# Advances in pediatric reference intervals: from discrete to continuous

**Victoria Higgins**[1,2], **Khosrow Adeli**[1,2]

[1]CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, M5G 1X8, Canada; [2]Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, M5S 1A8, Canada

*Correspondence to:* Khosrow Adeli. Clinical Biochemistry, Pediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, M5G 1X8 Canada. Email: khosrow.adeli@sickkids.ca

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The clinical utility of laboratory tests largely relies on availability of normative ranges (i.e., reference intervals) to enable objective interpretation and clinical decision making. Establishing accurate reference intervals is often beyond the capabilities of individual laboratories, requiring recruitment of a sufficiently large, healthy reference population, which becomes even more challenging in the pediatric population. Therefore, most laboratories interpret pediatric laboratory test results based on older and often inappropriate reference intervals, either established for a different population or using a different methodology. Pediatric reference intervals must reflect the physiological dynamic changes in biomarker concentration throughout the pediatric age range, particularly during the neonatal/infantile period and throughout pubertal development. To reflect these changes, the Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines on Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (1) recommend establishing separate reference intervals for subclasses that exhibit different normative ranges (e.g., age group, sex). If deemed clinically useful and physiologically relevant, statistical tests are then recommended to determine statistical significance, including the Harris and Boyd (2) or Lahti *et al.* (3) methods for Gaussian and non-Gaussian data, respectively. Reference interval initiatives have subsequently employed these methods to create both age- and sex-specific reference intervals (4-6). For example, the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) visually inspects reference value data and subsequently tests statistically significant partitions using the Harris and Boyd method (4). However, biomarker concentrations do not abruptly change with age, as is reflected by discrete age partitions, but rather continuously change throughout child growth and development. Therefore, partitioning reference intervals does not adequately reflect the true dynamic relationship between age and biomarker concentration.

A recent study published in Hepatology by Bussler *et al.* (7) established continuous pediatric reference interval based on healthy children and adolescents for hepatic enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). With the increased prevalence of non-alcoholic fatty liver disease in the pediatric population, particularly in obese subjects (8), accurate interpretation of hepatic enzymes has become increasingly important. This study used data from the Leipzig Research Centre for Civilization Disease (LIFE) Child study, one of the largest pediatric cohorts primarily comprised of healthy subjects in Europe. To further ensure the reference population was devoid of unhealthy subjects, exclusion criteria were applied including highly elevated liver enzymes, elevated C-reactive protein, intake of hepatotoxic drugs, or an elevated body mass index (BMI). Following exclusion criteria, data from 3,131 cases (1,746 individuals) aged 11 months to 16 years, were used to establish continuous reference intervals. The LMS-type method of Cole (9) was employed to determine the sex-specific 3$^{rd}$, 10$^{th}$, 50$^{th}$, 90$^{th}$, and 97$^{th}$ percentiles, with

the 3rd and 97th percentiles defined as the lower and upper reference limits, respectively. This method is widely used to establish reference percentiles of body measurements (10,11). To aid in knowledge translation and implementation of continuous reference intervals for hepatic enzymes, Bussler *et al.* also provided a package for R software (http://CRAN.R-project.org/package=childsds), which includes convenient functions to transform analyte concentration into age- and sex-specific standard deviation score (SDS) values for ALT, AST, and GGT. Therefore, by knowing the patient's age, sex, and liver enzyme concentration, the calculated SDS value will provide information not only regarding whether the results fall within or outside the age- and sex-specific reference percentiles, but where they fall within the reference value distribution.

Pediatric reference curves are also widely used to provide accurate reference data for body measures, including growth reference charts developed by the World Health Organization (WHO) (11) and the Centers for Disease Control and Prevention (CDC) (12), which are widely used for pediatric growth assessment. Smoothed centile charts have also been developed to interpret waist circumference and waist-to-height ratio values in pediatrics (10). These reference curves provide dynamic age- and sex-specific cut-offs, as a single cut point overlooks the profound physiological variation of these measures in children. As biomarker concentrations also exhibit dynamic changes throughout growth and development, smoothed centile curves more adequately represent normative values, providing more accurate laboratory test result interpretation. Continuous reference intervals for laboratory biomarkers have recently emerged in the field of laboratory medicine. For example, continuous age- and sex-adjusted reference intervals were established for urinary markers of cerebral creatine deficiency syndromes using laboratory data extracted from five laboratories and the developed model was subsequently verified using laboratory data from an additional laboratory (13). Furthermore, due to the highly invasive nature of cerebrospinal fluid (CSF) sampling, continuous reference intervals for CSF total protein (CSF-TP) were established by applying exclusion criteria to 20 years of patient data and using quantile regression to determine continuous 2.5th and 97.5th percentile curves (14). Specific to the pediatric population, Zierk and colleagues established continuous pediatric reference intervals for hematological and biochemical analytes by retrospectively analyzing clinical laboratory data using an indirect approach to estimate the supposedly healthy distribution from the whole data set containing both healthy and pathological subjects (15,16). Although data-mining approaches are more feasible due to circumvention of recruitment and blood collection from a large number of healthy subjects, direct studies of healthy populations are still regarded as the preferred method to determine accurate reference intervals (17). Establishing reference intervals through direct recruitment of reference individuals allows the application of defined inclusion and exclusion criteria, reduced variation of preanalytical and analytical factors, and minimizes errors of estimating reference values using data mining and retrospective analysis. However, limited studies have employed both direct sampling methods of reference individuals and continuous reference intervals to appropriately reflect age-related concentration dynamics. This highlights the importance of the recent contribution by Bussler and colleagues, which provides the first study of continuous pediatric reference intervals for all three transaminases in a healthy, normal weight pediatric cohort. Pediatric GGT reference percentiles were previously established by the KiGGS study in Germany (18) and ALT reference percentiles were previously established by England *et al.* (19), both studies exhibiting very similar reference trends to those by Bussler *et al.*

The ALT, AST, and GGT smoothed percentile curves established by Bussler and colleagues (7) further highlight the necessity for continuous reference intervals, rather than discrete age partitions, as hepatic enzyme concentrations exhibit dynamic and unique trends throughout the pediatric age range. For example, ALT is elevated in infancy, subsequently declines early in childhood, and increases until early adolescence, at which time values decline once again. In contrast, AST percentiles follow a continuous downward trend with increasing age. In addition to age and sex, the effect of additional covariates, including adiposity (assessed by BMI) and pubertal status (assessed by Tanner Stage), on these liver enzymes was also examined. ALT and GGT increase with increasing BMI-SDS, and this effect appears stronger in males than females. AST surprisingly decreases with increasing BMI-SDS, with the effect stronger in females than males. Puberty also significantly affects hepatic enzyme concentration, independently of age, with ALT and GGT positively associated and AST negatively associated with pubertal status. Examining the association of BMI and Tanner Stage on analyte concentration is often overlooked in reference interval studies, although these are

 

two important covariates that significantly affect several biomarkers.

Although continuous reference intervals better reflect the dynamic trend in analyte concentration throughout the pediatric age range, their implementation in clinical practice remains a hurdle. Currently, laboratory information systems are unable to accommodate a mathematical equation to allow test result interpretation based on continuous reference intervals specific for age, sex, and/or additional covariates. Therefore, while continuous percentile curves provide a more robust representation of normative values, age and sex bins are still required for implementation in clinical settings. Although these narrow bins will more accurately represent the dynamic concentration trends, they will again be reducing the complexity of the true age-related dynamics of biomarker concentration. Interpreting the influence of additional covariates (i.e., BMI, Tanner Stage, ethnicity, etc.) also remains a challenge. This could be achieved by simultaneously considering several variables in one mathematical model, although the practicality of implementation would again pose an obstacle. Furthermore, reference intervals for the neonatal/infantile age range also remains a challenge, which unfortunately was not addressed in this recent contribution, which only included children >11 months of age. Continuous reference intervals for reticulocyte parameters during the first 90 days of life were recently established by Christensen *et al.* (20), highlighting the highly variable levels early in life. Establishing continuous reference intervals would arguably be most valuable during this development period due to the extensive age-related changes evident week to week, and even during the first few days of life.

Overall, the recent contribution by Bussler *et al.* provides robust pediatric continuous reference intervals for three hepatic enzymes, as well as examined the effect of age, sex, BMI and pubertal status on their concentration. Providing percentile tables for 6-month intervals and a software package to easily determine the SDS values for each hepatic enzyme provides effective knowledge translation and clinical implementation of the established reference curves. A shift from discrete to continuous age- and sex-specific reference intervals will better represent the physiological, dynamic biomarker concentration throughout the pediatric age range. This in turn will provide more accurate pediatric laboratory test result interpretation and improve patient care. A key challenge is development of innovative interpretative tools in laboratory information systems to enable the application of continuous reference intervals in test result interpretation and clinical decision making.

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