PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
Volume 40 | Issue 1 |March 2021

FROM THE MIND OF THE CHAIR

Happy March to everyone!

Spring is just around the corner, and hope is in the air as more and more people get vaccinated. I am crossing my fingers that things continue to improve, and we can meet in person at the next AACC Annual Meeting, which was just moved to Atlanta.

Speaking of the Annual Meeting, don't forget that poster abstracts are due April 7th. If you are eligible for either of the two PMF Division Poster Awards, please check the box to indicate you would like to be considered. Our Division members continue to present outstanding scientific research, and we enjoy rewarding their endeavors.

If you are looking to become more involved in AACC, please check out the opportunity to volunteer on one of AACC's five core committees. Many committees are looking for new members, and information about each committee can be found on AACC.org.

In this issue of the newsletter, we discuss G in The ABCs of Pediatric Laboratory Medicine, and G is for Glomerular Filtration. Excerpts from the literature highlights an article on machine learning in the biochemical genetics laboratory. In our Interview with a Distinguished Colleague, we talk with Dr. Shannon Haymond, a past Chair of the PMF Division and the next President-Elect of AACC.

Angela Ferguson, PhD
Chair, AACC PMF Division

Table of Contents

THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

(G)lomerular Filtration

Emily L. Gill, PhD
Clinical Chemistry Fellow
Children's Hospital of Philadelphia
University of Pennsylvania

Measuring Glomerular Filtration Rate

Measured glomerular filtration rate (mGFR) provides an accurate patient specific value for assessing kidney function in both adult (> 18 years of age) and pediatric populations. To achieve an accurate mGFR, clearance markers specific to glomerular filtration are used, including inulin, inhexol and iothalamate. These markers are filtered solely via the glomerulus and are not secreted or reabsorbed by the renal tubules, synthesized or metabolized by the kidneys making them ideal for GFR measurement studies. The rate at which inulin is filtered by the glomerulus is defined by GFR x P_{in} and is equal to the rate at which inulin is excreted, U_{in} x V (equation 1), where P is the plasma concentration of inulin, U is the urine concentration of inulin and V is the volume of urine collected per set time often reported out as mL/day or L/day (equation 2) (1). GFR is measured in mL/min per the accepted body surface area of 1.73 m^2.

GFR (mL/min per 1.73 m^2) x P_{in} (mg/dL) = U_{in} (mg/dL) x V (mL/min) (1)

GFR (mL/min per 1.73 m^2) = (U_{in} (mg/dL) x V (mL/min)) / P_{in} (mg/dL) (2)

Renal inulin clearance provides an accurate measure of GFR; however, the need for a constant IV to achieve uniform levels along with the need for urinary catheterization in pediatric patients (especially in very young children who are not yet toilet trained) means that the procedure is both complex and time-consuming, making it impractical for routine clinical practice. Furthermore, this strategy presents a greater risk to patients as there is always a small risk of infection when placing a catheter. That said even in adults a 24-hour urine collection can be very challenging as patients will over- or under-collect and therefore catheterization may become necessary to achieve accurate results. Moreover, patients with bladder dysfunction are often unable to completely empty their bladder to provide a timed sample.

Estimating Glomerular Filtration Rate

Alternatively, there are many equations available for estimating glomerular filtration rate (eGFR). These equations utilize markers that are maintained at a constant concentration in serum to directly estimate kidney function. Creatinine a waste product of muscle metabolism is the most commonly used marker for eGFR with measurements traceable to isotopic dilution mass spectrometry (IDMS). Unfortunately, in pediatrics the lowest International Federation for Clinical Chemistry (IFCC) standard is 1 mg/dL, which is significantly higher than normal range for infants and children (1) as well as for patients with decreased muscle mass (e.g., oncology patients, patients with muscular dystrophy, patients with anorexia etc.). Creatinine is not used for mGFR because it undergoes tubular secretion (~10%) as well as glomerular filtration. Therefore, the resulting value is often an overestimate of mGFR. However, given that it should theoretically be maintained at a constant concentration in body fluids it is an ideal candidate for estimation equations.

The accepted equations used for calculating eGFR are different for adults (> 18 years old) and infants/children. Pediatric eGFR equations often utilize creatinine, cystatin C or a combination of the two markers. The most common creatinine-based equation in pediatric nephrology is the Bedside Schwartz equation which was updated in 2009 (2) (equation 3). The original Bedside Schwartz equation (equation 4) used k values based on age and sex where k is 0.33 for infants with low birth weight < 1 years old, 0.45 for infant at full term < 1 years old, 0.55 for children or adolescent girls and 0.70 for adolescent boys (3,4).

$$\text{eGFR (mL/min per 1.73 m}^2\text{)} = (0.413 \times \text{height (cm)})/\text{Scr (mg/dL)} \quad (3)$$

$$\text{eGFR (mL/min per 1.73 m}^2\text{)} = (k \times \text{height (cm)})/\text{Scr (mg/dL)} \quad (4)$$

The simplicity of the Bedside Schwartz equation means that it is ideal for bedside applications. Furthermore, it was validated using the creatinine enzymatic assay traceable to IDMS. The original Bedside Schwartz equation was developed and validated using the original Jaffe assay (alkaline picrate colorimetric assay, prior to IDMS standardization) (5). However, the Jaffe assay often falsely elevates serum creatinine levels due to interfering chromogens which are more likely to cause an issue in infant and child samples due to them having lower creatinine concentrations (6). The creatinine enzymatic assay which is more specific than the Jaffe assay and traceable to IDMS is becoming more common. Creatinine measurements performed using IDMS traceable enzymatic methods used with the original Bedside Schwartz equation will lead to an overestimate of eGFR (7). For this reason, the updated Bedside Schwartz equation has become the new gold standard creatinine-based equation.

Another marker that is less influenced by muscle mass is cystatin C. Cystatin C is freely filtered by the glomerulus but unlike creatinine is not secreted by the renal tubules, instead it is reabsorbed and metabolized. Therefore, the concentration of cystatin C during healthy kidney function should be maintained at a steady concentration in serum. It is therefore ideal marker for confirmatory eGFR in pediatric patients who have an unstable muscle mass. The univariate cystatin C-based equation was introduced in 2012 (8) (equation 5).

$$\text{eGFR (mL/min per 1.73 m}^2\text{)} = 70.69 \times [(\text{cystatin C (mg/L)}^{-0.931}] \quad (5)$$

Unlike the updated Bedside Schwartz equation, the cystatin C-based equation was developed using an unstandardized assay for measuring cystatin C. In 2010 an IFCC standard for cystatin C was introduced (9). Schwartz et al. recently recalibrated the cystatin C Siemens nephelometry assay using the new IFCC standard material (10). However, currently cystatin C measurements are more costly than creatinine and laboratories offering this testing is not as widespread as creatinine.
Although these univariate equations are accepted, multivariate eGFR equations that combine both markers have been proven to provide a better eGFR measurement (11). The CKiD Schwartz equations of 2009 (2) (equation 6) and the updated CKiD Schwartz equation of 2012 (8) (equation 7) contain variables for height and weight and incorporates creatinine, cystatin C and blood urea nitrogen (BUN).

$$\text{eGFR (mL/min per 1.73 m}^2\text{)} = 39.1 \times [\text{height (m)} / \text{Scr (mg/dL)}]^{0.516} \times [1.8 / \text{ScysC (mg/L)}]^{0.294} \times [30 / \text{BUN (mg/dL)}]^{0.169} \times [1.099^{\text{male}}] \times [\text{height (m)} / 1.4]^{0.188} \quad (6)$$

$$\text{eGFR (mL/min per 1.73 m}^2\text{)} = 39.8 \times [\text{height (m)} /\text{Scr (mg/dL)}]^{0.456} \times [1.8 / \text{ScysC (mg/L)}]^{0.418} \times [30 / \text{BUN (mg/dL)}]^{0.079} \times [1.076^{\text{male}}] [1.00^{\text{female}}] \times [\text{height (m)} / 1.4]^{0.179} \quad (7)$$

BUN only appears in multivariate equations in combination with creatinine and cystatin C. This is because BUN is a waste product of protein breakdown whereby amino acids undergo deamination to form ammonia, which enters the urea cycle. It is therefore heavily influenced by protein intake and also dependent on liver function and metabolic rate. It is therefore not an ideal standalone marker for eGFR

calculations when compared to creatinine and cystatin C.

The CKiD 2009 equation is recommended by current national guidelines. However, in situations where the creatinine concentration is unreliable a confirmatory estimation using the cystatin C-based equation could be beneficial providing that both equations agree to within ±15% (11). If the values fall within 15% of each other proceeding with the multivariate CKiD equation is ideal (11). There may be cases, however, where a discrepancy between the two markers occurs. Low creatinine with increased cystatin C is observed in patients with chronic kidney disease (CKD), low muscle mass, increased cystatin C production (hyperthyroidism) and following dialysis (12). Elevated creatinine with low cystatin C is observed in patients with increased muscle mass/creatinine supplementation, decreased cystatin C production (hypothyroidism) and recirculation of urinary creatinine (12).

When choosing an eGFR equation it is important to consider how it was validated and the details surrounding the population it was validated on to get an idea of its expected performance when it is applied in routine practice. The Chronic Kidney Disease in Children study cohort was used to validate both the updated Bedside Schwartz equation and the CKiD Schwartz equation. The validation population was aged between 1 – 16 years old with CKD (eGFR 15 – 75 mL/min per 1.73 m^2) and used a standardized serum creatinine assay traceable to IDMS (11). Cystatin C was measured using turbidimetry in the 2009 CKiD Schwartz equation and nephelometry in the 2012 updated CKiD Schwartz equation. Therefore, these equations may not provide an accurate estimation in mild CKD patients or individuals with normal kidney function. Also, this equation has not been validated in the 16-18yr age group and neonates.

Accuracy and precision are extremely important for the early detection of acute kidney disease (AKI), monitoring medication related nephrotoxicity, staging CKD and monitoring progression for classification and transplant among others. Moving forward pediatric eGFR calculations should be validated for all levels of kidney function, include multiracial populations and cover the full age spectrum (≤ 18 years old). It is also important to consider the changeover between pediatric and adult equations at the age of 18-year-old. Full age spectrum equations (13,14) have emerged recently in a bid to address biases in the latter issue, however there is still much work to do to ensure that eGFR values provide a true representation of kidney function.

References

1. Schwartz GJ, Work DF. Measurement and Estimation of GFR in Children and Adolescents. J Am Soc Nephrol, 2009, 4, 1832–43.
2. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New Equations to Estimate GFR in Children with CKD. J Am Soc Nephrol. 2009, 20, 629–37.
3. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr. 1984, 104, 849–54.
4. Schwartz GJ, Gauthier B. A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr. 1985, 106, 522–6.
5. Heinegård D, Tiderström G. Determination of serum creatinine by a direct colorimetric method. Clinica Chimica Acta. 1973, 43, 305–10.
6. Küme T, Sağlam B, Ergon C, Sisman AR. Evaluation and comparison of Abbott Jaffe and enzymatic creatinine methods: Could the old method meet the new requirements? J Clin Lab Anal. 2018, 32, e22168.
7. Srivastava T, Alon US, Althahabi R, Garg U. Impact of Standardization of Creatinine Methodology on the Assessment of Glomerular Filtration Rate in Children. Pediatr Res. 2009, 65, 113–6.
8. Schwartz GJ, Schneider MF, Maier PS, Moxey-Mims M, Dharnidharka VR, Warady B, et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. Kidney Int. 2012, 82, 445–53.

9. Grubb A, Blirup-Jensen S, Lindström V, Schmidt C, Althaus H, Zegers I. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. Clin chem lab med. 2010, 48, 1619-21

10. Schwartz GJ, Cox C, Seegmiller JC, Maier PS, DiManno D, Furth SL, et al. Recalibration of cystatin C using standardized material in Siemens nephelometers. Pediatr Nephrol. 2020, 35, 279–85.

11. Mian AN, Schwartz GJ. Measurement and Estimation of Glomerular Filtration Rate in Children. Adv Chronic Kidney Dis. 2017, 24, 348–56.

12. van Roij KGE, van der Horst HJR, Hubeek I, van Wijk JAE, Bökenkamp A. Discrepant Results of Serum Creatinine and Cystatin C in a Urological Patient. Clin Chem. 2017, 63, 812–4.

13. Pottel H, Hoste L, Dubourg L, Ebert N, Schaeffner E, Eriksen BO, et al. An estimated glomerular filtration rate equation for the full age spectrum. Nephrol Dial Transplant. 2016, 31, 798–806.

14. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, Dalton RN, Dubourg L, Gaillard F, Garrouste C, Grubb A, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate: A Cross-sectional Analysis of Pooled Data. Ann Intern Med. 2020

Excerpts from the Literature

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

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

Machine learning in the Biochemical Genetics Lab

Quantitative measurement of plasma amino acids (PAA) is important for detecting, confirming, and following several inborn errors of metabolism (IEM). The biochemical genetics labs (BGL) that offer PAA and other metabolic tests typically provide interpretations to aid with result comprehension and decision making. Training and competency in this service are usually acquired "on the job", rather than as a part of routine lab medicine training. For new BGL directors, or labs with heavy workloads, this can be a time-consuming task. As such, having automated clinical decision support tools to assist with PAA pattern recognition/interpretation selection is an attractive possibility to increase productivity.

The September 2020 issue of *Clinical Chemistry* includes a study titled: A Machine Learning Approach for the Automated Interpretation of Plasma Amino Acid Profiles by Wilkes et al (1). This study, examining the performance of 3 machine learning algorithms to identify disorders/conditions from authentic PAA results, was performed by researchers from Imperial College Healthcare in London, UK. The authors began by compiling 2,392 previously released PAA profiles, supplemented with select results from relatively uncommon IEM. Each result/interpretation was re-reviewed by two board-certified clinical biochemists and data was filtered so that only one profile per individual was included. Profiles with missing values were removed and individual amino acid measurements falling outside of the assay's linear range were transformed to absolute values. The remaining 2,084 profiles were imported into R statistical software for organization, training, and validation of the 28 included features (sex, 22 individual AA, and 5 AA ratios). Notably, 69% of the profiles included represented "no significant metabolic abnormality", whereas the remainder were classified as diagnostic for various IEMs or representative of other conditions ("abnormal").

The machine learning (ML) algorithms tested were all freely available forest-based approaches: random forests (RF), weighted-subspace random forests (WSRF), and extreme

gradient boosted trees (XGBT). Nested, stratified *k*-fold repeated cross-validation was applied to estimate the accuracy of each algorithm for interpreting results not included in the original data set (2). This type of cross-validation is ideal for tuning hyperparameters from an imbalanced data set (i.e. optimizing performance to ensure accurate identification of underrepresented IEM/conditions). Following model evaluation, the authors tested each algorithm's ability to differentiate abnormal profiles from those without significant abnormalities. Precision recall area under the curve (PRAUC) ranged from 0.921 (RF) to 0.953 (XGBT) and no significant improvement was achieved by including down-sampling (to further account for the imbalanced data set) and/or Bortuga feature selection (to reduce noise). PRAUC is a measure of the positive predictive value (precision) at various sensitivity (recall) thresholds. This is an appropriate metric for ML evaluations when the question of interest is binary, the data set is imbalanced, and there is interest in defining the performance of a model to identify underrepresented classes (abnormal results).

The next question addressed was: how well does each algorithm perform at identifiying individual IEM/conditions? This was achieved by applying the same cross-validation approach, applied to abnormal profiles only, and calculating macro-averaged F4 scores. Similar to binary discrimination, XGBT (0.776) was the most accurate while RF was not far behind (0.758). Feature selection with XGBT revealed that all data elements, except sex, contributed to recognition of individual IEMs. Finally, the authors evaluated how applying all three models, in concert, influenced class discrimination. This ensemble approach was performed by analyzing PAA results using all 3 algorithms, calculating the mean probability for all possible interpretations from the 3 classifiers, and assigning the interpretation with the highest probability as the predicted answer. This strategy accounted for variability between classifiers and resulted in modest improvement in accuracy relative to individual analyses.

Wilkes et al. effort demonstrates that ML algorithms can be tuned and optimized for accurate PAA result interpretation. Despite the impressive performance overall, the group points out several shortcomings of their method. For example, although optimal performance was observed using the ensemble approach, some false positives were noted (e.g.IEM/condition assigned when the correct classification should have been no significant metabolic abnormality). These false positives, however, were a consequence of appropriately tuning the algorithms so that more weight was placed on reducing false negatives. Another limitation was the lack of clinical information included in the original data set. While part of mastering PAA sign-out is pattern recognition, results should not be interpreted in isolation. Clinical findings, including but not limited to history/physical, medications, diet, routine lab results, radiographic findings, and newborn screening results, are important elements that help us fine-tune individual messages. Future studies, including more diagnostic profiles and incorporating other clinical information, may improve interpretation accuracy. For now, however, the authors acknowledge that a ML-approach for PAA interpretation does not negate the need for expert human result review. Rather, they suggest such tools could be used for "first pass analysis", to identify potentially informative profiles for intensive human review.

References

1. Wilkes EH, Emmett E, Beltran L, Woodward GM, Carling RS. A Machine Learning Approach for the Automated Interpretation of Plasma Amino Acid Profiles. Clin Chem 2020 Sep 1;66 9:1210-8.
2. Wilkes EH, Rumsby G, Woodward GM. Using Machine Learning to Aid the Interpretation of Urine Steroid Profiles. Clin Chem 2018 Nov;64 11:1586-95.

Interview with a Distinguished Colleague

By Angela Ferguson, PhD

Shannon Haymond, PhD, DABCC, FAACC
Vice Chair for Computational Pathology

Director, Mass Spectrometry, Ann & Robert H. Lurie Children's Hospital of Chicago

Associate Professor of Pathology, Northwestern University Feinberg School of Medicine

What changes do you see in the future of pediatric or maternal fetal laboratory medicine?

Laboratories, like many other industries, are still feeling the effects of the COVID-19 pandemic. A silver lining for our field was the increased public awareness of clinical laboratory testing and its role. I think this has created an unprecedented opportunity for laboratories to demonstrate their value and expertise. I hope that the future includes us building on this momentum to improve patient care and laboratory services. I also hope we continue to realize the benefits of lab analytics with better access to data and tools to help use those data for decision making. I am interested to see how expanded genomic and metabolomic testing changes the landscape for diagnosis of pediatric disease.

What development would you like to see occur in pediatric laboratory medicine over the next 3 years?

It would be great if we saw success from disruptive technologies for collection and analyses of small sample volumes. There have been recent advances in the development of micro collection devices and in using automated analyzers for analysis of microsamples, so it seems that we may be on the cusp. I would like to see this become more broadly available and scalable in the next 3 years.

Congratulations on your nomination to the next slate of AACC officers! What are you most looking forward to in your year as president?

Thank you! It is quite an honor to be selected as the next President-Elect of the association. I am looking forward to the opportunity to interact with AACC members and AACC's partner organizations. I am also excited to help advance AACC's strategic initiatives, particularly those related to data analytics.

2020-2021 PMF Division Executive Board

Chair: Angela Ferguson, PhD

Chair Elect: Stanley Lo, PhD

Past Chair: Alison Woodworth, PhD

Members At Large:
Van Leung-Pineda, PhD
Amy Karger, PhD
Jane Dickerson, PhD
Laura Smy, PhD

Secretary: Mark Kellogg, PhD

Treasurer: Joe Wiencek, PhD

Newsletter Editor: Sarah Wheeler, PhD

Newsletter Editorial Board:
Khushbu Patel, PhD
Stephen Roper, PhD

Fellow Representative: *Erin Schuler, PhD*