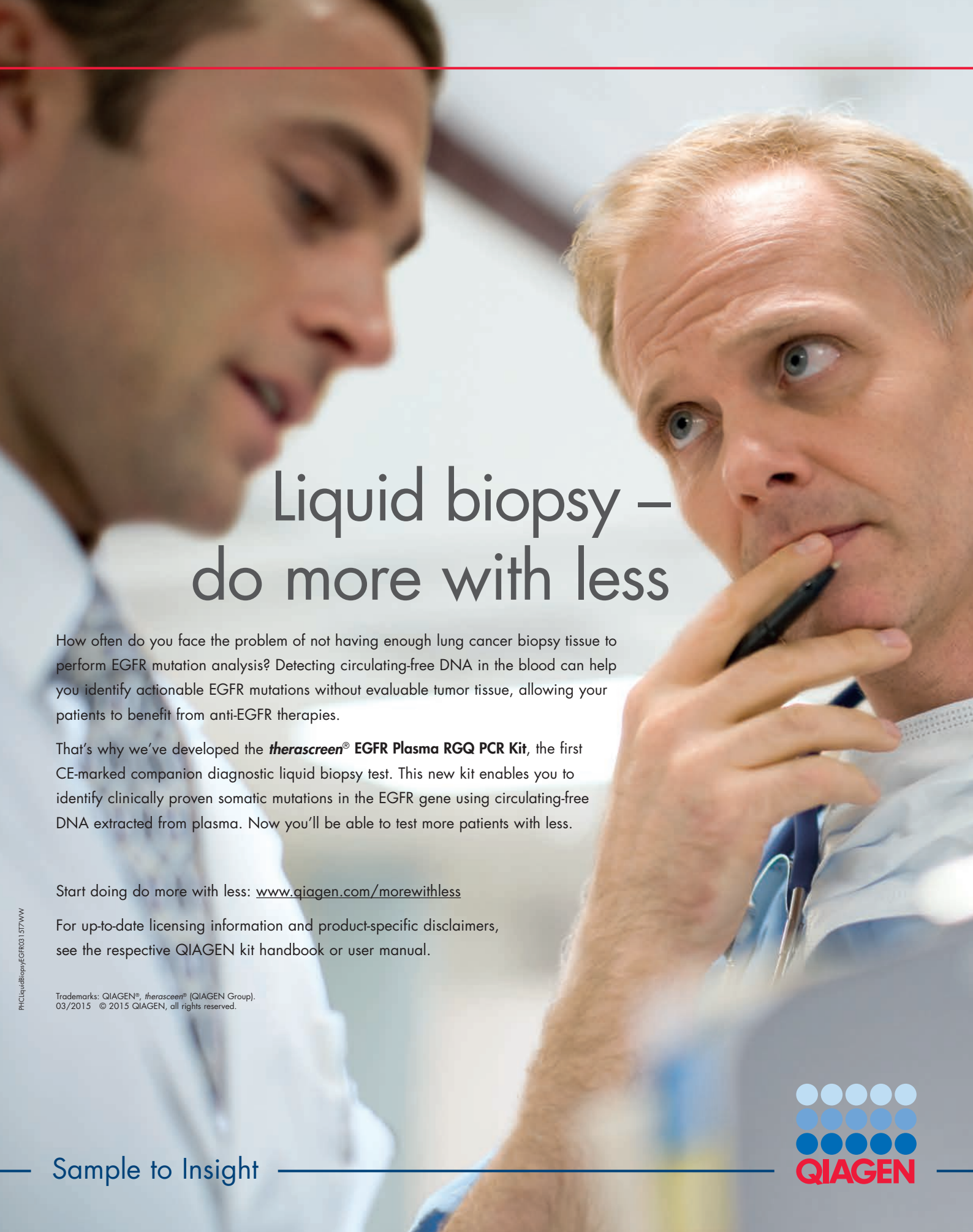
As a result of the group's 'pioneering' work, there is currently much more attention on the preanalytical phase among national societies, both European and international. For instance, during the last couple of years, many national societies dealing with laboratory medicine have established preanalytical working groups and the majority have delegated their representatives to the WG-PA.

During the last conference held in Portugal, EFLM members agreed that harmonization on preanalytical phase is necessary and they all declared a willingness to work with EFLM on this. Consequently, WG-PA will set up further projects aiming to improve harmonization at a European level, for example, on the preparation of guidelines for venous blood sampling. This clearly demonstrates the trust that others place in the WG-PA to carry out this important work and EFLM is very proud of that achievement.

"As a result of the group's 'pioneering' work, there is currently much more attention on the preanalytical phase among national societies."

How do you hope the work of the WG will impact the laboratory medicine community now and in the future?

I am sure that their work will greatly contribute to the overall harmonization of preanalytical phase testing in Europe and, possibly, even beyond. This will inevitably lead to a reduction in the overall error rate in TEP and, consequently, increase patient safety.



Liquid biopsy – do more with less

How often do you face the problem of not having enough lung cancer biopsy tissue to perform EGFR mutation analysis? Detecting circulating-free DNA in the blood can help you identify actionable EGFR mutations without evaluable tumor tissue, allowing your patients to benefit from anti-EGFR therapies.

That's why we've developed the ***therascreen***® **EGFR Plasma RGQ PCR Kit**, the first CE-marked companion diagnostic liquid biopsy test. This new kit enables you to identify clinically proven somatic mutations in the EGFR gene using circulating-free DNA extracted from plasma. Now you'll be able to test more patients with less.

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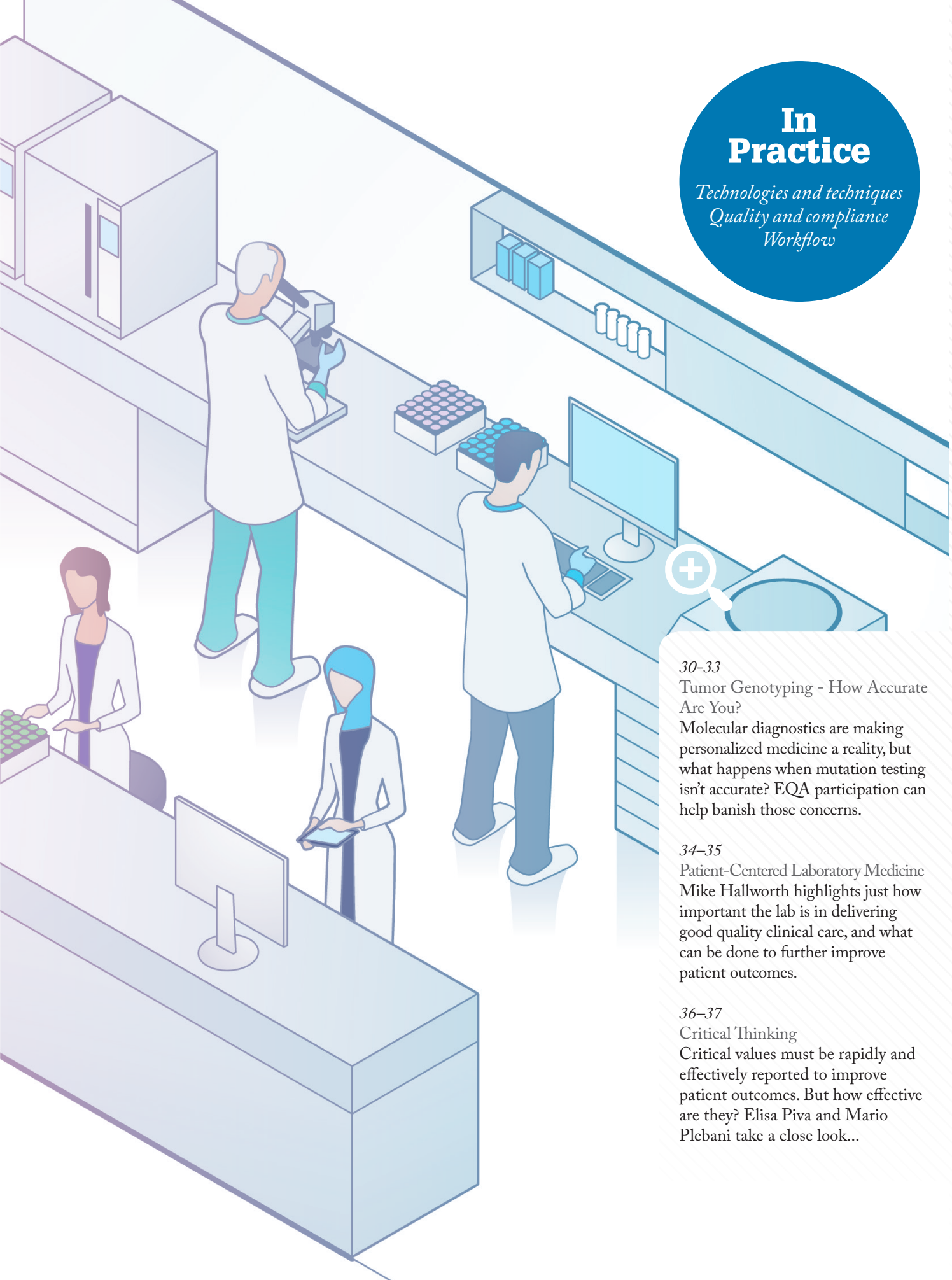
Sample to Insight

QIAGEN



In Practice

Technologies and techniques
Quality and compliance
Workflow

Tumor Genotyping – How Accurate Are You?

In the era of personalized medicine, the use of reference materials is more important now than ever.

By Joe Whittaker

Personalized medicine, with the aid of molecular diagnostics, is providing the exciting possibility of cost-effective tailored therapies, based on an individual patient's genetic code. This is particularly true in the case of cancer where a single nucleotide polymorphism (SNP) out of a three billion-base genome can be the difference between having, and not having, an actionable drug therapy. However, identifying this one-in-a-billion can be tricky; with the multiple steps of a diagnostic workflow (Figure 1), any variability that creeps into each step is further compounded downstream potentially leading to incorrect diagnoses. The need for consistent accuracy in order to provide a precise diagnosis and effective tailored therapy is therefore critical. So what progress is being made?

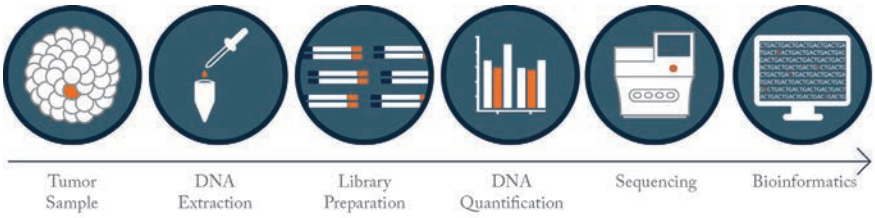


Figure 1. The multiple steps in a molecular workflow (example: NGS).

At a Glance

- *Molecular diagnostics are making personalized medicine a reality, with companion diagnostics supporting progress towards the goal of a precise diagnosis and a tailored therapy*
- *Europe has seen the approval of an EGFR tyrosine kinase inhibitor to have a label indicating the use of cell-free DNA obtained from a blood sample; the first of its kind, but large variances in concordance rates between cfDNA and tumor tissue have been reported*
- *More is clearly needed to ensure accuracy of mutation testing; an incorrect outcome could be potentially life-threatening*
- *Inaccuracies and errors made by diagnostic labs using a wide range of methodologies can be reduced though, using reference materials, and annual participation in EQA should be seen as the norm*

Companion diagnostic developments

Companion diagnostics are certainly making good headway towards achieving the ultimate goal. For example, the most recent collaboration between AstraZeneca and Qiagen provides the first companion diagnostic approach to guide the use of cell-free DNA (cfDNA) in the treatment of patients with advanced non-small cell lung cancer (NSCLC). The therapy, Iressa (gefitinib), is the first epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor to have a European label indicating the use of cfDNA obtained from a blood sample.

However, the clinical feasibility of using cfDNA to detect *EGFR* mutations was assessed in a recent Phase III trial of a Japanese subset of patients (1). The trial found that the proportion of patients identified with mutant *EGFR* was lower when assessed in cfDNA (23.7 percent) compared with tumor tissue (61.5 percent). A high rate of false negatives (56.9 percent) was also observed. The large variance in concordance rates for mutation results between cfDNA and tumor tissue are shown in Figure 2.

Although companion diagnostic technologies undergo thorough regulatory review before being released to the market, there is still a need to maintain clinical vigilance, particularly where limitations are identified within a workflow approach, sampling method or limit of detection. As with any clinical protocol, sample handling will require clinical vigilance through sound quality assurance and control methodologies, including routine validation activities.

Outside of cfDNA, the need for accuracy is shown in External Quality Assessment (EQA) schemes; for example, the worldwide EQA proficiency scheme (2014) reports that of laboratories tested, only 72 percent correctly identified *EGFR* mutations in patient samples (2).

While substantial advances continue to be made, it's clear that more is needed, and one technology that has seen an explosion in recent years is single-molecule sequencing (Figure 3). The new generation of these technologies (third-generation sequencing) is now emerging, with the potential for even higher throughput, longer reads and shorter time to result, which will lead eventually to a lower overall cost. However, as with any new technology, new challenges arise along with new workflow steps and therefore new sources of variability. Similarly, with all the data now being provided by

next-generation sequencing (NGS) technologies in greater quantities, volume and speed, how is it actually being used?

How is Big Data being used?

According to Boehringer Ingelheim's recent 'Let's Test Campaign' (4) – not enough. The survey, conducted between December 2014 and January 2015, found that, although 81 percent of newly diagnosed NSCLC patients received testing for *EGFR* mutations, only 50 percent of oncologists reported their treatment decision was effected by a patient's *EGFR* mutation subtype. It further found that they started one in four patients on first-line treatment before they had even received results on mutation status.

Cited reasons state lack of tumor histology and insufficient tumor samples. The lack of tissue samples has been a longstanding problem, particularly in hard-to-find lung cancers, hence the development of alternatives such as cfDNA tests. But lack of material for both clinical testing and validation and set up of diagnostic tests has always been an issue.

So what happens when therapies go wrong? Consider colorectal cancer as an example: *EGFR* targeting therapies have been developed for the treatment of patients with metastatic colorectal cancer to great effect. However, mutations within the *KRAS* gene are found in 30–40 percent of colorectal tumors (5) and people who have this particular mutation show a poor response to the popular therapies of cetuximab and panitumumab (6), with patients even experiencing worsening side-effects in some cases.

To put this into perspective; there are over 1.4 million people worldwide each year who are diagnosed with colorectal cancer (7). Combine this with the conservative number that 30 percent of these patients have a mutated *KRAS* gene, you can estimate that at a cost of $18,882 per treatment, it could potentially be costing payers over $8 billion worldwide per year because of incorrect tumor genotyping results in molecular diagnostics.

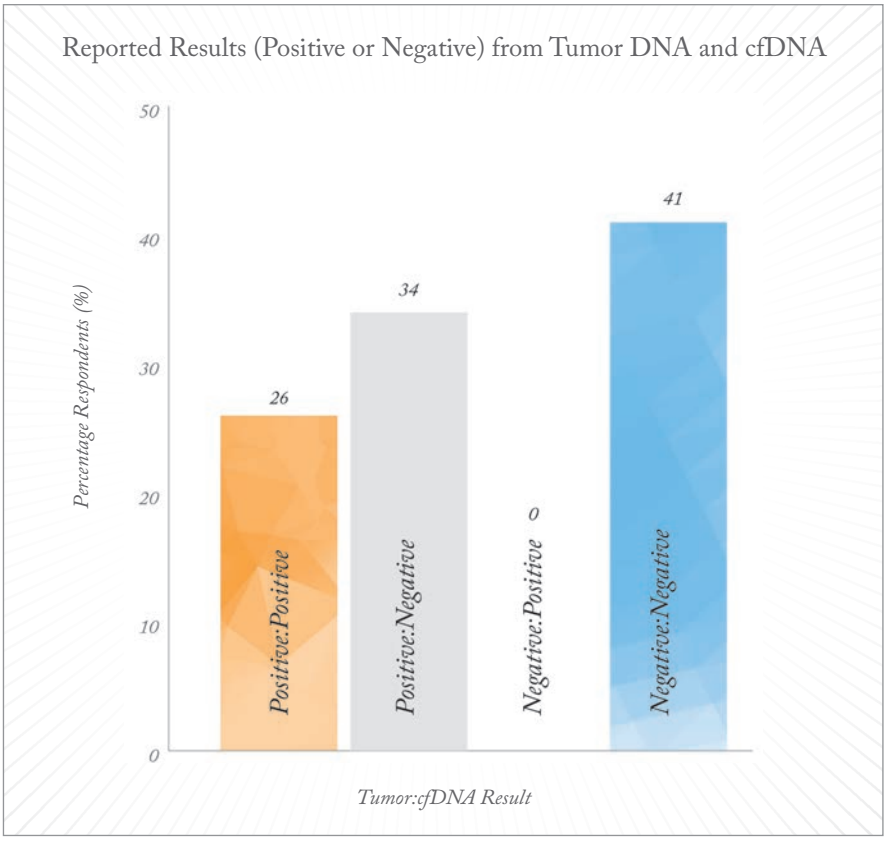


Figure 2. Adapted from Goto et al. (3) showing the variability between *EGFR* detection of tumor DNA and cfDNA. A 34% "mismatch" can be seen between mutation detection in tumor DNA and cfDNA (Positive:Negative, column 2).

As a result, since 2008, the use of *EGFR*-targeting antibodies in metastatic colorectal cancer has been restricted to patients with wild-type *KRAS* tumors by the European Medicines Agency, based on data showing a lack of efficacy and potential harm in patients with mutant *KRAS* tumors (Figure 5). To add complexity, *NRAS* has also been presented to be involved in the prognosis of inefficient treatment at ASCO (2013) (8), but that is another story. In any case, the variability between laboratories and methods means that some patients still receive medication when they do not need it, and more importantly, others do not receive potentially life-saving treatment when they do.

Aiming for accuracy

There are ways to increase and ensure the accuracy of a laboratories' tumor genotyping, including the use of reference material, EQAs and ISO standards. Simon Patton, Director of the European Molecular Quality Network (EMQN), believes that EQA proficiency testing schemes may be the answer. His organization is responsible for coordinating many EQA schemes including the most recent *EGFR* EQA scheme (2), which included three rounds. "EMQN has been organizing EQA schemes for rare single gene disorders for eighteen years. Because of this experience, we were approached by a number of clinical oncologists working in Europe to provide EQA for lung cancer testing," he says.

"We had evidence from a pilot scheme that the quality of lung cancer testing and reporting of the results to clinicians was in need of improvement. This area of diagnostics has evolved very fast, and

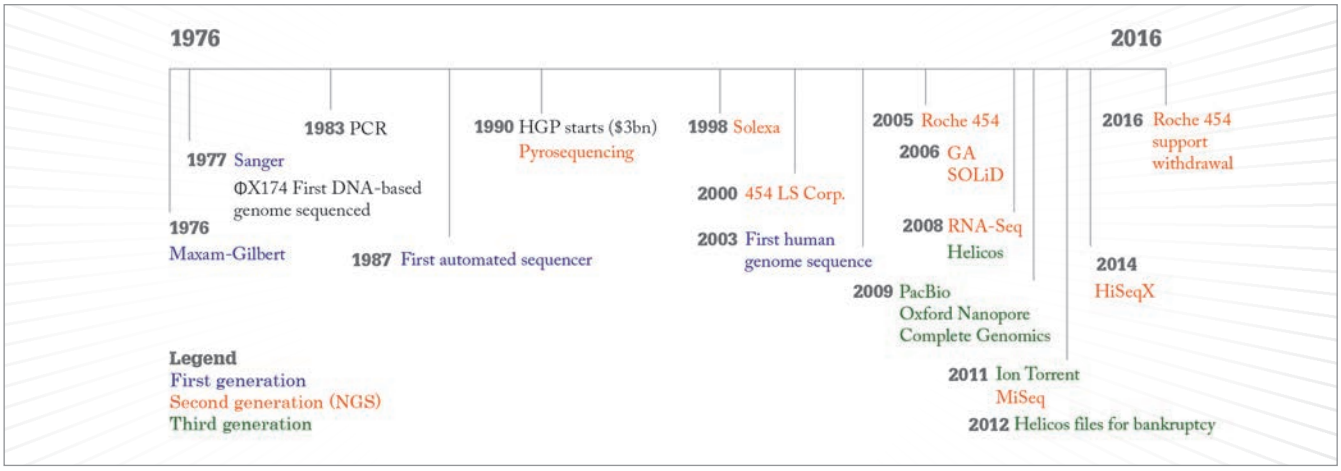


Figure 3. The rapid evolution of sequencing.

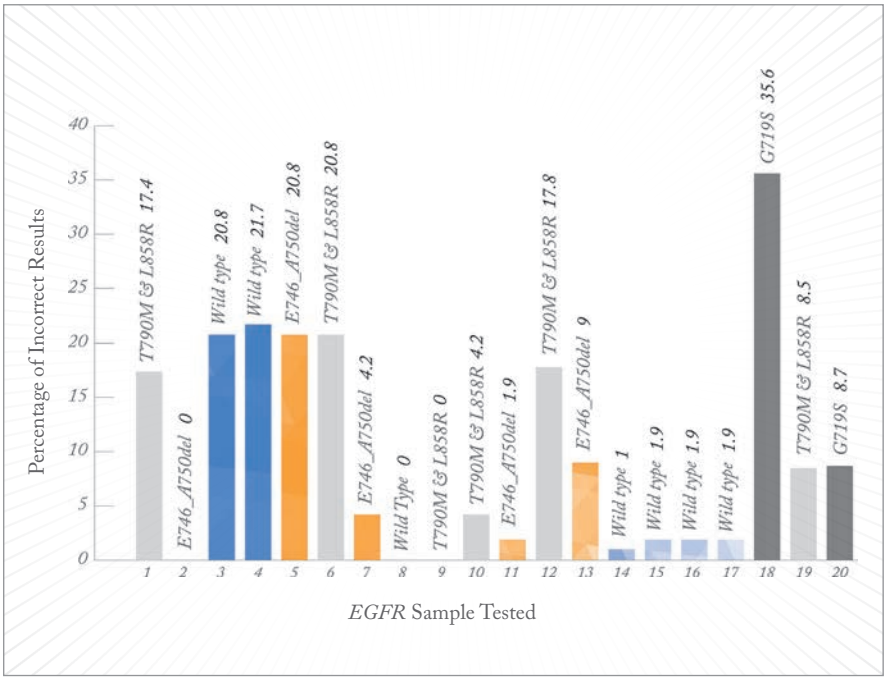


Figure 4. EQA results. Multiple samples were sequenced and reported according to the mutation status.

it's been driven by pharma's need to get their drugs into the clinical setting. This need has mainly been met by different diagnostic laboratories, predominantly genetics and pathology, which have been encouraged to set up testing for tumor markers, and the manufacturers have responded by developing new diagnostic kits and end-to-end diagnostic solutions. However working with compromised FFPE samples is challenging and EQA schemes are needed to ensure that the quality of testing delivers the right result, for the right patient at the right time," Patton adds.

The EQA scheme

A steering group of five individuals was formed who planned, designed and assessed the results of the pilot EQA scheme involved in NSCLC testing. It was coordinated and administered by the EMQN and three rounds were organized within a period of 18 months. The first was restricted to a maximum of 30 laboratories to establish proof-of-principle and validate the materials. A subsequent second round was organized with no restriction on participation. Laboratories that failed the second round were provided with another set of samples in a restricted third round. The steering group evaluated the results according to a predefined scoring system, which assigned two points to correct genotype and zero points to false-positive or -negative results (Figure 4).

Once the data were analyzed, false-negative results were found to account for 85 percent of all the genotype errors made in the scheme, which could be a result of the low sensitivity of the method used for mutational analysis. For example, the expected minimum level of sensitivity is 15 percent for Sanger sequencing, and 5.43 percent for the *p.(G719S)* mutation as defined in version 1 of the Qiagen Therascreen kit packaging insert. Genotyping *EGFR G719S* in particular showed a 35.6 percent error.

PCR/sequencing was the most common method used in the scheme for scanning to detect point mutations. The major disadvantage of sequencing though is that it is not very sensitive (9), especially in samples with low tumor cell content. Real-time allele-specific tests are much more sensitive and specific, but only test for a subset of common mutations.

Following the study, Patton commented, "There is still considerable room for improvement in the quality of genotyping of tumor genes and the diagnostic error rate [an incorrect genotype that leads to a misdiagnosis] remains stubbornly high at 3.65

Methodological combinations	*Count*
Pyrosequencing + fragment length analysis	3
Pyrosequencing + high-resolution melting	1
Pyrosequencing + high-resolution melting + fragment length analysis + SNaPshot kit	1
Pyrosequencing + NextGen sequencing	1
Pyrosequencing + Therascreen kit	1
Sequencing +AmoyDx kit	1
Sequencing + denaturing capillary electrophoresis	1
Sequencing + fragment length analysis	4
Sequencing + fragment length analysis + high-resolution melt analysis + restriction fragment legnth polymophism	1
Sequencing + fragment legnth analysis + high-resolution mely analysis + SNaPshot	1
Sequencing + fragment length analysis + restriction fragment length polymorphism	1
Sequencing + fragment length analysis + Taqman	1
Sequencing + high-resolution melting	4
Sequencing + MassArray analysis	1
Sequencing + pyrosequencing	1
Sequencing + pyrosequencing + high-resolution melting	2
Sequencing + restriction fragment length polymorphism	1
Sequencing + single-strand conformational analysis	1
Sequencing + Taqman + PNA clamp	1
Sequencing + Therascreen kit	5
Sequencing + Therascreen kit + CAST PCR	1
SNaPshot + single-strand conformational analysis	1
Therascreen kit + fragment length analysis + SNaPshot kit	1

Figure 5. The broad range of *EGFR* testing methodologies used by labs in round two of the EQA scheme: Only four methods were the same amongst 36 laboratories when identifying the same mutation.

percent (as measured by the EQA). Errors are made by laboratories using a broad range of methodologies (see Figure 5), but we do have evidence that poor validation and/or verification of new tests contributes significantly to this problem. This is especially true when implementing an NGS strategy, or using a 'black box' commercial diagnostic solution."

Not all doom and gloom

Although the inaccuracies and wide range of methodologies are evident in diagnostics, Patton does highlight some of the positives that have come from the EQA scheme: "We are seeing a significant improvement in clinical reporting with far less 'over interpretation' of the genotyping results with respect to treatment decision-making compared with previous EQA schemes. However, there still remains a tendency of participants to overstate the significance of the test result. EMQN has been pushing for standardization of reporting of sequence variants within the testing community by promoting best practice and the use of the Human Genome Variation Society (HGVS) mutation nomenclature guidelines. Both of these activities play an important role in improving the quality of the test result."

When asked about his overall recommendations and future plans for the scheme, Patton felt that although the improvement of the quality of testing is happening, there's still more to do: "Annual participation in EQA should be seen as the norm for all laboratories offering a diagnostic test if they are serious about ensuring that they offer a high quality testing service."

When applied correctly, personalized medicine can help identify not only patients who are most likely to benefit from a particular therapeutic product, but also those likely to be at increased risk of serious side-effects as a result of treatment. Furthermore, accurate diagnostics can also monitor a response to treatment with a particular therapeutic product, to achieve improved safety. In order to ensure the accuracy and achieve confidence of diagnostic testing/tumor genotyping, a myriad of options are available of which sustained evaluation and validation through reference materials, such as the EQA, are essential.

Joe Whittaker is diagnostics marketing manager at Horizon Discovery Group, Cambridge, UK.

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Patient-Centered Laboratory Medicine

Helping laboratories to understand their valuable role in the overall process of clinical care and the opportunities to directly impact, and improve, patient outcomes.

By Mike Hallworth

Laboratory testing is the single highest volume medical activity, and is essential for fast, accurate diagnosis of a vast array of clinical conditions. It is recognized as fundamental to clinically cost-effective delivery of healthcare, since it is so often the principal basis for costly downstream care – admission to hospital or high-cost investigative procedures, such as biopsy or complex imaging. Laboratory medicine also has a massive impact upstream of diagnosis, playing a key role in screening and risk assessment; areas which are becoming increasingly important with the recognition that early diagnosis and intervention reduce overall healthcare costs for a wide range of common diseases.

At a Glance

- *Laboratory medicine accounts for the highest volume of tasks conducted in a healthcare setting*
- *Evidence to support the contribution of the laboratory to the overall clinical process has, however, been difficult to obtain*
- *A task force set up by IFCC has recently published evidence supporting the impact of lab medicine in healthcare and highlighting gaps in understanding and deficiencies in use of lab tests*
- *A poster has been developed that provides a schematic of the opportunities for clinical laboratories to have a direct impact on clinical care and improve patient outcomes*

However, systematic evidence of the contribution of laboratory medicine to the clinical process has been difficult to obtain in the past – understandable, given the range of factors involved in reaching a diagnosis or planning treatment for an individual. The need for more specific and evidence-based measures of the added value that laboratory medicine brings has been recognized for many years, and the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) task force on the impact of laboratory medicine on clinical management and outcomes was established in 2012 to evaluate the available evidence supporting the impact of laboratory medicine in healthcare, and to develop new and prospective studies to demonstrate the contribution made by laboratory medicine to improving outcomes (1).

The task force has now published its report (2), which summarizes the existing evidence and indicates the gaps in our understanding. It also identifies deficiencies in the ways in which laboratory tests are used, suggests some potential solutions and offers a vision of a future in which laboratory medicine is used optimally to support patient care. As part of that vision, we have collaborated with The Pathologist to produce the infographic poster (see page 35 to find out how to get your copy). This presents in schematic form the multiple opportunities for clinical laboratories to have a direct impact on clinical care and improve clinical outcomes. The issues are also explored in a special issue of the eJIFCC published earlier this year (3).

Rapid, accurate diagnosis of the patient's condition is essential to obtaining the presenting condition, and there has been much emphasis in recent years on reducing misdiagnosis, underdiagnosis and overdiagnosis. When diagnostic error arises from laboratory testing, the pre- and post-analytical phases are much more vulnerable to error than the actual analysis, where laboratory workers have traditionally focused their error-reduction strategies. Epner et al. (4) have classified the laboratory-related causes of diagnostic error as:

- ordering the wrong test
- not ordering the right test
- misapplying the test result due to misinterpretation or failure of synthesis
- missing the test result – not getting it to the right place at the right time
- test result inaccurate.

"It represents a considerable challenge, but the rewards are immense"

The last of these causes is the least frequent and has least impact on patient outcome (5). The poster sets out the importance of influencing test requesting and ensuring correct interpretation and follow-up of critical or significant results, while still ensuring high analytical quality and reducing misleading results caused by poor specimen collection or handling.

Laboratories also have a vital role in

producing the evidence base that informs proper test utilization, which means ensuring that evaluations of biomarkers focus not just on analytical performance or diagnostic efficacy but on clinical effectiveness – a measurable impact on defined outcomes brought about by using the test (6). It is also crucial that these studies are continued as biomarkers come into clinical use, to ensure that the expected benefits are delivered in practice. This means effective participation in clinical audits – the equivalent of post-marketing surveillance in the drug field.

Laboratory doctors and scientists of the future must get out of their laboratories and become involved in the whole spectrum of clinical activity, including:

- producing guidelines for investigation
- advising clinicians on the best strategy for individual clinical presentations and the further tests needed to confirm a diagnosis
- ensuring that results are not misinterpreted or missed
- participating in audit of the effectiveness of testing strategies and using the resources of the service (human, technical and financial) effectively to do the right test on the right person in the right place at the right time.

It represents a considerable challenge, but the rewards are immense: better patient care, lower healthcare costs, improved job satisfaction for laboratory workers and enhanced ability to recruit and retain good scientists and pathologists. We hope that the new poster will help laboratory workers to better understand their role in the process, and to demonstrate the value of laboratory medicine to clinical staff, administrators, policymakers and patients.

Mike Hallworth is Chair, IFCC task force on the impact of laboratory medicine on clinical management and outcomes.

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To obtain a free copy of the poster discussed in this article, please visit the IFCC booth at Euromedlab Paris 2015, 21-25 June, or email tracey.nicholls@texerepublishing.com

Critical Thinking

Why it is vital to define critical values and establish standard procedures for reporting them.

By Elisa Piva and Mario Plebani

The classical definition of a laboratory critical value (CV) is any result for which an immediate, life-saving action must be both available and necessary. Clearly, failure to communicate such a result carries a high risk of adverse events including the death of a patient. It is widely recognized that the harmonization of this reporting is key to providing the best possible patient care – and yet there is no standardized method of reporting CVs to ensure maximum effectiveness. In an attempt to remedy this, we and our colleagues recently audited six months' worth of CVs to evaluate the effectiveness of reporting in relation to clinical decision-making and patient outcomes.

In our study (1), we investigated 200 consecutive inpatient CVs reported by the Department of Laboratory Medicine at the University-Hospital of Padua, Italy. In the same six-month period, we also audited 105 general practitioners (GPs) whose patients were referred to the Department of Laboratory Medicine and reported critical blood clotting rates or potassium levels. We asked doctors – clinicians and residents for inpatients; GPs for outpatients – what actions they undertook after being notified of their patients' CVs, using a standard set of questions that received a 100 percent response rate. Clinicians also gave us additional information, including their patients' clinical status, rates of expectation and triggering events for CVs, how they were notified of CVs, and whether or not they agreed with the values delimiting CVs.

As expected, CV notifications – which were unexpected findings in over 40 percent of cases – resulted in treatment changes for about 90 percent of patients in medical wards and 98 percent of those in surgical wards. Clinicians also further evaluated new complications in about 60 and about 70 percent of cases in medical and surgical wards, respectively, took additional patient care steps, and monitored patients' conditions more closely in over 25 percent of cases. Most surgeons were informed of their patients' CVs by information technology (IT) notification, whereas clinicians received IT notifications in 75 percent of cases but were also alerted in other ways (clinical records, text messages, reports from on-call doctors, or calls from the laboratory). Outpatients were grouped into two categories – those whose labs showed critical INR (international normalized ratio), and those with critically high potassium levels. For all patients with critical INR, GPs changed or stopped warfarin dosage; subsequently, 24 percent of patients were given an additional INR check and 5 percent were examined in a hospital setting. Hyperkalemic patients were all treated within four hours of physician notification and nine were admitted to the hospital for further treatment. In all instances, it is clear that the laboratory, and its role in CV reporting, is key to ensuring patient safety – and, as a result, the effectiveness of that process should be put under close scrutiny.

At a Glance

- *A sample of inpatient and outpatient lab critical values (CV) reports were examined to see the effects of CV reporting on doctors' decisions, actions and outcomes*
- *Our laboratory has been successful in using a computerized notification system that improves timeliness and avoids communication errors*
- *To ensure the best patient care, laboratories must clearly define CVs and adhere to standardized reporting procedures and improve patient outcomes*

Spotlight on CV reporting

It seems rather amazing that the concept of CVs, as George Lundberg originally defined it in 1972 (2), is still being used today. His findings indicated that patients with abnormally high or low laboratory values could die, or suffer irreparable physical damage, unless treated immediately. It's been more than 40 years and CV reporting is receiving more focus than ever from both the laboratory scientific community and several regulatory organizations. The body of evidence shows that timely notification of CVs is important for clinicians, and many accreditation agencies agree that CV reporting is one of the most essential tasks for laboratories. Unfortunately there is little information on the relationship between CV notification, doctors' decisions and improved patient outcomes. Our study aimed to determine whether or not CVs are still crucial in decision-making, but it is important to bear in mind that the testing cycle only serves its purpose if clinicians take action.

We performed a survey at our institution to evaluate the effectiveness of our computerized notification system for reporting CVs. Along with gauging the success of CV notification, we recorded the decisions and behaviors of clinicians after notification, and interviewed them about the importance of the results, as well as any medical actions they undertook or modifications they made to diagnostic and therapeutic approaches. We found that CV notification always leads to a change in patient management and outcomes – principally, the use of alternative drugs to address patients' health issues. Clinicians also make other calls, including ordering more lab tests and increasing patient monitoring. And wherever possible, with outpatients – especially those suffering critical hyperkalemia as a result of drugs interfering with potassium

homeostasis – doctors managed them safely in their homes, sparing them from hospitalization.

Advocating for automated notification

For all telephone calls made from laboratories, the literature indicates an average error rate of 3.5 percent (3). Automated communication improves the timeliness of notification and avoids potential errors. The use of IT is therefore of crucial importance in reducing the communication error rate, and improves the likelihood of reaching the on-call doctor – overall, it represents an efficient method of CV notification that supports effective clinical decision making. At our hospital, a computerized notification system has been implemented with the assistance of the IT department. The system was implemented not only because it meets the requirements of our clinicians and of accrediting bodies, but because we believe it has the potential to improve patient safety and provide context-sensitive reporting, something we consider to be of the highest priority.

Auditing patient outcomes has shown us that effective CV reporting is intrinsic to healthcare excellence – so now we need to get the message out. Laboratories should establish reliable value limits, chosen for true "life-threatening" analytes, and distinguish them from abnormal results. Policies should clearly describe the provider's responsibilities, for instance identifying the laboratory personnel in charge of CV notifications and the caregivers responsible for receiving those notifications. All of these measures are aimed at optimizing CV reporting, including the acceptable time interval between identification and notification of CVs – a gap that, in our laboratory, is now no more than 40 minutes. Finally, the notification, follow-up and documentation of CVs should all have quality indicators that can be regularly checked to ensure the best possible performance for our patients.

What's next?

In the future, we plan to conduct studies on the appropriateness of critical cutoff values and design more ways of harmonizing laboratory practices. There's a lot of work still to be done to make CV reporting more reliable – we need separate CV lists for neonatal, pediatric and adult care; we need to compare existing policies worldwide to promote cross-border changes and improvements; and we need to develop standard procedures for notifying treating physicians of their patients' CVs. To that end, we believe our main goals should be to harmonize CVs and the related procedures and practices among laboratories at an International level, as patient safety plays a key role in the mission of laboratory testing.

If pathologists and other laboratory professionals can rely on clear, universal CV definitions and notification procedures, we can all improve outcomes for our most vulnerable patient populations.

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Top five steps for CV reporting

1. Define CVs, highlighting the difference between critical values, critical tests and abnormal test results
2. Identify thresholds using reference evidence sources; cutoff values should reflect true life-threatening situations, according to Lunderberg's definition
3. Establish well written CV notification procedures, including:
   - data validation, avoiding the interference of potential preanalytical errors using automation systems
   - statement of the acceptable length of reporting, keeping in mind that the timeframe for reporting CVs should guarantee that the responsible physician is notified promptly, so that treatment can be started
   - communication tools, according to International Accreditation Standards
   - the identity of who notifies and personnel responsible for receiving results, keeping in mind that the physician is the individual who can really change patient management, while the person who notifies a CV needs to have sufficient clinical judgment to understand whether or not a true medical emergency exists
4. Track any phase of the CV notification
5. Establish procedures to evaluate and monitor the CV notification process and the outcomes.

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Infectious Disease Detective

A blood test to quickly decide if a viral or bacterial infection is the culprit causing disease could aid the quest to cut down on antibiotic misuse

By Kfir Oved and Eran Eden

The worrying problem of antibiotic resistance is frequently in the news, with global health forecasts over the last few years looking decidedly gloomy. Today, such predictions are becoming reality, and in some cases the situation is even worse than what was foreseen – the World Health Organization has named antimicrobial resistance a major threat to public health. A key factor in the rising resistance rates is antibiotic misuse. However, it must be acknowledged that misuse is not simply due to irresponsible prescribing, and is often driven by the difficulty in ascertaining the cause of the disease – bacteria or virus – especially in the early stages. The symptoms of many illnesses are similar, and lab tests such as C-reactive protein (CRP) or white blood cell count just aren't accurate enough to tell apart bacterial and viral infections.

A lesser known issue is that this diagnostic challenge not only results in overuse of antibiotics, but in underuse as well. It is estimated that roughly one in five cases of bacterial infection are misdiagnosed, with serious consequences for some patients, especially the elderly and those in the developing world. Taking into consideration the limitations of current diagnostic approaches, our team decided to attack this problem from a different angle. We reasoned that over thousands of years, the human immune system has evolved distinctive responses to different pathogens. Pioneering studies have shown the potential of the immune system to effectively tell the difference between different infection types (1). This inspired us to develop a test which harnesses the sensitivity of the immune system to quickly distinguish between viral and bacterial infections, to aid in the diagnosis and treatment of disease, and to tackle the issue of microbial resistance.

At a Glance

- *Antibiotic resistance is a growing problem which has many experts worried, with a variety of possible solutions being put forward*
- *Identifying which patients have a bacterial infection and which have a virus using a blood test could help physicians make informed decisions on treatment*
- *An ELISA-based test that computationally integrates the measurements of several circulating host-proteins showed high accuracy in clinical studies and is being piloted in several hospitals*
- *A point-of-care platform and further clinical studies are planned to expand application to make it more accessible*

Making theory reality

Now we had a theoretical starting point for developing our diagnostic test, we had to work out how to make it a reality, which took over four years. Firstly, we knew we wanted to identify immune response markers that are accessible and measurable even at the point of care. So we focused our search on proteins and other blood biomarkers, as these are easier and faster to measure than nucleic acids, especially in limited resource settings such as small hospitals and outpatient clinics. Secondly, we noted that most biomarker panels in use at that time contained bacterially-induced proteins, so we reasoned that including virally-induced proteins would create a more robust and accurate test.

With our demanding checklist of requirements in hand, we screened 600 human protein candidates. After multiple rounds of biomarker identification and validation, using both experimental and algorithmic approaches and a clinical study with over 1,000 participants, we identified several host-proteins that were differentially expressed. We then selected the most informative subset that included three proteins: TNF-related apoptosis-inducing ligand (TRAIL), interferon gamma-induced protein-10 (IP-10) and CRP. In line with our reasoning, the best subset included virally- and bacterially-induced proteins that display distinctive and complimentary dynamics during acute infections (Figure 1). We created an algorithm that integrates the levels of the three biomarkers to deliver a probabilistic score, which accurately predicts the cause of infection.

Putting it to the test

Our diagnostic test is fairly simple. Levels of the three proteins are measured in the hospital lab using ELISA, and our algorithm computes the likelihood of bacterial infection. We have shown the test to be robust across various pathogens, and our algorithm differentiates bacterial and viral infections with a sensitivity and specificity of over 90 percent (2). Today, the test is being piloted in several sites in Israel and Europe.

Now that the test is employed in working hospital labs, we're seeing that it impacts patient management in several ways. The obvious clinical decision it can influence is antibiotic prescription – we believe our test is improving the ability to accurately identify the cause of infection, empowering physicians to make better informed treatment decisions. But that's not all. Additional clinical decisions, such as requests for imaging data (e.g., chest radiographs, ultrasound and computed tomography)

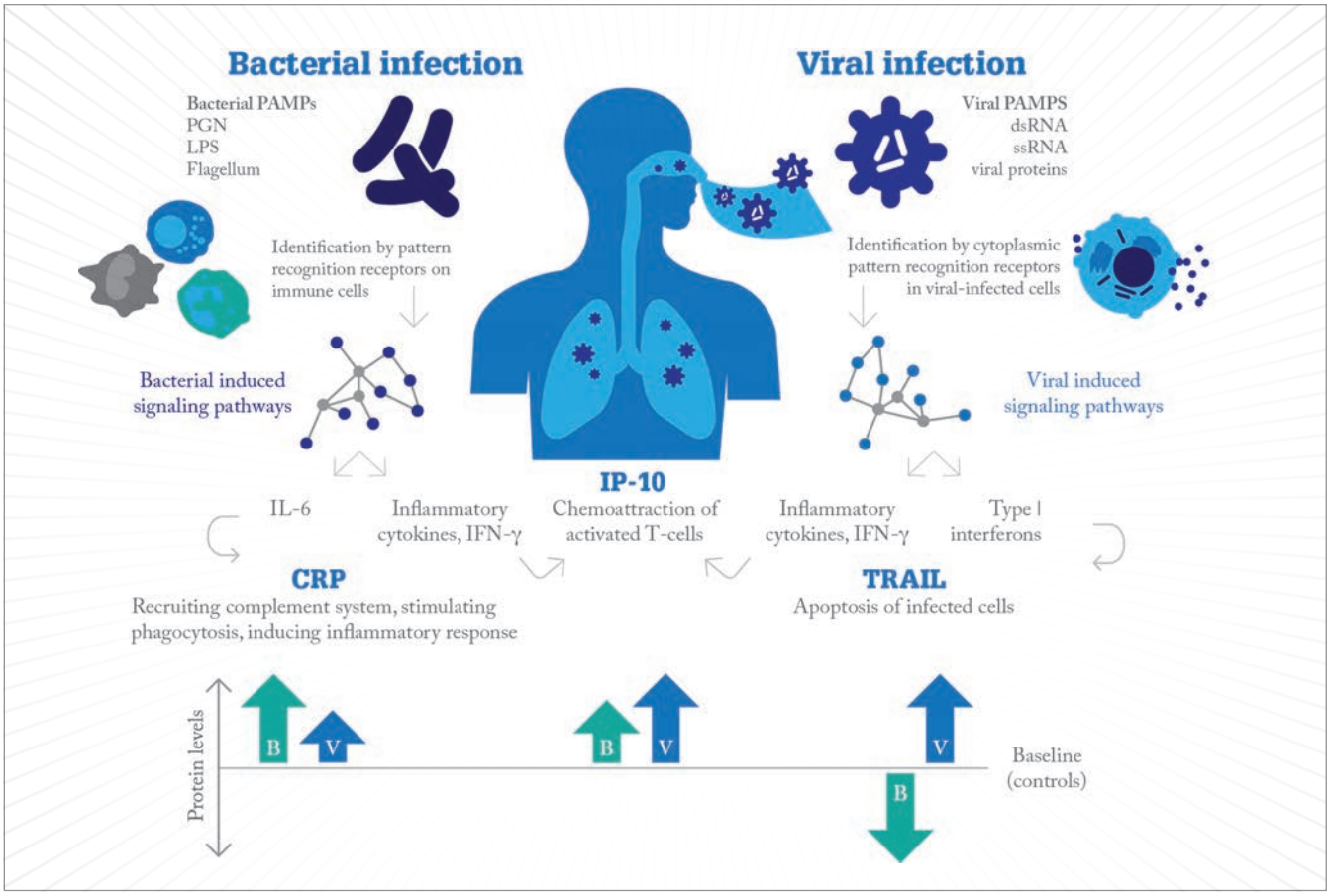

Figure 1. TRAIL, IP-10 and CRP participate in different signaling pathways and exhibit complementary dynamics in response to bacterial (B) and viral (V) infectious. PAMPS, pathogen-associated molecular patterns; PGN, peptidoglycan; LPS, lipopolysaccharide. Used under CC BY license, redrawn from original (2).

and work-ups, (e.g., multiplex PCR and lumbar punctures) are also influenced by the knowledge of the infection source. These procedures can be uncomfortable, and even pose risks to the patient, so the actionable information provided by our test has the potential to improve patient management and reduce healthcare costs beyond the "go/no go" decision about antibiotics.

Test results are currently available within a few hours. However, since we designed the test to require information on three proteins that can be readily quantified in the blood, we have the ability, and indeed are in the process of transforming it into a point-of-care test, which could return results in a matter of minutes. For pathologists and clinicians, we think our test will be regarded as a useful addition to the toolkit when piecing together the puzzle of infectious disease etiology.

Towards a superbugs solution

As our test is now CE marked and approved for clinical use in the EU, Switzerland and Israel, we intend to expand its availability in an early access program later this year. Further clinical studies are now underway across Europe and we are aiming to start clinical studies in North America. We believe our test has the potential to significantly impact antibiotic misuse, both by targeting those who need the drugs, and by preventing improper prescription. To quote the mission statement of the 2014 Longitude Prize; "We cannot outpace microbial evolution. A new broad-spectrum antibiotic, if applied with current methods, would eventually meet new forms of resistance. The overall solution involves a long-term path towards a more intelligent use of antibiotics enabling a future of more effective prevention, targeted treatments and smart clinical decision support systems."

We hope that our diagnostic test, especially the point-of-care version, will play an important role in the solution to the problem of antimicrobial resistance.

Kfir Oved is a co-founder and CTO of MeMed, a biotechnology company based in Israel.

Eran Eden is co-founder and CEO of MeMed, a biotechnology company based in Israel.

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Supercomputer Sequencing

The next step in cancer research may be the use of bioinformatics to analyze large amounts of RNA

By Michael Schubert

Over the past few decades, our understanding of cancer has grown increasingly advanced as we learn more about genetics, genomics and biochemistry. But in order to take advantage of this new knowledge, our methods of analyzing disease must progress rapidly as well. Rolf Skotheim, leader of the Genome Biology Group at Oslo University Hospital (Oslo, Norway), and his colleagues, use supercomputing to process the enormous amounts of raw data they gather.

Together with their collaborators at the University of Oslo, Skotheim's group studies the genetics of cancer. Their focus is on RNA transcription errors, particularly those that are involved in prostate and bowel cancers. "There are two main problems that can occur in transcription – either too much of it, which leads to the production of excessive levels of the given protein, or mistakes in it, which leads to RNA with the wrong composition of base pairs," says Skotheim. "One such mistake can result in fusion genes, hybrid stretches of nucleic acids where sections of two separate genes are erroneously joined. Fusion genes are commonly found in cancer cells, but can also be present in healthy tissue. In our case, we have been able to identify several fusion genes present only in prostate or colorectal cancers – which we may be able to use as biomarkers to determine the presence and severity of disease, or to offer patients future targeted treatment opportunities. Our aim is to identify and characterize those and other critical genes involved in cancer development."

Looking at genes in high volumes

The group's research differs from most types of genetic analysis because they are focused on RNA, and on analyzing it in large amounts. Each set of RNA molecules they analyze consists of about 100 million bases. They sequence millions of short sequences of 100 base pairs each, then run massive statistical analyses in order to localize each one to the correct region of the human genome. "We believe that RNA is the key to the genetic analysis of disease, because it allows us to easily read out the active parts of the genome," Skotheim says. "Additionally, most genes produce several variants of RNA and protein isoforms, and by taking this into account, we can make great strides in identifying cancer-specific molecules, including those caused by transcription from different promoters, alternative RNA splicing, and fusion with other genes – each of which may generate a totally different protein!" The kinds of readouts RNA offers aren't available from DNA, and proteins are even more difficult to examine, because they can't be analyzed as precisely, or in as unbiased a genome-scale manner. That's why Skotheim's research is focused on the analysis of cancer samples by high-throughput, paired-end RNA sequencing – though they gain added value by combining that work with DNA sequencing of the same samples for comparison.

"I think it's important to note that this isn't the kind of work that can be done by a single laboratory," Skotheim says of his group's studies. "We're part of a consortium of researchers that includes surgeons, pathologists, oncologists, geneticists, bioinformaticists and more. It's clear to us that multidisciplinary involvement, even within the research groups, is crucial for good translational genomics." But flesh-and-blood colleagues aren't the only valued collaborators on the project – Skotheim also works with Abel and Colossus, two supercomputers at the University of Oslo. They are Linux clusters and shared resources for research computing, designed to run many concurrent tasks with large datasets and memory requirements. Abel is a powerful cluster – with 650 computers, it runs on a total of 10,000 central processing units. (And you thought your quad-core laptop was powerful!) At the time of its installation, Abel was the 96th most powerful computer system in the world, offering the Genome Biology Group a huge advantage other cancer researchers may not have. "Needless to say, we wouldn't get much done without them – you could spend your entire life crunching numbers and still not map a single nucleotide to its correct location in the genome. At 10,000 times the speed of an ordinary computer, we rely on Abel and Colossus to crunch those numbers for us."

At a Glance

- *To keep up with the amount of genetic information coming in, our analysis techniques must be high-speed and high volume as well*
- *Supercomputers offer realistic timeframes for analyzing large amounts of genomics data*
- *Researchers at Oslo University now use supercomputing to examine RNA transcription errors that cause fusion genes in prostate and colorectal cancers*
- *So far, cancer researchers have found numerous fusion proteins present only in prostate tumors – which may one day result in better diagnostics and targeted treatment*

A new approach to disease research

Supercomputers like the ones at the University of Oslo are revolutionizing cancer research. The approach used by Skotheim's group allows them to parallelize certain tests – like checking the expression level of a gene, or whether or not it is mutated – by analyzing all genes in a single experiment. That saves the researchers from having to make educated guesses as to which genes should be scrutinized in a particular set of disease samples – and means that, ultimately, doctors won't have to guess which genes to test in patients, either.

The Genome Biology Group is particularly interested in fusion genes in cancer, as they are usually specific to the cancer cells and thus particularly useful in diagnostics. Some might even encode a fusion protein that can be targeted therapeutically! But this is where the supercomputers really come into play. If scientists were to test the RNA in a cancer sample for fusions between every possible combination of two genes among the 20,000 genes present, taking into consideration the fact that the genes can be fused anywhere along their sequences, they would have to set up a virtually infinite number of tests to check for all possible fusions in a cancer sample. And then, on top of that, they would ideally test multiple samples! Of course, sequencing all of the RNA in a cancer sample helps researchers know what to look for when they're searching for fusion genes – but doing that typically generates about 20 million short RNA sequences. Then comes the job of understanding where in the genome each of those sequences originates and when they reliably match two separate genes. Ambitious studies like these involving large volumes of sequence data require heavy parallelization and combinatorics – things that the supercomputers can facilitate.

"So far," says Skotheim of his group's work, "we have identified and published new fusion transcripts from both prostate and colorectal cancers. Combining whole-genome and RNA sequencing of colorectal cancers revealed novel fusion transcripts and splice variants of the WNT effector gene *TCF7L2* (1), which acts as a transcription factor in its native form." He adds that they have also identified three other fusion transcripts, *AKAP13-PDE8A*, *COMMD10-AP3S1,* and *CTB-35F21.1-PSD2,* as novel intrachromosomal fusion transcripts – and not only that, but the most highly recurring chimeric transcripts for colorectal cancers (2). Though they don't yet know the functional and clinical significance of these chimeric RNA molecules, they hope to elucidate that in the future, because the main goal is to develop some of these new, cancer-specific transcripts into clinically useful biomarkers or drug targets. "It would be great to see a successful clinical implementation of our work, and to know that our research has contributed to improved medical care for cancer patients around the world."

Supercomputers in the laboratory

But the Genome Biology Group's studies aren't the only research that can benefit from supercomputing – advanced technology and bioinformatics have the potential to support all kinds of benchtop work. Wet lab experimentation is often done in only one or a few samples at a time, testing for anomalies in only a few genes or proteins. This kind of selectivity requires deep insight to design the best experiments, and can only be done at low throughput. With the advent of new genome technologies, together with computational techniques, some hypotheses can be generated on a whole-genome scale. They can even be tested on all 20,000 or so human genes at once! Subsequent experimental validation can then be based on data about all of the genes or transcripts in a particular specimen, rather than just a few pieces of information.

"I'm optimistic about the future of computational genomics in cancer research, because I think it's a key component of modern biology," Skotheim says. "After all, the amount of data generated in genetics is already overwhelming to many geneticists. Every time you run a high-throughput sequencer, you generate terabytes of data. Current trends indicate that the data flow into genetics will just continue to grow – which means that people who are highly skilled in computer technologies are absolutely essential at this point. Not only do we need people who are able to handle the infrastructure for storing and processing large amounts of data, but ideally they'll combine this competence with an understanding of the genome biology of cancers. Only by developing these skills in new researchers can we expect future genomics data to be handled in an insightful manner – and that's what we'll need if we want to continue to turn that data into reliable, innovative and beneficial research for the cancer patients we hope to support."

Rolf Skotheim leads the Genome Biology Group in the Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet. He is also an associate professor in the Department of Informatics, University of Oslo, Norway.

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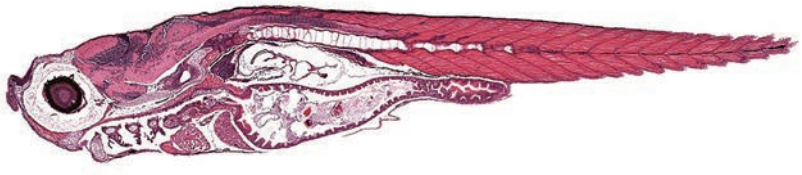
A "Model" Career

Curiosity's road to pathology, zebrafish genetics, genomics and imaging

By Keith Cheng



Zebrafish sagittal section, 14 to 20 days post-fertilization (6.2 mm). Credit: Zebrafish Atlas (zfatlas.psu.edu).

I am often asked how, after choosing pathology as a specialty, I ended up with a research career as topically divergent as mine. The breadth of interests has led to a fascinating – and incredibly fun – exposure to an array of disciplines ranging from genetics and model systems to genomics and imaging. So what resulted in this diversity? I think it's all thanks to the accident of my birth: my genetic nature, my family, our time in history, and being at the right place at the right time. The makeup of my genes has given me a strong sense of curiosity and wonder. None of these factors is unique or surprising on its own, but the way they came together to give me such a breadth of research interests tells a story that I hope is intriguing, fun, and most importantly, useful to my colleagues.

I was introduced to the idea of research by my father, who was a synthetic organic chemist. When I asked what had motivated him to make the drastic jump from navigator in the Chinese navy to a PhD in chemistry, he told me that the brilliant stars of the night sky made him realize how small we are in the universe and inspired him to make a difference. The desire to help others was natural to him, so he thought it would be interesting to synthesize new chemicals that could be useful to humanity. From this story, I learned how profoundly a sense of curiosity and a desire to help humanity could motivate a lifetime of work – and, from that day forward, I found myself inspired to do the same.

But how did that lead me to pathology? I discovered an affinity for the field early on in my career. It was during second-year pathology lectures in medical school that I learned about cancer, and was struck by the stark aggression of malignant cells as they invaded host tissues. That was impactful, but the true moment of decision came during my surgery rotation at Bellevue Hospital, when I discovered an entirely new meaning of cancer. This time, what I noticed was the variation between different cells in the same tumor – and that moment fixed my interest in devoting my career to the cellular aspects of human biology and disease, inspiring more and more research questions to pursue. On my other rotations, I couldn't help being deeply affected by my patients' suffering, which made research seem an especially good way to bring benefit to others as broadly as possible.

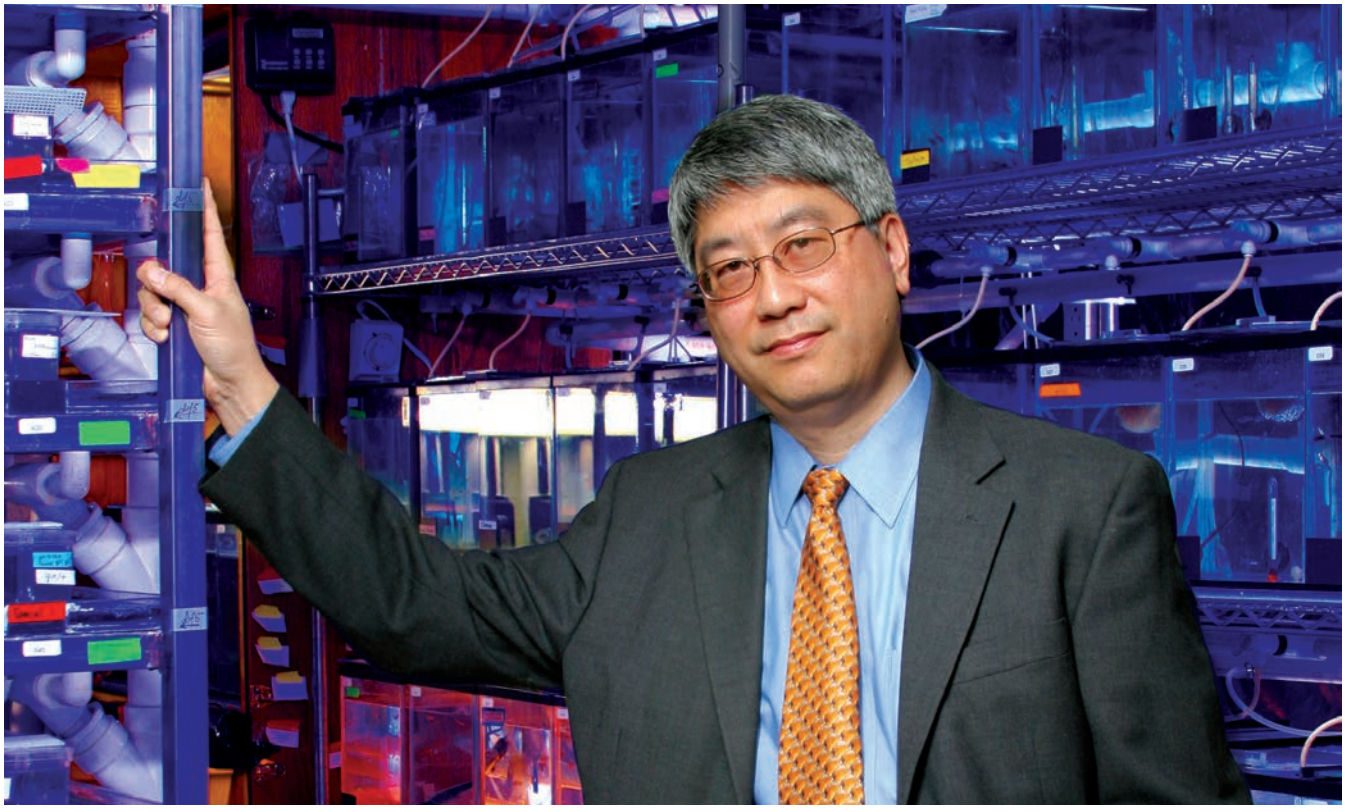
"[Pathology] was a way for me to make research discoveries that could have an impact on many people, rather than just one at a time."

By the year's end, pathology had become an obvious choice – it was a way for me to make research discoveries that could have an impact on many people, rather than just one at a time.

I found magic when I started my residency training in anatomic pathology.

At a Glance

- *More than any other discipline, pathology offers the ability to explore a wide variety of scientific and medical interests*
- *I found my way to the field through a fascination with the inner workings of the cell and their effects on human biology and disease*
- *The path of my career has led me through mutator phenotypes, skin pigmentation, 2D and 3D imaging, and now phenomics*
- *For younger researchers with similar interests, I recommend cultivating a knowledge of biology and computational sciences – as well as curiosity, motivation and a good work ethic*



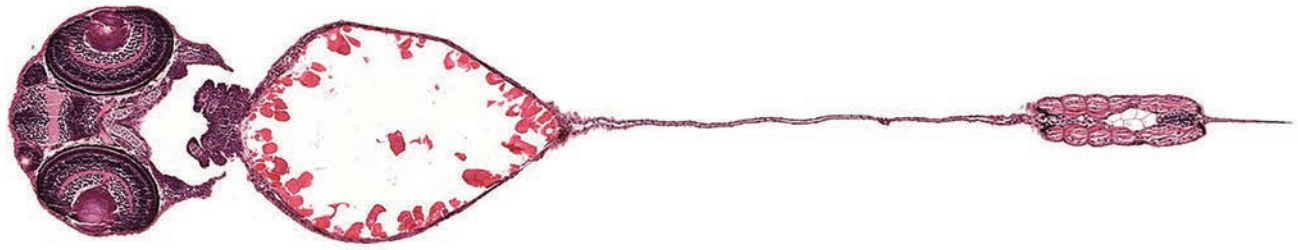
The more I studied disease under the microscope, the more I wanted to understand it from a basic scientific perspective – so, during my residency at the University of Washington in Seattle, I decided to pursue a doctoral degree. A fellowship put together by Larry Loeb allowed me to research in any laboratory I chose. By first rotating through several laboratories, I found that I loved abstract thinking and, in particular, genetics – an affinity for which I thank my mentor, Gerald Smith, a leader in the study of recombination. At last, I saw a path to addressing medical problems on a larger scale than the one-on-one.

The science of skin color

My experiences in medical and graduate school highlighted the importance of the "mutator phenotype," an elevated rate of spontaneous mutation, in the development of cancer. The mutator hypothesis states that this phenotype is necessary to explain the accumulation of mutations that causes human cancer, and during my postdoctoral studies, I opted to test this hypothesis by screening for mutations in a vertebrate model system. Thanks to *golden*, a recessive pigment mutant, I discovered two new interests: the zebrafish as a model system and, eventually, skin pigmentation as a model phenotype.

My goal in setting up my own research laboratory was to explore the possibility of a forward genetic screen in zebrafish. To do that, though, I needed an institution that could understand the boldness of this initiative – one with the temerity to allow me to pursue the idea despite low funding and high aims. I was lucky enough to find such an institution at the Penn State College of Medicine (Hershey, PA, USA), where I began my work in 1992. It took me four years to obtain my first *genomic instability (gin)* mutants – one of which did appear to cause an order-of-magnitude increase in cancer susceptibility among heterozygotes. Along the way, I began to wonder about the cellular basis of the decreased pigmentation in *golden* mutant zebrafish, so I investigated that as well. Curiosity led me to pursue the project outside of my funding, on a shoestring budget deeply dependent on collaboration.

It turned out that the human orthologue of *golden*, *SLC24A5*, contributes to human skin color. In fact, it is a determining contributor to pigmentation differences between people of European and of African descent. We suspect that it's a modulator, rather than an "on-off" switch, which means that the more active this gene is, the greater the number, size and density of melanosomes in the skin



Zebrafish coronal section, three days post-fertilization (3.5 mm). Credit: Zebrafish Atlas (zfatlas.psu.edu).

cells and the darker the resulting skin color. Our discovery attracted a lot of attention – people wanted to know if *SLC24A5* was "the gene for white skin color." They hoped it would explain one of the most contentious issues in the last half-millennium of human civilization. I regularly get asked how people can modify their skin color, or cure vitiligo or other depigmentation diseases. Unfortunately, I can't help with either – but I am interested in why people want so badly to change the color of their skin, and I'm hopeful that a more complete understanding of skin color genetics can demystify race. Perhaps that would allow humanity to focus less of its energy and resources on skin color-based discrimination and more on making the world a better place.

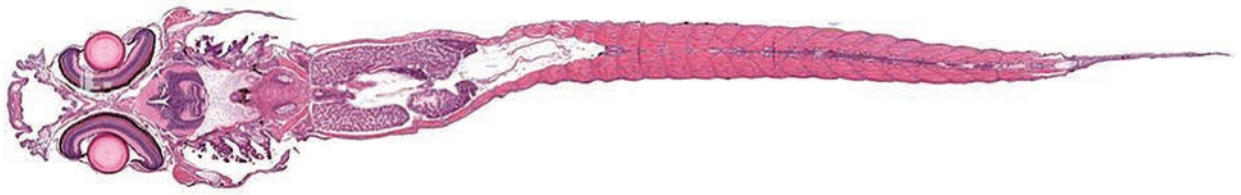
Looking at the whole organism

Skin pigmentation and cancer aren't the only areas where genetically altered zebrafish are useful – they've become a powerful model for studying all manner of vertebrate biology and human disease. Just as physicians must learn normal anatomy and microanatomy so that they can recognize abnormality, the first step in conducting zebrafish experiments is to understand their normal gross and microscopic anatomy. To help, we're generating a web-based 2D histology and 3D atlas of zebrafish microanatomy (1). It's the first full lifespan atlas of its type, and we hope that it will someday provide a scaffold for gene expression and morphological data generated both in our laboratory and globally. We'd even like to expand the project to include comparisons with genetic, reverse genetic, and disease abnormalities; other types of imaging; cross-disciplinary development of new imaging technologies in collaboration with engineers and computer scientists; and integration with websites for other model systems. Most recently, we've begun working with scientists at the University of Chicago and Argonne National Labs to develop a high-throughput way of 3D imaging optically opaque tissues at histological resolutions, so that all cell types can be studied at once. Our plan is to involve pathologists around the world in providing a high-quality atlas that is well-connected between all model systems.

I consider "functional genomics" to be an approach that begins with phenotype and then uses a combination of genetics, genomics, bioinformatics and proteomics to solve biological problems. This kind of work is always exciting to me because I'm drawn to integrative solutions – and I admit I love the gadgets, too. Zebrafish functional genomics has a unique place in the study of genes and phenotype in the context of the whole organism. It's great that we have a vertebrate model with a sequenced genome – one that develops *ex vivo* (meaning that embryos don't need to be excised from the mother), is transparent during its early development (meaning any cell type can be visualized with fluorescence), produces many offspring very quickly (meaning that forward genetic and chemical screens are easy), and offers opportunities to use excellent, well-established reverse genetic tools. These unique features are the reason I have dedicated my career to the development of the zebrafish atlas, new imaging tools, and now a functional genomics core facility to encourage other laboratories to explore the possibilities for themselves. I've worked to foster coordinated cross-genomic activities that take advantage of the strengths of different model organisms, genomics approaches, proteomics, and high-throughput chemical screens, all with the goal of addressing important biological and medical problems.

The phenomics appeal

Lately I've become very interested in the idea of phenomics, which uses high-throughput phenotypic profiling as a tool to understand biology and disease. As pathologists, we are well familiar with the fact that multiple phenotypes are associated with individual genes (pleiotropy) and diseases (syndromes). Phenomics is a highly collaborative endeavor – so I'm trying to contribute from the perspective of an anatomical pathologist with the



Zebrafish coronal section, 21 to 29 days post-fertilization (7.8 mm). Credit: Zebrafish Atlas (zfatlas.psu.edu).

"Our plan is to involve pathologists around the world in providing a high-quality atlas that is well-connected between all model systems."

zebrafish phenome project.

What is this project? To enable morphological phenotyping at cell resolutions, I'm trying to create X-ray-based, micron-scale computed tomography as a 3D imaging tool – something that will require whole-animal examination of a small, vertebrate model. The fact that phenotype can affect any cell type, and commonly affects multiple organ systems simultaneously, drives the need for whole-animal phenotyping. Multiple factors (the work involved in covering large tissue areas, the geometric increases in file sizes, and the need for speed) demand the use of a small model organism. And to increase relevance to humans, we need a vertebrate model – the zebrafish, which is the smallest vertebrate model is well-developed as a genetic system. By using cutting-edge technologies from computer science, engineering, materials science and bioinformatics, we hope to place each genetic and environmental impact in the spatial, temporal and physiological context of the whole organism. The tools we're developing for the zebrafish phenome project will be applicable to many human tissue samples, and in instances where we learn clinically relevant information, may even enter the realm of standard of care. To me, that's a very exciting possibility, and that's what makes phenomics so enticing as an area of study.

Advice for the new generation

So why is it important for me to share my story? A pathology researcher must have a strong sense of curiosity, self-motivation, and work ethic – those are the best predictors of success. You need a passion for science and a commitment to excelling in your field, because these things will encourage you to ask questions in a deeper way, engage in discussion and be open to learning new things. For today's budding pathologists who are interested in going off the beaten path to find a productive career and make a difference in our discipline, I would encourage the study of both genetics and computer science; after all, we're moving more and more toward high-throughput analyses and large-scale studies that involve a lot of data processing. We also need to become skilled at sharing our work – not only are good laboratory and computational skills important, but once a set of successful experiments is complete, effective packaging and communication of the message become critical. Ideally, your enthusiasm for your work can inspire more pathologists to get involved in research!

I've learned from my career that it's exciting to culture a sense of wonder, passion, and curiosity. I've found that the key ingredients for happiness and a rewarding career are to stay motivated not just for yourself, but for the benefit of science and medicine, and to make passion and curiosity an engine for your activities. And if you keep an open mind while you explore, even serendipity can be a tool to answer the questions that arise.

Keith Cheng is director of experimental pathology, director of the Penn State Zebrafish Functional Genomics Core, and a distinguished professor of pathology at the Penn State Hershey College of Medicine, USA.

Reference

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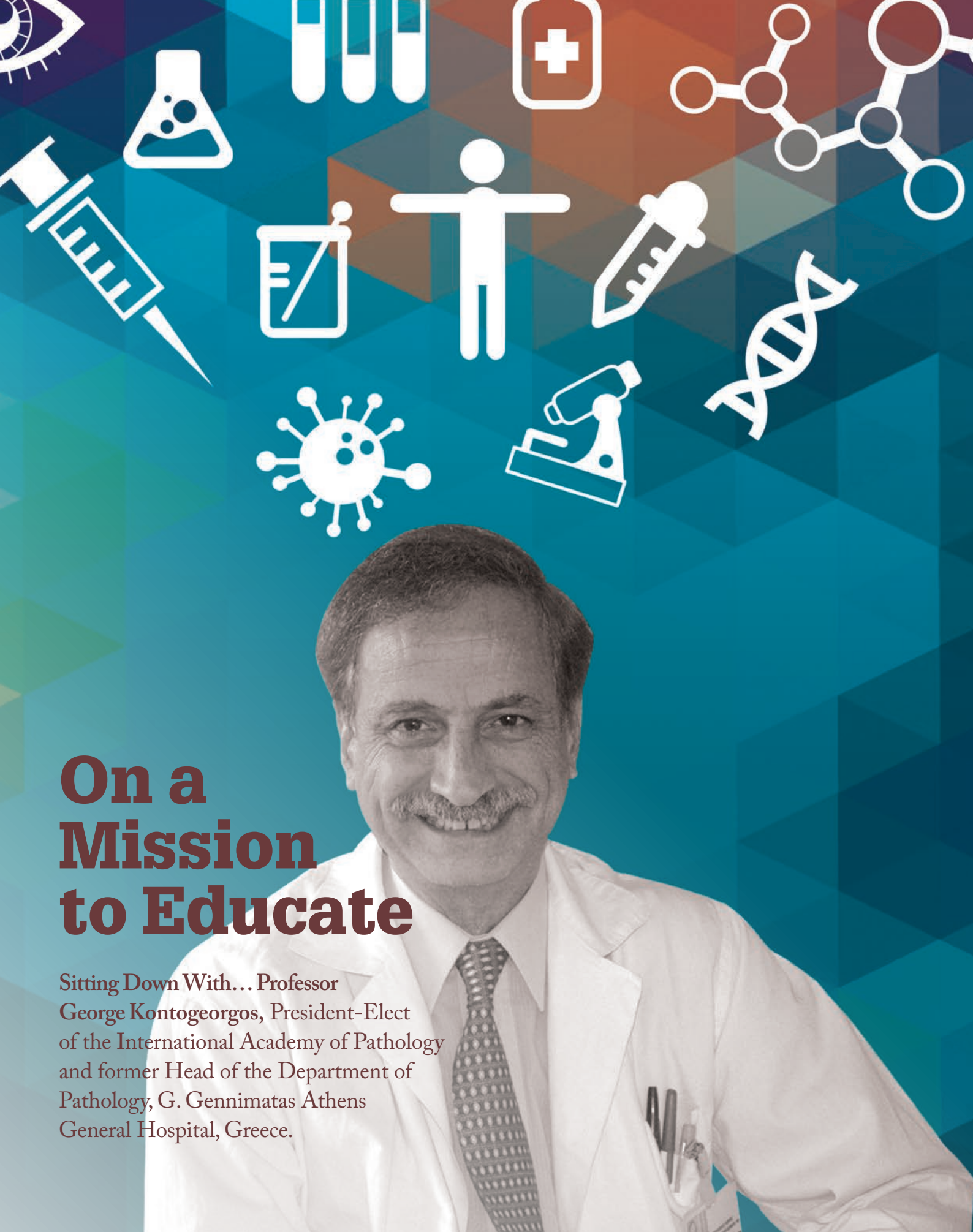

On a Mission to Educate

Sitting Down With… Professor George Kontogeorgos, President-Elect of the International Academy of Pathology and former Head of the Department of Pathology, G. Gennimatas Athens General Hospital, Greece.

Why pathology?

Pathology derives from the combination of the Hellenic words: *"pathos - πάθος"* meaning suffering, and the ending "*-logy*" deriving from *"logia" – λέγειν"* meaning the study of a certain science. It's the basis of medicine, the study of the essential origin and nature of diseases, based on the structural and functional changes produced by them.

I first used a microscope as a first year medical student. During my biology lab work, I had to observe a specimen of *Borrelia Obermaier*, a spirillum species causing relapsing fever, transmitted by bedbugs. I was deeply impressed, entering a wonderful microcosm, and I felt that I was ready to explore it. When I began clinical courses I realized that through pathology you can have a deep insight into the causal factors and explore the mechanisms of disease. Pathology is the ultimate tool to prove the truth, the final diagnosis that, as a rule, cannot be disputed. These thoughts led me, without any hesitation, to start my residency in pathology.

What areas of research have most interested you during your career?

I mostly focused on pituitary tumors, and contributed to book chapters and to the WHO classification of endocrine tumors. My work included pharmacodynamics experiments on octreotide effect of dispersed tissue cultures of somatotroph adenomas and investigation of their somatostatin receptors, in relation to clinical treatment. I would count recognition of double and multiple pituitary adenomas as one of my most important contributions. I would also consider my studies on apoptosis in pituitary adenomas important, because at the time this process was not recognized, so this was pioneering work. I was able to describe in detail the spectrum of apoptotic processes at histologic and electronic microscopic levels. Finally, my systematic investigation of null cell adenomas gave me the chance to prove that the substantial majority of these tumors represent gonadotroph adenomas.

As President Elect of the International Academy of Pathology, what do you see as the key issues facing the field?

The International Academy of Pathology is the largest pathology society with approximately 20,000 members and 52 divisions worldwide. I served for the last 10 years as Vice President for Europe, and I have seen a wide diversity of education levels in pathology among countries. Some countries are underserved in pathology and they deserve support; education needs to be improved upon as much as possible. A crucial way to offer assistance is through ambassadors – these are highly respected pathologists who are also experienced teachers. I had the chance to undertake an educational mission as IAP ambassador in separate visits to Turkey and Georgia. I found this extremely important with high participation, enthusiasm and active discussion. So, I am committed to expanding these activities and aim to recruit internationally recognized tutors who will offer education to countries that need it.

How can the education issue be addressed in the long term?

I want to motivate pathologists from countries, such as Turkey, Georgia and Serbia, to form their own national divisions. In this way, they can seek funds from the Education Committee of the International Academy of Pathology (where I have continuously served for the last six years) and plan their own scientific events. Without doubt, congresses, slide seminars, long and short courses are all very important for education. But we have to ask ourselves: do all pathologists have the opportunity to attend if they are to pay their own travel and accommodation expenses? How do we help people from disadvantaged countries? Certainly, the Education Committee helps young pathologists to participate in the international congresses by providing a number of bursaries. But I think we can do better. I believe it is time for an educational revolution, to give power to all and make education possible everywhere. We are now living in the Internet era, with a tremendous number of applications and facilities available to us. We must open more education channels and take advantage of the benefits this offers.

Telepathology, including digital images, virtual microscopy, teleconferences and real-time broadcasting of scientific sessions would help pathologists from all countries to participate in the educational activities of the IAP, without having to travel. Webinars should be planned and made available with a reasonable range of time zones with the presenter, for free. This gives pathologists the chance to attend meetings and to actively participate in discussions. The first priority of the Academy is education, and the challenge for global pathology in the years to come is to harmonize it.

How will the role of the pathologist evolve in the next 10 years?

Multidisciplinary collaboration with biologists, chemists and other scientists has become very important. Molecular pathology is expanding rapidly, and in most instances is mandatory for clinical practice. But molecular testing alone, without the use of morphology, may be misleading and result in inappropriate treatment. The pathologist should be the leading scientist coordinating clinical investigation and research, both to reduce budgets and to ensure best clinical practice.

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