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CLSI-based transference of the CALIPER database of pediatric reference intervals to Beckman Coulter DxC biochemical assays

Petra A.T. Araújo [a,1], Dylan Thomas [a,1], Tara Sadeghieh [a,1], Victoria Bevilacqua [a], Man Khun Chan [a], Yunqi Chen [a], Edward Randell [b,c], Khosrow Adeli [a,*]

[a] CALIPER Program, Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada
[b] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
[c] Eastern Health, St. John's, NL, Canada

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ABSTRACT

Background: The CALIPER program has established a comprehensive database of age- and sex-stratified pediatric reference intervals for over 85 common biochemical markers, largely using the Abbott ARCHITECT assays. To allow a broader application of the CALIPER database, we examined transference to 36 Beckman Coulter Synchron Unicel DxC800 assays, based on the CLSI C28-A3/EP9-A3 guidelines.

Methods: Patient sample comparisons were performed for 36 biochemical assays using 200 serum specimens obtained from pediatric patients on the Abbott ARCHITECT ci8200 and the Beckman Coulter DxC800. For each analyte, R^2 values were calculated to assess the quality of correlation between the platforms. Statistical criteria used to assess transferability included a) regression analysis to create the equation of the line of best fit, b) standardized residual, c) Bland–Altman, and d) quantile–quantile plots. Transferred reference intervals were further verified by analyzing serum samples from 100 healthy children from the CALIPER cohort on the Beckman Coulter system.

Results: The reference intervals for most of the assessed analytes were transferable to Beckman Coulter assays (31 out of 36 studied) and the newly calculated reference intervals were verified through analysis of CALIPER reference samples (28 out of 31). Eighteen assays demonstrated excellent correlation ($R^2 \geq 0.95$), and 13 assays showed strong correlation ($0.77 \leq R2 \leq 0.94$).

Conclusion: The current study allowed successful transference of a large number of biochemical markers from the CALIPER database to assays on the Beckman Coulter DxC800 platform. Transference should facilitate broader application of CALIPER reference intervals at pediatric centers using DxC biochemical assays.

Introduction

Reference intervals represent the interval of analyte concentrations observed in a population of healthy individuals, and are helpful to facilitate biochemical test interpretation and proper diagnosis. In order to be reliable, reference values must reflect a target population, as well as the effects of covariates such as age, sex, and ethnicity, which can significantly impact analyte levels [1]. It is thus important that these covariates are taken into account when determining reference values, and that reference intervals are partitioned accordingly. Consequently, the task of establishing accurate reference intervals can be quite challenging [2]. It is even more difficult to establish reference intervals for pediatric populations, since important physiological changes occur during childhood and adolescence, including hormonal fluctuations, which may result in variations in analyte levels according to age and gender [3]. Since a sufficient number of healthy participants must be included in each age and gender partition to allow for proper statistical analysis, a larger number of participants are often needed from pediatric populations. Additionally, the recruitment of healthy children and teens from the community requires ethical considerations, and parental consent is necessary. Thus, determining pediatric reference intervals from healthy community children often requires great financial and time investments, resulting in very few high-quality studies in this field, and inadequate pediatric reference intervals for many analytes [4].

Abbreviations: ALB and ALBm, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY7, amylase; apoB, apolipoprotein B; ASO-, antistreptolysin-O; AST, aspartate aminotransferase; BUN and BUNm, blood urea nitrogen; C3, complement C3; C4, complement C4; CALC, calcium; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; CHOL, total cholesterol; CLSI, Clinical and Laboratory Standards Institute; CO_2E, carbon dioxide enzymatic; CR-E, enzymatic creatinine; CRP, C-reactive protein; DBIL, direct bilirubin; FE, iron; CRPH, high sensitivity C-reactive protein; GGT, gamma-glutamyl transferase; HDLD, direct high-density lipoprotein; HPT, haptoglobin; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LD, lactate dehydrogenase; MG, magnesium; PHS or PHOSm, phosphorus; PAB, prealbumin; TBIL, total bilirubin; TG, triglycerides; TP and TPm, total protein; TRFN, transferrin; URIC, uric acid.

* Corresponding author at: Clinical Biochemistry, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, M5G 1X8 Canada.
E-mail address: khosrow.adeli@sickkids.ca (K. Adeli).

[1] These authors contributed equally to this manuscript.

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The CALIPER (Canadian Laboratory Initiative for Pediatric Reference Intervals — http://www.caliperdatabase.com/cdb/) program is an ongoing collaboration between pediatric centers across Canada, which aims to address the current gaps in pediatric reference intervals. To date, CALIPER has established a comprehensive database of pediatric age- and sex-specific reference intervals for over 85 biochemical markers, which is now being used internationally [5–7]. Many of the CALIPER reference intervals, however, were established using the Abbott ARCHITECT analytical system, and thus have been largely applicable only to laboratories using Abbott assays. A key objective of the CALIPER project is to make the new reference interval database applicable to other analytical platforms in order to ensure that the maximum number of laboratories, pediatricians and children can benefit from the established reference intervals. Nevertheless, due to the associated complexity, challenges, and cost, it would not be feasible for CALIPER to conduct full reference interval studies for all analytes on each of the major analytical systems. To ensure a broader application of the database, a reliable and convenient alternative is to conduct a transference study between different analytical systems. The Clinical and Laboratory Standards Institute (CLSI) guidelines emphasize the feasibility of transferring reference intervals established in one laboratory (donor) to other (receiving) laboratories [8]. According to the CLSI, transference significantly decreases the costs and challenges associated with a full study. This is possible because the first step of transference – a comparison of the performances between the two systems – does not require samples from healthy subjects, and laboratories can use sera from pediatric patients, which are substantially easier to obtain. The second step – verification of the transferred reference intervals – requires samples from healthy participants, but as few as 20 reference samples can be sufficient to verify [8].

Previously completed studies have used the transference method to create pediatric reference intervals for the Dade Behring Dimension RxL analyzer [9], and to demonstrate that reference intervals for immunoassays on the Siemens ACS:Centaur are equivalent to those on the Siemens ACS:180 system [10]. The CALIPER project has also completed several transference studies, where reference intervals for 28 basic chemistry analytes were transferred from Abbott ARCHITECT to Siemens Vista 1500 platforms, 16 to the Beckman Coulter DxC800, 21 to Ortho Vitros 5600, and 19 to Roche Cobas 6000 [11].

In the present study, pediatric reference intervals (from birth to 18 years of age) for 31 biochemical markers were transferred from the Abbott ARCHITECT assays to the Beckman Coulter Synchron Unicel DxC800 assays. Assessment of transferability was based on strict statistical criteria. It is important to note that the comparisons performed between the Abbott and Beckman Coulter assays make no assumption as to assay accuracy, and the differences are largely due to differences in calibration of assays or specific reagents utilized on the two different analytical platforms.

Methods

Transference approach

In order for a reference interval to be considered transferable between different biochemical assays, the relationship between measurements obtained from both assays must follow a series of statistical criteria and meet specific assumptions. The assessment of transferability of the reference intervals was based on the CLSI guidelines C28-A3 [8] and EP9-A3 [12], and the statistical protocol has been described in a previous CALIPER transference study (Fig. 1) [11]. The Institutional Review Board (IRB) of the Hospital for Sick Children approved this study and the collection of blood samples from healthy children and adolescents from the community. All testing on the DxC800 was performed blinded in laboratories at Beckman Coulter, Inc., Brea CA USA.

Assessment of correlation and other statistical assumptions

200 serum samples obtained from pediatric patients at the Hospital for Sick Children (ON, Canada) were tested for 36 assays on both the Abbott ARCHITECT ci8200 and on the Beckman Coulter Synchron Unicel DxC800 systems. The tests included chemistry assays (total (TBIL) and direct bilirubin (DBIL), calcium (CALC), carbon dioxide (CO_2E), enzymatic creatinine (CR-E), iron (FE), magnesium (MG), phosphorus (PHS, PHOSm), urea nitrogen (BUN, BUNm), uric acid (URIC)), enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY7), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), lactate dehydrogenase (LD)), lipids and lipoproteins (apolipoprotein B (ApoB), total cholesterol (CHOL), triglycerides (TG), HDL cholesterol (HDLD)), and protein markers (albumin (ALB, ALBm), antistreptolysin O (ASO-), complement C3 (C3), complement C4 (C4), high sensitivity c-reactive protein (CRPH), haptoglobin (HPT), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), prealbumin (PAB), total protein (TP, TPm) and transferrin (TRFN)). For some analytes, two different Beckman Coulter assays exist for the same marker, which employ different reagents or methodologies. In those cases, the reference intervals previously determined on the Abbott ARCHITECT [5] were compared to both Beckman Coulter methods. This was the case for albumin (ALB and ALBm), urea (BUN and BUNm), phosphorus (PHS and PHOSm) and total protein (TP and TPm).

In order to assess transferability of the reference intervals, concentration or activity measurements obtained from the Beckman Coulter assays were plotted against the corresponding Abbott ARCHITECT values, and the quality of correlation between the two systems was assessed through R^2 values, calculated using the statistical software R [13]. Reference intervals for assays with corresponding $R^2 < 0.70$ were automatically considered non-transferable, since the quality of correlation was poor and, therefore, the transferred reference intervals would not be accurate.

For analytes with $R^2 > 0.70$, the equation of the line of best fit was calculated. Simple linear regression using the least squares approach was used in order to calculate the equation of the line of best fit in cases where $R^2 \geq 0.95$, while Deming regression was the method of choice to determine the equation in cases where $0.70 \leq R^2 < 0.95$.

The next step was to investigate if there was bias in the relationship between the systems, and to assess whether residuals followed a normal distribution and were randomly scattered across all of the levels of Abbott measurements. These assumptions were assessed by visual inspection of three different graphs: the quantile–quantile (Q–Q), Bland–Altman, and residual plots. In order to assess normality, the Q–Q plot was visually inspected and distribution was considered normal if the real distribution was close to the theoretical normal distribution (that is, if the points fell on or close to the line that represented normality). The random distribution of residuals was assessed using the residual plot. In this graph, the values for Abbott were plotted on the x-axis, while the corresponding residuals for individual Beckman Coulter methods were plotted on the y-axis. In cases where trends, patterns or clusters were detected, the reference interval was considered non-transferable.

Finally, the Bland–Altman plot (difference plot) was inspected. This method is used to compare two different techniques, and the percent differences between Beckman Coulter and Abbott values were plotted against the averages of the two techniques. Again, a random distribution of points, absent of patterns, clustering or trends, was necessary for a reference interval to be considered transferable, since this suggested that there was no bias in the relationship between the methods.

The reference intervals for any given analyte were only considered transferable when all of the three graphs were compatible with the required assumptions. It is important to note that an analyte was considered non-transferable in cases where one of the graphs suggested biased or non-normal distribution of the residuals, in spite of how strong the correlation may have been. For analytes that followed all of the assumptions, the equations of the line of best fit were then used to calculate the corresponding Beckman Coulter reference intervals, by

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