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Validity of establishing pediatric reference intervals based on hospital patient data: A comparison of the modified Hoffmann approach to CALIPER reference intervals obtained in healthy children

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ABSTRACT

Objectives: To compare pediatric reference intervals calculated using hospital-based patient data with those calculated using samples collected from healthy children in the community as part of the CALIPER study.

Methods: Hospital-based data for 13 analytes (calcium, phosphate, iron, ALP, cholesterol, triglycerides, creatinine, direct bilirubin, total bilirubin, ALT, AST, albumin and magnesium), measured on the Vitros 5600, collected between 2007 and 2011 were obtained. The data for each analyte were partitioned by age and gender as previously defined by the CALIPER study. Outliers in each partition were removed using the Tukey method. The cumulative distribution function (cdf) was then determined for each analyte value following which, the inverse cdf values of a standard Gaussian distribution were calculated. The analyte values were plotted against the inverse cdf of the standard Gaussian distribution. Piece-wise regression determined the linear portion of the resulting graph using the statistical software R. Linear regression determined an equation for the linear portion in each partition and reference intervals were calculated by extrapolating to identify the 2.5th and 97.5th centiles in each partition based on the inverse cdf values (which would correspond to the values −1.96 and 1.96 of the Gaussian distribution). Using the 90% confidence intervals for the reference intervals defined by CALIPER and the Reference Change Value (RCV) as the criteria, these calculated reference intervals were compared to those reported previously by CALIPER. Reference samples were also measured on the Vitros 5600 analyzer in an attempt to validate the calculated reference intervals.

Results: In general, the reference intervals calculated from hospital-based data were generally wider than those calculated by CALIPER. None of the reference intervals calculated using the Hoffmann approach fell completely within the 90% confidence intervals calculated by CALIPER.

Conclusions: These results suggest that calculating pediatric reference intervals from hospital-based data may be useful, as a guide, in some cases but will likely not replace the need to establish reference intervals in healthy pediatric populations.

Introduction

The majority of clinical decisions in medicine are based on laboratory measurements and their associated reference intervals. In pediatric medicine there exists a paucity of reliable and accurate reference intervals for analyte levels in patients [1]. The use of adult reference intervals in pediatric medicine is not appropriate and can lead to the under or over-estimation of an analyte level which can result in misdiagnosis of patients as well as in costly, and often unnecessary, medical follow-ups [2].

Reference intervals generally consist of a statistically derived range of values denoting the central 95% of values taken from healthy population [3]. Reference intervals are of significant importance to modern medicine. Establishing accurate reference intervals is theoretically sound but, practically, relatively challenging. It is difficult to define an individual as “normal” or “healthy”, ensuring that there are no subclinical issues present. Furthermore, differences in analyte values between certain populations and the use of different laboratory methods by clinical laboratories hamper the use of standard reference intervals, requiring that individual institutions calculate their own intervals.

The CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) initiative is a collaborative project between several pediatric hospitals across Canada. This initiative aims to update and fill gaps

Abbreviations: BMI, body mass index; CALIPER, Canadian Laboratory Initiative in Pediatric Reference Intervals; CLSI, Clinical Laboratory Standards Institute.

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that currently exist for pediatric reference intervals. CALIPER has recently published age and gender-specific pediatric reference intervals for 40 general chemistry markers. These reference intervals were established through the recruitment of more than 2000 healthy children, aged 0–18 years from the community [3].

Establishing reference intervals through recruitment of healthy individuals can be costly and very time consuming. The recruitment of pediatric reference individuals is particularly challenging due to the dynamic changes occurring with child growth and development. This often results in the need for age and gender-specific partitioning of reference intervals which requires a large number of reference samples. An alternative method for establishing reference intervals was first proposed by biostatistician, Robert G. Hoffmann. In his original 1963 paper, Hoffmann proposed an indirect a posteriori method in which reference intervals for analytes could be calculated using hospital in and out-patient data [4]. The approach proposed by Hoffmann requires two assumptions: 1) that hospital data for a particular analyte forms a Gaussian distribution and 2) that the majority of measurements made in the hospital represent normal individuals. Hoffmann began by plotting the cumulative frequency of a particular result against the analyte value on normal probability paper. He then chose the linear portion of the resulting graph, centered on the 50th percentile, therefore giving the most weight to these values. By extrapolating the linear portion of the graph, the 2.5th and 97.5th centiles could be calculated, representing the range which should include only apparently healthy individuals, if the assumptions made are valid. Hoffmann used this approach with a relatively small number of patient results ($n = 60$) for glucose as a proof-of-concept. Today, with the use of computers, much of the subjectivity of the Hoffmann approach can be eliminated and very large numbers of samples can be analyzed.

The obvious advantage to this approach is that it removes the need to recruit healthy individuals, instead taking advantage of hospital data which has already been collected and is readily available. Here, to test the validity of this approach, we calculated pediatric reference intervals for 13 biochemical markers (albumin, creatinine, ALP, ALT, AST, HDL, calcium, magnesium, phosphate, iron, cholesterol, triglyceride, unconjugated bilirubin) using a modified version of Hoffmann's original method. Calculated reference intervals were compared to those recently published by CALIPER [3], as a gold standard. A validation study was also performed to definitively assess the feasibility of this approach in a pediatric setting.

Methods

Patient data

Five years (2007–2011) of hospital-based data from children aged birth to 18 years was requested for the following analytes: albumin, ALP, ALT, AST, total bilirubin, calcium, creatinine, cholesterol, HDL-cholesterol, iron, magnesium, phosphate and triglycerides. These analytes were all measured on the Vitros 5600 analyzer at the Hospital for Sick Children in Toronto. These data were filtered using a unique identifier for each patient so that only one result from an individual patient was used in the analysis. The total number of results obtained, for each analyte, from the hospital database is shown in Table 1. The total number of results used to calculate each reference interval after partitioning, outlier removal and piece-wise regression is also listed in Table 1.

Partitioning

The data were partitioned by age and gender based on the partitions that were identified by CALIPER [3].

Statistical approach for reference interval calculation

The steps in the approach taken to calculate reference intervals for each analyte are outlined in Fig. 1.

Table 1
Percentage of patient analyte results used to calculate reference intervals using the modified Hoffmann approach.

Analyte	Age	Gender	Number of patient results before outlier removal	Number of patient results after outlier removal and piece-wise linear regression	% results included in final analysis
Albumin (g/L)	0–14 days	Both	4730	10	0.2
	15 days–1 year	Both	28784	15	0.1
	1–8 years	Both	67472	25	0.0
	8–15 years	Both	62610	20	0.0
	15–19 years	Female	20464	16	0.1
	15–19 years	Male	19656	29	0.1
ALP (U/L)	0–14 days	Both	2873	59	2.1
	15 days–<1 year	Both	15178	147	1.0
	1–<10 years	Both	58593	130	0.2
	10–<13 years	Both	17217	108	0.6
	13–<15 years	Female	6647	43	0.6
	13–<15 years	Male	7366	62	0.8
	15–<17 years	Female	7959	34	0.4
	15–<17 years	Male	8399		0.0
	17–<19 years	Female	4376	36	0.8
	17–<19 years	Male	4431	40	0.9
ALT (U/L)	0–1 year	Both	48432	21	0.0
	1–13 years	Both	130177	20	0.0
	13–19 years	Female	35159	15	0.0
	13–19 years	Male	33541	19	0.1
AST (U/L)	0–14 days	Both	9967	30	0.3
	15 days–1 year	Both	36865	37	0.1
	1–7 years	Both	67456	20	0.0
	7–12 years	Both	45030	16	0.0
	12–19 years	Female	38803	12	0.0
	12–19 years	Male	37161	16	0.0
Calcium (mmol/L)	0–<1 year	Both	23403	75	0.3
	1–<19	Both	63189	32	0.1
Cholesterol (mmol/L)	0–14 days	Female	N/A		
	0–14 days	Male	N/A		
	15 days–<1 year	Both	691	71	10.3
	1–<19 years	Both	21250	231	1.1
Creatinine (mmol/L)	0–14 days	Both	18584	27	0.1
	15 days–2 years	Both	97184	15	0.0
	2–5 years	Both	65637	12	0.0
	5–12 years	Both	109483	14	0.0
	12–15 years	Both	56246	35	0.1
	15–19 years	Female	34407	29	0.1
	15–19 years	Male	33496	41	0.1
HDL-C (mmol/L)	0–14 days	Both	N/A		
	15 days–<1 year	Both	171	69	40.4
	1–<4 years	Both	485	111	22.9
	4–<13 years	Both	2605	92	3.5
	13–<19 years	Female	3198	59	1.8
	13–<19 years	Male	1563	31	2.0
Iron (mmol/L)	0–<14 years	Both	6794		0.0
	14–<19 years	Female	1500		0.0
	14–<19 years	Male	1042		0.0
Magnesium (mmol/L)	0–14 days	Both	11765	26	0.2
	15 days–1 year	Both	47119	53	0.1
	1–19 years	Both	200627	37	0.0
Phosphate (mmol/L)	0–14 days	Both	1516	137	9.0
	15 days–<1 year	Both	3900	47	1.2
	1–<5 years	Both	6321	64	1.0
	5–<13 years	Both	8657	40	0.5
	13–<16 years	Female	3292	58	1.8
	13–<16 years	Male	3031	72	2.4
	16–<19 years	Both	3997	59	1.5
Triglycerides	0–14 days	Both	45		0.0