

Longitudinal Studies of Cardiac Troponin I in a Large Cohort of Healthy Children

Gus Koerbin,^{1,2} Julia M. Potter,^{1,3} Walter P. Abhayaratna,^{3,4} Richard D. Telford,³ Tony Badrick,⁵ Fred S. Apple,⁶ Allan S. Jaffe,⁷ and Peter E. Hickman^{1,3*}

BACKGROUND: There is little information available on cardiac troponin concentrations in healthy young children.

METHODS: Using a precommercial high-sensitivity assay from Abbott Diagnostics, we measured cardiac troponin I (cTnI) in longitudinal blood samples collected at ages 8, 10, and 12 years from a cohort of healthy, community-dwelling children. The 99th percentile values were calculated and estimates of the long-term biological variation were made.

RESULTS: cTnI concentrations were above the limit of detection in 87%, 90%, and 98% of the children at ages 8, 10, and 12 years. The 99th percentiles were lower compared to a healthy adult population in both male and female children at all ages studied. At the 3 periods of study assessment, different children had cTnI concentrations above the 99th percentile. The calculated 99th percentile varied markedly depending upon whether the lowest or highest cTnI measurement for an individual child was included in the calculation. Biological variation varied markedly between 0% and 136%, the index of individuality was low at 0.36, and the reference change value was an increase of 147% or a decrease of 59%.

CONCLUSIONS: In this longitudinal study of cTnI concentrations in healthy children as determined by a high-sensitivity assay, different children had concentrations of cTnI above the 99th percentile at the 3 episodes of assessment. These results suggest that in children the 99th percentile may not be a reliable index of silent cardiac disease, but rather may be indicating low-grade intercurrent illness.

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The 99th percentile is well established as an essential component of the definition of myocardial infarction (1, 2, 3). It has been demonstrated on innumerable occasions in adult populations, with both conventional and high-sensitivity assays, that persons with cardiac troponin concentrations above the 99th percentile are at a markedly increased risk of death from myocardial infarction (4–9). Studies defining the 99th percentile are always cross-sectional in nature, with samples being taken at a single time point. To our knowledge there have been no studies in which the same population has had repeat sampling to look at the reproducibility of the 99th percentile.

Studies of cardiac troponin concentrations in children are uncommon. We have had the opportunity to collect blood from the same cohort of 800 healthy children on 3 separate occasions at 2-yearly intervals and measured cardiac troponin I (cTnI)⁸ and cTnT (10) in these samples. All children were closely monitored during the study period, had physical examinations and serial echocardiographic confirmation of normal cardiac structure and function, and were free of any identified disease. For the current study we report our longitudinal investigations of the cTnI concentrations measured with a high-sensitivity assay in this cohort of healthy children.

Materials and Methods

This study was approved by the ACT (Australian Capital Territory) Health Human Research Ethics Committee. Informed consent was obtained from the parents or guardians of all study participants.

STUDY PARTICIPANTS

The Lifestyle of Our Kids (LOOK) study is a multidisciplinary study examining the beneficial effects of exercise in healthy young children. In 2005 a cohort of

¹ ACT Pathology, Garran, Australia; ² University of Canberra, Canberra, Australia; ³ Australian National University Medical School, Canberra, Australia; ⁴ Department of Cardiology, Canberra Hospital, Garran, Australia; ⁵ Faculty of Health Sciences and Medicine, Bond University, Gold Coast, Queensland, Australia; ⁶ Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, and University of Minnesota School of Medicine, Minneapolis MN; ⁷ Cardiovascular Division, Department of Internal Medicine, and Core Clinical Laboratory Services, Department of Laboratory Medicine and Pathology, Mayo Clinic and Medical School, Rochester, MN.

* Address correspondence to this author at: Canberra Hospital, Gilmore Crescent Garran, AC, Australia, 2605. Fax +61-2-62443398; e-mail peter.hickman@anu.edu.au.

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⁸ Nonstandard abbreviations: cTnI, cardiac troponin I; LOOK, Lifestyle Of Our Kids; hs, high sensitivity; LoB, limit of blank; LoD, limit of detection; CV_T, total CV; CV_i, biological variation for each individual child; RCV, reference change value.

healthy 8-year-old children was enrolled from Canberra schools, assigned to intervention and control groups, and followed for 4 years. The details of this study have been published elsewhere (11).

As part of this study, serum was collected in 2005, 2007, and 2009, when the children were 8, 10, and 12 years old. The children were requested to fast overnight and refrain from vigorous exercise on the morning of the sample collections. Details of the sample-handling procedures have been published elsewhere (12). After the initial analyses were performed, serum was stored frozen at -80°C .

ECHOCARDIOGRAPHY

Echocardiography was performed on all children at ages 8, 10, and 12 years. Cardiac structure and function were assessed by 1 of 2 experienced sonographers using transthoracic echocardiography (Vivid 7, General Electric Healthcare) according to a standardized protocol. Measurements were made on-line and recorded digitally with study number as their only identification. A cardiologist, blinded to the participants' clinical data, interpreted the echocardiogram after review off-line. Cardiac chamber sizes and left ventricular mass were quantified and indexed for body size according to current American Society of Echocardiography guidelines (13). Left ventricular ejection fraction was quantified by the biplane disc summation method (Simpson's rule) using the 2-dimensional echocardiography images from the apical 4- and 2-chamber views. A small number of children with abnormal cardiac structure, or function, including congenital or valvular heart disease, were excluded from this study.

TROPONIN ANALYSES

Samples used in this study were subjected to only 1 freeze-thaw cycle. The long-term stability of cTnI with both the Beckman Coulter high sensitivity (hs)-TnI (14) and the Abbott ARCHITECT STAT hs-cTnI assays (15) has been demonstrated under these conditions. Assays were performed in singlicate. The 30 highest results from each year were repeated and no discrepancies were found.

Analytical studies of the ARCHITECT STAT hs-cTnI assay from Abbott Diagnostics, which was made available in a precommercial form by the manufacturer (Abbott USA) showed that the limit of blank (LoB) was 0.5 ng/L, the limit of detection (LoD) was 1.0 ng/L, the concentration corresponding to the 20% CV was 1.8 ng/L, the concentration corresponding to the 10% CV was 3.9 ng/L, and the concentrations corresponding to the 99th percentile values were 14.0 ng/L in adult males and 11.1 ng/L in adult females (15).

STUDIES WITH hs-cTnI IN LOOK CHILDREN

Not all children had blood collected on every occasion. Thus we have some children who had only 1 sample collected, some with 2 samples, and some with 3 samples collected. We calculated the 99th percentile value for males and females separately at ages 8, 10, and 12 years, and looked to see what the effect would be on the calculated 99th percentile if we aggregated data for children who had more than 1 sample collected, using either the highest or lowest concentration obtained. All study participants had baseline hs-cTnT measurements (10). Where there was any discordance between the results for cTnT and cTnI, samples were checked for possible heterophile antibody interference, and 1 sample was excluded as a result.

For children who had a result above the 99th percentile and had more than 1 sample collected, we looked at whether such increases were reproducible, i.e., if a person had a result above the 99th percentile on one occasion, we investigated the likelihood of it being above the 99th percentile on a subsequent occasion. To enable some statistical comparison of that reproducibility of results, we partitioned results arbitrarily using 3 cut points, the 99th, 95th, and 75th percentiles (1 tail). We calculated concordance between the repeat measures as κ values using MedCalc™ version 9.2 software. A κ value of 0.21–0.40 was considered to be fair agreement, 0.41–0.60 moderate agreement, and 0.61–0.80 good agreement.

As we explain below, we were able to eliminate a peripubertal growth spurt as a source of variation. Hence the only contributions to the total variation were analytical variation and biological variation. Because this cohort was large, with multiple measurements made for many of the study participants, the total variation for each child was calculated as the SD of the 2 or 3 measurements taken over the course of the study and expressed as SD and total CV (CV_T) for each individual. We fitted a power function to our precision profile for this hs-cTnI assay (15) ($y = 26.457x^{-0.6466}$; $r^2 = 0.92$) and calculated the assay CV at the mean cTnI concentration for that child. This in turn allowed us to calculate the biological variation for each individual child (CV_i) as the difference between the CV_T and the analytical variation:

$$CV_i = \sqrt{(CV_T^2 - CV_A^2)}. \quad (1)$$

We independently verified these data by performing ANOVA. We used a Mann–Whitney test to compare data for those study participants for whom there were 2 measurements against those for whom we had 3 measurements to see if these study participants were members of the same population.

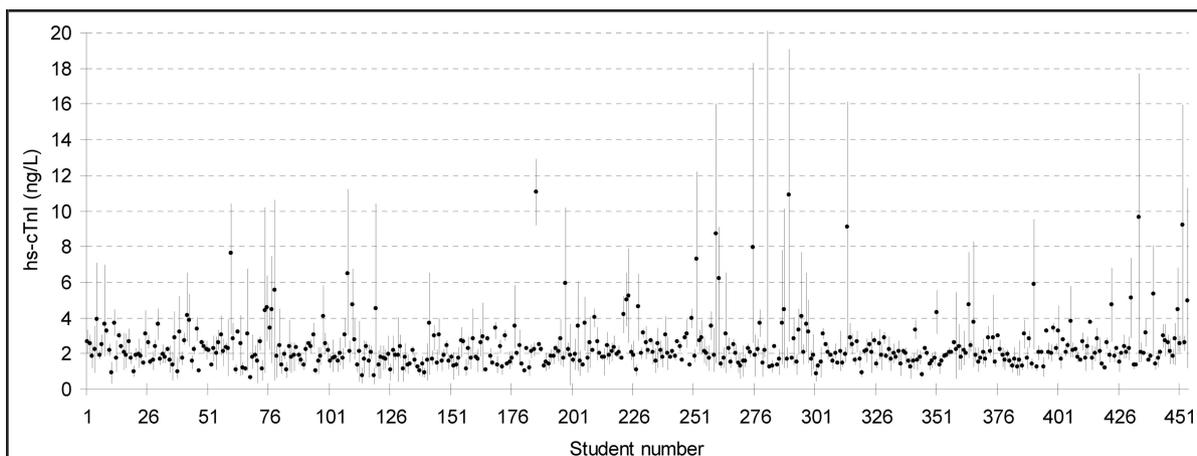


Fig. 1. Within- and between-child cTnI concentrations for 453 children who had more than 1 measurement made. The dots represent mean cTnI concentrations and the bars show the spread in cTnI concentrations. The children are ordered according to school starting with the school that was coded AR and finishing at the school coded WH. The results from 1 child with a peak cTnI concentration of 59 ng/L have been excluded from this figure.

We used our data from the multiple measurements on the children to calculate the index of individuality and the reference change value (RCV) as described by Fokkema et al. (16).

Results

Fig. 1 shows the distribution of cTnI for both within-subject and between-subject results. The great majority of results lie below 4 ng/L, with occasional results that are higher. Table 1 shows the 99th percentiles for boys and girls separately at the ages of 8, 10, and 12 years. These are substantially lower than comparable values in adults. Although there was a difference in troponin concentrations between the 8-year-old boys and girls ($P = 0.02$), for the older children there was no significant difference between the sexes. In addition, Table 1 shows the proportion of children who had cTnI concentrations above the LoB, LoD, 20% CV, 10% CV, and

adult 99th percentiles. Over the 3 sampling periods, 87%, 90%, and 98% of the children had results above the LoD ($P < 0.001$ for 2005 vs 2007 and 2007 vs 2009; $P < 0.05$ for 2005 vs 2009, Mann–Whitney nonparametric analysis). Between the ages of 8 and 12 years, median left ventricular mass had increased by 65% (data not shown).

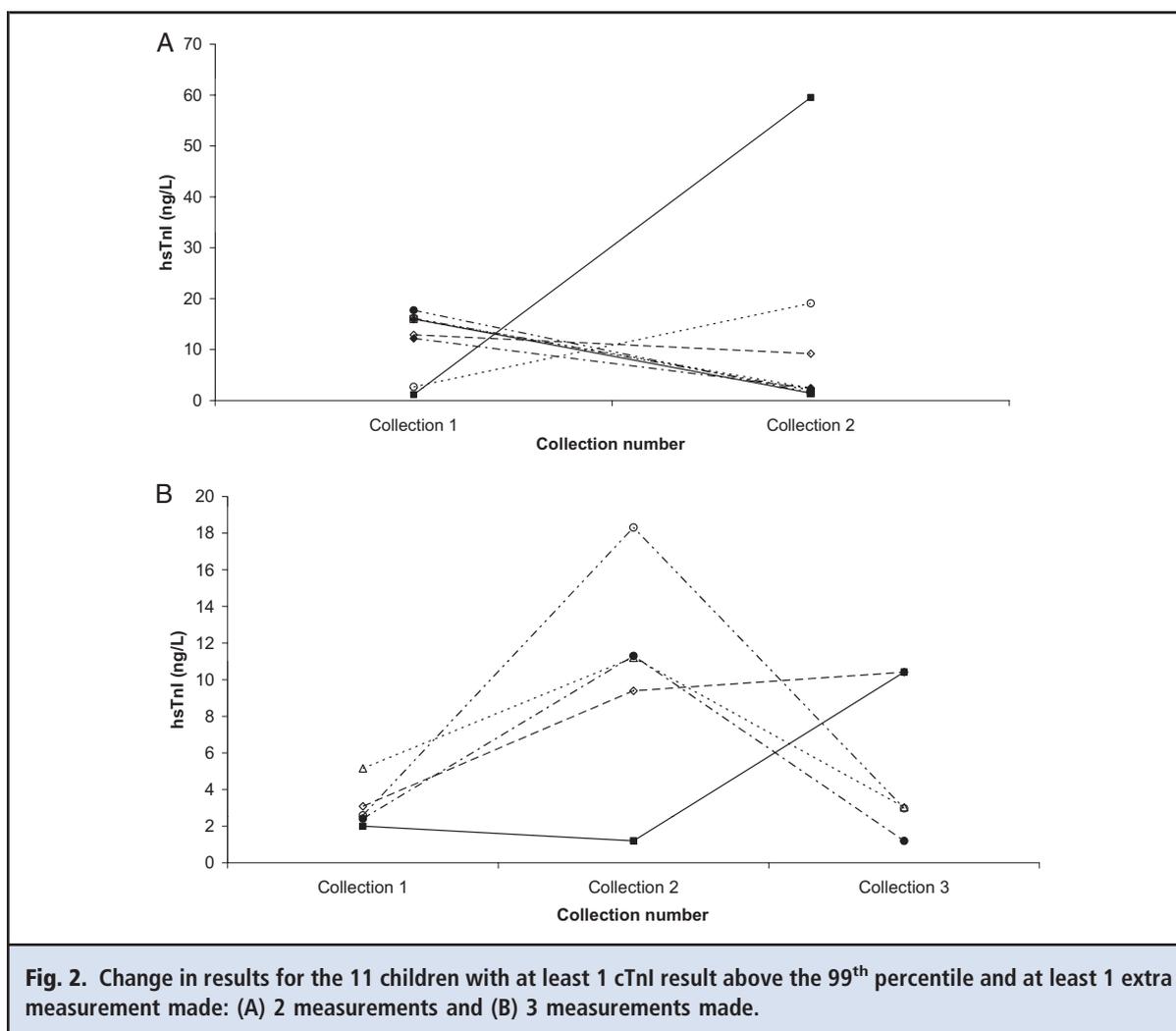
In a post hoc examination we explored the relationships between cTnI and the inflammatory markers (creatine kinase and C-reactive protein) and found no evidence of any association (data not shown). Furthermore, an examination of the physical activity records of this cohort showed that none of the children were involved in training or physical activity of the intensity or volume likely to markedly raise inflammatory markers and no child had undertaken any form of strenuous exercise in the 12 h before providing the blood sample.

We assessed the reproducibility of a high value in an individual child. We had a total of 11 children who

Table 1. 99th Percentiles by year and number and percentage of LOOK children from each year of the study who had cTnI concentrations above the indicated cut point.

Year of hs-cTnI	n	Boys 99th percentile	Girls 99th percentile	Boys vs girls, P	LoB 0.5 ng/L, n (%)	LoD 1.0 ng/L, n (%)	20% CV 1.8 ng/L, n (%)	10% CV 3.9 ng/L, n (%)	Adult 99th percentile 13.6 ng/L, n (%) ^a
2005	386	9.4	8.2	0.02	376 (97.4%)	337 (87.3%)	265 (69.4%)	45 (11.7%)	3 (0.7%)
2007	452	10.5	10.1	0.45	450 (99.5%)	407 (90.0%)	186 (41.2%)	24 (5.3%)	2 (0.4%)
2009	451	10.2	7.9	0.36	450 (99.8%)	444 (98.4%)	348 (85.1%)	32 (7.1%)	2 (0.4%)

^a The number under Adult 99th percentile refers to the number of children who were above the adult 99th percentile [Koerbin et al. (15)]. Male and female data have been combined.



were above the 99th percentile on at least 1 occasion and had at least 2 measurements made. We show these data for the individual children in Fig. 2, divided on the basis of whether they had 2 or 3 measurements made. There was no predictable pattern, with some children having an initial high concentration which fell or vice-versa. The κ statistic, a measure of concordance between measurements, was 0.00, indicating that if a child had 1 measurement above the 99th percentile it offered no predictive value on any other measurement in this child.

With our longitudinal data we investigated the effect of utilizing, in turn, the highest and lowest measurements for which children had repeated values. These data are shown in Table 2. For both males and females, there was an approximately 2-fold increase in the apparent 99th percentile when the highest value was used as opposed to the lowest ($P < 0.001$).

For children with more than 1 cTnI measurement, we were able to derive an estimate of total CV, and with our data for assay CV, estimate the biological CV for each child. These data are shown in Fig. 3. The results ranged from 0% through to 136%, with a median value of 33%. Table 3 also shows the spread of both cTnI concentrations and the biological CVs. Because one possible reason for a large biological variation might be the peripubertal growth spurt, we also calculated biological variation for children who had 2 samples collected at ages 8 and 10, compared to children who had 2 samples collected at ages 10 and 12; that is, we compared relatively younger with relatively older children. Using Mann–Whitney testing, we found no significant difference between these 2 groupings. For this population CV_I was 33%, CV_G (interindividual biological variation) was 106%, the index of individuality was 0.36, and the RCVs were 147% for a significant rise and 59% for a significant fall.

Table 2. Variable 99th percentiles based upon whether the highest or lowest cTnI concentration was used for children aged 8–12 years from whom multiple blood samples were collected.^a

	cTnI, ng/L		Sex		P (M/F)
	Male	Female	Male, n	Female, n	
cTnI high	10.54	14.77	343	340	0.68
cTnI low	5.71	6.47	343	340	0.84
P (high/low)	<0.001	<0.001			
Children with multiple samples and results changed			229	225	

^a Approximately two-thirds of children had more than 1 sample collected and their highest and lowest results were used in our calculations.

Discussion

Our study is unique in 2 regards. First, we have had a unique opportunity to assess a cohort of truly healthy community-dwelling children in whom cardiovascular health had been monitored during the study period and cardiac structure and function had been carefully evaluated by serial echocardiography. Other studies such as CALIPER (Canadian Laboratory Initiative on Paediatric Reference Interval Database) have used samples collected from hospital outpatients (17). We have demonstrated that these latter data are probably showing subtle effects of the ill health of the study participants (12). The other unique element to our study

was our opportunity to collect longitudinal data, which we did 3 times over a period of 4 years.

A high proportion of our cohort of children had cTnI above the LoD. At age 8 years this proportion was 87%, at age 10 years it was 90%, and at age 12 years it was 98%. These results compare with 14.8%, 20.3%, and 14.0% at ages 8 years, 10 years, and 12 years, respectively, using hsTnT (10) and >98% of a population of healthy adults having a cTnI concentration above the LoD using this same assay (15). This healthy population has either no cardiac disease or an extremely low level of disease. The fact that nearly all members of this population have detectable troponin strongly suggests that cardiac troponin release is not

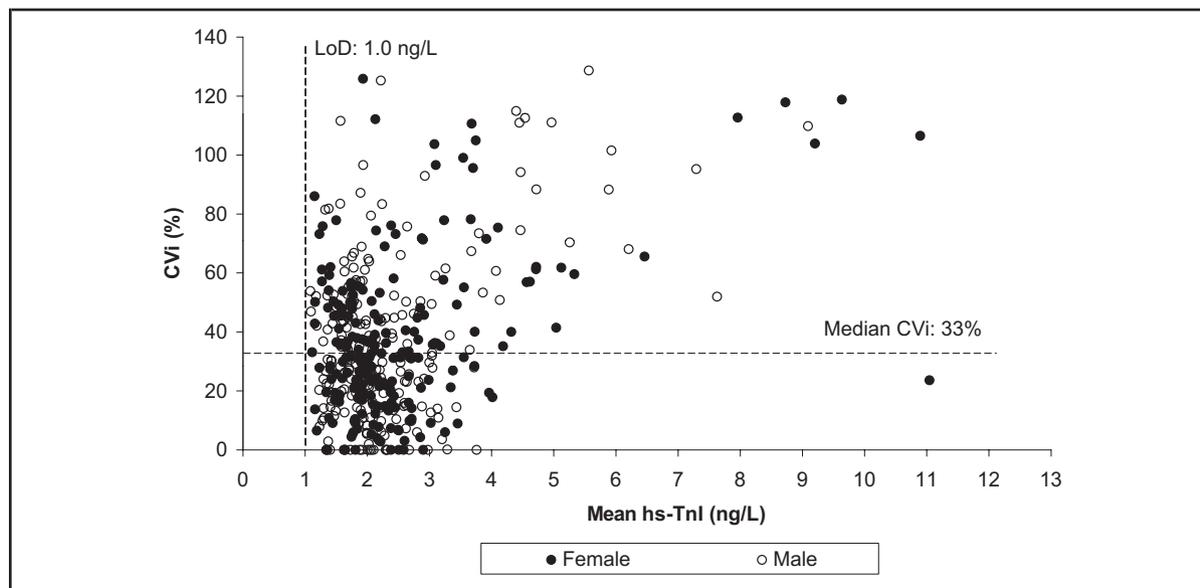


Fig. 3. Long-term biological variation of cTnI in healthy children.

On the x axis is plotted the mean hs-cTnI concentration for children who had either 2 or 3 samples collected and assayed. Using Eq. 1, we calculated CV_i from the CV_T and the analytical variation for the assay at the specified concentration. Data for 11 children with mean cTnI <1.0 ng/L (LoD) are excluded from this figure.

Table 3. Medians and 2.5–97.5 percentiles for cTnI concentrations in the different groups, and for the observed biological variation of cTnI (CVi) in these groups.

	n	cTnI (ng/L)			SD	n ^a	CVi			
		Median	2.5th Percentile	97.5th Percentile	Median	TnI (>1.0 ng/L)	Median	2.5th percentile	97.5th percentile	
3 Measurements	153	2.1	1.2	5.5	0.7	153	37%	5.1%	111%	
2005 and 2007	66	1.9	0.8	5.7	0.6	62	37%	4.0%	110%	
2005 and 2009	78	1.9	1.0	5.7	0.6	77	37%	4.0%	111%	
2007 and 2009	156	2.0	1.1	4.8	0.5	151	29%	5.1%	96%	
Mann-Whitney		P								
3 vs 2 measurements		0.14								
2005/7 vs 2007/9		0.55								

^a The numbers are less for the biological variation data because there were a small number of individuals who had mean cTnI concentrations <1.0 ng/L (LoD for the assay) who were excluded. One sample was excluded from the 2005–2009 cohort because it exceeded the criteria of Dixon [Horowitz et al. (27)].

always pathological, as was widely believed until very recently. Bergmann et al. have shown (18) that there is a slow but significant turnover of cardiac myocytes each year, and this finding can explain the very high prevalence of low concentrations of cardiac troponin in healthy persons. It is of interest that the increase with age in cTnI detectability we observed in children was accompanied by a 65% increase in median left ventricular mass between the ages of 8 and 12 years, suggesting that physiological myocardial growth may have been an important contributor. On the basis of our findings, the performance of the cTnI assay used in this study meets the guideline-acceptable and level 4 third-generation high-sensitivity assay criteria according to the troponin scorecard (19), with imprecision $\leq 10\%$ at the 99th percentile and with $\geq 95\%$ of samples providing a measureable concentration above the assay's LoD.

In both the preadolescent males and females at the 3 ages in our study the 99th percentile values were lower than for adults. Male 99th percentiles changed little over the 3 sampling periods, although the 99th percentile in girls was higher in the 10-year-olds than the 8- or 12-year-olds, owing to 3 children having high cTnI concentrations. However, the 97.5th percentile was slightly lower in this group (7.4, 5.9, and 6.5 ng/L in 8-, 10-, and 12-year-old females, respectively), suggesting that the 99th percentile was distorted by an unidentified event, as we discuss below.

Interestingly, it was not the same children whose results were repeatedly above the 99th percentile. There were 11 children who had at least 1 result above the 99th percentile and who had at least 1 more sample collected. None of these 11 children had a repeat result above the 99th percentile. Review of these data shows a

wide scatter, with no consistent pattern of increased or decreased concentrations.

Why is there this wide scatter? One possibility is that preanalytical or analytical factors may be responsible. However, sample collection was meticulous, with trained phlebotomists taking blood from the children, and the serum being separated, centrifuged, and frozen at -80°C within 4 h. Assays were performed only after 1 freeze–thaw cycle, with recentrifugation before analysis, and we have documented the stability of cTnI under these conditions. The assay shows excellent between-run imprecision down to low concentrations (15). All assays on the 3 separate sample collections of the study were performed over a short time frame, with the same lot numbers of reagents. For these reasons we believe preanalytical or analytical causes are very minor contributors to the total variation in results and that the scatter is a reflection of the children in their environment. Previous studies to determine the 99th percentile have all used cross-sectional data. Our study is unique in having longitudinal data available, and when longitudinal data are available it is a moot point as to which value should be used, the highest, lowest, or mean. Where we have multiple samples from the 1 child, it is of interest to assess the 99th percentile depending upon whether the lowest or highest result is used (Table 2). This matter will require careful consideration.

We have been able to estimate the long-term biological variability of cTnI in children who had more than 1 measurement made. The range is substantial, from 0% to 136%, with a median of 33%. However, Fig. 1 shows that the majority of children had only a small variation in cardiac troponin concentrations, with a small number of children having a much larger

variation. Nearly all of the children with a high cardiac troponin concentration had only a single high result, and in most cases at least 1 result that was quite low. Our analysis suggests that the peripubertal growth spurt is not a major contributor to this biological variability. We have shown previously with hs-cTnT that there is a significant clustering of children with detectable cTnT within the same school and same year, suggestive of an infective etiology (10). Whether this cardiac troponin rise reflects minor degrees of cardiac necrosis unrelated to coronary artery disease, or whether cardiac troponin is being released by some non-necrosis-related mechanism (20) cannot be determined from this study. The children with the larger biological variation and those with results above the 99th percentile may simply be a reflection of their having a minor episode of subclinical disease associated with cardiac troponin release.

Short-term studies have shown the index of individuality to be low (21), indicating that monitoring serial changes in an individual is of greater value than using reference intervals. A longer-term study of 9 months duration, conducted in a population at high risk of cardiovascular disease, showed that the index of individuality was still only 0.45 (22). Our study, conducted over an even longer time span (4 years), confirms this low index of individuality (0.36). The CV₁ for our population was higher, and as a consequence the RCVs were also higher, as shown in Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol58/issue12>.

What is the significance of a cardiac troponin concentration above the 99th percentile in healthy children? Although we will have to wait for many years to see the ultimate outcomes in these children, this lack of reproducibility of a high cardiac troponin concentration suggests that something other than primary cardiac disease may contribute. What do these data from children tell us about the 99th percentile in adults? Although in adults it is clear that the 99th percentile identifies a population at an increased risk of adverse cardiac events (4–9), it is also the case that persons with cardiac troponin concentrations that are below the 99th percentile are also at a risk, albeit lower than in those with cardiac troponin concentrations above the 99th percentile (2, 23, 24). In contrast with previous

studies in adults, our study is unique in being longitudinal. It may be that if longitudinal sampling were to be conducted in adult populations we might see a greater variability as well. The situation for hs-cTnI measurements may be similar to that for C-reactive protein, for which there is substantial biological variability (25) and for which therefore the CDC/American Heart Association has recommended that 2 measurements be made (26). Using such a strategy might greatly amplify the predictive power of the 99th percentile by removing some of the background noise due to the biological variation associated with apparently minor illnesses.

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