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MEDICAL LABORATORY OBSERVER

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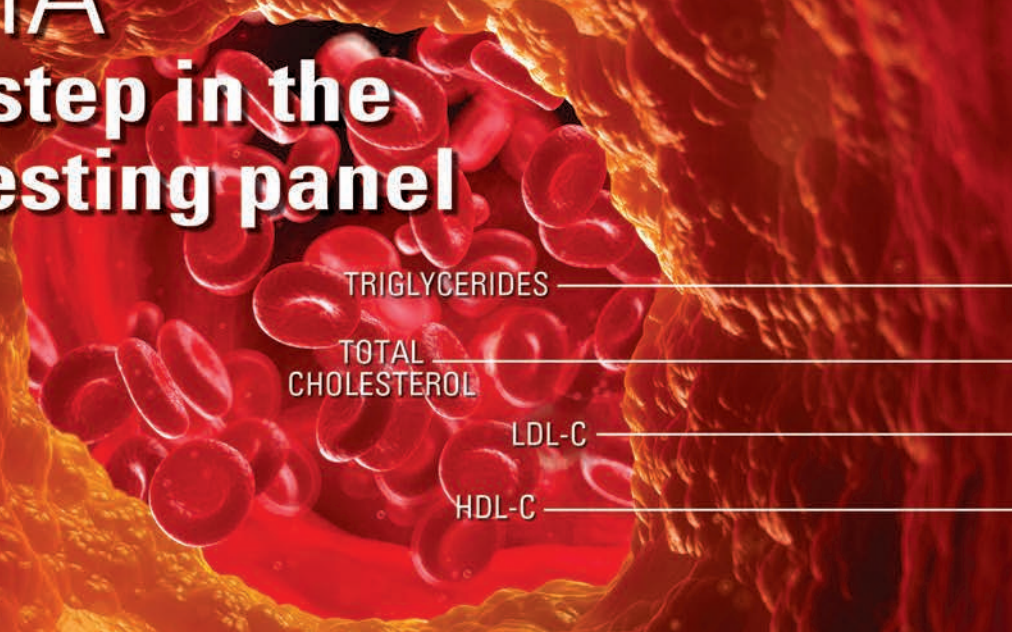
CE

sPLA$_2$-IIA
The next step in the cardiac testing panel



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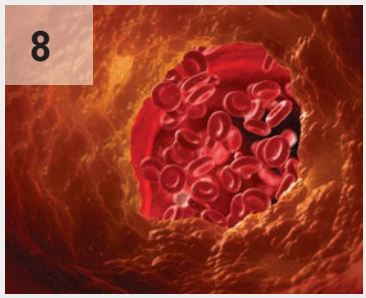

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Networking at KnowledgeLab 2019

By Lisa Moynihan
Editor

The 2019 Clinical Laboratory Management Association (CLMA) annual conference was held in Grapevine, TX this year. The CLMA KnowledgeLab is one of my favorite conferences to attend based on several reasons: its intimate size, its quality vendors, and its regular attendees (a special shout-out to my SAFMLS friends!). I've highlighted a few of my favorites, below:

In its fourth year attending CLMA, the Society of American Federal Medical Laboratory Scientists (SAFMLS) is a non-profit organization established in 1971. Membership is open to qualified military (active and reserve), Public Health Service, and Veteran's Administration personnel, and their affiliated scientists that support healthcare via laboratory research and evaluation. Their primary objective is, "maintaining and enhancing high professional standards through improved laboratory policies and technology in support of the healthcare delivery systems."[1] Explore SAFMLS, here: https://www.safmls.org/.

Always on the lookout for something interesting, I happened upon Bobby Tschann, US-Fungitell Account Manager at the Associates of Cape Cod, Inc. Their tagline is, "Your Endotoxin & Glucan Experts." They were displaying their Fungitell Assay (FDA-cleared and CE marked) for rapid invasive fungal infection (IFI) screening. Their assay is manufactured using horseshoe crab blood. (Interesting fact: horseshoe crabs use hemocyanin to carry oxygen through their blood. Because of the copper present in hemocyanin, their blood is blue.) They have a cool Horseshoe Crab Sustainability Project which focuses on supporting fisheries worldwide and ensuring the genetic diversity of the horseshoe crab. Learn more, here: http://www.acciusa.com/.

If you've ever had to undergo an invasive colonoscopy, you'll appreciate this next product—Epi proColon. Epi proColon is an FDA approved colon cancer screening blood test. Marketing Director David Bull explained to me that their goal is addressing non-compliant patients (those unwilling or unable to be screened by stool-based, direct visualization, and/or serology tests). In a clinical study, 99.5 percent of twice non-compliant persons completed the blood test.[2] Discover more, here: https://www.epiprocolon.com/us/.

I made a new acquaintance at the Center for Surveillance, Epidemiology, and Laboratory Services (CSELS), a Division of Laboratory Systems (DLS) at the Center for Disease Control (CDC) in Atlanta, Georgia. James Bratton, fellow editorial guru and Japanophile, exposed me to their government funded programs, including laboratory eLearning tools, courses, and resources—many of which are FREE. Browse their full catalog of lab-centric opportunities at https://www.cdc.gov/csels/dls/ and DLS training courses at https://www.cdc.gov/labtraining/.

Last, but not least, I connected with the dedicated team at Visiun, among them, Technical Support Specialist, Vanessa Hawrylak. With Visiun's support, Vanessa is seeking a lab who can donate a waived chemistry analyzer to help build a small STAT lab in her crisis-stricken home country of Venezuela. Any other lab supplies that are no longer needed are also being accepted. Read more about Vanessa's honorable efforts in our chemistry feature found on page 16.

Looking forward to seeing old and new faces next year in Louisville, Kentucky for KnowledgeLab 2020 from March 29 – April 1, 2020!

References available at mlo-online.com.

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FAST FACTS

PCOS

Polycystic ovary syndrome (PCOS) is caused by an imbalance of reproductive hormones that creates problems in the ovaries and is one of the most common causes of infertility. With PCOS, the egg may not develop properly, or it may not be released during ovulation.

1 in 10
women of childbearing age are affected by PCOS.

5-10 percent
of women between 15 and 44 have PCOS.

5 million
is the number of women in the U.S. that have PCOS.

20s and 30s
is the age most women discover they have PCOS.

70 percent
of women have hirsutism (male-pattern hair growth) as a symptom.

10 percent
loss of body weight encourages regular menstrual cycles, improving successful fertilization.

2 of these 3
symptoms determine a PCOS diagnosis: irregular periods or no periods as a result of anovulation; abnormal male hormone levels resulting in excess hair, acne, or thinning scalp hair; and/or multiple small ovarian cysts.

• **Sources:** https://www.womenshealth.gov/a-z-topics/polycystic-ovary-syndrome, https://www.cdc.gov/diabetes/library/spotlights/pcos.html

Tickborne illness

CDC offers new resource for tick season. Tick season is here and that means it's time once again for people to protect themselves and their loved ones (including pets) from tick bites. The Centers for Disease Control and Prevention (CDC) has an updated digital press kit available with the latest information about the increasing number of reported tickborne illnesses, newly discovered disease-causing germs, expanding ranges of ticks, and a novel tick species recently found in the United States.

Last year, nearly 60,000 cases of tickborne disease were reported to the CDC by state health departments and the District of Columbia. Though it can't be predicted how bad any particular season will be, it is known that reducing exposure to ticks is the best defense against Lyme disease, Rocky Mountain spotted fever, and other tickborne infections.

The CDC has more information available here: https://www.cdc.gov/media/dpk/diseases-and-conditions/lyme-disease/index.html.

Infectious disease

Decline in measles vaccination is causing a preventable global resurgence of the disease. In 2000, measles was declared to be eliminated in the United States. Today, however, the U.S. and many other countries are experiencing concerning outbreaks of measles because of declines in measles vaccine coverage. Without renewed focus on measles vaccination efforts, the disease may rebound in full force, according to the *New England Journal of Medicine* by infectious diseases experts at NIAID, part of the NIH, and the Penn State University College of Medicine's Milton S. Hershey Medical Center.

Measles is an extremely contagious illness transmitted through respiratory droplets and aerosolized particles that can remain in the air for up to two hours. Most often seen in young children, the disease is characterized by fever, malaise, nasal congestion, conjunctivitis, cough, and a red, splotchy rash. Most people with measles recover without complications within a week. However, for infants, people with immune deficiencies, and other vulnerable populations, the consequences of a measles infection can be severe. Rare complications can occur, including pneumonia, encephalitis, other secondary infections, blindness, and even death. Before the measles vaccine was developed, the disease killed between 2 and 3 million people annually worldwide. Today, measles still causes more than 100,000 deaths globally each year.

Measles can be prevented with a vaccine that is both highly effective and safe. Each complication and death related to measles is a "preventable tragedy that could have been avoided through vaccination," the authors write. Some people are reluctant to vaccinate their children based on widespread misinformation about the vaccine. For example, they may fear that the vaccine raises their child's risk of autism, a falsehood based on a debunked and fraudulent claim. A very small number of people have valid medical contraindications to the measles vaccine, such as certain immunodeficiencies, but almost everyone can be safely vaccinated.

When levels of vaccine coverage fall, the weakened umbrella of protection provided by herd immunity—indirect protection that results when a sufficiently high percentage of the community is immune to the disease—places unvaccinated young children and immunocompromised people at greater risk. This can have disastrous consequences with measles. The authors describe a case in which a single child with measles infected 23 other children in a pediatric oncology clinic, with a fatality rate of 21 percent.

If vaccination rates continue to decline, measles outbreaks may become even more frequent, a prospect the authors describe as "alarming." This is particularly confounding, they note, since measles is one of the most easily prevented contagious illnesses. In fact, it is

possible to eliminate and even eradicate the disease. However, they say, achieving this goal will require collective action on the part of parents and healthcare practitioners alike.

Leadership

Strong laboratory leaders—a key asset for global disease control. Laboratories play an essential role in the detection, diagnosis, prevention, and control of diseases. To strengthen this role, ECDC (European Centre for Disease Prevention and Control) and five partner organizations have developed the Global Laboratory Leadership Program (GLLP) to support and sustain national laboratory systems under a One Health approach.

Reliable laboratory services continue to be limited in many low- and middle-income countries. Although there have been examples of effective laboratory responses to outbreaks, a well-documented number of such events have shown how a lack of robust laboratory systems can impede disease control and prevention efforts.

To strengthen laboratory systems and address gaps in laboratory leadership competency across sectors, the GLLP was established. ECDC collaborated with five partners to develop the GLLP: Association of Public Health Laboratories (APHL); Centers for Disease Control and Prevention (CDC); Food and Agriculture Organization of the United Nations (FAO); World Organization for Animal Health (OIE); and World Health Organization (WHO).

The GLLP targets professionals working in human and animal health laboratories, as well as laboratories with public health functions such as environmental, agricultural, food, chemical, and aquatic laboratories.

The development of the GLLP represents the first global effort involving input and support from multiple organizations and institutions to create a core set of competencies for laboratory leaders working in support of health systems and a related flexible and adaptable learning package.

HIV

Novel antibody may suppress HIV for up to four months. Regular infusions of an antibody that blocks the human immunodeficiency virus (HIV) binding site on human immune cells may have suppressed levels of HIV for up to four months in people undergoing a short-term pause in their antiretroviral therapy (ART) regimens, according to a report published online in *The New England Journal of Medicine*. Results of the Phase 2, open-label study indicate the antibody, known as UB-421, was safe and did not induce the production of antibody-resistant HIV. The study was supported in part by NIAID, a component of the NIH, and United Biopharma.

The study was conducted in Taiwan and led by Chang Yi Wang, PhD, Chief Scientific Officer and Chairperson of United BioPharma. Twenty-nine volunteers with well-controlled HIV discontinued their normal regimens of daily oral ART at the time of their first infusion or one week later, depending on their ART regimen. Fourteen study participants received eight regular weekly infusions of UB-421, while 15 received eight higher-dose infusions every other week. At the end of the eight- or 16-week treatment period, all volunteers restarted their previous ART regimen and were evaluated in follow-up visits up to eight weeks later. Apart from a single participant who discontinued the study because of a mild skin rash, volunteers in both groups maintained HIV suppression throughout the treatment period in the absence of ART.

Previous experimental infusions of broadly neutralizing antibodies (bNAbs) have suppressed HIV for about two weeks by targeting proteins on the virus itself, but the rapid mutation rate of HIV induces antibody-resistant strains that render the treatment ineffective. UB-421 theoretically avoids this possibility by blocking a stable human protein that HIV uses to infect T cells.

Indeed, resistance to UB-421 was not seen in this study. Because the small study did not include a comparator group receiving a placebo infusion, further studies have been planned in Taiwan and Thailand to evaluate the safety and efficacy of UB-421 as a treatment for HIV.

HPV

HPV rates for women under 40 increasing, putting them at higher risk of related cancers. Human papillomavirus (HPV) infection rates are increasing in women born after 1980 who did not receive the HPV vaccine—putting them at higher risk for HPV-related cancers, according to a University of Michigan study.

While more than 90 percent of HPV-related cancers are preventable, HPV causes more than 40,000 cases of cancer in the U.S. each year, including cervical, oropharyngeal, anal, and other genital cancers. The CDC estimates that at least half of all sexually active men and women will acquire HPV in their lifetime.

Because testing for genital HPV started in 2003 for women and in 2013 for men, there are no direct measurements of how HPV incidence and prevalence have changed over the past decades—before the vaccine became available for women in 2006 and for men in 2009.

Researchers said previous analyses focused only on measures of current HPV infection (viral DNA) or past HPV infection (antibodies), producing sometimes competing results, making it difficult for experts to predict current and future trends.

For their analysis, researchers developed a model that uses both HPV infection and past infection data, as well as mathematical representations of the underlying mechanisms of infection, recovery, and the generation of antibodies, to paint a better picture of HPV prevalence in the present and past.

Their model indicates that while there may be a substantial increase in HPV prevalence in more recent birth cohorts, HPV vaccination may ultimately control adverse HPV-related outcomes, including genital warts and cancer. Questions still remain, such as why there is a peak in HPV infection among 45-to-55-year-olds.

$sPLA_2$-IIA—the next step to enhancing your cardiac testing panel

By Rebecca Kirby

Cardiovascular disease (CVD) is the number one cause of death globally, with an estimated 17.7 million people dying each year. This figure represents approximately 31 percent of all global deaths.[1] Worryingly, this figure is set to increase to over 23.6 million by 2030. These startling statistics highlight the urgent need for better and earlier identification of at-risk individuals; this is especially true as many CVD cases can be prevented with appropriate lifestyle changes. This article will discuss the utility of $sPLA_2$-IIA, a novel biomarker for use in CVD risk assessment.

Traditional and routinely run biomarkers for CVD risk include lipid assays such as Total Cholesterol, HDL Cholesterol (HDL-C), LDL Cholesterol (LDL-C), and Triglycerides. There is, however, a growing body of research and evidence indicating that additional risk assessment biomarkers need to be considered. Conventional risk assessment markers like those mentioned previously detect a mere 20 percent of all CVD patients. As the prevalence of CVD continues to rise worldwide, the need for reliable risk markers has never been more important.

$sPLA_2$-IIA has been proven to have clinical utility as a biomarker of inflammation. Inflammation is a process by which the body launches an attack utilizing our white blood cells. This response results in redness and swelling to either eliminate the pathogen or rid the body of an intruder.[2,3] Inflammation is a common response in many disease states, in some cases the body's immune system triggers an inflammatory response when there are no invaders to fight off. Inflammation is also associated with CVD. Although not a direct cause of CVD, inflammation is common in heart disease and stroke patients and is thought to be a sign of an atherogenic response. It is believed that in CVD the body perceives the plaque as abnormal or foreign and in response the body tries to prevent the plaque from entering the blood.[3] However, in some circumstances, the plaque may rupture and come in contact with the blood triggering clot formation.[3]

Early risk assessment helps to reduce the risk of a cardiac event occurring. Identifying those at highest risk of CVD and ensuring they receive appropriate treatment can prevent premature death.[4] Early risk assessment is particularly important in people who present with one or more risk factors including hypertension, diabetes, or hyperlipidemia. As stated before, by 2030 it is estimated that almost 23.6 million people will die from CVD, with heart disease and stroke projected to remain the leading causes of death.[4] This provides further confirmation that early diagnosis is an essential step in reducing the number of individuals affected.

The financial burden that CVD places on health services makes the development of a diagnostic biomarker even more essential. $sPLA_2$-IIA, a member of the secretory phospholipase A_2 family, has been found to have clinical utility as an inflammation biomarker specifically in the diagnosis of CVD risk. The addition of $sPLA_2$-IIA could compliment the labs existing cardiac risk panel, providing a different outlook and method of assessing cardiac concerns in patients.

Biological significance

The phospholipase A_2 family encompasses a wide range of enzymes that are all capable of hydrolyzing the sn-2 ester bond of a natural phospholipid substrate. This reaction produces lysophospholipids and free fatty acids. Despite similar structural features, catalytic mechanisms, and evolutionary relationships, the superfamily of PLA_2

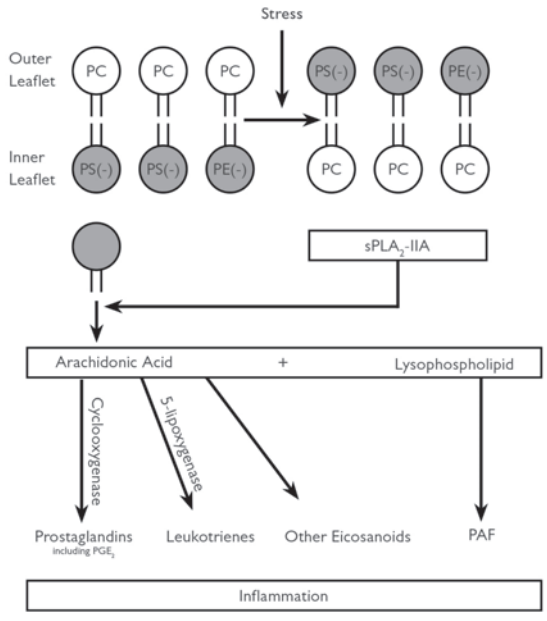

Figure 1: Inflammation[5]

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Discuss disease and mortality statistics of cardiovascular disease (CVD).
2. Recall the new biomarker being studied for CVD and its utility in early risk assessment.
3. Describe the chemical process by which $sPLA_2$-IIA acts on the coronary artery wall.
4. Discuss other diseases that $sPLA_2$-IIA is being used on.

enzymes is divided into fifteen separate groups and a number of subgroups.[5] Representing one of the groups is the secretory PLA_2 ($sPLA_2$) family, which consists of ten catalytic active enzymes. These enzymes share the same characteristics as the PLA_2 superfamily but have additional unique features.

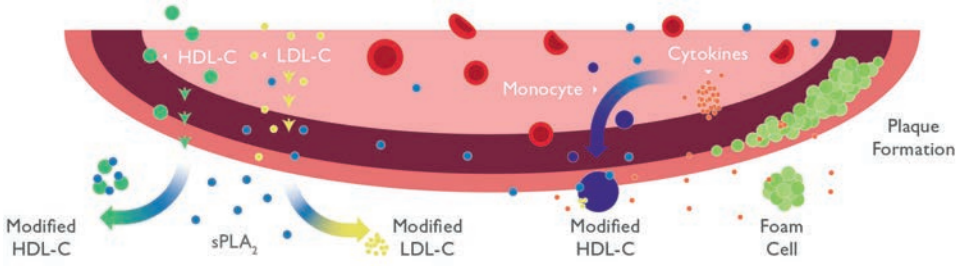

Figure 2. Atherosclerosis[6]

$sPLA_2$-IIA

$sPLA_2$-IIA is the prototypic member of the group II $sPLA_2$ subfamily and has been shown to be induced by pro-inflammatory stimuli in a wide variety of cells and tissues. It has been found to be associated with a number of inflammatory diseases including coronary artery disease and atherosclerosis.[5] These factors have contributed to its nickname, "inflammatory $sPLA_2$."

Pro-inflammatory $sPLA_2$-IIA

$sPLA_2$-IIA can also be referred to as a bactericide and is involved in the degradation of the bacterial membrane providing a host defense mechanism against microbial infection. $sPLA_2$-IIA is highly cationic and has a higher affinity for anionic phospholipids, such as phosphatidylethanolamine (PE). This affinity means that $sPLA_2$-IIA preferentially binds to PE over phosphatidylcholine (PC). PE is a phospholipid that is abundantly found on bacterial membranes. This allows the enzyme to penetrate the cell and hydrolyze membrane phospholipids causing bacterial death.[5]

Figure 1 illustrates how $sPLA_2$-IIA binding to anionic phospholipids leads to its activation and promotes inflammation.[5] The oxidation of phospholipids produces stress. This stress causes the anionic phospholipids phosphatidylserine (PS) and PE to be transported to the outer leaflet. This reaction activates cationic $sPLA_2$-IIA.

The higher activity of $sPLA_2$-IIA promotes the hydrolysis of the outer leaflet phospholipids into arachidonic acid and lysophospholipids. Through the cyclooxygenase and 5 – lipoxygenase enzymes, arachidonic acid is converted into prostaglandins, leukotrienes, and other eicosanoids. The lysophospholipids are converted into platelet-activating factors (PAFs). All four of the products produced have been linked to inflammation. Detecting the release of $sPLA_2$-IIA could therefore be a good starting point for the diagnosis of diseases related to inflammation.[5]

$sPLA_2$-IIA hydrolysis reaction

$sPLA_2$-IIA production of fatty acids and biologically active phospholipids plays an important role in platelet, monocyte, and endothelial activation, processes known to be critical steps in atherogenesis.[6] Unlike traditional cardiac biomarkers used to predict adverse outcomes in patients with acute coronary syndrome (ACS), $sPLA_2$-IIA has been shown to act at multiple pathways involved in atherogenesis, from lipid oxidation to modulation of vascular and inflammatory cell activation and apoptosis.[7]

Key observations through research found that $sPLA_2$-IIA mediated modification of lipoproteins plays a role in the development of atherosclerosis. The surface of both LDL Cholesterol (LDL-C) and HDL Cholesterol (HDL-C) is surrounded by phosphatidylcholine (PC)—a type of phospholipid which has been scientifically proven to

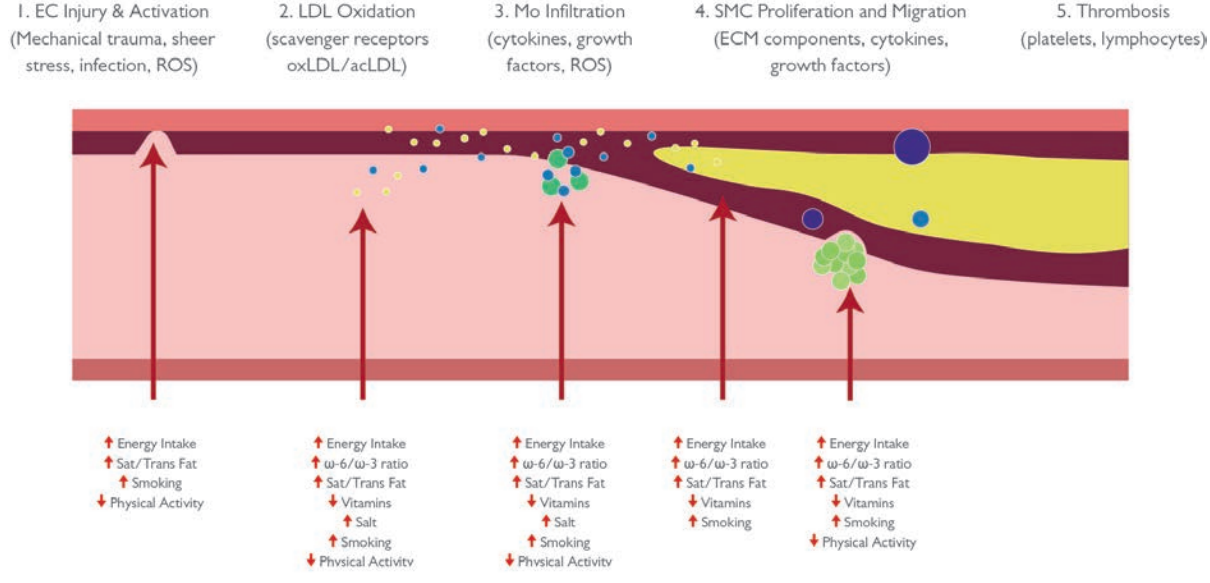

Figure 3. Atherogenesis leading to the atherosclerosis[20]

serve as a good extracellular target for several isoforms of $sPLA_2$-IIA. $sPLA_2$-IIA works by hydrolyzing these phospholipids resulting in the production of free fatty acids and lysophosphatidylcholine (LPC) which can generate

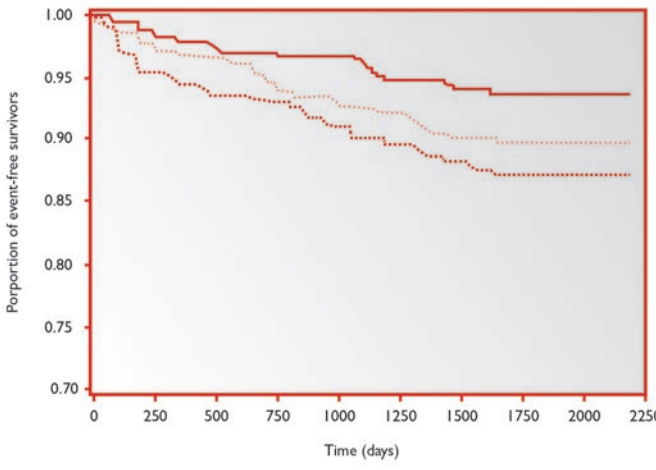

Figure 4. Kaplan-Meier estimates of secondary fatal and non-fatal CVD events during follow-up according to tertiles of sPLA2-IIA mass at baseline[13]

pro-inflammatory actions, accelerating atherosclerosis.[6] Hydrolysis of LDL-C correlates with the production of the more atherogenic, small dense LDL cholesterol (sdLDL-C) particles. The $sPLA_2$-IIA -processed LDL-C particles contain a large amount of lysophospholipids and exhibit the property of "small-dense" or "modified" LDL-C, which facilitates foam cell formation from macrophages. Research has shown that high levels of sdLDL-C compared to less dense, larger LDL-C increases the risk of coronary heart disease.

Figure 2 illustrates the proposed role of $sPLA_2$-IIA in the development of atherosclerosis. The diagram highlights the role $sPLA_2$-IIA has in the hydrolysis of LDL-C into the more atherogenic sdLDL- C.[6]

Comparison of $sPLA_2$-IIA with the traditional Lp-PLA_2 test

Lp-PLA_2 is a cardiac biomarker, sharing similarities with $sPLA_2$-IIA as it too is a member of the phospholipase A_2 enzyme family. Both $sPLA_2$-IIA and Lp-PLA_2 have associations with LDL-C. LDL-C carries Lp-PLA_2 to the coronary artery walls where it activates an inflammatory response. This process makes plaques, if present, more prone to rupture. This enzyme is associated with causing inflammation of the coronary artery walls, indicating that high levels of Lp-PLA_2 would increase the risk of a heart attack or stroke.[8] In contrast, in blood, $sPLA_2$-IIA can hydrolyze LDL-C producing sdLDL-C which is highly atherogenic.[9] $sPLA_2$-IIA is linked to the formation and destabilization of atherosclerotic plaques. Research has found that increased $sPLA_2$-IIA protein expression increases with atherosclerotic lesion development.

Figure 3 illustrates the process of atherogenesis and atherosclerosis and the formation of plaques. These are processes that both $sPLA_2$-IIA and Lp-PLA_2 are involved in.[20]

Though involved in similar mechanisms, research has found that among biomarkers of inflammation $sPLA_2$-IIA mass improved risk discrimination for identifying patients that have an increased risk of major adverse cardiovascular events.[10] The biological role of Lp-PLA_2 has been controversial, with contradictory antiatherogenic and proatherogenic functions.[9]

Research conducted found a significant association between $sPLA_2$-IIA mass and diagnosis of CVD while the Lp-PLA_2 activity appeared to not be associated with CVD diagnosis. Furthermore, patients with $sPLA_2$-IIA at presentation in the highest quartile had a statistically higher incidence of cardiac death and myocardial infarction (MI) however, no association was observed with Lp-PLA_2 activity.[11] Although Lp-PLA_2 activity has been associated with some cardiovascular diseases, researchers question whether Lp-PLA_2 has clinical utility and its role as a risk prediction biomarker.[12]

Relevant studies

1. Association between type II secretory phospholipase A_2 plasma concentration and activity and cardiovascular events in patients with coronary heart disease (2009).[13]

Figure 4 provides an estimation of event free survivors over a number of days depending on the level of $sPLA_2$-IIA mass present in the patient. As shown in the graph, the lower line represents the patients with the highest levels of $sPLA_2$-IIA. The proportion of event free survivors is lower for this tertile in comparison. This proportion further confirms the importance of measuring $sPLA_2$-IIA for preventing secondary coronary events.

2. Lipoprotein associated phospholipase A_2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress (2006).[9,14]

A study carried out by KAROLA involved measuring $sPLA_2$-IIA mass and $sPLA_2$-IIA activity in stable coronary disease. $sPLA_2$-IIA mass and $sPLA_2$-IIA activity showed a relatively good correlation ($r = 0.63$), and both measurements were significantly associated with an adverse outcome. $sPLA_2$-IIA mass appeared to perform better as a risk predictor than $sPLA_2$-IIA activity when extreme tertiles were compared.

3. Prognostic value and the changes of plasma levels of secretory type II phospholipase A_2 in patients with coronary artery disease undergoing percutaneous coronary intervention (2003).[9,15]

A small study was carried out on patients who were angiographically proven stable coronary artery disease (CAD). Circulating levels of $sPLA_2$-IIA mass were higher in these patients than in control individuals. In addition, elevated baseline levels of $sPLA_2$-IIA were independently associated with adverse outcomes including; coronary death, MI, and coronary revascularization.

Research has shown that levels of $sPLA_2$-IIA increase immediately after mechanical disruption of the coronary artery plaque by percutaneous coronary intervention (PCI). A PCI is a non-surgical procedure that uses a catheter to place a stent in the blood vessels in order to open vessels that have been blocked by atherosclerosis. $sPLA_2$-IIA responds rapidly to the inflammatory activity in atherosclerotic arteries and plaque rupture. Also, the higher levels of $sPLA_2$-IIA after PCI could be used to predict risk of future coronary events.

Additional uses of $sPLA_2$-IIA

Scientific research has consistently highlighted the potential of $sPLA_2$-IIA as a risk marker for CVD. However, there is also a growing body of evidence to support the clinical use of $sPLA_2$-IIA as a diagnostic marker in other disease

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areas. Sepsis is a serious complication of infection and without quick treatment it can lead to multiple organ failure and death.[16] Survivors of sepsis suffer from various complications that arise from organ dysfunction, which can result in severe impairment in their quality of life. The key to sepsis management relies on prompt and accurate diagnosis. This is because every hour appropriate treatment with antibiotics patient mortality increases by five to 10 percent. There has therefore been considerable effort among researchers to discover the most reliable sepsis biomarker.[17]

The current guidelines for sepsis diagnosis follows the Systemic Inflammatory Response Syndrome (SIRS) criteria. This involves monitoring the patients' temperature, heart rate, respiratory rate, and WBC count. Several blood tests may also be employed including procalcitonin (PCT) which is the most commonly used biomarker for the diagnosis of sepsis. The accuracy and reliability of PCT however is questionable. Research was conducted to compare different biomarkers of sepsis and their accuracy in early rule in/out of sepsis. The study involved testing 168 different biomarkers (including $sPLA_2$-IIA and PCT) in pediatric and adult intensive care units. Of the 168 markers tested, only five biomarkers were found to have greater than 90 percent specificity for diagnosis, this included $sPLA_2$-IIA but excluded PCT.[18]

PCT is the current diagnostic and prognostic biomarker utilized in emergency departments. Although PCT and $sPLA_2$-IIA are correlated, the accuracy of PCT as a biomarker alone is significantly lower than $sPLA_2$-IIA.[18]

$sPLA_2$-IIA has been found to demonstrate significant association with the presence of sepsis. Studies have found that $sPLA_2$-IIA as an early marker of sepsis carries substantial clinical significance. Although further studies are required for confirmation, this provides another potential use of this biomarker. $sPLA_2$-IIA has also shown clinical utility as a biomarker for chronic inflammatory conditions such as rheumatoid arthritis.

Methodology

Randox utilizes the latex enhanced immunoturbidimetric methodology in the development of the $sPLA_2$-IIA assay. Traditionally, ELISA-based methods were developed for the detection of $sPLA_2$-IIA levels. The main downfall of the ELISA method is that it is time-consuming for practical clinical diagnostic use.[19] Conversely, immunoturbidimetry is the most popular methodology as it can be easily adapted for high volume testing in automated analyzers.[21]

Conclusion

CVD continues to be the leading cause of death worldwide.[1] Consequently, it is vital that superior assays, such as $sPLA_2$-IIA are implemented to detect the disease in its earliest stages for the early and effective implementation of treatment plans, reducing the financial burden on health care systems. As $sPLA_2$-IIA offers clinical utility as an inflammatory biomarker, specifically in the diagnosis of CVD, the implementation of $sPLA_2$-IIA is a good starting point for the diagnosis of diseases related to inflammation.[5] Not only can $sPLA_2$-IIA be utilized in the diagnosis of CVD, but also in the prevention of secondary coronary events.[13]

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Rebecca Kirby, serves as a Marketing Executive for **Randox Laboratories**, provider of diagnostic solutions globally.

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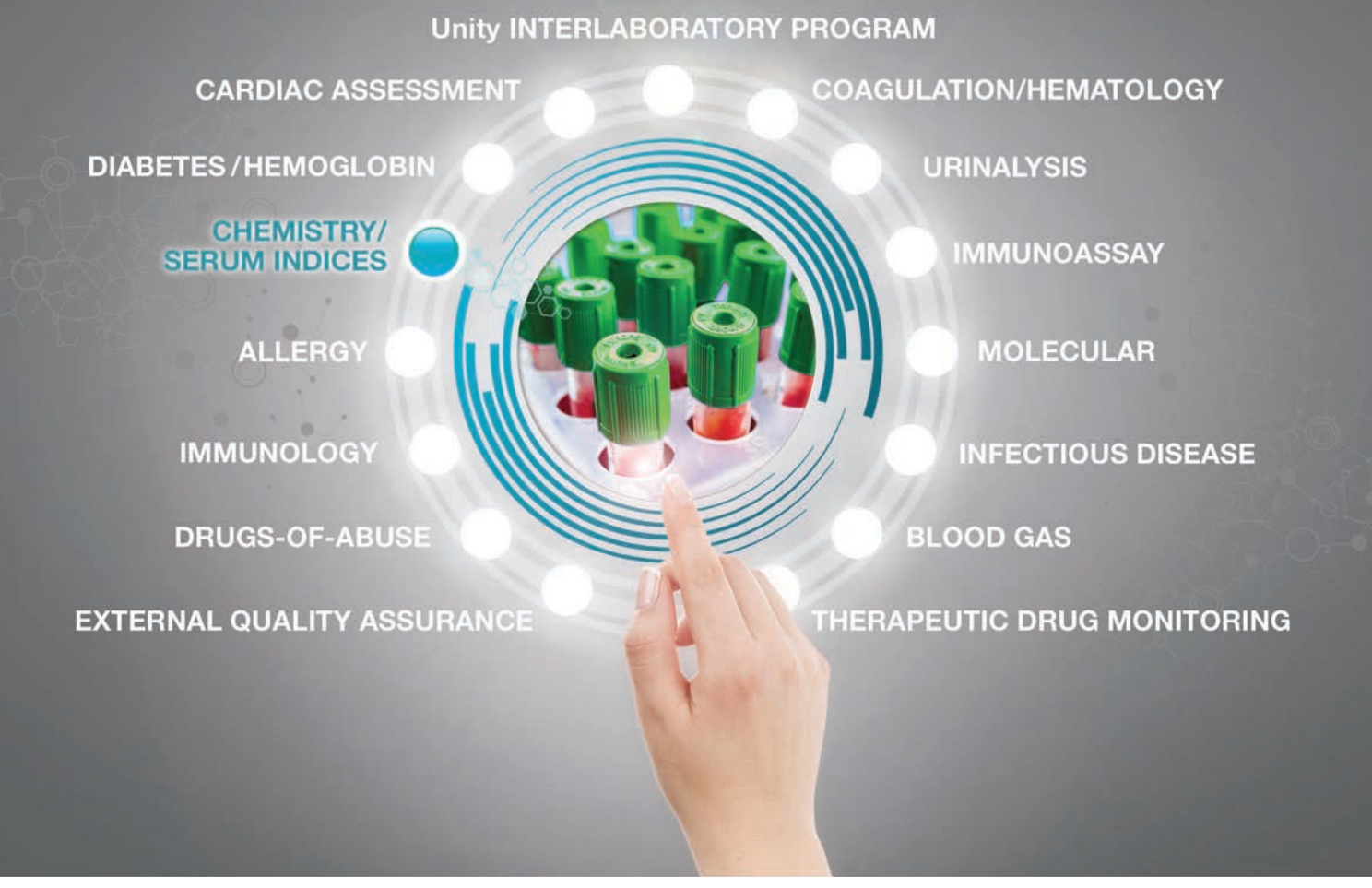

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BIO-RAD

sPLA$_2$-IIA—the next step to enhancing your cardiac testing panel

June 2019 *[This form may be photocopied. It is no longer valid for CEUs after December 31, 2020.)*

TEST QUESTIONS

Circles must be filled in, or test will not be graded. Shade circles like this: ● Not like this: ⊗

1. **The continued rise of cardiovascular disease (CVD) each year represents about _____ percent of all global deaths.**
   - a. five
   - b. 13
   - c. 24
   - d. 31

2. **What percentage of CVD patients does conventional risk assessment biomarkers detect disease in?**
   - a. five
   - b. 10
   - c. 15
   - d. 20

3. **The clinical utility of the sPLA$_2$-IIA test has been proven to be a biomarker for**
   - a. infection.
   - b. inflammation.
   - c. toxin exposure.
   - d. all of the above

4. **Inflammation has been found to be a direct cause of CVD.**
   - a. True
   - b. False

5. **As little as how many risk factors are taken into consideration when determining early risk assessment in individuals?**
   - a. one
   - b. three
   - c. five
   - d. seven

6. **The sPLA$_2$-IIA test has been specifically useful in determining the**
   - a. prognosis of current CVD disease.
   - b. cause of CVD.
   - c. diagnosis of CVD disease risk.
   - d. all of the above

7. **Which family of phospholipase does sPLA$_2$-IIA belong to?**
   - a. A_2
   - b. A_1
   - c. B
   - d. C

8. **As well as being an inflammatory marker, sPLA$_2$-IIA also functions as a**
   - a. herbicide.
   - b. fungicide.
   - c. bactericide.
   - d. all of the above

9. **The final product(s) produced by the binding of sPLA$_2$-IIA to phospholipids is/are**
   - a. platelet activating factors (PAFs).
   - b. leukotrienes and other eicosanoids.
   - c. prostaglandins.
   - d. all of the above

10. **Traditional cardiac markers are used to predict adverse outcomes, whereas the sPLA$_2$-IIA measurement can be used to predict the inflammatory processes that can potentially lead to a cardiac event.**
    - a. True
    - b. False

11. **sPLA$_2$-IIA modification of lipoproteins has been found to play a direct role in the development of**
    - a. atherosclerosis.
    - b. arteriosclerosis.
    - c. pulmonary embolisms.
    - d. petechiae.

12. **sPLA$_2$-IIA acts on the LDL cholesterol membrane to produce small-dense LDL cholesterol particles through a _____ reaction.**
    - a. oxidation
    - b. hydrolysis
    - c. enzymatic
    - d. reduction

13. **Compared to its less dense form, small particles of which type of lipid has been demonstrated in increasing the risk of coronary heart disease?**
    - a. Lipo-a
    - b. HDL
    - c. LDL
    - d. triglycerides

14. **Lp-PLA$_2$ and sPLA$_2$-IIA both have association with LDL, however sPLA$_2$-IIA mainly**
    - a. hydrolyses LDL to produce atherogensis.
    - b. acts to promote antiatherogenic functions.
    - c. is carried by LDL to the coronary artery wall and activates inflammation.
    - d. all of the above

15. **Research has proven that Lp-PLA$_2$ has a high association with CVD diagnosis, cardiac death, and MI.**
    - a. True
    - b. False

16. **Studies have shown that sPLA$_2$-IIA has a prognostic value and when percutaneous coronary intervention is used, the sPLA$_2$-IIA level responds by**
    - a. decreasing immediately.
    - b. increasing immediately.
    - c. decreasing about four hours after the procedure.
    - d. increasing about four hours after the procedure.

17. **What other disease has sPLA$_2$-IIA been researched on that shows promising results?**
    - a. sepsis
    - b. diabetes
    - c. transient ischemic attack
    - d. pneumonia

18. **sPLA$_2$-IIA has been shown to be a more accurate biomarker than _____ in sepsis patients.**
    - a. CRP
    - b. WBC count
    - c. PCT
    - d. all of the above

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Chemistry instrumentation—a critical need in Venezuelan labs

By Vanessa Hawrylak, MS, MT(ASCP)

Venezuela's story is a sad one—it went from being the richest country in South America in the 70's and 80's to being one of the world's poorest in 2019. The peace and prosperity that once filled the country has been replaced with violence and extreme suffering. Venezuela is now facing one of the worst humanitarian crises ever to occur in the Western Hemisphere. Nearly 90 percent of the country's population lives below the poverty level and more than half of the families are unable to meet their most basic needs for food and hygiene.[1] **In fact, malnutrition levels are so critical that the Secretary General of the Organization of American States is quoted as saying, "Newborns in Syria have a better chance of survival than those born in Venezuela today."**[2]

Entrance of oncology clinic

While the dictatorial government regime argues that there is not a crisis, the United Nations (UN) and the United Stated of America (USA) have confirmed the existence of the crisis and the terrible impact it is having on the people of Venezuela. Both the UN and the World Health Organization (WHO) have reported alarming statistics that confirm the magnitude of the problem. According to the *Washington Post*, in 2018, the United Nations Food and Agricultural organization indicated that between 2015 and 2017, 11.7 percent of the Venezuelan population (around 3.7 million), was undernourished. Unofficial statistics indicate that 80 percent of Venezuelans are food insecure with a high percentage of children under five with severe malnutrition surpassing the WHO threshold for crises.[2]

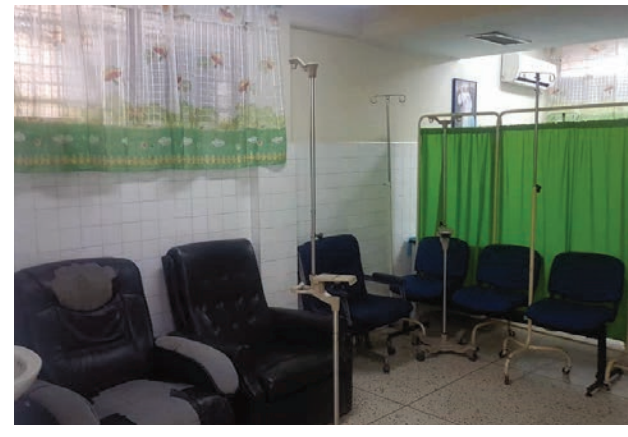
Building a small STAT lab

As a native of Venezuela, and a healthcare professional in the laboratory field here in the United States, it has become my mission to help those in need. Utilizing my Venezuelan family and friends as intermediaries, we've identified two organizations that focus on helping children. One organization focuses on providing children with food, education, and comfort while they recuperate from intense malnutrition. The other is an oncology clinic that provides accessible cancer services for pediatric patients. They also provide treatment for patients with leukemia among other blood disorders.

However, the crisis in Venezuela is so critical that these two organizations are struggling to find supplies to help the children. The shortage is so bad in the oncology laboratory that patients may experience a lack of services for up to three months. Currently, the lab can only perform manual hematology tests and they are without a chemistry analyzer. Basic testing is not available, and doctors have been forced to treat patients "blindly." Mortality rates have gone through the roof. The situation is so dire that on many occasions, the medical facilities find themselves without access to electricity or even water. Compounded by the absence of basic sanitation, diseases that were once eradicated long ago are now on the rise—including leprosy, tuberculosis, and typhoid fever. I've been told lab testing has now become a commodity; only those with money can afford to go to a private laboratory for testing.

POCT chemistry equipment need

Thanks to generous contributions and the full support of my employer, Visiun, I have been able to prepare a shipment to facilitate a point-of-care instrument for hematology testing. Soon, I will be able to open a GoFundMe page to help regions that are the most hardly hit. Chemistry analyzers have been the most challenging to find. I've been focusing on locating a (new or used) point-of-care test (POCT)



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Laboratory of oncology clinic

or small chemistry analyzer. At minimum, an analyzer can help pediatric oncology patients get basic chemistry tests. My goal is to supply the lab with as many basic needs as possible, focusing on the supercritical patients with the hopes of saving lives.

Through interviews I've learned POCT analyzers are not very common and only a few private laboratories have the ability to support a range of chemistry analyzers—even if the range is just a few. However, a POCT chemistry instrument could be a vital solution to the existing problem. It provides great flexibility for minimizing calibrations and linearities studies that could become costly when using a regular analyzer. It is flexible enough for transportation and support. And most of all, it provides results that can be accurate and reliable for basic testing.

As a medical technologist, I once argued about the reliability of POCT in chemistry testing. However, after experiencing a great deal of instrument training and seeing many technological advancements, I now feel confident enough to say that POCT innovations are demonstrating a great improvement in the medical technology field. In fact, a recent article by Saif Ali Bepari said, "POCT devices in the chemistry industry are considered [important to] support targeted innovations across various disease types." Bepari argues that POCT devices are, "crucial in achieving universal healthcare targets and the usage of POC testing devices is set to increase at higher-tier health and laboratory settings across developed and emerging economies in the coming years."[3]

Waiting room of oncology clinic

How you can help

As a profound activist of accurate and reliable chemistry test results, I believe that the flexibility of training, transportation, and cost effectiveness of POCT chemistry devices will help resolve major laboratory crises in underdeveloped and crisis-ridden countries such as Venezuela.

Overall, I am very grateful that I work for a company that supports my humanitarian efforts. And most of all I am grateful to family and friends, including my Venezuelan medical technology colleagues, who have put in time and effort to support this cause.

According to many studies, at least 70 percent of medical decisions rely on lab results that give important information on an individual's best course of treatment.[4] Imagine being a sick, poor child lost in the shuffle of a crisis-ridden country, void of any laboratory testing. Imagine a sick child in the midst of extensive treatment, such as chemotherapy, without any lab tests being performed to monitor their condition. Lab tests are a necessary and fundamental part of a patient's diagnosis and treatment. Due to the current situation in Venezuela, every medical situation has become a guessing game. Will I live? Or will I die?

Should you wish to learn more and/or contribute to my cause of finding medical laboratory equipment and/or supplies for the underserved children of Venezuela, please contact me directly at vanessa.hawrylak@visiun.com or visit one of the organizations directly, Fundacion Kapuy: https://kapuy.org.ve\.

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PCR: a versatile tool—from detecting genetic diseases and cancers to monitoring stability after bone marrow transplants

By Teresa Snyder-Leiby, PhD

Polymerase chain reaction (PCR) is the foundation for hundreds of molecular DNA tests for the detection and monitoring of a wide range of genetic diseases. Molecular testing methods and examples of the diseases detected include:[1-12]

- Qualitative fluorescence PCR (QF-PCR): Detection of aneuploidy (such as Down syndrome) and repeat expansion diseases (such as Fragile X and Huntington's disease).
- Multiplex Ligation-dependent Probe Amplification MLPA® and Methylation Specific (MS-MLPA®) (MRC-Holland reference): Detection of diseases from insertions/deletions/point mutations; including predisposition to cancer (BRCA 1), neuromuscular disorders (Duchenne muscular dystrophy), and aneuploidy.
- Microsatellite instability: Detecting cells deficient in DNA repair capabilities, microsatellite instability-high (MSI-H), or mismatch repair deficient (dMMR) in solid tumors.
- QF-PCR using human identity chemistries is also an essential tool in monitoring engraftment post-transplant.

QF-PCR - aneuploidy

Aneuploidy (an abnormal number of chromosomes) is the most frequent genetic disorder observed in live births and miscarriages, with trisomies being the most prevalent, accounting for approximately 53 percent of all chromosome abnormalities. QF-PCR is quantitative, and the laboratory and data analysis steps can be partially automated for accurate results of more samples in less time than the traditional karyotype method. QF-PCR uses chromosome-specific primers to amplify DNA fragments for each of the chromosomes of interest. DNA fragments are separated with capillary electrophoresis and analyzed by fragment size and number of fragments. The peak height or area, is used to quantify the amount of DNA amplified, determining if the expected pair of each chromosome or aneuploidy is present.

Three DNA fragments—visualized as peaks of approximately the same height in an electropherogram, or peak ratios of 2:1 and 1:2—are indications that the sample is in the trisomic range. Custom chemistries or commercial PCR kits provide primer sets that will detect chromosomes 13, 18, 21, X, and Y. In many cases these multiplexes are sufficient for conclusively detecting and quantifying aneuploidy.

The peak pattern shown in **Figure 1** is an example of a sample that is in the trisomic range for Down syndrome. Peak ratios at loci D21S11 (1:1:1), D21S1437, and IFNAR (1:2) are typical indicators of trisomy 21. Loci D13S634 and D21S1311 contain only single peaks (non-informative). The remaining loci have high heterozygous balance, which is typical of two copies of the homologous chromosomes.

Repeat expansion diseases

Expansions of simple sequence repeats, mainly but not limited to tri-nucleotide repeats, are responsible for over 40 human diseases. In general, an increasing number of repeats results in more severe phenotypes and the number of repeats increase (expand) as the disease gene is inherited. PCR primers are used to amplify the triplet or hexanucleotide repeats. These fragments are separated by capillary electrophoresis. Large repeats are in the high molecular weight range. Molecular weight and number of repeats do not have a strictly linear relationship for larger fragments. Reporting requires a correction for the non-linear relationship. DNA control(s) with fragments of a known number of repeats are included in the PCR and capillary electrophoresis. The size and known number of repeats of the control DNA are used to calculate correction and mobility factors for converting the sample peak size (molecular weight) into a number of repeats.

Figure 2 is a report containing the electropherogram of a Huntington's control sample used to calculate the correction and mobility factors. **Figure 3** is a report of a sample with a single fragment in the normal range and detection of a full expansion of 176 CAG repeats.

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Figure 4 is an example of a sample with no duplications or deletions for a spinal muscular atrophy (SMA) test. The red trace in the electropherogram is the normal sample. The blue trace is the test sample. **Figure 5** illustrates heterozygous deletions at the two SMN_2 probes, as the test sample peak heights are half the height of the normal sample.

Microsatellite instability

Microsatellites are stretches of DNA where a 1-5 base pair sequence is repeated several times. The most common microsatellite in humans is a dinucleotide repeat of CA which occurs tens of thousands of times across the genome.

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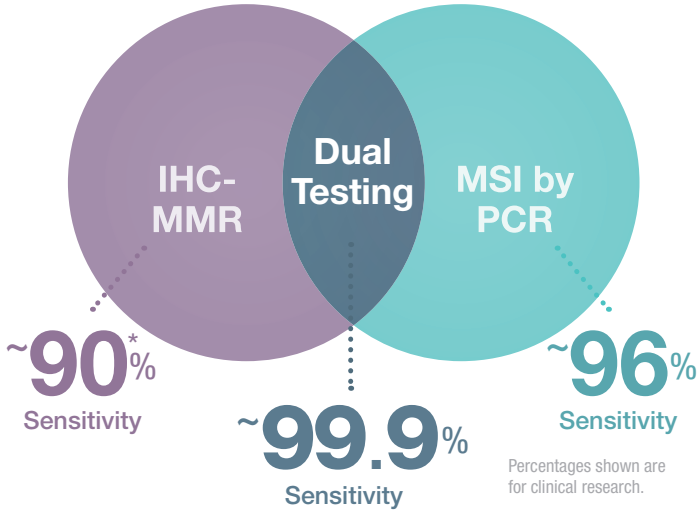

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*Dudley *et. al* (2016) *Clin. Cancer Res.* **22**, 813-820.

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MSI Analysis System, Version 1.2 chemistry is For Research Use Only. Not for Use in Diagnostic Procedures.

Microsatellite instability (MSI) is a key factor in several cancers including colorectal, endometrial, ovarian, and gastric cancers. Colorectal cancer studies have demonstrated two mechanisms for MSI occurrence. The first is in hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, where an inherited mutation in a mismatch-repair gene causes a microsatellite repeat replication error to go unfixed. The replication error results in a frameshift mutation that inactivates or alters major tumor suppressor genes. These genes are essential in the regulation of the cell cycle and, ultimately, the prevention of cancer.

The second mechanism whereby MSI causes colorectal cancer is an epigenetic change which silences an essential mismatch-repair gene. In both cases, microsatellite insertions and deletions within tumor suppressor gene coding regions result in uncontrolled cell division and tumor growth. Five markers have been recommended by the National Cancer Institute to screen for MSI in HNPCC tumors (often called Bethesda markers). Generally, MSI detection in two of the markers is considered a positive result or high probability of MSI (MSI-H).

Figure 6 is an example of a sample with MSI-H. The tumor sample fragments are compared to the fragments from non-tumor tissue of the same source by overlaying the electropherograms. The normal tissue is the red trace; the other traces are the tumor sample. Each of the polymorphic loci have additional microsatellite fragments in the tumor sample. The summary table highlights any of the markers with an increase in the number of microsatellite fragments.

Monitoring chimerism

Monitoring post bone marrow transplant chimerism, the extent to which the donor cells have engrafted in the recipient, is essential to confirm stability or detect any signs of graft failure. The analysis uses the same QF-PCR human identity chemistries which amplify short tandem repeats (STRs) used for forensic human identity testing. Highly polymorphic STRs are amplified using a multiplex of STR primers and separated by capillary electrophoresis. Peak heights or areas of fragments from donor and recipient are used to calculate chimerism, the percent of the DNA from the donor.

Figure 7 is an electropherogram from a control 3:1 mixture. Peaks attributed to donor, recipient, or shared are indicated by the flag labels (D, R, D/R). **Figure 8** contains the corresponding result table of the percent donor at each locus, total percent donor chimerism, and quality metrics.

Editor's note: Figures 1-8 and references can be found online at www.mlo-online.com.

Teresa Snyder-Leiby, PhD has served as product manager for DNA fragment analysis software at **SoftGenetics** for 11 years. Teresa was a biology professor at the State University of New York (SUNY New Paltz) prior to joining the support and development team at SoftGenetics.

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Enterprise vs. purpose-built lab RCM systems

Considerations for lab directors

By Lâle White and David Nichols

The laboratory industry has experienced tremendous change in the past few years. New compliance obligations and continued fee compression place an economic burden on many labs. In an attempt to save money, some labs—in particular those associated with hospitals and health systems—are using an enterprise revenue cycle management (RCM) module that is offered as part of the hospital's electronic health record (EHR) software. This article explores what lab leaders need to consider when it comes to the capabilities of enterprise RCM systems vs. those purpose-built for laboratories. The health system may feel the enterprise RCM system is the most cost-effective option, but that fails to take into account the specific needs of the laboratory to maximize its revenue contribution and effectively manage compliance risk.

Compliance

The increasing complexity of compliance obligations for laboratories can seem unsustainable. In the first PAMA (Protecting Access to Medicare Act) data reporting period, few hospital labs were required to report. The definition of applicable lab has been adjusted, so many hospital lab leaders should be collecting the required data and preparing to deliver an accurate dataset during this second reporting period.

> Failure to report PAMA data accurately can result in severe penalties. Applicable labs that fail to report or report incomplete or incorrect data face potential penalties of up to $10,000 per day, per line item.

Although the definition of applicable lab has been modified, it's still unclear how many hospital labs will be required to report. Nor is it well-understood whether the additional data will have a meaningful impact. It is also unknown whether hospital labs will be able to successfully distinguish outreach data from outpatient data, as is required. According to the American Hospital Association, it is a significant burden on hospitals to effectively distinguish this data and is especially difficult if the lab is using the hospital's enterprise RCM system, as the level of detail required for PAMA reporting is often not collected.

To properly support compliance requirements, labs need RCM and financial management technology that not only helps improve cash collections, but delivers visibility into and control over financial operations. Optimizing billing and accounts receivable processes, intelligently automating workflow, facilitating claim and appeal filing, and removing clerical decision making also reduce regulatory compliance risk.

Payor billing rule changes

The most extensive change to payor billing rules of late is related to the Eliminating Kickbacks in Recovery Act of 2018 (EKRA). The Substance Use-Disorder Prevention that Promotes Opioid Recovery and Treatment for Patients and Communities Act (SUPPORT Act) is intended to address the national opioid crisis. EKRA, one of its accompanying bills, however, has potential impact on many laboratories, including hospitals that provide no services related to substance abuse treatment.

There are things that laboratories can do within their billing and RCM processes to help with EKRA compliance. For example, laboratories need to review policies related to writing off co-pays and deductibles. Under EKRA, it is now a federal felony to write-off these patient responsibility balances as a standard of practice for private payors, as it already is under Medicare. This can be extremely difficult to do with an enterprise RCM, not designed specifically for laboratories.

For example, when a lab offers waivers and discounts, these cannot be fixed amounts, nor have a stated upper limit. It cannot be a one-size-fits-all approach. There needs to be bona fide financial assistance counseling for each patient and attempts need to be made to collect on any balances due. This is especially relevant for hospitals using an enterprise RCM. Because the average in-patient invoice is dramatically higher than the typical outreach lab invoice, there is a tendency for many hospitals to allocate their collection resources toward the higher value balances, and therefore, smaller lab-based balances tend to be written-off.

A purpose-built lab RCM solution provider, with lab-specific operational expertise, can advise you on mitigating the impact of EKRA by optimizing reimbursements and pursuing a secondary payor strategy to further reduce the patient out-of-pocket. In addition, the purpose-built solution provider can also advise on taking the best advantage of an organization's data to formulate a strategy for maximizing the organization's "in-network" footprint.

Data visibility

Another impact of running a lab on an enterprise RCM system is that typical enterprise RCM systems do not provide the data visibility required to effectively operate a lab. Purpose-built RCM systems for laboratories use web services to deliver capabilities and information wherever they are needed across the system, including connections with patients and clients through portals as well as interoperating with external systems. The best web services for labs have HIPAA and internet security protocols built in. It is also essential for labs that these two-way data exchanges operate in real time, removing redundancies and data latency.

The information held within lab billing and information systems is useful and powerful. Diagnostic services influence most medical and therapeutic decisions, and the data from the patient's medical record, most relevant for optimizing care, is also diagnostic data. Labs need to leverage these important data assets, but if they are using enterprise RCM systems, they may not be able to fully access, visualize, and thereby benefit from this valuable information.

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Data visibility is truly essential, yet just as important are the analytics to help make the data actionable. Analytics help an organization leverage its data assets to understand how the lab is operating, by payor, by specialty, by test, by territory, etc. Analytics also help lab leaders better understand the business they earn from each referring physician. This helps identify opportunities for increasing the value of the relationship. There is also tremendous value to this data beyond the billing department. Clinical data is a treasure trove for analyzing and improving population health.

Business intelligence

Hospital outreach, outpatient, and other diagnostic labs have specific business intelligence (BI) needs that cannot be met by most enterprise RCM systems. Data on the efficiency and effectiveness of lab operations are essential. It is also critical to have fast access to reporting and analytics without impact to production system performance.

Some of the most important BI components and capabilities for labs include:

- Dashboards: view information summaries with ease, and drill down for further detail, if necessary.
- Auditable: ensure data integrity and GAAP/Sarbanes-Oxley compliance. Easily address PAMA and FASB 606 accounting requirements.
- Benchmarking: provide metrics that enable tracking and compare the organization's financial and operational performance to your industry peer group.
- Key performance indicators: use data to understand trends and establish and track key performance indicators.
- Drill-through data: view contextual details related to the data elements by drilling up/down in the summary reports.
- Ad hoc reporting: enable end users to easily build his/her own reports.

Precision medicine

As the healthcare industry continues to adopt precision medicine approaches, labs need informatics technology to integrate clinical, diagnostic, and financial data to enable care teams to gain better insight into the patient's medical situation, make better diagnostic and therapeutic decisions, and improve outcomes. Frankly, this is something that most enterprise RCM systems just cannot do. They are not designed to improve the efficiency of patient care coordination, deliver data visibility or quality metrics reporting for reimbursement, nor to effectively discover actionable insights.

The advancement of precision data creates more complex tests and thus more complex billing. It tends to result in more rejected claims, and thus more appeals. The appeal process can be challenging for labs using an enterprise RCM system, since access to and the attachment of the data needed for a successful appeal is not automated. This results in manual intervention, which is costly and opens the laboratory up to new risks—including compliance risk.

ADDITIONAL CONSIDERATIONS

Can the RCM system:

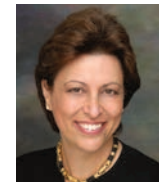
- distinguish between billing on order, billing on result, and manage its ramifications?
- identify when the performing location is different than the billing location?
- report collected and expected revenues on a per salesperson basis for commission calculation purposes?
- easily handle bifurcated and multiple fee schedules?
- accurately track and report how much volume is being written-off rather than worked?

Lab-specific RCM solutions provide more value

In short, enterprise RCM systems just don't have many of the capabilities required for labs. We see hospital outreach labs struggling to use the hospital system. From a compliance perspective, in today's environment where new regulatory and payor requirements are being introduced regularly, making a commitment to compliance and obtaining the technology solutions that an organization can rely on to proactively support their compliance needs and programs are imperative. Otherwise the risk is just too great, and it is even harder for hospital outreach labs to succeed.

Too often hospital and health system lab administrators have enterprise RCM systems pushed on them in a misguided attempt to save money. They are told the enterprise system will meet the lab's needs and comes bundled with the cost of the hospital or health system software. Unfortunately, what comes with the enterprise RCM system is the potential for compliance risk, additional labor costs to create the "workarounds" the lab needs, and subpar capabilities that fail to maximize reimbursement for the highly valuable work the lab performs. These labs leave over half their potential profit margin uncollected, because the cost to collect is too high. Only laboratory-specific automation can cost-effectively maximize both AR collections and profitability.

Lab leaders need to rationalize data strategies with business strategies. A lab-specific RCM system can go a long way to making this happen. Value-based pricing is dependent upon the ability to demonstrate financial and economic benefit, which is not possible in an enterprise system. Without leaders focused on technology, data, and strategy alignment, it will be challenging to thrive in today's environment of reimbursement compression and increased regulation.



Lâle White serves as CEO and founder of **XIFN**, a company dedicated to the vision of optimizing the economics of healthcare. A nationally recognized expert in medical financial management and regulatory compliance, with over 35 years of experience in information systems development and medical billing.

David Nichols, MBA, serves as President and founder of **The Nichols Management Group**, leading laboratory consulting for a number of healthcare systems, independent laboratory, biotechnology, private equity, and managed care clients. Nichols has a clinical and anatomic lab consulting practice based in York Harbor, Maine.

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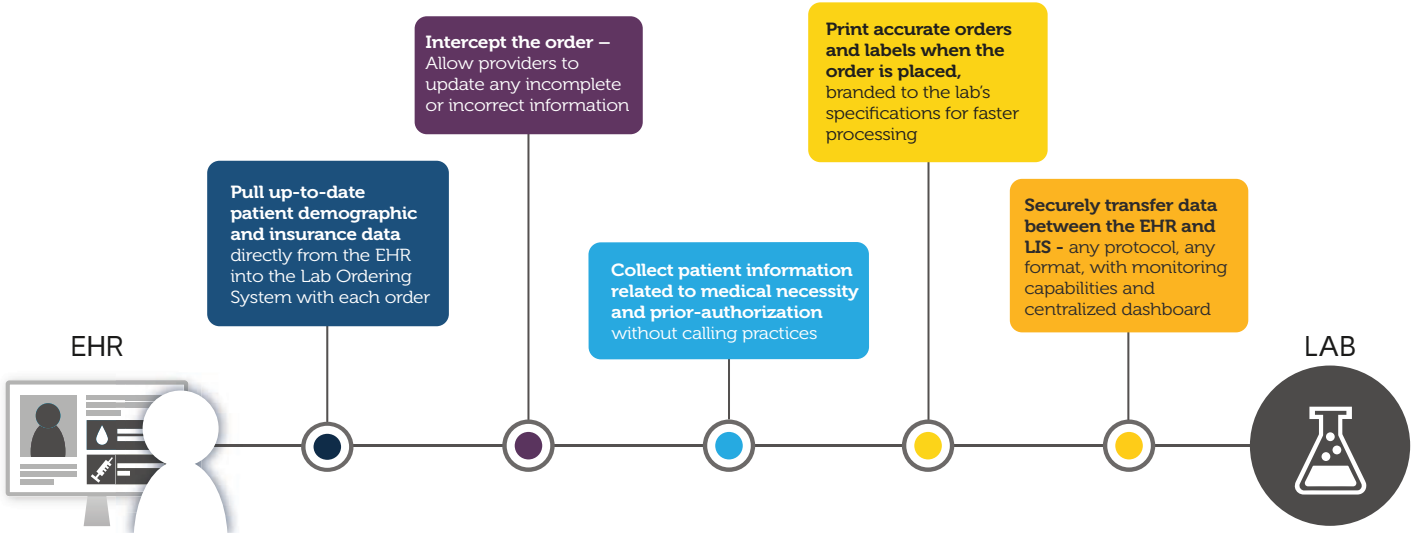

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Diamond-Blackfan anemia: a familial case with emphasis on multi-diagnostic approach to diagnosis and treatment

By Floyd Josephat, EdD, MT(ASCP) and Katrina Goff, MS, MLS(ASCP)

Diamond-Blackfan anemia (DBA) is an autosomal dominant disorder of the bone marrow where an insufficient amount of red blood cells are produced leading to anemia. The condition is named after the pediatricians Louis K. Diamond and Kenneth Blackfan, who described congenital hypoplastic anemia in 1938. This type of anemia usually presents within the first year of life and can lead to a multitude of secondary conditions which will be discussed within this case study.[1] DBA is rare and affects five to seven live births per million worldwide. Corticosteroids is the initial treatment but the Diamond Blackfan Anemia Registry found that 36 percent of those on steroids also require monthly blood transfusions.[2] This case study will explore a multi-diagnostic approach to testing and treating a familial case of DBA, including hematology, chemistry, and immunohematology. Secondary disorders and diseases such as myelodysplastic syndrome, hemochromatosis, and organ failure will be discussed, as well as gene therapy, bone marrow transplants, and leucine therapy treatments.

Anemia

According to the National Heart, Lung and Blood Institute (2012), anemia is any condition leading to a shortage of red blood cells.[3] There are different types of anemia, some not life threatening and some that are very serious. DBA is a type of serious anemia that is chronic and has a strong genetic component. DBA is a failure of the bone marrow to produce an adequate supply of red blood cells. This shortage causes the anemic state in patients and leads to an overall depletion of oxygen throughout the body.[4] There are some physical abnormalities that are sometimes associated with this disorder but will not be discussed here, since the patients involved did not demonstrate any such abnormality. Several mutations of ribosomal proteins have been found in those suffering from DBA with 25 percent showing a mutation on the RPS19 gene. This was surprising since the mutation was thought to be due to a deregulation in erythropoiesis.[6] This disorder is also autosomal dominant which means that only one copy of the gene is needed to cause the disease.[4]

Diagnostic criteria

Diagnostic criteria for DBA from the 2008 International Clinical Care Consensus Document are: age less than one year, macrocytic anemia with no other cytopenias, reticulocytopenia, normal bone marrow cellularity with a decrease in red cell precursors, and normal platelet and neutrophil counts."[2] Treatment options for those suffering from DBA initially start with corticosteroid treatment. A large dose is given for the first two weeks after diagnosis to initiate the bone marrow to produce more red blood cells though the mechanism behind this is unknown. After the initial dose, a sustainable dose is given and the possible adverse side effects that come with steroid use are monitored (upset stomach, increased blood sugar, increased blood pressure, and increased risk for infection). The Diamond Blackfan Anemia Registry found that 82 percent were initially responsive to steroids. Thirty-six percent of that 82 percent also received monthly blood transfusions.[2]

Most patients suffering from DBA have to receive blood transfusions every three to six weeks. The patients hemoglobin is monitored (usually monthly) to ensure it is not dropping to a dangerous level. People with DBA usually have scheduled transfusions to ensure this doesn't happen. These patients sometimes present a problem for the healthcare worker since getting good access to a vein may be difficult in someone who receives chronic transfusions. It is also a challenge for the patient to receive appropriate blood when they begin developing antibodies to prior blood that they have received. Chronic transfusions are also dangerous since they increase the risk of a transfusion reaction. Those who are transfusion dependent are also at an increased risk of developing a disorder called hemochromatosis, which is essentially iron overload.[2]

Hemochromatosis is monitored by ferritin levels which indicate the levels of iron in the body. If iron overload is found to be an issue, chelation therapy can be done to remove the excess iron. There are currently two chelation drugs approved by the FDA: Exjade and Desferal. Exjade is an oral drug that binds the iron and removes it through the stool. Desferal is an injectable treatment over eight hours that removes the excess iron through urine.[2] Stem cell, or bone marrow transplants are also a possible treatment for those suffering from DBA. If successful, the bone marrow then functions normally and makes an adequate amount of red blood cells. The risks for a bone marrow transplant are numerous, including death if a rejection occurs.[2]

Laboratory diagnosis

Hematology

Numerous tests performed in the hematology department are vital in a doctor's treatment of a DBA patient. Those who are transfusion dependent have a CBC done every three to six weeks, usually coinciding with their transfusion protocol. Those on steroid therapy need fewer CBC's, mainly to check for infections due to lowered immune response. Elevated adenosine deaminase is an enzyme that breaks reticulocytosis. When thrombocytopenia is seen, a bone marrow aspirate is usually performed to check for bone marrow failure or the development of myelodysplastic syndrome or aplastic anemia.[5]

Macrocytosis (red blood cells that are larger than normal) is often seen with DBA. A study done by Pesciotta et al researched whether the macrocytosis was due to protein translation malfunctions by looking at the proteomes in patients with DBA versus samples from healthy patients.

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Most DBA patients also have an increase in fetal Hgb, a decrease in adenosine deaminase activity, and reticulocytosis. Ribosomal protein S19 accounts for up to 25 percent of the 11 proteins that undergo mutations in DBA. Of the four DBA patients who underwent proteomic analysis, abnormal proteins were found in all four including dysferlin and MHC class 1 proteins. Findings suggest that DBA protein mutations on RBC's are special unto themselves.[7]

Incidence of cancer in DBA is highly elevated, more specifically acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS). Clinical presentations and therapies vary greatly between individuals. Vlachos, Atsidatos, Alter, and Lipton did a retrospective study where they took 608 individuals from the DBA registry in North America and measured incidence of MDS and AML. Of the 608 total patients studied, 17 patients with DBA had more than one type of cancer with 15 demonstrating solid tumors and two with AML. Four patients developed MDS. Eight patients were transfusion dependent at the time of cancer diagnosis, two were in remission, and four had never been treated for anemia. Of the 18 who had cancer, ten had a mutation in a ribosomal protein gene known to be associated with DBA. Three of the four most common genotypes were represented: five with RPS19; two with RPL11 (mother and daughter); and two with RPL5. The median overall survival for all patients was 56 years.[8]

Immunohematology and chemistry

Iron is an important metal within the body responsible for DNA synthesis as well as oxygen binding. There is no method for ridding excess iron naturally so iron overload can be problematic in those receiving multiple transfusions. One unit of red cells has 200 mg of iron, which is more than double the daily recommended dose.[10] Ferritin is often used as an indicator for overall iron storage in the body. Generally, ferritin levels are therapeutic in DBA patients at levels between 1,000 and 1,500 ng/mL. These levels are measured every three months and if instability is seen, an MRI is usually ordered to rule out liver and/or tissue damage.[5] Liver and cardiac damage is not uncommon in those receiving multiple transfusions since the excess iron leads to deposits (called hemosiderins) accumulating in liver and cardiac tissue.[9] Shander, Cappellini, and Goodnough reported on a retrospective study in 2009 that studied 152 patients who had a total of 4,875 units of red cells transfused from 1987-1998. The study found that those who demonstrated iron overload had a much higher organ failure and mortality rate than those who did not show elevated ferritin.[10]

Treatment

Leucine is an amino acid that aids in protein synthesis regulation and is a possible future treatment for those suffering from transfusion dependent DBA. Jaako et al in 2012 looked at the effects of leucine in a mouse model who was RPS-19 deficient. Double the amount of leucine found in serum was given in drinking water to the mice. The study concluded that of those given the leucine there was a significant increase in red blood cells as well as hemoglobin concentrations. There appeared to be no adverse side effects.[11] This has huge implications for those with DBA since the disease carries with it so many adverse secondary complications.

CBC		REFERENCE RANGES
WBC	3.1	4.5-11.0 thou/ul
RBC	3.5	4.5-5.9 mill/ul
HGB	10.1	13.5-17.5 gm/dl
HCT	28.6	41.0-53.0 %
MCV	82.7	80-95 fl
MCH	29.1	25-35 pg
MCHC	35.2	25.0-35.0 gm/dl
RDW	18.6	11.5-14.5 %
PLT	300	150-450 thou/ul
NEUT	54	40-70 %
LYMPH	32	15-45 %
MONO	10	1-8 %
EO	3	0-6 %
BASO	0	0-2 %
MORPH	MOD ANISO	
PT	15.4	11.8-15.2 sec
INR	1.18	0.00-1.49
PTT	40.9	22.1-35.7 sec
CHEM		**REFERENCE RANGES**
GLU	116	75-110 mg/dl
Na	137	137-145 mmol/L
K	4.1	3.6-5.0 mmol/L
Cl	104	101-111 mmol/L
CO2	29	21-31 mmol/L
ANION GAP	8.1	7.0-14.0
BUN	12	9.0-21.0 mg/dl
CREA	0.8	0.40-1.30 mg/dl
Ca	8.7	8.5-10.5 mg/dl
TP	6.4	6.0-8.4 gm/dl
ALB	3.7	3.0-5.0 gm/dl
A/G RATIO	1.4	1.0-2.2 gm/dl
GLOBULIN	2.7	1.5-3.8 gm/dl
TBILI	1.2	0.2-1.3 mg/dl
AST	41	5-49 IU/L
ALK	92	38-126 IU/L
ALT	65	7-56 IU/L
LACTIC ACID	13.5	6.5-19.3 mg/dl

Table 1: Patient's CBC profile and coagulation parameters
Lab values: Father

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CBC	PRE-TRANSFUSION	POST-TRANSFUSION	REFERENCE
WBC	2.9	2.3	5-14.5 thou/ul
RBC	3	3.8	4-5.2 mili/ul
HGB	8.2	11.1	11.5-15 g/dl
HCT	24.5	32.1	35-45 %
MCV	82.4	84.7	77-95 fl
MCH	27.7	29.4	25-33 pg
MCHC	33.6	34.7	31-37 g/dl
RDW	16.5	15.5	11.5-14 %
PLT	259	303	150-450 thou/ul
NEUT	33	26	34-56 %
LYMPH	49	55	24-54 %
MONO	11	11	3-10 %
EO	6	7	0-5 %
BASO	1	1	0-2 %
FERRITIN		**REFERENCE**	
14-Aug	2484	23.9-336.2	
27-Jun	2109		
25-Apr	2712		
6-Jan	2180		
19-Nov	1677		
24-Oct	2238		
14-Aug	1907		

Table 2: Patient's CBC profile and coagulation parameters
Lab values: Son

Case study presentation

A 29-year-old man presented to the ER with complaints of fever, chills and coughing. His initial exam showed a temperature of 101.1 and a pulse oximetry of 94 percent. During the course of taking the patients history, it was noted that he has DBA and has had iron overload. His current medications included Exajade taken by mouth daily. A PT, PTT/INR, CMP, CBC were ordered, and Ibuprofen was given for the fever. His CBC results showed low WBC, RBC, HGB, HCT, and lymphocyte levels with elevated RDW and neutrophils. His CMP showed high glucose and ALT. (**Table 1**) His immunohematology records were checked and the patient received a full antigen typing of his red cells, and has since been transfused monthly.

This patient also has two sons, one six and the other five years of age. Both inherited DBA. Their records indicate that they get labs drawn and transfusions on a bi-monthly basis. The oldest son's labs consistently show low RBC, HGB, HCT, and reticulocyte levels with extremely high ferritin levels. (**Table 2**) Both children have now started to show signs of autism, but no correlation has been made with autism spectrum disorder and DBA. The children are responding well to the transfusions and no plans have been made for bone marrow transplants.

Conclusion

DBA is a disease of the bone marrow that causes insufficient red cell production. This case study was about a family that suffers from DBA. The case study was developed to provide an overview of the disease with emphasis on a multi-diagnostic approach to help diagnose and treat this disease. DBA presents differently in every person affected and has a variety of treatments. Unfortunately, all of those treatments as well as the disease itself carries many secondary risks.

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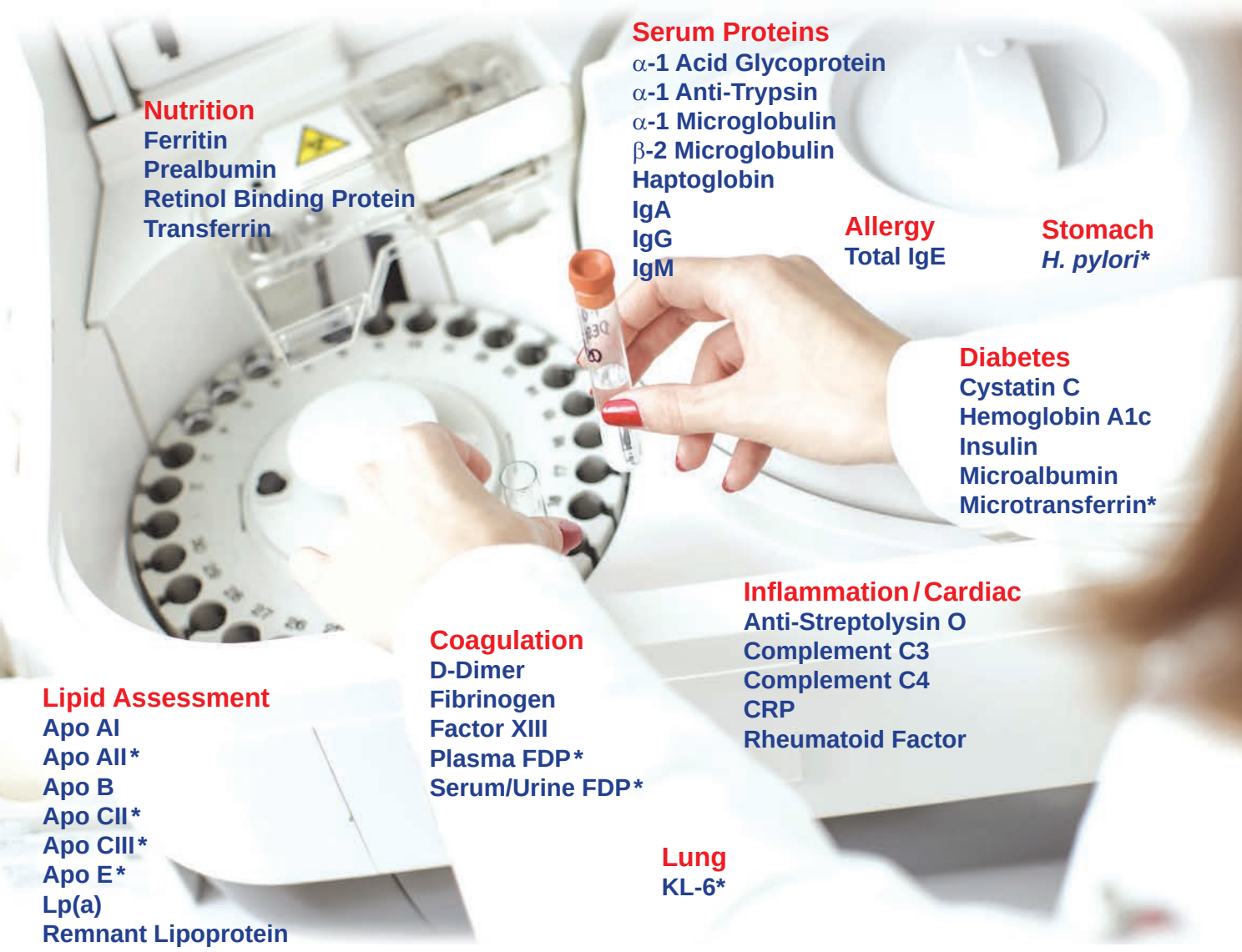

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Quantitative trait loci—uncovering genes for continuously variable traits

By John Brunstein, PhD

In this month's episode, we're going to take a bit of a detour out of front-line molecular testing methods and delve a bit into something more theoretical but with impacts on molecular testing. It's likely something you haven't encountered unless you have a specialized genetics background, and it's also something readers of this column may have wondered about in some form and may find of interest.

Mendelian isn't everything: QTLs

Let's start by considering something even the non-genetics specialists have some familiarity with—that is, simple Mendelian genetic traits. These are phenotypic (physical appearance or behavioral) traits which can be classified in discrete and mutually exclusive bins, like eye color. You might have green, blue, or brown eyes, but each of these is readily distinguishable—it's not like there is a continuous rainbow spectrum of natural human eye colors. Other examples of discrete Mendelian traits in humans would be hairy pinna (earlobes) or widow's peak (hairline). There are many more possible examples of less visibly obvious traits such as enzyme isoforms with discrete Mendelian states having biochemically measurably different behavior. However, for a great many phenotypic traits we actually have a continuous spectrum of outcomes rather than discrete states. Think for example height, or longevity, or resting blood pressure. In fact, a great many of the phenotypic behaviors we'd like to know more about express themselves in this quantitative—as opposed to quantized—form. An example, which probably nobody cares about but which serves illustrative purpose might be, "rate of fingernail growth."

The immediate complexity which springs to mind when we consider these types of measurements is that the end result observed in each individual is based both on genotypic and environmental factors, or "nature and nurture" as it's sometimes called. A second level of complexity arises when we consider that these sorts of traits are likely to be influenced to a larger or smaller degree by multiple genes working in combination (a polygenic trait). Additional confounding issues could also possibly be epigenetic modifications to genes through mechanisms such as base methylation or histone acetylation influencing expression levels, or finally "variable penetrance" which is something of a catch-all term applied in genetics to cases where although a particular allele of a gene has a known effect, the scale of that effect is variable for reasons we don't have a firm grasp on. What we'll discuss in this month's article is "QTL (Quantitative Trait Loci) mapping," the approach taken to help identify genes and their alleles influencing quantitative loci of interest, with strategies to deal with our first and second confounding issues (and quite possibly our fourth issue). Epigenetic modification is a bigger topic and one we'll leave out of the mix for now.

Step 1: Genetic markers and association statistics

Our first step in this puzzle is to have a relatively high density of randomly distributed genetic markers across the genome. These can take the form of any sort of identifiable "tag" which allows us to track its closely physically associated (linked) DNA. Single Nucleotide Polymorphisms (SNPs) are one of the most common type of such tag—single nucleotides at known locations in the genome, which exists in more than one form in the population. We might for instance note one location which is "A" in 70 percent of genomes making up our population and "C" in the remaining 30 percent. It doesn't matter whether this is in a coding region or not (statistically, it probably isn't) or whether the two alleles have any actual physical significance (even more unlikely)—all we care about is we now have a differentiable physical spot in the genome. As genetic recombination is a stochastic process, DNA sections near this marker stay attached to it more frequently than DNA sections further removed. If we have enough of these markers then we can track the movement of fairly small sections of DNA as they reassort through recombination and sexual reproduction to create individual genomes.

With these densely and randomly scattered markers on hand, we can now take sample individuals from our population and essentially do nothing more than look for statistical association of particular marker(s) with our trait of interest—in this case, rate of fingernail growth. We're looking for one or more sections of DNA, whose inheritance seems to track with a measure of our phenotype, such that we can make statements like "With regard to this SNP, we observe faster fingernail growth in 97 percent of 'C:C' genotype individuals as compared to 'A:A' genotype individuals." What we are actually observing is that there is some gene near to our marker, and at some time in the past on a chromosome which carried the allelic form of that gene which contributes to faster fingernail growth, there was a mutation at the nearby SNP transverting an A to C residue. (The temporal reverse is also possible, such that A and C SNP alleles came into existence and then the gene near one of these mutated. The result is the same, a functional gene allele is linked to a detectable marker.) Because these are physically closely linked, recombination between the marker and the allele of interest is rare and the marker now moves around as a surrogate for that allelic variant. Note that 'rare' doesn't mean 'never,'

Sepsis? Septic Shock?

Measure Lactate *Bedside*

New Study Shows Early Lactate Measurement Improves Outcomes for Sepsis

Sepsis remains a leading cause of morbidity and mortality. Because of a strong association between elevated lactate levels and increased mortality, sepsis guidelines call for lactate testing soon after the onset of sepsis. The U.S. Centers for Medicare and Medicaid Services Severe Sepsis and Septic Shock Early Management Bundle (SEP-1) mandates an initial lactate be drawn between 6 hours before and 3 hours after severe sepsis presentation.

,QDQHZ VWXG\ RI SDWLHQWV WKDW PHW 6(3 FULWHULD for sepsis, only about 60% of patients had an initial lactate drawn within the SEP-1 window.[1]

(DFK KRXU RI GHOD\ LQ LQLWLDO ODFWDWH PHDVXUHPHQW ZDV associated with delayed antibiotics and an increase in mortality.[1]

³6\VWHPDWLF HDUO\ ODFWDWH PHDVXUHPHQW ZKHQ D SDWLHQW presents with sepsis may thus be useful in prompting earlier, potentially life-saving interventions."[1]

Fast, Easy Bedside Testing

StatStrip Lactate is a hand-held, true point-of-care system that brings lactate testing directly to the patient's bedside. Lactate is currently measured on blood gas analyzers, which creates numerous problems IRU EHGVLGH WHVWLQJ %ORRG JDV DQDO\]HUV DUH W\SLFDOO\ LQ D ¿[HG ORFDWLRQ 7KH\ UHTXLUH ODUJH VDPSOHV DV PXFK DV PLFUROLWHUV DQG VDPSOH labeling and transport. They have analysis times as long as 3 minutes, DQG DUH H[SHQVLYH WR SXUFKDVH DQG UXQ

As easy as bedside glucose testing, StatStrip Lactate uses a disposable biosensor to provide the fastest turnaround time (13 seconds) on the smallest whole blood sample (0.6 µl) with lab-like accuracy.

[1] 0DUJD $ HW DO ,PSOLFDWLRQV RI &HQWHUV IRU 0HGLFDUH 0HGLFDLG 6HUYLFHV 6HYHUH 6HSVLV DQG 6HSWLF 6KRFN (DUO\ 0DQDJHPHQW %XQGOH DQG ,QLWLDO /DFWDWH 0HDVXUHPHQW RQ WKH 0DQDJHPHQW RI 6HSVLV *Chest*

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and a first statistical value we get is strength of association (here, 97 percent) which also is a surrogate measure of what the actual distance is between the marker and the gene. If we're particularly lucky we may even have two or more genetic markers which demonstrate this sort of linkage association and based on their relative frequencies of association, we can narrow down the general area of DNA the gene likely resides in based on relative closeness to these markers. Luck in this case is greatly increased as marker density increases, of course—high density marker maps make the entire QTL mapping process easier than lower density maps.

More statistics: effect sizes

There is an unrelated second statistic we should look for with our now-linked marker, and that is effect size. Imagine for instance when we looked for markers associated with fingernail growth rate, we found three widely separated markers with statistical relevance. All have one allele which clearly shows accelerated fingernail growth rate, but compared to some baseline reference value, Marker A shows a three percent increase in growth rate, Marker B shows a 22 percent increase, and Marker C shows a seven percent increase. (Strictly speaking the statistics would be more complicated than that, such as having a 95 percent confidence interval range of effect, but we're ignoring these nuances here because they can—and do—fill entire textbooks. There are even multiple completely different statistical approaches to identifying our linked markers. For the sake of brevity, we're avoiding all of that as the basic concepts as presented here remain valid across all these approaches.) These values suggest the relative scale of impact each gene has on the final phenotype.

And the candidate gene is…

Now armed with the knowledge of what regions have the biggest impact on fingernail growth rates, we can proceed (probably from the biggest impact markers to lowest) to look for likely candidate genes. If we find an ORF (open reading frame, a potential protein coding region) near one of our markers, and we find its translated amino acid sequence for example has a high degree of similarity to a known keratin synthase gene, that would make sense and be a good candidate. Our evidence for its involvement would get even stronger if we find this gene expressed as an RNA (or even just a fragment of it, in parlance an EST, Expressed Sequence Tag) telling us that it's an active gene. If we could then find either amino acid variations in this candidate gene, or variances in its RNA expression level, which correlate with our phenotypic observations of fingernail growth rate, we can become increasingly certain that we've found our actual gene contributing to the continuous phenotype. A full proof would then best be done by targeted gene modification in whatever our model organism is for fingernail growth (the genetic equivalent of fulfilling Koch's Postulate, by making the genetic change in an otherwise controlled background and environment and observing the expected effect). If there is no well established model system, then at least cloning of the variant gene forms, in-vitro protein expression, and enzyme kinetics studies can be nearly as helpful by demonstrating that yes, the protein version as coded for by gene found associated to the "C" SNP has faster enzymatic behavior than the version found associated with the "A" SNP. Follow these approaches through on the other identified linked loci and candidate genes to rule them in or out, and we have now fulfilled our lab's lifelong academic ambition of understanding multiple genes influencing fingernail growth rate.

Resolving complexities

We mentioned above that this approach would attempt to address the challenge of "nature versus nurture." Part of this is through the effect size values discussed above; generally, if the genetic effect is big enough, "nature" is more important than "nurture" and an effect is visible even with disparate environmental factors. Other approaches however can include things like linking in family pedigree information, where sibling studies can be done on the hopes that environments will be similar; things like dizygotic twins can be particularly useful here. Alternatively, if there are suspected particular environmental factors, these may be accounted for in collected and paired metadata for each genetic subject. The second complexity—polygenic traits—was directly dealt with above, as we were able to identify multiple loci. The fourth complexity—our fudge factor of "variable penetrance"—to some extent may be explained away once we have our data in hand, as we start to show that all of loci A, B, and C are involved but we don't see all of the impact of a particular allele at A unless we also have a particular allele at C. This is no longer variable penetrance, it's a definable epistatic gene interaction. Finally, although we said we'd ignore the third complexity of epigenetics, it's becoming increasingly possible from a laboratory technical perspective to capture data on things like DNA methylation during sequencing. Analogous statistical approaches to identify differential patterns of epigenetic labeling of particular gene regions correlating to phenotype are of course possible and will likely become more commonplace as the underlying data becomes more commonly available.

Having complete genomes of organisms in orders of magnitude is cheaper and easier than it was only a few years ago. Making sense of all of that information, by understanding how these genes contribute not just to discrete Mendelian traits but to all the complex polygenic continuously variable metrics we can imagine, is the next step in making good use of the data. Hopefully, the forgoing has demystified the process for those of you not already familiar with it. Should it merely have wet your appetite for the subject, there are numerous good up to date texts on the subject available.

John Brunstein, PhD, serves as an Editorial Advisory Board member for MLO. John is also President and CEO for British Columbia-based **PathoID, Inc.**, which provides consulting for development and validation of molecular assays.

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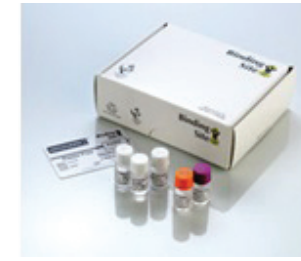

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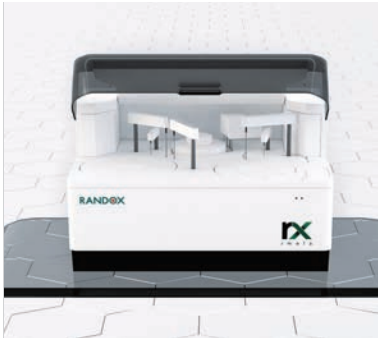

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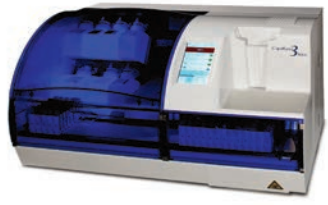

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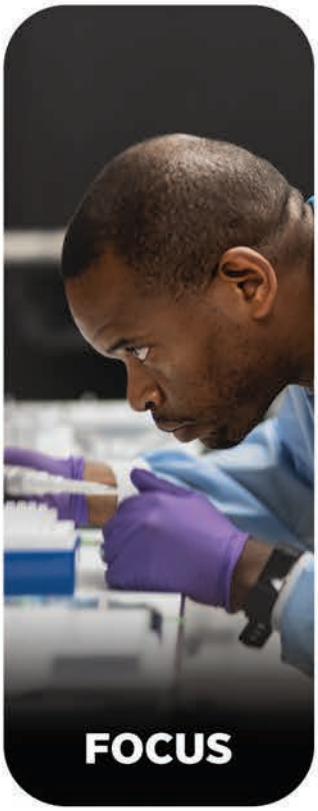

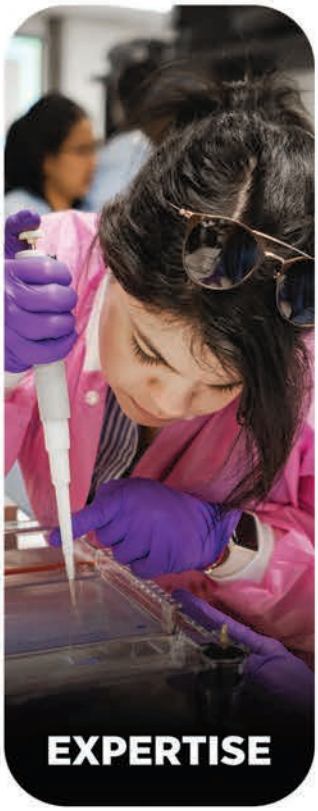

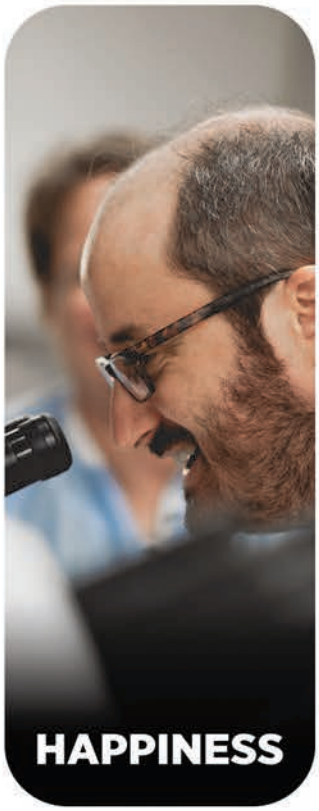

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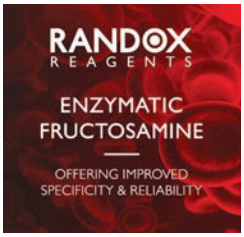

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A conversation with Lior Hod, ELLKAY's Beekeeper, Chief Culture Officer, Founder & President

ELLKAY was founded in 2002. Can you tell us the story behind its foundation? Kamal Patel, ELLKAY's CEO, and I started ELLKAY in the basement of my home in 2002, where we started building demographic bridges for laboratories. It all started with a soda business… I started working with Kamal at a company back in 1997 as Senior Developers and we shared an office. In fact, we still share an office. We thrived working as a team on complex projects. During our time there, the company increased the price of soda from $1.00 to $1.10. We decided to put a small fridge in our office and fill it with sodas. We advertised 50¢ sodas and everyone in the company started coming to our office for them. We added other items: water, candy, chips, ice cream, and free watermelon on Thursdays. We made $3,000 profit that year… all of which went to charity. We loved it and it felt great. So, we decided to start our company with the same energy and culture as our little "side venture." My wife Janet came up with the company name ELLKAY after our first name initials ELL for Lior and KAY for Kamal.

What kept you occupied prior to 2002? I started my career in the early '90s in the clinical laboratory testing space as a consultant for a company called MetPath Laboratory (known today as Quest Diagnostics). During my time at MetPath, I helped design and build one of the first electronic lab order and results management systems, called CCLink. I then went to the 3M Health Information Division, and they were just starting out with healthcare. I was one of the lead developers for their All Patient Refined Diagnosis Related Groups (APR DRG) solution.

How did the acquisition of the CareEvolve Laboratory Portal and Connectivity Platform benefit ELLKAY and its customers? CareEvolve was a great fit for ELLKAY—both companies have a high focus and strength in technology and innovation. While ELLKAY already had a good footprint in the market before the acquisition, this allowed us to offer more advanced laboratory outreach solutions to those markets and expand our level of service and partnership in the hospital market. More importantly, it increased our whitespace for all our other product lines (non-lab) in the hospital market. I feel it was a good transaction for both sides.

Please explain how ELLKAY is a "client-first" company. At ELLKAY, we are very proud of our 'client-first' focus. What it means is that we value our clients and recognize that strong relationships are the foundation for a strong company. Our customer-centric approach drives results. We understand that every business is different and there is no 'one size fits all' model, therefore we work with our clients to identify the best strategy for their success, while providing innovative, scalable solutions, and unparalleled service.

Your title has Chief Culture Officer in it. What does this mean at ELLKAY? I take this title very seriously. I view my life as one long journey, forming friendships along the way. Due to ELLKAY's growth, we purchased our new building in 2017. We moved from a 13,000-square-foot building to one with 74,000 square feet. I personally participated in designing and building the best workplace I could think of. We went with an open floorplan design to encourage collaboration and innovation, glass office partitions, standing corner desks, LED lights, gaming chairs, and a whole lot more.

Since the time we started the business in my basement, we have always paid for lunch for our employees. We also provide cold drinks, the best coffee, bagels, fresh fruits, fresh-baked cookies, ice cream, and beer on tap. I like to create an environment that rewards the hard work of our employees. I believe that in creating an atmosphere that celebrates their dedication, it encourages them to not only have fun, but also strive for excellence.

Receiving ELLKAY honey is always a pleasant surprise! I heard you tend honeybees on the roof of ELLKAY's HQ. Tell me how this all started. In early 2015, Kamal and I were driving for a meeting with our product design team. That morning, I had happened to read an article in the *New York Times* about how the honeybees were dying throughout the country. With it fresh in my mind, I brought up the subject and mentioned how I had always wanted to raise honeybees and harvest the honey for our friends and clients. The group loved the idea, so I contacted my beekeeper friend and the process started.

We owned our building, so we were able to set up six hives (which initially housed 36,000 bees) on our flat roof. Everyone volunteered to build the hives and care for them and within months, the bees multiplied to more than 300,000. When we moved offices, we also (carefully) relocated the hives and its residents. We now have 16 active hives and we produce and package our own honey on premises. The honey demand is so high that we are in the process of expanding to a remote location in upstate NY.

Besides tending to the honeybees, what do you like to do off-the-clock? My passion is basketball. We are a big basketball family; I played college ball with my brother at Yeshiva University and we are still currently ranked as No. 2 and No. 3 for scoring. I am also blessed that my three boys also played for the same school. In fact, all three played in the same game in 2017, marking the first time in NCAA Division III men's basketball history that three siblings from the same team were on the floor at the same time. I am a proud grandfather to six-month-old twins. I'm also fortunate that two of my children work with me at ELLKAY, so I get to spend time with them and mentor them regularly.

Read the full interview at mlo-online.com.

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