PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
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AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Greetings to all! I hope 2023 has greeted you warmly and you are remaining safe and healthy.
Though it is early in the calendar year, division leadership has begun planning for the 2023 AACC Annual Scientific Meeting. The PMF leadership group worked to submit several division-sponsored proposals with the hope that at least one will be accepted for the event. This is also a great time to remind you that the due date for abstracts is fast approaching, February 16th. If you are planning to submit an abstract, please consider to throwing your hat into the ring for the PMF Division Poster Awards.

This issue of the division newsletter continues in its journey through The ABC's of Pediatric Laboratory Medicine as we explore the letter "K". The featured title is "K is for Kleihauer-Betke test: A brief review" authored by Abhinav Grover, MBBS, MD, MS and Laura Smy, PhD, MLS (CSMLS), DABCC. There's also a well-written Excerpt from the Literature addressing universal pediatric lipid screening brought to us by Stephen Roper, PhD . And lastly, we feature an Interview with a Distinguished Colleague, our 2022 awardee for Outstanding Contributions to Pediatric and Maternal-Fetal Clinical Chemistry, Nathalie Lepage, PhD from the University of Ottawa.

This is my first newsletter as Chair of the division. I am the honored recipient of all the hard work the preceding Chairs have accomplished for this division. I would like to specifically acknowledge Angela Ferguson, PhD for her leadership, patience and support, and hope that I am able to carry on these valuable characteristics throughout my term.

Sincerely,

Stanley F Lo, PhD
Chair, AACC PMF Division

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THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

K is for Kleihauer-Betke test: A brief review

Abhinav Grover, MBBS, MD, MS

Resident, PGY-3
Pathology and Laboratory Medicine
Medical College of Wisconsin

Laura Smy, PhD, MLS (CSMLS), DABCC

Assistant Professor

Co-Director Clinical Chemistry/Toxicology, Wisconsin Diagnostic Laboratories

CLIA Laboratory Director, Froedtert Community Hospitals – New Berlin, Pewaukee, Oak Creek, Mequon
Medical College of Wisconsin

Fetomaternal hemorrhage (FMH) is a complication of pregnancy and defined as the transplacental passage of fetal blood into the normally separate maternal blood. As a result, the fetal blood, containing a high percentage of fetal hemoglobin (HbF) in the red blood cells, mixes with maternal blood and may need to be quantified by the Kleihauer-Betke (KB) test.
The cause for FMH is a disruption in the placental barrier that can occur for several reasons, for example, altered placental microarchitecture, abdominal trauma, ectopic pregnancy, or preeclampsia [1, 2]. Although FMH can happen as early as 4th week of gestation, it occurs more commonly after 12 weeks' gestation, when the uterus rises above the pelvis and becomes susceptible to direct trauma. Massive FMH exposes the mother to possible Rhesus incompatibility but also imperils the fetus as it causes severe fetal anemia or fetal distress [1], and possible exsanguination. A minimal amount of exposure, 0.01 mL to 0.03 mL of FMH, is sufficient to cause isoimmunization of the mother, such as activation of the maternal immune system of a Rhesus-D protein (RhD) negative mother carrying an RhD positive fetus [3]. Isoimmunization may result in maternal formation of anti-RhD antibodies generating the risk of RhD disease, a type of hemolytic disease of the fetus and newborn, in future pregnancies if the fetus is RhD positive. These maternal antibodies bind to RhD positive erythrocytes of the fetus that may set off a series of events for the fetus including hemolysis, anemia, hydrops fetalis, and possibly death. Administration of Rh-immune globulin (RhIg) is used to prevent the formation of the maternal antibodies [3].

Upon delivery, the dose of RhIg to administer to the mother must be determined. The rosette screen may be performed first. A small amount of FMH is not sufficient to cause a positive rosette screen. In cases where a significant volume of fetal blood has entered the maternal circulation, a positive rosette screen indicates that a quantitative test, such as the KB test, is required to determine whether the bleed was sufficient to warrant a larger dose of RhIg [4].

The Kleihauer-Betke (KB) test can quantify the level of FMH to determine the RhIg dose needed. KB testing can also aid in the management of several other obstetrical conditions such as fetal demise and preterm labor. Traditionally, major trauma in Rh negative pregnant patients has been the only indication for the KB test although what constitutes major trauma has not been well defined in the literature [3]. There are other factors outside of magnitude of trauma or blunt force that can increase the risk of FMH including anterior placental location and coagulopathies [3]. Further, a positive KB test was reported to accurately predict the chance of preterm labor subsequent to trauma, whereas clinical evaluation did not [5]. The association with risk of preterm labor has prompted various authors to

suggest routine use of the KB test in pregnant trauma patients, irrespective of Rh status and the method or magnitude of the trauma [5]. The result, in these cases, is used more for guiding management and prognosis [3].

The basis of the KB test is that HbF is resilient to acid elution, whereas adult hemoglobin is not. Therefore, this test is also referred to as the Kleihauer Betke Acid Elution test. The result is fetal cells are stained bright pink and maternal cells pale pink (Figure 1), which are manually counted using a microscope to determine the percentage of fetal cells. The percentage of fetal cells is used to calculate the dosage of RhIg to be administered to prevent sensitization to the "D" antigen. The dose is based on the following calculations [3].

Volume (mL) of fetal blood = Percentage of fetal cells x 50

Number of Vials of 300 mcg RhIG required = Volume of fetal blood/30 mL

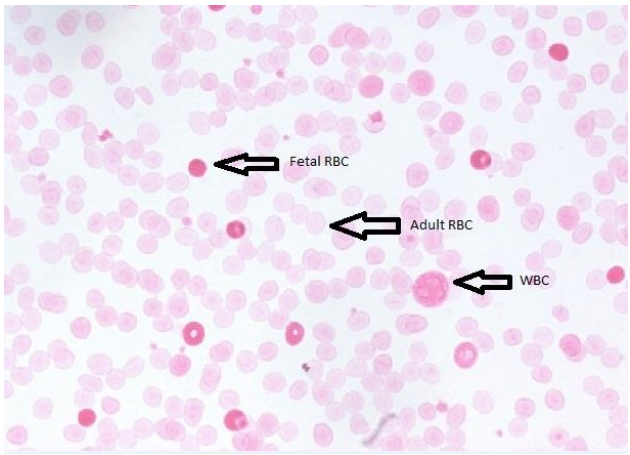

Figure 1: Microscopic depiction of KB test that shows fetal cells, adult cells, and a neutrophil white blood cell (WBC). (Figure modified from [6]).

Aside from the inherent variability in manual methods, which also apply to the KB test [7], reasons for overestimation of FMH using the KB test include F-cells and lymphocytes. Fetal red cell contains predominantly HbF while an F-cell contains a mixture of HbF and other types of hemoglobin. Therefore, F-cells can lead to erroneous interpretations of the KB test in pregnant women with conditions associated with increased HbF, such as hereditary persistence of fetal hemoglobin [8]. More specific technical methods, such as flow cytometry, may be required to quantitate the FMH in these patients, but these technologies may not be readily available [4, 9]. Lymphocytes can also resemble fetal red cells, but the lymphocyte may be differentiated by its granularity, slightly larger size, and visualization of the nucleus [10]. Also, there are several limitations of the KB test. An ABO incompatibility between mother and fetus may conceal a large transplacental hemorrhage [7, 8]. Additionally, weak D testing should be performed on samples from the mother who tests positive for the rosette screen but negative for the KB test [7]. Because of the limitations and variability of the KB test, results should always be interpreted in the context of clinical findings [7] and adding one additional dose of RhIg has been recommended to ensure adequate dosing [4].

In conclusion, the KB test is an adequate test to estimate the amount of FMH and is still widely used [7, 9]. However, alternative methods for detecting FMH have been tried, including gel agglutination, the enzyme-linked antiglobulin test (ELAT), or flow cytometry [11], and institutions are transitioning towards a primary flow cytometric approach for quantification of FMH, which may reduce RhIg over-administration [9].

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Excerpts from the Literature

Stephen Roper, PhD
Associate Medical Director of Pediatric Laboratory Services, St. Louis Children's Hospital

Associate Professor of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO

Universal Lipid Screening in Children: Time for a checkup

In 2011 the National Heart Lung and Blood Institute (NHLBI) released a summary report on cardiovascular disease (CVD) risk reduction in children and adolescents [1]. Consistent with earlier guidelines, the 2011 document advocated for lipid testing of at-risk children as early as age 2, promoted lifestyle change/nutritional management as first-line intervention, and provided age-specific lipid goals. In a controversial change from past guidelines, the 2011 report also introduced a recommendation for universal lipid screening (ULS) starting between ages 9-11. The rationale being that a significant number of children with dyslipidemia are missed when providers rely on a targeted approach based on obesity, family history, or other criteria. ULS was further justified by pointing out that this strategy would likely improve early detection of relatively common genetic conditions (e.g. familial hypercholesterolemia) and secondary hyperlipidemias that predispose to accelerated CVD. However, the NHBLI report did not comment on practical challenges ULS might introduce; like pediatrician comfort identifying/treating dyslipidemia, parental willingness to participate, access to testing, or the logistics of follow-up/referrals for abnormal results. Fast forward a decade and studies examining provider attitudes towards ULS, as well as the impact of screening, have identified barriers that are keeping these recommendations from reaching their full potential [2, 3].

A recent study in Clinical Pediatrics investigated ULS practices during well-child visits in a single academic clinic system from 2014-2017 [3]. This retrospective, observational study sought to define the proportion of abnormal lipid results obtained in 9–11-year-olds and examine provider follow-up behavior immediately after a 1 year effort to increase screening. Using electronic medical record review, the researchers queried demographic and clinical information, as well as lipid results, in 1,039 children. They analyzed associations between lipid orders and results versus body mass index (BMI), patient ethnicity, health insurance, age, family history, and disposition of follow-up. The authors observed a relatively high rate of lipid panel orders (n=719, ≈69%) during well-child visits, however the rate of test completion was drastically lower (n= 343, ≈33%). Examination of variables associated with testing status revealed no relationship to BMI, family history of CVD, gender, or type of insurance, yet some ethnicities were significantly more likely to complete testing. Of those individuals that

followed through (n=343), non-fasting specimens were predominant (≈80%) and the overall rate of dyslipidemia (defined as any abnormality on an initial or follow-up fasting lipid panel) was approximately 10% (n=35). The only patient characteristic significantly associated with dyslipidemia was obesity, however a substantial proportion of individuals without family history of CVD or elevated BMI had abnormal results. Examining provider follow-up behavior, most children with dyslipidemia received nutrition counseling/weight management resources and/or dietician referral. More than 2/3 of these patients returned for a follow-up visit over the study period, yet only 34% (n=12) had another lipid panel ordered during that time.

This study and others demonstrate unanticipated challenges to ULS adherence and highlight areas where clinical labs may be able to help [4, 5]. First, pediatric provider awareness of NHLBI recommendations. Even after the yearlong effort to increase screening in this study, only 69% of eligible children had a lipid panel ordered. Pediatric labs may be able to support guideline compliance through educational activities, building reflex orders to assist with suggested follow-up testing, and provide comments to aid with result interpretation [2, 6]. Second, promoting parental/child willingness to participate in ULS. Well child visits occur primarily in clinic locations, some of which may not have a central lab nearby. Waived, capillary whole blood point of care (POC) devices are available and NHLBI guidelines allow for the use of non-fasting specimens for initial screening. Although POC lipid measurement is not as accurate as central lab methods, having this option available on-site may decrease parental/child anxiety related to phlebotomy, allow for real-time decision-making, and increase the likelihood that testing is completed. Finally, labs may be able to improve systematic lipid screening by promoting the availability of add-on testing for lipids from blood obtained for other routine lab studies. For example, building electronic medical record notifications to alert providers of ULS recommendations and offering the addition of a lipid panel in 9–11-year-olds.

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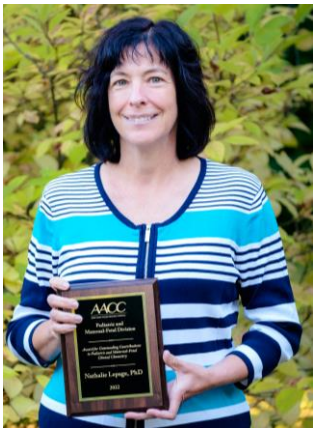
Interview with a Distinguished Colleague: 2022 Award for outstanding contributions to pediatric and maternal-fetal clinical chemistry

By Stanley F Lo, PhD



Nathalie Lepage, PhD, FCCMG, FCACB

Laboratory Head, Biochemistry – Newborn Screening Ontario
Children's Hospital of Eastern Ontario

Full Professor, Department of Pathology and Laboratory Medicine, University of Ottawa

When you reflect upon your professional career in clinical chemistry, do you have any words of wisdom for those beginning their own career?

I am proud that so many younger fellows have chosen the wonderful field of clinical chemistry. Welcome!

The field of clinical chemistry includes an enormous number of sub-specialties. Obviously, the priorities are analytical excellence and consultation for interpretation of laboratory results. But a career in clinical chemistry entails so much more. What makes this field so wonderful is all the skills that one can develop. It could be related to the use of technologies, quick examples are the use of laboratory automation for pre-, analytical, post-; the utilization of artificial intelligence for post-analytical designs; the integration of samples collected on filter paper to help coordinate self-collection, etc. It could be communication to a wide variety of audiences, including the peers, the clients of our laboratories, the general public, the decision-making authorities. It could be mastering the quality assurance aspects of the laboratories, including accreditation, regulatory, continuous improvement initiatives, and maintenance of competency. It could be fruitful involvement in research. The laboratory is a prime example where new markers, new analytical platforms, new interpretative algorithms have direct impact on patient care, morbidity and mortality.

Fellows-in-training need to design their training rotations to ensure exposure to all aspects of clinical chemistry. After completion of your fellowship, keep your habits and be a keen learner throughout your career. All of us need to be involved at the local, regional and national levels early in our career. Building and maintaining a network of colleagues will be an asset each of us will always cherish. Whenever a collaboration is proposed, ensure you assess its implication and potential long-term benefits. Once you are involved, be passionate about the projects and become an advocate. Colleagues will participate and help with completion of tasks.

With the COVID-19 pandemic, what has been the most significant changes to your practice?

COVID pandemic was challenging as many of our loved ones passed away too rapidly. However, to my surprise, the COVID pandemic brought positive changes. In our laboratory, there were hypothetical procedures for Emergency Preparedness. Once the pandemic was upon us, it was clear that the plan did not match the reality. Hence a sustainable Emergency Preparedness plan has been designed and would be available, should the need arise again in the future.

One major change in our department has been the implementation of a working from home policy. This change provided means of communication, much more efficient that the old “pager”. It also created collaborative virtual tools that have direct impact of team efficiency. It also increased awareness that on-site interactions and ad-hoc discussions should be nurtured. A “corridor” discussion with a colleague has always potential to become a continuous improvement project.

On a personal note, I realized that the work-life balance is essential. At beginning of working from home, it was difficult to have defined working hours as the office is just a door away. With trial and error, I was able to incorporate a calendar for physical fitness. I am an avid runner, hence I now have, booked in my calendar, two evenings and one 90-minute week-end time out blocked for this activity. One suggestion is that each of us should be passionate for a limited number of extra-curricular activities and have time committed to embrace them.

In the future, what sort of changes are you expecting?

It is always difficult to predict, but there are hints that some changes will continue to take place in clinical chemistry. Population screening and prevention should be offered throughout the life span, rather that scattered at some age intervals. Diversity, equity and inclusion will be at the forefront of laboratory medicine, rather than being a goal to achieve. Laboratory automation for unique populations, including pediatrics, will become available. Analytical methods will allow non-invasive specimens (saliva, urine and/or whole blood samples on filter papers) as recommended and approved specimens. Molecular genetics will continue its integration into primary care in health. Artificial intelligence will be incorporated where clinical skills and interpretation are required, to bring other dimensions to the pattern recognition. Unmanned aerial vehicles will become additional tools for specimen transportation. A unique electronic chart will be available for all of us from coast to coast, instead of multiple limited electronic charts managed by several regional jurisdictions. One mundane example that should be mentioned is that faxes will be discontinued!

Exciting times are waiting for us. We should ensure our field has great visibility and continues to attract the resourceful younger generations.

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