Original Article

Specific reference interval for high-sensitivity cardiac troponin I among healthy children in Wuhan, China

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Background: Troponin (Tn) is of an important biomarker for the diagnosis and prognosis of myocardial injury and for evaluating the severity of cardiac involvement due to other systemic diseases in children. Unfortunately, high-sensitivity cardiac troponin I (hs-cTnI) specific reference intervals (RIs) are extremely limited. This study aimed to establish a preliminary pediatric hs-cTnI RI for newborns, children, and adolescents in Wuhan, China.

Methods: A total of 1,355 healthy participants (1 day to 19 years) were recruited from a cross-sectional study implemented in Wuhan Children's Hospital from September 2022 to August 2023. Serum hs-cTnI levels were detected via the Mindray automated chemiluminescence immunoassay analyzer (CL-6000i). Specific serum hs-cTnI RIs were established according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The RIs were defined by the nonparametric median (P50), and 2.5th, 97.5th [P50 (P2.5–P97.5)] intervals.

Results: Of the 1,355 pediatric participants, serum hs-cTnI concentrations of 1,332 children were measured. The serum overall P50 and 95% interval range (P2.5–P97.5) of serum hs-cTnI was 0.41 (0.00, 44.31) ng/L. This was higher in males of 0.47 (0.00, 44.90) ng/L than in females of 0.36 (0.00, 44.17) ng/L (P<0.01). Age- and sex-specific differences in hs-cTnI levels were observed. The levels were highly variable in children under 1 year of age (especially in newborns), deriving a P50 (P2.5–P97.5) of 22.06 (1.04, 154.22) ng/L, and gradually narrowed and decreased with increasing age, with a markedly lower established P50 (P2.5–P97.5) of 0.36 (0.00, 2.16) ng/L. However, the levels began to increase slightly at the age of 9–12 years and reached a small peak at the age range of 15 to 18 years in males with 0.71 (0.03, 3.29) ng/L, while females were less affected by puberty. Sex- and age-specific RIs for hs-cTnI were established: 5 age-specific RIs in males, 1 day–1 month: 30.16 (8.67, 171.81) ng/L; >1–12 months: 13.20 (0.63, 61.91) ng/L; >1–15 years: 0.36 (0.00, 1.86) ng/L; >15–18 years: 0.71 (0.03, 3.29) ng/L; >18–19 years: 0.52 (0.00, 1.92) ng/L; and 4 age-specific RIs in females, 1 day–1 month: 43.93 (18.82, 146.38) ng/L; >1–12 months: 5.22 (0.92, 42.54) ng/L; >1–6 years: 0.54 (0.00, 2.74) ng/L; >6–19 years: 0.23 (0.00, 1.56) ng/L.

Conclusions: This study preliminarily established age- and sex-specific serum hs-cTnI RIs using the Mindray CL-6000i system in healthy children in Wuhan, China.

Keywords: High-sensitivity cardiac troponin I (hs-cTnI); myocardial injury; reference intervals (RIs); newborn; children

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Introduction

Although acute myocardial infarction (AMI) and coronary artery disease are extremely rare in children, myocardial injury caused by various primary and secondary diseases is also an important contributor to childhood morbidity and mortality (1). For example, cardiac myocarditis, which is a common heart disease in children, is often misdiagnosed owing to its atypical early clinical manifestations, diverse electrocardiographic changes, and few clinically specific markers. Severely ill children can experience cardiogenic shock, heart failure (HF), and even death (2). Therefore, early diagnosis and assessment of myocardial injury remains a major challenge for pediatric patients.

Troponin (Tn) is an important regulator of striated muscle contraction, which is expressed in both skeletal and cardiac muscles and is composed of three subunits: TnC (the Ca^{2+} binding subunit), TnI (the inhibitory subunit), and TnT (the tropomyosin binding subunit) (3). Compared to traditional kinase isoenzymes (CK-MB) and myoglobin, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) show better specificity and sensitivity to myocardial cell injury and are considered the gold-standard biomarkers for the diagnosis of AMI (4). At present, cTn is widely used in the diagnosis and risk stratification of acute and chronic myocardial injury in adult. For children, due to the physiological changes and common diseases during their growth and development being different from those of adults, the clinical application and interpretation of cTn testing are also different. cTnI/cTnT has important value in the auxiliary diagnosis and prognosis assessment of cardiovascular diseases such as acute chest pain, myocarditis, congenital heart disease (CHD) and HF in children, and can be used to assess the severity of non-cardiovascular diseases and the degree of cardiac involvement (5-8).

With the promotion of high-sensitivity cTn (hs-cTn) testing, its clinical application in children is also more extensive. In pediatric CHD, hs-cTn may provide a better prognostic value when compared to an adult population (7). Unfortunately, there are limited data on high-sensitivity cTnI (hs-cTnI) in healthy newborns, children, and adolescents, leading most laboratories to take reference of adult-based cutoff values to interpret the results. However, the age-specific reference intervals (RIs) for laboratory test results are an essential prerequisite for determining a child's health status, because children and adolescents have different blood concentrations of various clinical laboratory analytes at different stages of growth and development. The lack of accurate pediatric RIs is a major issue that limits the use of the hs-cTn approach for the diagnosis and clinical care of cardiac diseases in these populations. Some studies have reported the pediatric hs-cTnI RIs, but most were based on small samples (9-11). For example, Bohn *et al.* (11) analyzed 271 serum samples (1 day–18 years old) from the Canadian Laboratory Initiative on Pediatric Reference

Highlight box

Key findings

- This study established age- and sex-specific serum high-sensitivity cardiac troponin I (hs-cTnI) reference intervals (RIs) for newborns, children, and adolescents in Wuhan, China.

What is known and what is new?

- hs-cTnI has increasing application in the diagnosis and prognostication of cardiac conditions in children (e.g., for myocarditis, congenital heart disease, heart failure). However, there are limited data on pediatric reference standards of hs-cTnI, leading most laboratories to take reference of adult-based cutoff values to interpret the results.
- This analysis provides age- and sex-specific serum hs-cTnI RIs in 1 day–19 years healthy children in Wuhan, China. RIs of serum hs-cTnI established based on the Mindray CL6000i was relatively reliable, and might have sufficient comparability among the instruments.

What is the implication, and what should change now?

- These findings will provide more accurate RIs for clinical use to better distinguish the status and degree of myocardial injury in pediatrics.
- Further multi-center and large sample validation is needed. The validity, sensitivity, and specificity of these RIs for the diagnosis of myocardial injury in the real world need to be further clarified and optimized.

Intervals (CALIPER) cohort to establish age-specific RIs for hs-cTnI on the Abbott Alinity I system. However, the establishment of an RI for cTn should also consider the influence of ethnicity, region, population size, inclusion/exclusion criteria, statistical methods and detection methods (12-15). Age- and sex-specific 99th percentile upper reference limits (URL) for hs-cTnI levels have been determined in healthy Chinese adults (16). Therefore, accurate RIs are essential for clinical decision making in the pediatric population.

In this study, we measured serum hs-cTnI concentrations in 1,332 recruited healthy children aged 1 day–19 years via the Mindray automatic chemiluminescence immunoassay analyzer CL-6000i. We then assessed and analyzed its distribution in these populations using appropriate statistical methods according to the C28-A3 guidelines of Clinical Laboratory Standards Institute (CLSI) document (17), and accurate serum hs-cTnI RIs for Chinese children were established. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-24-98/rc).

Methods

Sampling methods

Based on CLSI C28-A3 guidelines (17), reference individuals were selected by direct sampling techniques according to specific, well-defined inclusion and exclusion criteria. Reference individuals covered as many age groups as possible (1 day–19 years), and the number of male and female individuals was equal.

Sample size

According to the recommendation of CLSI C28-A3 guidelines (17), a minimum sample size of 120 cases was necessary for RI calculation (95% RI, two-tailed) to obtain a robust estimate of the 90% confidence interval using nonparametric methods. In addition, at least 120 cases per group were required for grouping. For newborns and infants, a minimum sample size of at least 40 cases was recommended. Gender ratio of overall and each group was designed as 1:1.

Research participant recruitment

Overall, 1,355 healthy children who visited the physical examination center and out-patients of Wuhan Children's Hospital from September 2022 to August 2023 were selected as the research participants. Clinical pediatricians conducted physical examination and asked about past medical history for screening. Inclusion criteria were as follows: (I) apparently healthy and metabolically stable children in the physical examination center and out-patients in our hospital; (II) healthy full-term neonates with a birth weight of 2.5–4.0 kg, an Apgar score of 8–10, and no abnormality in routine screening for genetic diseases. Exclusion criteria included: (I) participants with a history of heart disease, cardiovascular disease or a family history of cardiovascular-related diseases; (II) participants taking cardiovascular drugs or anticoagulants; (III) participants with a history of severe respiratory diseases, systemic acute or chronic diseases, or infection in the past six months; (IV) participants having a history of chronic illness, acute illness within 7 days of sample collection. Children with blood specimen abnormalities, such as hemolysis were also excluded (*Figure 1*). After screening, 1,332 samples from healthy individuals (653 males and 679 females, aged 1 day to 19 years) were included in the establishment of the RIs for hs-cTnI. Our reference population was divided into the following nine age groups according to the age distribution: 1 day–1 month, >1–12 months, >1–3 years, >3–6 years, >6–9 years, >9–12 years, >12–15 years, >15–18 years, >18–19 years.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) for research on human participants, and this study was approved by the Ethics Committee of the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital, No. 2022R040-E01/2024R042-E01). Written informed consent was obtained from all participants or their guardians (provided by at least a guardian).

Sample collection and measurement

Subjects were required to have an overnight fast of 8–10 h. In brief, neonates and infants were examined before their next feeding. Children aged over 1 year were required to abstain from food and water for at least 8 h but no more than 10 h. Considering a person's biological rhythms, venous blood (2 mL) was drawn from each participant between 6 and 10 a.m. As in our previous study (18), a Roche Cobas P612 pretreatment system (Roche

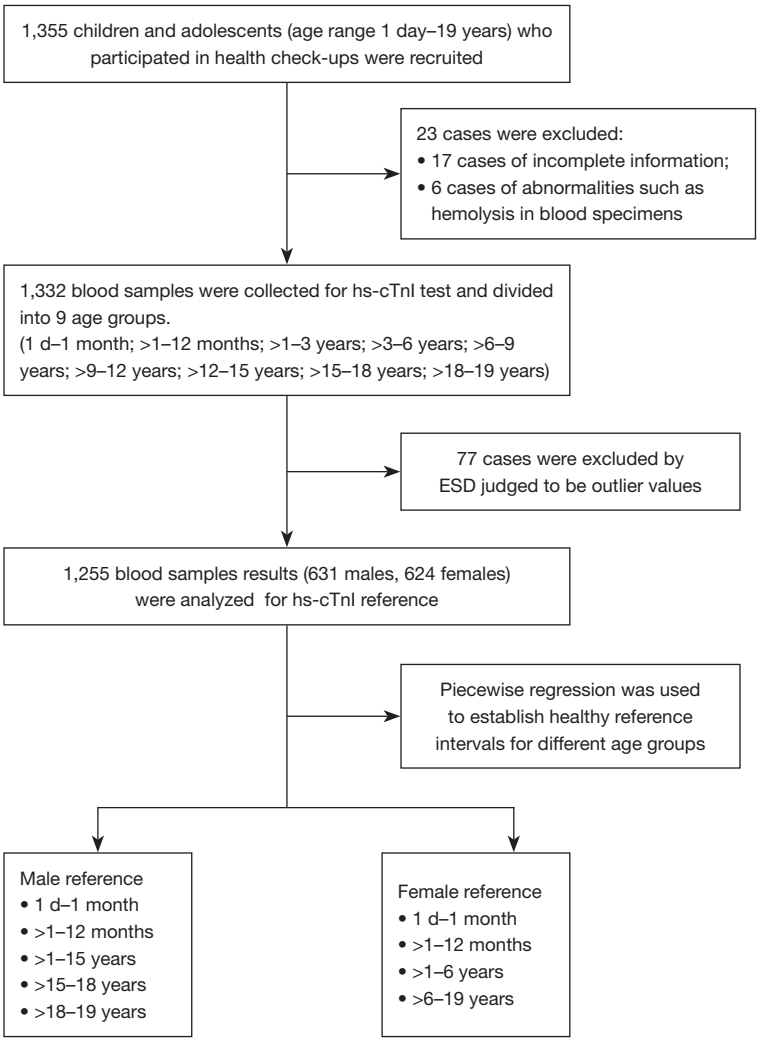

Figure 1 Flowchart of recruitment and exclusion used to establish hs-cTnI healthy reference interval. hs-cTnI, high-sensitivity cardiac troponin I; ESD, extreme studentized deviation.

Diagnostics, Germany) was used to separate serum within 6 h from sample collection. An hs-cTnI (CLIA) assay (Mindray, Shenzhen, China) was immediately conducted to detect serum hs-cTnI levels via the Mindray automated chemiluminescence immunoassay analyzer (CL-6000i). Serum samples that could not be detected on the same day were immediately stored in a −80 ℃ ultra-low-temperature refrigerator for detection to be carried out on the next day. Samples can only freeze and thaw once, not repeatedly. Standard methodologies, and dedicated reagents (Mindray, Shenzhen, China) were used according to the manufacturer's instructions, and routine maintenance was performed.

Quality control

In previous research (19), the analytical and clinical diagnostic performance of hs-cTnI assays has been thoroughly evaluated. All the results were in accordance with the requirements of International Federation of Clinical Chemistry and Laboratory Medicine for hs-cTn detection (20). The analytical method was strictly controlled by routine maintenance, calibration, and quality control according to the manufacturer's instructions and clinical laboratory standards and routine operating protocols, and samples were analyzed only when all analytical parameters were acceptable. The linear range of the hs-cTnI kit was

Table 1 Comparison of age distribution between males and females

Age group	Outlier (n)	Total, n (%)	F, n (%)	M, n (%)	G	P value
1 d–1 m	6	59 (4.70)	29 (4.65)	30 (4.75)	0.08	0.78
>1–12 m	2	68 (5.42)	35 (5.61)	33 (5.23)		
>1–3 y	6	134 (10.68)	65 (10.42)	69 (10.94)		
>3–6 y	11	199 (15.86)	102 (16.35)	97 (15.37)		
>6–9 y	18	199 (15.86)	101 (16.19)	98 (15.53)		
>9–12 y	12	171 (13.63)	77 (12.34)	94 (14.9)		
>12–15 y	9	158 (12.59)	70 (11.22)	88 (13.95)		
>15–18 y	11	199 (15.86)	110 (17.63)	89 (14.10)		
>18–19 y	2	68 (5.42)	35 (5.61)	33 (5.23)		

d, day; m, month; y, year; M, male; F, female.

2.4–25,000.0 ng/L. The limit of detection (LoD) for this assay was 0.50 ng/L.

Statistical analysis

Statistical analysis was constructed by R program V.3.6.3 and R-studio (version 1.4.1106, R Foundation for Statistical Computing, Vienna, Austria), as described in our previous study (18). Frequencies and percentages were used to describe the categorical variables. According to the CLSI EP09-A3 guidelines (21), outliers were eliminated by combining the generalized extreme studentized deviation (ESD) method and box diagram (outlier ratio ≤5%). Normal distribution of the data was checked using the Shapiro-Wilk normality test and skewness/kurtosis tests. The sex differences in hs-cTnI distribution were evaluated by the Kruskal-Wallis test. The Goodman-Kruskal gamma test was used for the distribution test in the age group × sex crosstabs. The "ggplott2" package was used to represent the probability density curves of hs-cTnI in the males, females, and total populations. A scatter plot with linear fitting of the distribution of hs-cTnI levels at different ages was constructed using GraphPad Prism V.8.00 (GraphPad Software, CA, USA). Age division of the reference values of the detection indicators was performed using a piecewise regression analysis. We used the nonparametric method to estimate the RIs for hs-cTnI. The hs-cTnI levels were presented as the 2.5th percentile (P2.5), median (P50), and 97.5th percentile (P97.5). The RI was calculated using P2.5 for the low reference limit and P97.5 for the high reference limit. A two-sided P value <0.05 was considered significant.

Results

Characteristics of the study population

Based on the generalized ESD method and box diagram, 77 hs-cTnI values were eliminated as outliers. A total of 1,255 healthy individuals were ultimately included in this study, and were divided into subgroups according to age: 59 newborns (4.70%) were aged under 1 month, 68 infants (5.42%) were aged >1–12 months, 134 subjects (10.68%) were aged >1–3 years, 199 subjects (15.86%) were aged >3–6 years, 199 subjects (15.86%) were aged >6–9 years, 171 subjects (13.63%) were aged >9–12 years, 158 subjects (12.59%) were aged >12–15 years, 199 subjects (15.86%) were aged >15–18 years, 68 subjects (5.42%) were aged >18–19 years. The female-to-male ratio was 1:1.01, with 49.72% (624/1,255) being female, and the age distribution differences between the male and female groups were not statistically significant (G =0.08, P=0.78). Detailed characteristics of the study populations are shown in *Table 1*.

Distribution characteristics of hs-cTnI

The proportion of samples with hs-cTnI concentrations above the LoD was 44%. The probability density distributions of serum hs-cTnI levels in the overall, male, and female populations are shown in *Figure 2*. The distribution of serum hs-cTnI levels in the pediatric population was markedly skewed. The median and 95% interval range (P2.5–P97.5) of hs-cTnI levels in the population divided according to age and sex are shown in *Table 2*. The overall serum median and 95% interval range

(P2.5–P97.5) of hs-cTnI was 0.41 (0.00, 44.31) ng/L. This was higher in males [0.47 (0.00, 44.90) ng/L] than in females [0.36 (0.00, 44.17) ng/L] (P<0.01, *Table 2*). Age-specific differences were also observed. We found that hs-cTnI was highly variable in children under 1 year of age (especially in newborns), with a P50 (P2.5–P97.5) of 22.06 (1.04, 154.22) ng/L, which gradually narrowed and decreased with increasing age, with a markedly lower established P50 (P2.5–P97.5) of 0.36 (0.00, 2.16) ng/L (*Figure 3*). However, serum hs-cTnI levels began to increase slightly at the age of 9–12 years and reached a small peak at the age range between 15 and 18 years in males at 0.71 (0.03, 3.29) ng/L. Conversely, females were less affected by puberty.

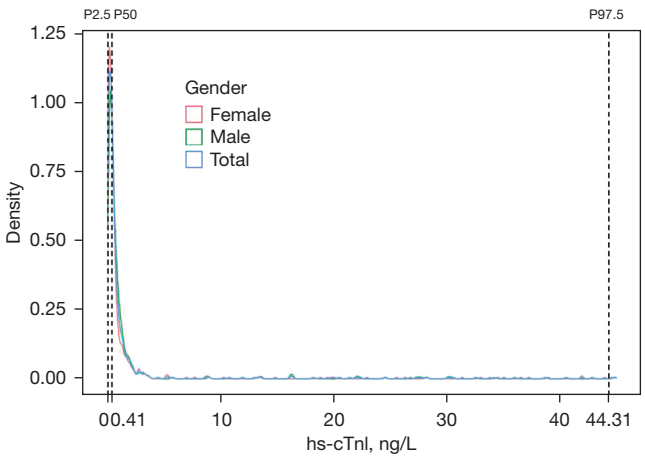

Figure 2 The probability density distributions of sex-specific hs-cTnI. The red, green, and blue curves represent the female, male and total distributions, respectively. Three dashed lines represent the P2.5, P50 and P97.5, respectively. hs-cTnI, high-sensitivity cardiac troponin I; P2.5, 2.5th percentile; P50, median; P97.5, 97.5th percentile.

Pediatric RIs for hs-cTnI

Differences in serum hs-cTnI levels were observed in different age groups according to the Kruskal-Wallis analysis and Nemenyi test. Age- and sex-specific pediatric RIs for hs-cTnI are presented in *Figure 4* and *Table 3*. We found that hs-cTnI levels were higher in newborns, resulting in a wide variation in the distribution during this period, which gradually narrowed and decreased with increasing age. Small sex-specific differences were observed at 12–19 years, with higher concentrations in males. In addition, hs-cTnI levels increased and reached a small peak again between 15 and 18 years in males. Thus, age partitioning was performed at this age. In females, as there was little change in the hs-cTnI concentrations from 6 to 19 years of age, no partitioning was required.

Table 2 Serum hs-cTnI concentrations according to age and sex in the study population P50 (P2.5, P97.5) (ng/L)

Age group	Total	M	F	P
1 d–12 m	22.06 (1.04, 154.22)	22.06 (0.85, 170.71)	21.5 (1.16, 140.59)	0.71
1 d–1 m	37.23 (8.74, 176.69)	30.16 (8.67, 171.81[a])	43.93 (18.82, 146.38[a])	0.07
>1–12 m	5.46 (0.84, 61.75)	13.20 (0.63, 61.91[a])	5.22 (0.92, 42.54[a])	0.16
>1–19 y	0.36 (0.00, 2.16)	0.41 (0.00, 2.18)	0.30 (0.00, 2.09)	<0.01
>1–3 y	0.85 (0.00, 3.20)	0.90 (0.00, 3.17)	0.76 (0.01, 3.34)	0.76
>3–6 y	0.38 (0.00, 2.41)	0.31 (0.00, 2.57)	0.40 (0.00, 2.49)	0.13
>6–9 y	0.26 (0.00, 0.94)	0.27 (0.00, 0.92)	0.24 (0.00, 1.00)	0.47
>9–12 y	0.36 (0.00, 1.03)	0.38 (0.00, 1.02)	0.34 (0.00, 1.11)	0.14
>12–15 y	0.30 (0.00, 1.69)	0.38 (0.00, 1.81)	0.19 (0.00, 1.72)	<0.01
>15–18 y	0.40 (0.00, 2.86)	0.71 (0.03, 3.29)	0.22 (0.00, 2.74)	<0.01
>18–19 y	0.27 (0.00, 1.92)	0.52 (0.00, 1.92)	0.15 (0.00, 0.98)	<0.01
1 d–19 y	0.41 (0.00, 44.31)	0.47 (0.00, 44.90)	0.36 (0.00, 44.17)	<0.01

Data were analyzed by the Kruskal-Wallis test. [a], 95th percentile presented and 97.5th not available. hs-cTnI, high-sensitivity cardiac troponin I; P2.5, 2.5th percentile; P50, median; P97.5, 97.5th percentile; m, month; d, day; y, year; M, male; F, female.

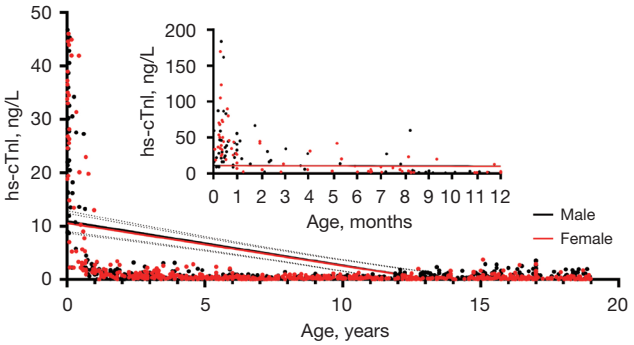

Figure 3 The distributions for hs-cTnI concentrations graphed by age and color-coded by sex. Inset: hs-cTnI concentrations in infants aged 0–12 months. The red and black dots represent the female and male values, respectively. The fitted curve based on a linear regression model adjusted for age represents the linear distribution of hs-cTnI, and the red and black curves represent females and males, respectively. hs-cTnI, high-sensitivity cardiac troponin I.

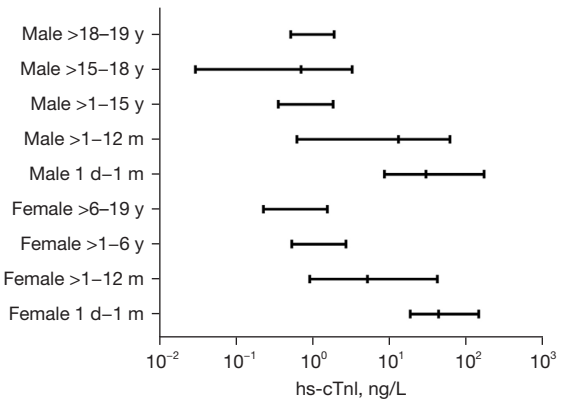

Figure 4 The group distributions of the RIs stratified by age and sex for hs-cTnI. The line exhibits the 95% interval range, and the three short vertical lines represent the P2.5, P50, and P97.5 from left to right. RI, reference interval; hs-cTnI, high-sensitivity cardiac troponin I; P2.5, 2.5th percentile; P50, median; P97.5, 97.5th percentile; d, day; m, month; y, year.

Discussion

Appropriate medical diagnosis and treatment of children largely depend on the availability of accurate laboratory tests and applicable RIs. However, the lack of a large study population representative of healthy children makes it challenging to establish RIs in pediatric populations (22). Currently, many programs are devoted to the development

Table 3 Sex- and age-specific pediatric RIs for hs-cTnI (ng/L)

Group	RI, P50 (P2.5, P97.5)
M	
1 d–1 m	30.16 (8.67, 171.81[a])
>1–12 m	13.20 (0.63, 61.91[a])
>1–15 y	0.36 (0.00, 1.86)
>15–18 y	0.71 (0.03, 3.29)
>18–19 y	0.52 (0.00, 1.92)
F	
1 d–1 m	43.93 (18.82, 146.38[a])
>1–12 m	5.22 (0.92, 42.54[a])
>1–6 y	0.54 (0.00, 2.74)
>6–19 y	0.23 (0.00, 1.56)

[a], 95th percentile presented and 97.5th not available. RI, reference interval; hs-cTnI, high-sensitivity cardiac troponin I; P2.5, 2.5th percentile; P50, median; P97.5, 97.5th percentile; d, day; m, month; y, year; M, male; F, female.

of pediatric RIs, such as the KiGGs study in Germany (23), CALIPER study in Canada (24), and Harmonizing Age Pathology Parameters in Kids (HAPPI Kids) in Australia (25), Pediatric Reference Intervals (PRINCE) in China (26); but the RIs of hs-cTnI in children remain largely unexplored. Although the 2018 American Association for Clinical Chemistry/International Federation of Clinical Chemistry (AACC/IFCC) standard does not recommend the establishment of a ethnicity-specific 99th percentile URL for hs-cTn, some studies have suggested that African-Americans have higher levels than Caucasians (27), Vietnamese have higher levels than Americans (28), and Chinese have lower levels than Koreans (29), Pakistanis (30) and Germans (31). However, given the diet, upbringing and environment of Chinese children may be different, it has been reported that the levels of creatine kinase and alkaline phosphatase in Chinese children are significantly lower than that of European and American children, and Chinese children develop elevated sex hormones at an earlier age than North American children (32,33). Thus, age- and sex-specific RIs for serum hs-cTnI in a Chinese pediatric population were preliminary established using the Mindray automatic chemiluminescence immunoassay analyzer CL-6000i in this study. This serves to fill the gap in the long-term lack of RIs in pediatrics in China after the National Health

Commission of China first issued health standards for RIs of blood cell analysis (WS/T779-2021) and commonly used clinical biochemical tests (WS/T780-2021) in children aged 28 days to 18 years (http://www.nhc.gov.cn, WS/T779-2021, and WS/T780-2021).

With the advent of highly sensitive methodologies, cTnI is detectable in the peripheral blood of most healthy children from birth to adolescence (9,10,34). Of all serum samples, the detection rate of hs-cTnI was 44% (LoD claimed by reagent manufacturers: 0.50 ng/L), and the detection rate could reach to 73% (LoD values reported in the latest performance validation of the Mindray hs-cTnI instrument by Li *et al.*: 0.21 ng/L) (19). The detection data in this study were adhered to the 2018 AACC/IFCC standard, which suggest that it is able to detect cTnI at concentrations at or above the LoD in at least 50% of healthy men and women. Further verification is required in the later stage (20). The distribution of hs-cTnI in the entire pediatric population in our study is as follows: a wide and high distribution during the newborn period (under 1 month), a rapid decline during 1 year, followed by a slow decrease. This trend is consistent with previous reports, including the CALIPER study (9-11) (*Table 4*), which suggests that the physiological release of cTnI occurs in healthy children. Differences and high variability of hs-cTnI concentrations have been reported in neonates within the 1st and in the 2nd week of age [median (interquartile range) 21.5 (31.2) *vs.* 9.3 (93.5) ng/L, respectively] by Clerico *et al.* (36). Elevated neonatal hs-cTnI levels may be due to transient hypoxia, perinatal cardiovascular remodeling in the process of adapting to extrauterine life, physical stress at birth and proliferating and enlarging cardiomyocytes with different metabolism and faster troponin turnaround after birth (1,36-38). The shift of the afterload pressure from the right ventricle to the left ventricle associated with reduced pulmonary resistance may be in connection with programmed apoptosis and cardiomyocyte proliferation in early infancy, which also leads to the release of small amounts of cTn (37,38).

Numerous studies have shown that hs-cTnI concentrations are higher during the first year of life (35,36), and this large variation is associated with the constant changes that children undergo, which further illustrates the influence of age on the establishment of RIs. In our study, the P97.5 URLs in children aged 1–19 year (total: 2.16 ng/L, female: 2.09 ng/L, male: 2.18 ng/L) were consistent with a larger pediatric study (n=4,029) reported by McEvoy *et al.* on Ortho hs-cTnI assay (total: 3.00 ng/L, female: 2.00 ng/L, male: 4.00 ng/L) (14) (*Table 4*), but when infants were included, we found that the overall P97.5 URL increased significantly to 44.31 ng/L. Further detailed analysis of the participants within 1 year old showed that the established P97.5 for newborns and infants were 176.69 and 61.75 ng/L, respectively, which are similar to that reported by Caselli *et al.* (139.40 and 85.15 ng/L) (10) (*Table 4*). Therefore, all these indicate that the RIs of serum hs-cTnI for newborns, infants, children, and adolescents established by us are reliable and age-specific.

Sex also has a huge impact on the establishment of RIs (24). Therefore, we further explored whether there is a difference in serum hs-cTnI levels between different sexes. The results showed that there was no statistically significant difference between males and females before the age of 12 years, but there were sex differences in adolescents (12 to 19 years). Regarding this observation, there are still some debates among studies (9-11,39). For example, no sex correlation was found for hs-cTnI levels in the CALIPER cohort study (n=271) (11), whereas sex differences in P97.5 was reported in the National Health and Nutrition Examination Survey (NHANES) study (n=4,029) (14). The small sample size may be the main reason for the lack of a comprehensive evaluation of the differences in hs-cTnI levels in terms of sex and age. In addition, unlike the two broad age partitions (0–6 months and 6 months–19 years) in the CALIPER cohort study (11), we captured some short time periods with highly dynamic changes between males and females by detailed age partitions in a larger study sample (n=1,255). We observed a slight increase in hs-cTnI levels in male adolescents starting from puberty (age of 9–12 years) and these reached a small peak between 15 and 18 years; however, hs-cTnI levels in females were less affected by puberty and were always lower than those in males. Thus, we further established different age groups for the RIs in males and females. Previously, the LIFE Children study has reported that there were sex differences in P97.5 for hs-cTnT, and that hs-cTnT also showed an upward trend in males in the second stage of puberty (40). Sex differences in hs-cTn levels in healthy adolescents may be driven by left ventricular mass, sex hormones, enzymes (such as alkaline phosphatase) and metabolic factors (such as body mass index and blood lipids levels) (1,33,40,41). Therefore, the RIs of hs-cTnI in different adolescents still require a large sample size for multicenter verification to eliminate disputes and ensure the accuracy and reliability of results.

The comparability of RIs between different instruments and reagents is of great significance for clinicians when

Table 4 Summary of pediatric reference intervals studies for hs-cTnI (ng/L)

Study	Year	Manufacturer	Specimen	Partition	N		P2.5	P25		P50		P75		P95	P97.5		P99	
					M	F		M	F	M	F	M	F		M	F	M	F
Kavsak *et al.* (9)	2014	Abbott	Serum	1–19 y	315										11.70		30.90	
Caselli *et al.* (10)	2016	Abbott	Plasma	1 d–1 m	19	17		12.85		21.45		44.00		139.36				
				1–12 m	36	21		5.70		11.50		21.65		85.15				
				1–10 y	32	33		1.60		2.20		2.83		38.98				
				10–18 y	108	113		1.50		2.00		2.80		6.30			41.29	
				1 d–18 y	379			1.70		2.90	2.10	6.20		46.84			115.77	
Caselli *et al.* (35)	2016	Abbott	Plasma	1 d–1 m	18	6		3.30		9.30		93.80						
				1–12 m	15	11		4.55		13.05		52.05						
				1–12 y	19	11		4.00		11.45		48.10						
				13–18 y	13	13		2.10		2.60		3.90						
				1 d–18 y	65	41		3.40	1.70	5.10		13.42	2.80					
Mondal *et al.* (34)	2021	Abbott	Cord	Term infants	130	126				6.00				41.45			166.30	
Bohn *et al.* (11)	2023	Abbott	Serum	1 d–6 m	49		1.10			9.10					48.00		55.80	
				6 m–19 y	222		0.10			1.00					4.80		5.50	
McEvoy *et al.* (14)	2023	Abbott	Serum	1–7 y	438										6.00		9.00	
				8–12 y	1,207										6.00		14.00	
				13–18 y	2,384										8.00		17.00	
				1–18 y	2,022	2,007									9.00	5.00	16.00	17.00
		Siemens	Serum	1–7 y	438										11.00		18.00	
				8–12 y	1,207										14.00		26.00	
				13–18 y	2,384										20.00		46.00	
				1–18 y	2,022	2,007									21.00	11.00	43.00	24.00
		Ortho	Serum	1–7 y	438										3.00		5.00	
				8–12 y	1,207										3.00		9.00	
				13–18 y	2,384										3.00		7.00	
				1–18 y	2,022	2,007									4.00	2.00	9.00	5.00

hs-cTnI, high-sensitivity cardiac troponin I; P2.5, 2.5th percentile; P50, median; P97.5, 97.5th percentile; P99, 99th percentile; d, day; m, month; y, year; M, male; F, female.

identifying diseases. The current establishment of pediatric RIs for hs-cTnI are mostly based on the Abbott system (*Table 4*). Owing to the lack of standardization or consistency between assays, instrument-specific RIs are not easy to transfer between different laboratories. As shown in *Table 4*, the distribution of Ortho and Mindray hs-cTnI were slightly shifted to the left compared to that of Abbott or Siemens hs-cTnI. Moreover, applying performance criteria (a mean imprecision of ≤3.7%) based on biological variation (42), we found only Mindray showed acceptable imprecision (coefficient of variation, CV ≤3.7%) in all samples, Siemens and Ortho produced relatively high CVs at the lower concentrations, and Abbott assay results exceeded the criteria for all three concentrations (*Table 5*). This showed that Mindray's precision may be better. These results indicated that the RIs of serum hs-cTnI established based on the Mindray CL6000i might have sufficient comparability between the instruments.

Table 5 Imprecision evaluation for hs-cTnI

Manufacturer	hs-cTnI-L		hs-cTnI-M		hs-cTnI-H	
	Concentrations (ng/L)	CV (%)	Concentrations (ng/L)	CV (%)	Concentrations (ng/L)	CV (%)
Mindray	10.51±0.22	2.07	36.05±0.84	2.32	10,679.00±163.40	1.53
Abbott (14)	8.00–16.00	6.40	169.00–314.00	3.50	2,758.00–4,444.00	6.70
Siemens (14)	12.00–28.00	3.80	–	–	9,000.00–21,000.00	2.60
Ortho (14)	6.00–16.00	4.20	–	–	17,511.00–21,403.00	2.80

Data are mean ± SD or range. L, M, H were quality controls based on serum pools (low value levels, median value levels, high value levels). hs-cTnI, high-sensitivity cardiac troponin I; CV, coefficient of variation; SD, standard deviation.

In theory, the 99th percentile URL has a higher confidence in the truth value, but compared with the 95th percentile, it has the disadvantages of a larger interval width, higher imprecision and being susceptible to outliers. Below the 99th percentile URL is probably a more reliable estimate (43). Therefore, choosing the right percentile URL is an important consideration in setting RIs. Apple *et al.* found that the sex-specific 99th percentile URL varied according to differences in detection and statistical methods by comparing nine hs-cTnI and three hs-cTnT assays, and underscored the importance of continuing to update RIs as new analytical systems are adopted (44). The 99th percentile is chosen to minimize false positives in the diagnosis of myocardial infarction. However in pediatrics, McEvoy *et al.* recommended the use of the more stable and statistically accurate sex-specific P97.5 URL to assess myocardial injury as myocardial infarction is rare in children and adolescents (14). In this study, we used P97.5 as the high RIs limit for calculating serum hs-cTnI levels. However, downgrading the 99th percentile URL remains controversial and requires extensive prospective research. The value of RIs requires further verification, the clinicians must also interpret cTn concentrations and their variations based on the clinical presentation of each patient.

Although we preliminarily established relatively reliable RIs for serum hs-cTnI in pediatrics and conducted a multisystem evaluation, there are still some limitations in this study. First, this was a single-center study, and multicenter validation is required. Second, although this study included a large sample of healthy Chinese children covering all ages, the sample size of some groups was still slightly insufficient, and it needs to be improved in the future. Third, the validity, sensitivity, and specificity of these RIs for the diagnosis of myocardial injury in actual clinical work need to be further clarified and optimized.

Conclusions

We preliminarily established relatively reliable RIs for serum hs-cTnI in healthy Chinese pediatrics using the Mindray CL-6000i system and conducted a multisystem evaluation with the currently established pediatric RIs, which can provide more accurate RIs for clinical use to better distinguish the status and degree of myocardial injury in pediatrics.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-24-98/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) for research on human participants, and this study was approved by the Ethics Committee of the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital, No. 2022R040-E01/2024R042-E01). Written informed consent was obtained from all participants or their guardians (provided by at least a guardian).

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