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Plasma cardiac troponin I concentrations in healthy neonates, children and adolescents measured with a high sensitive immunoassay method High sensitive troponin I in pediatric age

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ABSTRACT

Over the past 10 years cardiac troponin (cTn) immunoassays have been improved in analytical sensitivity and precision thereby allowing the measurement of cTn in adult healthy subjects. However, there are currently substantial gaps in our knowledge on circulating levels of cTn in healthy children. The aim of this study is to evaluate the distribution of plasma troponin concentration in apparently healthy pediatric subjects using a high sensitive immunoassay for cTnI measurement (hs-cTnI). Blood samples were obtained from 357 healthy pediatric subjects [204 males; age range 0–18 years; mean (SD): 8.7(6) years], including 36 subjects aged <1 month (neonates), 57 between 1 and 12 months (infants), 65 between 1 and 10 years (toddlers), and 223 between 10 and 18 years (adolescents). The percentages of healthy population with cTnI values equal or less than the calculated and LOD value were 13.1%. cTnI plasma levels were highest in the first month of life with a progressive decline in the next years and were lower in female. At multivariate analysis, only age was predictor of hs-cTnI plasma levels. The age and sex of children influence normal and physiologically released circulating concentrations of hs-cTnI, suggesting the need of reference intervals specific for age and sex.

1. Introduction

A critical tool for care of adult patients with cardiovascular disease is represented by circulating biomarkers, which could help clinicians in diagnosis, risk stratification and therapeutic monitoring [1]. Even if some important biomarkers have been validated in pediatric settings, such as natriuretic peptides [2–3], there are currently substantial gaps in our knowledge on circulating levels of cardiac biomarkers in children. Among them, cardiac troponin I (cTnI) and T (cTnT) are the marker of choice for the detection of myocardial injury and the diagnosis of myocardial infarction, as recommended by the most recent international guidelines [4–6]. Over the past 10 years, cTn immunoassays have been improved in analytical sensitivity and precision thereby allowing the measurement of circulating levels of protein in the major part of healthy subjects [7–8]. Thus, it has been suggested that troponin release may be related to both physiological and pathological mechanisms [8–9]. In healthy adult subjects, age- and gender-dependent effects have been observed: cTn levels are lower in women than men and increase with age in both genders [8,10–11].

There are several limitations and critical points in design and interpretation of studies as well as in evaluating analytical performance of a laboratory test in pediatric age, particularly in neonates [12–14]. Moreover, studies concerning cTnI concentrations in pediatric age are extremely difficult due to the very low troponin concentrations in children [15,16], which are usually below the analytical sensitivity of the most part of commercially available immunoassay methods. Therefore, there are no data on the behaviour of cTnI concentrations from birth to adult age.

The study aim was to evaluate the distribution of plasma cTnI concentrations in apparently healthy pediatric subjects aged from 0 to 18 years. Due to the very low cTnI concentrations in healthy children

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Table 1
Age and sex of the study population subdivided in groups according to age.

	Newborns ≤1 month	Infants 1 < months ≤ 12	Children 1 < years ≤ 10	Adolescent 10 < years ≤ 18	Total 0 < years ≤ 18
N	36	57	63	219	375
age, days	5.9 ± 1.3	143.4 ± 12.5	2083.4 ± 158.1	4829.2 ± 49.6	31.92 ± 112.5
males, n	19	36	32	108	195

Continuous variables are presented as media ± SE, categorical variables as absolute N.

[15,16], an accurate assessment of analytical performance of cTnI immunoassay used in this study was also performed to confirm that the most part of cTnI concentrations of pediatric subjects enrolled in this study was measured with statistical confidence.

2. Materials and methods

2.1. Patients

A group of 375 healthy pediatric subjects (52% males; age range 0–17.9 years; mean (SD): 8.7 (5.9) years) was enrolled at the G. Monasterio Tuscany Foundation (Massa, Italy) and the Giannina Gaslini Institute (Genoa, Italy). Informed consent was given by all parents of subject enrolled in this study. The whole group of healthy subjects was subdivided according to age: 36 (9.6%) neonates, aged ≤1 month, 57 (15.2%) infants, between 1 and 12 months, 63 (16.8%) toddlers, between 1 and 10 years, and 219 (58.4%) adolescents, between 10 and 18 years (Table 1).

Among subjects, newborns had routine screening for genetic disorders, while infants, children and adolescents underwent blood sampling during follow-up after an intervening disease or endocrine work-up for normal variant of growth. All newborns were delivered at term (from 37 to 41 weeks of gestation) and with body weight at birth ranging from 2.5 to 4.1 kg with an Apgar score > 8. In infants, children, and adolescents, clinical examination excluded the presence systemic acute/chronic diseases, and laboratory tests were within the reference limits. The presence of cardiac diseases was excluded by careful clinical examination and also by echocardiography, when necessary.

2.2. Blood sampling and laboratory analysis

To minimize the blood collection, only the residual plasma volume after routine laboratory test was used. The residual volume of EDTA blood samples, collected in polypropylene tubes, was stored at −80 °C until the cTnI assay. cTnI concentration was measured at the Fondazione Toscana G. Monasterio with the STAT Architect high Sensitive TnI using the Architect i1000SR platform (Abbott Diagnostics, Ref. B3P250) [17]. The limit of blank (LoB) and the limit of detection (LoD), determined by the manufacturer, were 1.3 and 1.9 ng/L, respectively (Table 2). Moreover, the 99th percentile value reported by the manufacturer for the reference adult population was 26.2 ng/L for the whole population, while 34.2 ng/L for male and 15.6 ng/L for females, respectively.

2.3. Statistical analysis

Because cTnI circulating levels are not normally distributed, both non-parametric and parametric tests after logarithmic transformation of data were used for statistical analysis. All statistical analyses were performed with the Stat-View 5.0.1 program (1992–98, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Univariate and multivariate linear regression analysis were used to evaluate the clinical variables independently associated with hs-cTnI plasma levels. Probability values were 2 tailed and values of 0.05 were considered significant.

Table 2
LoB, LoD and LoQ values of hs-STAT Architect immunoassay for cTnI.

Reference	LoB, ng/L	LoD, ng/L	LoQ CV 10%, ng/L
Manufacturer	0.7–1.3	1.1–1.9	4.7
Krintus et al. [19]	0.7–1.3	1.1–1.9	4.6–8.1
Present study	0.7	1.3	5.0

3. Results

3.1. Evaluation of analytical performance of hs-cTnI immunoassay

The limits of blank (LoB) and detection (LoD) were calculated following the CLSI EP17-A protocol [18], using the Architect i1000SR platform. The 0 calibrator of STAT Architect method, which does not contain cTnI, was considered as the blank of the method and it was repeatedly measured using two lots of reagents throughout two months (mean 121.08 RLU, SD 15.59 RLU, CV 12.88%, n = 48). We also estimated the error in the measurement of 0 point of the standard calibration curve by taking into account the 48 measurements of 0 calibrator and those of 130 samples collected from healthy subjects of cardiac patients with measured cTnI values in the range from 0 to 75 ng/L, using 2 lots of reagents during a two month period. The relationship between the signal of instrumentation, assessed as RLU, and the measured cTnI concentration was linear (RLU = 150.51 + 45.41 cTnI, R = 0.9917). These results were then used for the calculation of LoB value according to the EP17-A protocol (LoB = estimated method blank value + 1.645 SD) [18]. The LoD value was calculated following the formula: LoD = LoB + 1645 SD [18], where SD was estimated by the distribution of cTnI values measured in a plasma sample with a very low cTnI concentration (mean 1.335 ng/L, SD 0.393 ng/L, CV 29.41%, n = 40). LoD and LoB values, calculated in this study, are reported in Table 2; for comparison, the LoB and LoD values respectively reported by the manufacturer and by a multicenter study [19] were also indicated in the table.

The reproducibility of the STAT Architect method was evaluated according to the CLSI EP5-A2 protocol [20], using two heparinized plasma samples with mean cTnI concentrations of 3.4 ng/L (pool A) and 42.1 ng/L (pool B); within-run and total imprecisions were 7.8% and 12.7% for pool A and 3.7% 4.6% for pool B, respectively. The imprecision

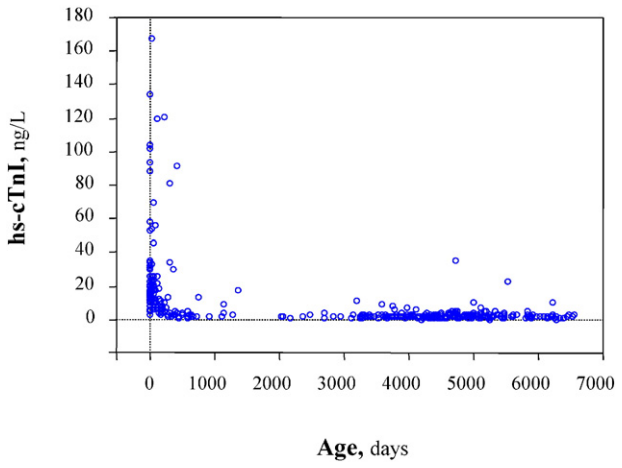

Fig. 1. Time-course of hs-cTnI values in healthy pediatric subjects throughout the first 18 years of life.