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Pediatric Reference Value Profiling of Essential Trace and Toxic Elements in Healthy Children and Adolescents Using High-Resolution and Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry.

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Guests: Dr. Mary Kathryn Bohn is a post-doctoral fellow in Clinical Chemistry at the University of Toronto in Ontario, Canada. Dr. Matthew Nichols is a clinical biochemist at London Health Sciences Centre and an assistant professor at Western University in London, Ontario, Canada.

Randye Kaye:

Hello, and welcome to this edition of *JALM* Talk, from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host Randye Kaye.

Essential trace elements, such as copper, selenium, and zinc, play critical roles in metabolism and neurocognitive function. Measurement of essential trace element concentrations in blood can be especially important in children, such as those with inborn errors of metabolisms, chronic kidney disease, oncologic disorders, or those on low protein diets, as these groups are particularly at risk for elemental deficiencies and their complications.

Children are also more sensitive to toxic metal exposure, as certain elements, such as lead are more readily absorbed relative to adults, increasing risk of organ and neurocognitive damage. Despite the clinical importance of trace element measurements in these scenarios, age- and sex-specific pediatric reference intervals are lacking for these measurements.

The July 2023 issue of *JALM* features a study that established reference intervals for 13 plasma and 22 whole blood trace elements using the CALIPER cohort of healthy children and adolescents. Two different inductively coupled plasma mass spectrometry techniques were used and compared for the study.

Today, we're joined by two of the article's authors, Dr. Mary Kathryn Bohn and Dr. Matthew Nichols. Dr. Bohn is currently a postdoctoral fellow in Clinical Chemistry at the University of Toronto, and she's actively involved in international reference interval harmonization efforts. Dr. Nichols is a clinical biochemist at London Health Sciences Center and an assistant professor at Western University in London, Ontario, Canada, where he oversees several clinical mass spectrometry areas,

including toxicology, therapeutic drug monitoring, and trace metals. Welcome Drs. Bohn and Nichols.

First of all, Dr. Bohn, I'll ask you this one. Why are pediatric reference intervals important in interpreting trace element results?

Mary Kathryn Bohn: So, reference intervals are age- and sometimes sex-stratified standards that serves as help associate and benchmarks for the interpretation of many biomarkers of health and disease, prompting potential diagnosis, monitoring, or treatment. And adult reference intervals are really commonly available either in the literature or also are sometimes provided by the manufacturer for a specific test. Unfortunately, this is not always the case in pediatrics, particularly for specialized testing such as trace elements.

We know that children are not small adults. There are many physiological adaptations that occur in childhood that may influence the concentrations of a given biomarker. For trace elements in particular, we see dynamic changes in intestinal maturation and also in hormonal status, and this is arguably very important, considering the clinical utility of trace and heavy elements and diverse clinical settings, such as nutritional, metabolic, as well as genetic disorders.

Randye Kaye: So, what's challenging about establishing pediatric reference intervals?

Mary Kathryn Bohn: There are many challenges in establishing pediatric reference intervals, and I'll maybe highlight two for the purpose of this podcast. First, is really simply resources. It is incredibly challenging and expensive in both cost as well as time to set up an infrastructure to collect sufficient samples from children and adolescents with informed consent. And this has really prevented, I would say, the study of many biomarkers in the pediatric population. And for trace elements in particular, samples do need to be collected in a special tube, known as a royal blue top tube, which are element free in order to prevent any type of contamination.

And this is really an added layer of complexity on an already challenging issue. In our study, we collected samples from approximately 320 children to allow specifically for the examination of differences in various covariates, such as age, sex, and also differences between analytical technology.

And this brings me to the second challenge I'll highlight, which is partitioning. It can be very challenging to make decisions regarding when to partition, or separate by age or sex. Do I partition at five years versus six years? It can be really challenging to make that decision. And some groups will do it arbitrarily, whereas ours and a lot of others use

recommended statistical tests to be able to justify where to place these partitions. However, for biomarkers that change significantly with age, this can be very challenging even when using statistics.

It also requires a sufficient sample size of a minimum of 40, ideally 120, samples per group, which can be very difficult to achieve in pediatrics. Altogether, this has really created a lot of barriers to the establishment of pediatric reference intervals.

Randye Kaye: All right. Thank you. Everything's more challenging with children. Your study used samples collected from the CALIPER initiative. Can you tell us a bit more about CALIPER?

Mary Kathryn Bohn: Definitely. So, CALIPER stands for the Canadian Laboratory Initiative on Pediatric Reference Intervals. It was developed in 2009 by Dr. Khosrow Adeli at The Hospital for Sick Children, as well as many collaborating Canadian clinical chemists. The main objective of CALIPER is really to develop an accurate and robust set of reference intervals for various biomarkers of health and disease, using several analytical technologies to close this evident gap that we have currently in pediatric practice, such that clinical labs have access to the most up-to-date information to inform pediatric test result interpretation.

And to date, CALIPER has established pediatric reference intervals for over 200 biomarkers, including initially more general chemistry and immunoassay tests and now expanding to hematology, coagulation, special endocrinology, and also trace elements. This is really only possible, thanks to the hard work and dedication of several clinical research coordinators, students, and volunteers, who actually will go to Canadian schools and set up on-site clinics for blood collection and recruit children into our program, applying very strict exclusion criteria.

Over 13,000 children and adolescents have actually participated in CALIPER in the past 10 years and we're very grateful for their contribution to research. The data that CALIPER has generated, including these trace elements data, are both published in peer-reviewed journals but they're also available free for access on our online data base, which has been accessed by over 8,000 users in over 100 countries. And I think this really speaks to the importance and global reach of this project. Excitingly, this study marks the first time that CALIPER has studied trace elements in children.

Randye Kaye: Thank you, Dr. Bohn. Let's turn to you for a minute, Dr. Nichols. The pediatric samples were measured by two different mass spectrometry techniques triple quadrupole inductively coupled plasma tandem mass spectrometry, or

ICP-MS, and high-resolution sector field ICP-MS. How do these methods differ and why did you use both in your study?

Matthew Nichols: So, the overall goal of this study was to measure trace elements on two of the more sophisticated mass spectrometry platforms that you would find in clinical labs. So, trace elements testing by mass spectrometry is not a standardized form of testing. So, this has sometimes led to differences in local reference intervals at different labs. The platforms themselves can vary by vendor, complexity, throughput, as well as the quality of results that they pull out. So, a triple quadrupole instrument measures in unit resolution and it has a certain set of tools that it will employ to deal with common interferences such as polyatomic interferences. It uses things like the introduction of various gases, hydrogen, helium, oxygen, ammonia being common ones to deal with polyatomic interferences through modes like kinetic energy discrimination or to produce reactions with elements of interest resulting in a mass shift.

Comparing this to a high-resolution sector field ICP-MS, the high resolution means we are getting subunit resolution. So, kind of fractions of the dalton. So, when it encounters polyatomic interferences, it uses its superior resolving power to use very small changes in mass in order to be able to tell things apart. It is a significantly more complex, as well as expensive, machine, and those are the two tools that we used in the study.

Randy Kaye: All right, thank you so much. Dr. Bohn, did you find that the elements that you measured differed by age or sex?

Mary Kathryn Bohn: Yes. So, we established pediatric reference intervals and exposure limits for a very comprehensive panel of approximately, I would say 20 elements, either in whole blood, plasma, or both, and eight of those elements demonstrated significant age-specific differences, which included cadmium, copper, manganese, mercury, molybdenum, strontium, valium, and zinc. We did not see any sex-specific differences, and of these age differences, some we expected and some we did not.

For example, we saw a decrease in copper concentrations throughout the pediatric age range. And we anticipate that lower adolescent copper may be explained by maturing intestinal absorption, as well as potential dynamics in circulating binding proteins, such as ceruloplasmin, from birth to adolescence.

In contrast, we saw slightly higher reference values for some toxic or heavy elements, including mercury. And this could be explained by bioaccumulation over the course of the pediatric age range. In addition, dietary consideration, such

as fish consumption was not assessed and may influence these values for elements such as arsenic as well as mercury. I also wanted to point out that samples could not be collected in our study, prospectively in healthy children and young children less than four years of age. Therefore, we were not really able to evaluate age-specific differences in infancy and early childhood in the CALIPER cohort.

However, we did pull approximately five to ten years of data from each institution that was completing the study for commonly ordered elements, such as copper, zinc and selenium to try and address this gap. Particularly, as this testing is commonly ordered in the zero to 1 age range. We use the procedure called indirect reference interval techniques in order to harness the power of these data to try and derive early infantile and also reference intervals in young childhood. And these techniques essentially use a little bit more complicated statistical method to exclude disease populations in a mixed population data set.

From this subanalysis, we did see lower copper and selenium concentrations, as well as higher zinc concentrations in infants, relative to older children. However, it was really unclear to us whether these changes result from normal physiology or if they're due to patient population characteristics and indirect data. As many tasks were ordered from departments such as pediatric general medicine or gastroenterology or hepatology, which have a higher incidence or pre-likelihood of disease, than clinical chemistry and or immunoassay tests where these indirect techniques that I just spoke of have been primarily validated. So, we really do feel like further studies are needed to better elucidate health associated reference values and trace elements in the infantile population in particular.

Randye Kaye: All right, thank you. So finally, now that your study has established these reference intervals, how do you anticipate they'll be useful in clinical practice?

Mary Kathryn Bohn: So, this study was really the first to report pediatric reference intervals for this comprehensive panel of trace elements, using the two techniques that Dr. Nichols described. And our findings do suggest that some elements do require age-specific interpretation for appropriate clinical decision-making. As we talked about at the beginning, children are not small adults. We also demonstrated, as Dr. Nichols suggested as well, that these evaluated methods that we were using were pretty concordant for most elements, supporting the feasibility for common trace elements reference intervals, maybe for select assays in this population.

Therefore, I think these findings altogether will be most useful to pediatric labs who offer trace element testing, serving as a benchmark of what should be expected in a normal population, in the investigation of traditional deficiencies, as well as toxic metal exposure, altogether aiming to really improve clinical decision-making in this population. And at our hospital, we're taking steps to integrate these data into patient care, however, it's important to note that they should be validated in other populations if you're looking to adopt in your own setting.

Randy Kaye:

That was Dr. Mary Kathryn Bohn and Dr. Matthew Nichols, discussing their *JALM* article, "Pediatric Reference Value Profiling of Essential Trace and Toxic Elements in Healthy Children and Adolescents Using High-Resolution and Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry."

Thanks for tuning in to this episode of *JALM* talk. See you next time and don't forget to submit something for us to talk about.