Normative Values of High-Sensitivity Cardiac Troponin T and N-Terminal pro-B-Type Natriuretic Peptide in Children and Adolescents: A Study from the CALIPER Cohort

Emily Lam,[a] Victoria Higgins,[b,c] Liyong Zhang,[d] Man Khun Chan,[b,c] Mary Kathryn Bohn,[b,c] Karin Trajcevski,[b,c] Peter Liu,[d] Khosrow Adeli,[b,c] and Paul C. Nathan[a,*]

Background: Cardiac troponin (cTn) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are increasingly used clinically to evaluate and prognosticate acute myocardial infarction and heart failure, respectively. Pediatric reference intervals and cut-offs have not been established for Roche's Elecsys Troponin T hs (high sensitive) assay. Although pediatric reference intervals exist for NT-proBNP, cut-off values do not exist. In this study, we report reference intervals and 99th percentile cut-offs in a large, healthy Canadian pediatric population using the CALIPER cohort.

Methods: Blood samples from 484 healthy children and adolescents between 0 and <19 years old were recruited from hospital outpatient clinics and community settings. Serum samples were analyzed using Roche's Cobas e411 and evaluated for high-sensitivity cTnT (hs-cTnT) and NT-proBNP concentrations. 95% reference intervals and 99th percentile cut-off values were established.

Results: Three hs-cTnT age partitions were established (0 to <6 months, 6 months to <1 year, and 1 to <19 years) with highest concentrations observed in children under 1 year. Two NT-proBNP age partitions were established (0 to <1 year, and 1 to <19 years), also with higher concentrations in infants under 1 year of age. For each of these age partitions, the 99th percentile cut-off, 95% reference interval, and proportion of detectable concentrations were determined.

Conclusions: This is the first study to examine hs-cTnT and NT-proBNP reference values together in a healthy pediatric cohort without other clinical indications. We present 99th percentile cut-offs, which will allow clinicians to appropriately evaluate cardiovascular disease in children and adolescents.

[a]Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, Toronto, ON, Canada; [b]CALIPER Program, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; [c]Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada; [d]Cardiac Function Laboratory, University of Ottawa Heart Institute, Ottawa, ON, Canada.
*Address correspondence to this author at: The Hospital for Sick Children, 555 University Ave. Toronto, Ontario, M5G 1X8 Canada. Fax 416-813-5327; e-mail paul.nathan@sickkids.ca.
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IMPACT STATEMENT

High-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are clinically important biomarkers in the diagnosis and prognosis of cardiovascular disease. However, pediatric reference values have not been established for Roche's widely used Troponin T hs (high sensitive) assay, and pediatric cut-offs do not exist for NT-proBNP. In this study, we present age-partitioned reference intervals and cut-offs for these two cardiac markers in a large cohort of healthy Canadian children and adolescents. These age partitions underscore the importance of using pediatric-specific reference values and cut-offs in the diagnosis, prognosis, and treatment of pediatric cardiovascular disease.

INTRODUCTION

In healthy adults, certain blood biomarkers are clinical correlates and prognostic indicators of cardiovascular disease. Elevated levels of cardiac troponin (cTn) are observed in acute myocardial infarction, while N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations are used to evaluate heart failure *(1,2)*.

Over the past decade, there has been a rise in the clinical use of high-sensitivity cardiac troponin assays, which are defined as having a coefficient of variation (CV) of ≤10% at the 99th percentile concentration and can detect analyte concentrations above the limit of detection (LoD) in >50% of a healthy reference population *(3)*. Most of these assays have been developed to measure levels of the cardiac troponin I isoform; to date, only Roche Diagnostics has developed high-sensitivity cardiac troponin T (hs-cTnT) assays *(4)*. B-type natriuretic peptide (BNP) and NT-proBNP are formed from the cleavage of pro-B-type natriuretic peptide (proBNP) in response to an increased cardiac load. While BNP is an active peptide, NT-proBNP is biologically inert *(5)*. Although both BNP and NT-proBNP assays are used clinically, NT-proBNP is more stable and is a better biomarker for detecting heart failure than BNP *(6)*. Numerous platforms can run NT-proBNP assays (e.g., Siemens, bioMérieux, Ortho-Clinical Diagnostics, and Roche Diagnostics), but most of these use Roche NT-proBNP antibodies *(5)*.

Reference intervals and 99th percentile cut-offs for hs-cTnT and NT-proBNP have been established in the adult population *(7, 8)*, although published data vary due to the use of different assays and/or analytic platforms *(9)*. Age- and sex-specific reference ranges for hs-cTnT have recently been established in a pediatric population using Roche's Elecsys Troponin T Gen 5 STAT assay *(10)*. However, pediatric ranges and cut-offs have not yet been determined for Roche's Elecsys Troponin T hs (high sensitive) assay, which is considered equivalent to the Gen 5 STAT assay *(11)* and continues to be widely used around the world. Previously published pediatric reference ranges for NT-proBNP are limited by small sample sizes *(12)* and the use of surrogate samples (e.g., umbilical cord blood for neonate values *(13)*), and 99th percentile cut-offs do not exist. Here, we present normative values for these two blood biomarkers in children and adolescents from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) cohort *(14)*.

MATERIALS AND METHODS

This study was approved by the Research Ethics Board at The Hospital for Sick Children (Toronto,

Canada). Healthy individuals between 0 and <19 years old were recruited from hospital outpatient clinics, schools, and community programs. Exclusion criteria were determined using a health questionnaire and included current pregnancy, any chronic illness, or metabolic disease. Participants who were acutely ill in the month prior to sample collection and those who reported the use of prescribed medication in the 2 weeks prior were also excluded.

Each participant or their substitute decision maker gave informed written consent, completed a short health questionnaire, and provided a venous blood sample. Samples were collected using serum separator tubes (SST^{TM}; BD) and checked for hemolysis, stored at -80 °C, and analyzed using Roche's Cobas e411 in accordance with the manufacturer's instructions. Roche's CalSet and PreciControl sets were used to calibrate the assays prior to running the samples.

The LoD of the Elecsys Troponin T hs assay (Roche Diagnostics) is 5 ng/L, and the limit of blank (LoB) is 3 ng/L. The limit of quantitation (CV ≤ 10%) is 7 ng/L, and CV is ≤20% at 4 ng/L *(15)*. The assay has a detection range of 3–10 000 ng/L and a CV < 10% at 14 ng/L *(7)*. The NT-proBNP (Roche Diagnostics) LoD is 5 ng/L with a measuring range of 5–35 000 ng/L, and the functional sensitivity is 50 ng/L *(16)*. Concentrations of hs-cTnT and NT-proBNP were measured and 95% reference intervals (i.e., 2.5th and 97.5th lower and upper reference limits) as well as 99th percentile values were established according to the Clinical and Laboratory Standards Institute (CLSI).

Statistical Analyses

Reference intervals were established in accordance with CLSI EP28-A3c guidelines *(17)* using R software, as described previously *(18)*. Prior to analysis, all values below the analytical measuring range were recorded as the LoD for NT-proBNP. For hs-cTnT, values below the measuring range were recorded as the LoB to ensure consistency with published data for Roche's Gen 5 hs-cTnT assay *(10, 19)*. Scatterplots of hs-cTnT and NT-proBNP concentrations by age and sex were generated in Microsoft Excel, allowing visual inspection of the data and manual removal of extreme outliers. Age and sex partitions identified by visual inspection were confirmed using the Harris and Boyd method *(20)*. Additional outliers were removed for each partition using the Tukey method *(21)* twice or the adjusted Tukey method *(22)* twice, for Gaussian and skewed data, respectively. Reference intervals (i.e., 2.5th and 97.5th percentiles) and 99th percentile cut-offs were calculated using the nonparametric rank method for partitions with sample size ≥120 or the robust method of Horn and Pesce for partitions with sample size between 40 and 120 *(23)*. Corresponding 90% confidence intervals were calculated for all upper and lower reference limits, as well as for 99th percentile values.

RESULTS

Serum samples from 484 pediatric participants (birth to <19 years old, 229 females, 255 males) were included in this study. All samples were analyzed for NT-proBNP levels, while a subset of 245 samples were analyzed for hs-cTnT, as this was a sufficient sample size to statistically determine age partitions for hs-cTnT reference intervals. Serum concentrations of hs-cTnT and NT-proBNP were examined by age and sex. No differences between males and females were observed for hs-cTnT (118 females, 127 males) or NT-proBNP (229 females, 255 males) (Figs. 1 and 2). Following visual inspection, 3 hs-cTnT and 2 NT-proBNP outliers were manually removed due to very high values. Analysis by the Tukey and adjusted Tukey methods removed an additional 2 and 6 outliers for hs-cTnT and NT-proBNP, respectively.

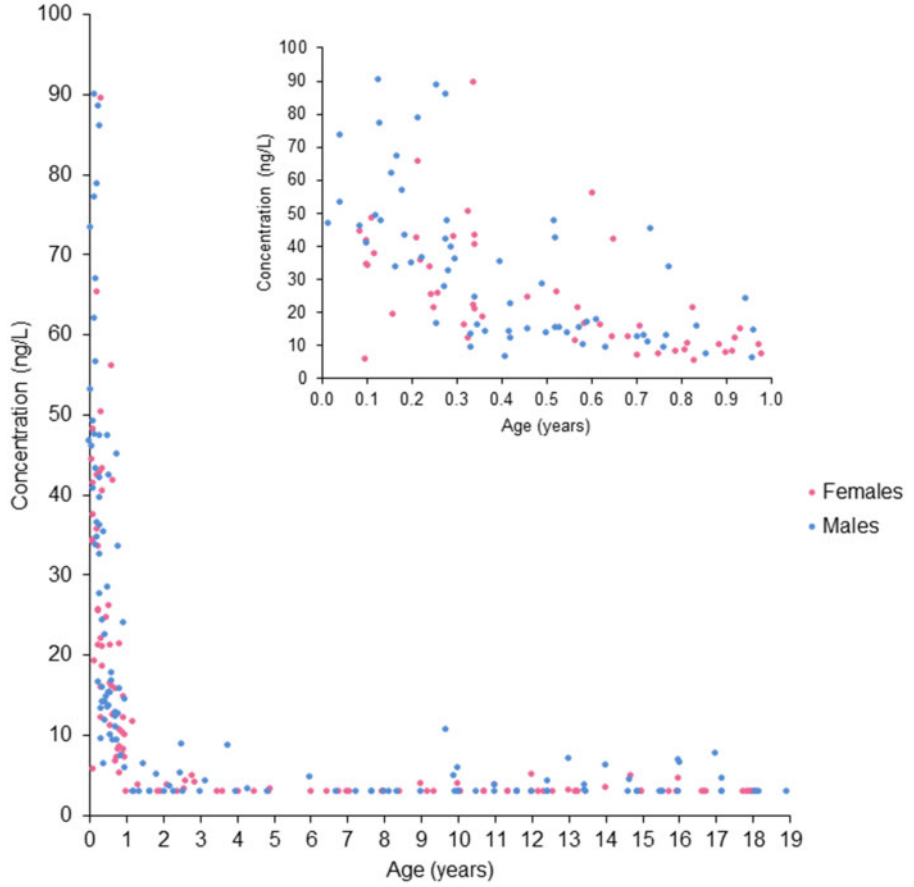

Fig. 1. Concentrations of hs-cTnT in children and adolescents under 19 years of age by sex (females: pink, males: blue). Inset: hs-cTnT concentrations in infants aged 0–1 year.

Following outlier removal, reference intervals were calculated for hs-cTnT (n = 240) and NT-proBNP (n = 476) with the upper reference limits calculated at the 97.5th percentile (Table 1). Since no sex differences were found, results for males and females were consolidated for all reference interval calculations. For lower reference limits that were below the measuring ranges of the assay, we report the hs-cTnT lower reference limit as <3 ng/L and the NT-proBNP lower reference limit as <5 ng/L. For each partition, upper and lower reference limits, as well as 99th percentile cut-offs, were calculated with 90% confidence intervals. The proportion of detectable results for each marker is also reported.

Partitioning of the hs-cTnT concentrations revealed 3 distinct age groups: 0 to < 6 months, 6 months to < 1 year, and 1 to < 19 years with higher concentrations in the 2 youngest age groups. NT-proBNP concentrations were partitioned into 2 distinct age groups: 0 to < 1 year and 1 to < 19 years; similarly, concentrations are higher in infants aged 0 to < 1 year. While NT-proBNP concentrations were visibly higher in the first month of life and rapidly declined thereafter, with stable concentrations between 1–12 months,

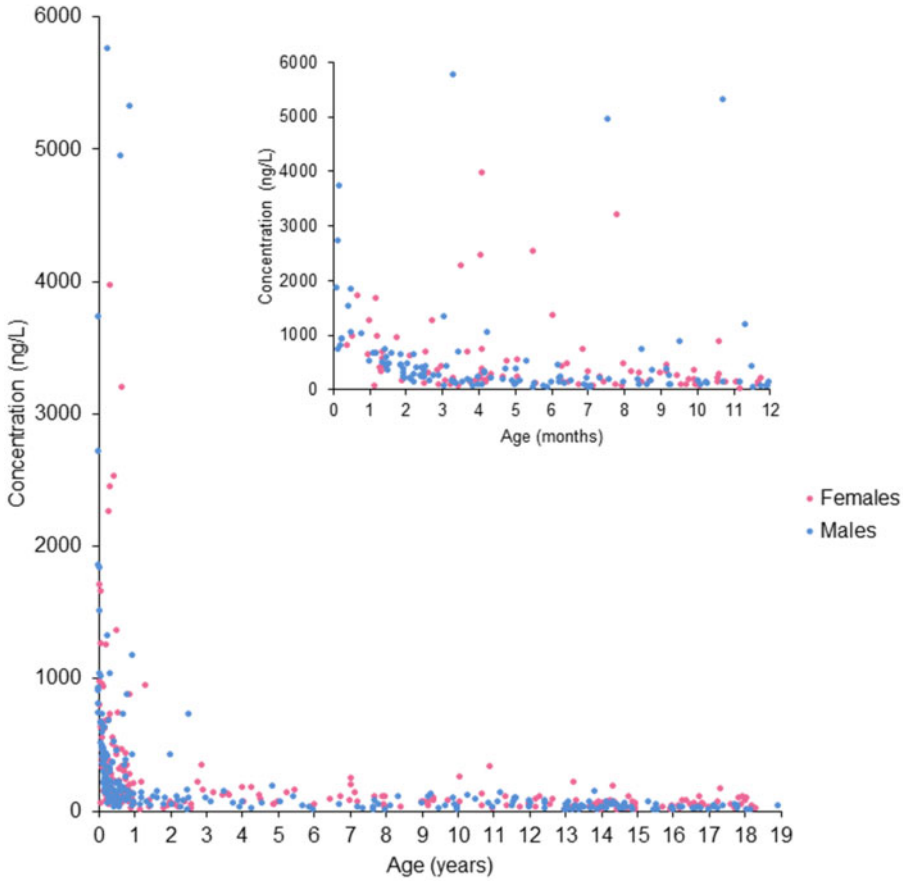

Fig. 2. Concentrations of NT-proBNP in children and adolescents under 19 years of age by sex (females: pink, males: blue). Inset: NT-proBNP concentrations in infants aged 0–1 year.

the sample size was insufficient to calculate a reference interval accurately for 0–1 month.

DISCUSSION

Cardiac troponin assays are widely available and, in particular, high-sensitivity assays are becoming critical in the diagnosis of acute myocardial infarction *(24)*. In children, these assays are used to detect cardiac disease such as acute myocarditis and dilated cardiomyopathy. Roche's Troponin T hs assay has a 99th percentile cut-off value of 14 ng/L, but this cut-off was determined in adult populations and is not age-specific *(7)*. This study is the first to present reference intervals for the Troponin T hs assay in a large pediatric population that includes children from birth to age <19 years.

Members of our group previously reported pediatric hs-cTnT reference ranges and 99th percentile cut-offs in the CALIPER cohort using Roche's Gen 5 hs-cTnT assay *(10)*. Our findings are generally similar, with increased levels in the first 6 months of life, followed by low levels throughout childhood and adolescence. However, Bohn et al.

Table 1. Pediatric reference intervals and cut-offs for high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic protein (NT-proBNP).

Analyte	Age range	n	Lower limit (90% CI[a]) (ng/L)	Upper limit (90% CI) (ng/L)	99th percentile Cut-off (90% CI) (ng/L)	% > 3 ng/L	% > 5 ng/L
hs-cTnT	0–<6 months	64	7 (4, 10)	78 (68, 87)	87 (76, 97)	100%	N/A
	6 months–<1 year	45	6 (5, 7)	34 (28, 42)	39 (32, 47)	100%	N/A
	1–<19 years	131	<3 (<3, <3)	9 (6, 11)	11 (11, 14)	30%	N/A
NT-proBNP	0–<1 year	211	39 (26, 57)	3569 (2188, 4876)	5272 (4788, 6937)	N/A	100%
	1–<19 years	265	<5 (<5, 7)	178 (144, 203)	216 (182, 250)	N/A	97%

[a] CI, confidence interval.

(10) observed a more rapid decrease during the remainder of the first year of life, with a 99th percentile value of 21 ng/L between 6 months and <1 year of age, while we found a 99th percentile value of 46 ng/L during the same age range. Interestingly, Bohn et al. observed sex-specific differences in children and adolescents from age 1 to <19 years. It is possible that we were not able to detect any sex-specific differences in this present study due to a smaller sample size in this age range.

Previous studies using a non-high-sensitivity cTnT assay have inferred that cTnT levels are higher in newborns and infants compared to older children and adult reference values *(25, 26)*. However, these studies did not concurrently evaluate cTnT levels in infants and older children, which limits the interpretation of infant cTnT levels in relation to those of older children because results vary depending on the assay and analytic platform used. Franzini et al. *(26)* calculated the 99th percentile upper reference limit in an adolescent cohort (10–14 years of age) as 8.1 ng/L, which agrees with the upper reference limit of 9 ng/L in our 1 to <19 year partition.

Levels of NT-proBNP in infants reported here are consistent with those reported for other NT-proBNP assays, which also show elevated levels in neonates *(27, 28)*. Similar to our results and using the Roche platform, Nir et al. *(28)* evaluated NT-proBNP in 690 individuals from birth to 18 years of age and found markedly higher plasma levels of NT-proBNP in newborns with levels decreasing from birth to two years of age. They constructed 7 age partitions: 0 to 2 days, 3 to 11 days, >1 month to ≤ 1 year, >1 to ≤ 2 years, >2 to ≤6 years, >6 to ≤14 years, >14 to ≤18 years. We included more infants from birth to 1 year of age (n = 210) than their 3 lower age partitions (n = 177 across their 0 to 2 days, 3 to 11 days, and >1 month to ≤ 1 year partitions), which may have strengthened the robustness of our reference intervals. In addition, Nir et al. included samples collected from individuals who had blood taken for other clinical indications, while samples from the CALIPER cohort were taken from individuals who did not have any suspected clinical conditions. Albers et al. *(27)* analyzed NT-proBNP on a Roche platform in 408 individuals aged 1–29 years and constructed 12 age partitions, although the sample sizes for almost all partitions fell below the minimum sample size of 40 recommended for constructing reference intervals *(23)*. These authors also analyzed samples from pediatric patients with other clinical conditions. However, consistent with our findings, both previous studies did not find any differences between males and females in NT-proBNP concentrations.

Since our study population includes only apparently healthy newborns and infants, the elevated hs-cTnT and NT-proBNP levels likely do not indicate myocardial damage. The physiological mechanisms behind these post-natal concentrations are currently unknown, although animal studies have shown that programmed cell death occurs during growth of the atria and ventricles *(29)*, so biomarker evidence of myocardial apoptosis would not be surprising during the rapid development of the first year of life. cTnT and proBNP are released into the blood during myocardial apoptosis and cardiac wall stress, respectively *(30, 31)*, which may explain the increased levels of these markers circulating in the blood.

Hs-cTnT and NT-proBNP have been used to examine subclinical cardiac damage in adults, and those with elevated levels of these biomarkers appear to have an associated decrease in left ventricular function as determined by echocardiography *(32)*. However, it remains unknown whether these biomarkers might also reflect subclinical cardiac damage in a pediatric population, and if so, whether adult cut-offs are applicable to children. For example, Soongswang et al. *(33)* suggested that the cTnT cut-off in adults with acute myocarditis (>100 ng/L using a conventional cTnT assay) may be different than that in children (52 ng/L). Cantinotti et al. (*34)* noted that the use of NT-proBNP in the pediatric population is limited by a paucity of large prospective studies evaluating the clinical utility of this marker in the diagnosis, prognosis, and treatment of children with heart disease. Various studies of pediatric myocardial injury and heart disease have observed elevated levels of these cardiac biomarkers in newborns and older children *(35, 36)*, but without comparisons to pediatric cut-offs or a sufficiently large reference population, these findings must be interpreted with caution. Importantly, these studies must take into consideration that hs-cTnT and NT-proBNP are naturally higher in infants aged <1 year, and as such, findings of elevated markers in infants should be interpreted using reference intervals and cut-offs specific to this age group.

This study is limited due to the use of only a screening questionnaire to determine participant eligibility without including imaging, other surrogate biomarker data, or a physical examination. Recent guidelines published by the American Association for Clinical Chemistry Academy and the International Federation of Clinical Chemistry and Laboratory Medicine suggest that sex-specific 99th percentile upper reference limits for high-sensitivity cTn assays should be determined by including samples from a minimum of 300 men and 300 women *(3)*. As such, sex-specific pediatric reference ranges may need to be further refined using larger study cohorts.

In healthy adults, the intra-individual variability of hs-cTnT and NT-proBNP has been noted to be higher with weekly sampling compared to hourly or daily sampling *(37, 38)*. For hs-cTnT, the CV ranges from 1.2–48.2% for 4- to 6-hour sampling, to 8.3–94% for 4- to 10-week sampling *(37)*. The CV of NT-proBNP ranges from 9% for daily sampling to 35% for weekly sampling *(38)*. The index of individuality, defined as the ratio of intra-individual variability to inter-individual variability, is considered to be low (and therefore indicates strong individuality) if it is <0.6, and it is considered high (which supports the use of reference intervals) if it is >1.4 *(37)*. The index of individuality appears to be moderate for hs-cTnT, varying from 0.20 to 1.4 depending on study methodology *(37)*. Previous studies have reported a low index of individuality for NT-proBNP ranging from 0.5 to 0.64 *(39, 40)*. Thus, it is important to note that these indices may suggest that reference intervals for hs-cTnT and NT-proBNP have limited clinical utility; instead, serial measurements may be more useful in guiding clinical decisions. However, intra-individual variability of these two biomarkers have not yet been studied in a pediatric population.

Here, we are the first to present hs-cTnT and NT-proBNP data together in a single healthy cohort of children and adolescents using the same analytical platform. In addition, although members of our group have previously reported hs-cTnT cut-offs for Roche's Troponin T Gen 5 STAT assay, the Troponin T hs assay is still widely used globally, which supports the importance of these reference data.

In summary, use of hs-cTnT and NT-proBNP as biomarkers to assess cardiovascular disease in children should consider pediatric-specific reference intervals and cut-offs rather than adult values. We have provided these here for the Roche platform in a large sample of healthy children and adolescents representing the full range of ages from 0 to <19 years. Further research is needed to elucidate the physiological mechanisms of these biomarker levels, which will guide the appropriate diagnosis, risk-stratification, and treatment of children at high risk of cardiovascular disease.

Nonstandard Abbreviations: cTn, cardiac troponin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CV, coefficient of variation; LoD, limit of detection; hs-cTnT, high-sensitivity cardiac troponin T; BNP, B-type natriuretic peptide; proBNP, pro-B-type natriuretic peptide; hs, high sensitive; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; LoB, limit of blank; CLSI, Clinical and Laboratory Standards Institute.

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