How to interpret elevated plasmatic level of high-sensitive troponin T in newborns and infants?

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Short title: High sensitive troponin T in newborns and infants

Summary

Background: Research and clinical implications on novel cardiac biomarkers has intensified significantly in the past few years. The high-sensitive troponin T (hscTnT) assay plays a dominant role in diagnostic algorithm regarding myocardial injury in adults. Despite generally accepted use of hscTnT there are no data about physiological concentrations and cut-off limits in neonates and infants to date.

Objective: The aim of this study is to assess hscTnT levels in healthy newborns and infants.

Methods: Consecutively 454 healthy full termed newborns and 40 healthy infants were enrolled in the study. Samples of cord or venous blood were drawn and tested for hscTnT concentrations with high-sensitive TnT assay (Roche Cobas e602 immunochemical analyser). The 97.5 percentile of hscTnT concentration was assessed and correlation analysis was performed in neonates.

Results: Two hundred and thirteen samples (47%) were excluded due to blood haemolysis of various degrees in neonates. Finally, the group of 241 healthy newborns was statistically analyzed. The median concentration of hscTnT was 38.2ng/ml, 97.5 percentile reached 83.0 ng/l (confidential interval 74.1 to 106.9 ng/l). HscTnT concentrations were statistically decreased in haemolytic samples when compared to non-haemolytic samples (34.3 ng/l [26.7 to 42.0 ng/l] and 37.1 ng/l [30.5 to 47.9 ng/l], respectively, p = 0.003). Elevated plasma concentrations of hscTnT decreased to adult level within six months.

Conclusion: This study has confirmed the higher reference levels of hscTnT in neonates and young infants when compared with adult population. Many extracardiac factors as haemolysis and age may affect the hscTnT level. Based on presented results, a careful clinical interpretation of hscTnT is recommended.

Key words: high sensitive troponin T, infants, myocardial damage, newborns

Introduction

Cardiac troponins are sensitive markers of myocardial damage and are also powerful prognostic indicators of adverse cardiac events. With advances in technology, a new era in troponin assays has started. The ability of the high-sensitive troponin T (hscTnT) assay to detect troponin T elevation in blood within two hours and with higher sensitivity after a myocardial insult led to the replacement of former troponin assays. However, conventional cardiac troponin T and I concentrations might be elevated in newborns despite a normal cardiovascular state (*Adamcová et al. 1995*). To date, there is limited information on high-sensitive troponin I in children (*Caselli et al. 2016*). However, there are no reliable data regarding hscTnT reference levels in newborns and infants. The primary aim of this study was to determine the hscTnT distribution in healthy neonates. The secondary objective was to correlate hscTnT with birth weight, blood gases, degree of haemolysis in neonates and to assess changes of hscTnT concentrations during early infancy.

Methods

This study was approved by the local Ethics Committee and performed according to the principles of the Declaration of Helsinki. After written informed consent was obtained from legal guardians, 454 full-term healthy newborns were enrolled in the study. All subjects were recruited from the single neonatology department at the university hospital. Exclusion criteria included an Apgar score less than 8 points performed at standard intervals of time after birth, the presence of congenital defects or infection, and a history of chronic medication or drug abuse in the mother.

Samples of cord blood were drawn in the tube with lithium heparin as a blood anticoagulant and tested for hscTnT concentrations and blood gases. The electrochemiluminescent (ECLIA) method was used to assess the hscTnT concentration (Roche Cobas e602 immunochemical analyser). The level of haemolysis was measured by a photometrical Cobas 8000 analyser. Samples with haemolysis higher than 1 g/l (haemolysis index $\geq$ 2) were excluded from further analysis, respecting the recommendations of the analytical manufacturer. Blood gases and acid-base balance analysis were

performed using a GEM 3500 analyser according to the standardised methodology of the university hospital.

HscTnT concentrations were not normally distributed and therefore the medians and interquartile ranges are reported and non-parametric comparisons were made. We constructed a reference range of cardiac troponin T concentrations and calculated the upper limit in the population (97.5th centile). Differences among groups were examined by the Mann-Whitney test. Correlations among hsTnT and other parameters (birth weight, level of haemolysis, umbilical cord acid base status) were analysed using the rank correlation coefficient. A p-value of 0.05 or less was considered statistically significant.

Results

Cord blood samples were taken from 454 healthy newborns; 213 samples were excluded due to a blood haemolysis index ≥ 2. The subset of 241 samples was statistically analysed (**Table 1**). The median hsTnT concentration was 38.2 ng/l [interquartile range 31.3 to 48.0 ng/l]. The distribution of hsTnT values in the study group is shown in **Figure 1**. The lower and upper limits of the hsTnT reference range were calculated as 20.1 ng/l (95% CI 15.3 to 21.8 ng/l) and 83.0 ng/l (95% CI, 74.1 to 106.9 ng/l), respectively. The difference between hsTnT concentrations in boys and girls tended to be statistically significant (38.7 ng/l [33.0 to 52.8 ng/l] ng/l and 36.7 ng/l [29.2 to 47.1 ng/l], respectively, p = 0.052). The concentration of hsTnT was found to be significantly lower in newborns delivered via Caesarean section when compared with vaginal delivery (35.0 ng/l [28.5 to 43.4 ng/l] and 38.9 ng/l [32.6 to 48.4 ng/l], respectively, p = 0.0084), **Figure 2**. HscTnT values were significantly lower in haemolytic blood samples (haemolysis index ≥ 3) when compared with non-haemolytic samples (34.3 ng/l [26.7 to 42.0 ng/l] ng/l and 37.1 ng/l [30.5 to 47.9 ng/l], respectively, p = 0.003). The hscTnT level correlated inversely with the base status (BE: rho = -0.14, p = 0.03) and with the pH level of the umbilical cord blood (rho = -0.14, p=0.03). Elevated plasma concentrations of hscTnT decreased to adult range within six months, **Figure 3**.

Discussion

Our study presents the reference range of hscTnT concentrations calculated for healthy newborns. The upper limit of hscTnT was significantly higher when compared with adult population (83.0 ng/l and 13.5 ng/l respectively) (Giannitsis *et al. 2010*). This finding is in agreement with studies using conventional troponin T and I assays in healthy neonates (Baum *et al*. 2004, Clark *et al.* 2001). The reason for this elevation remains questionable. The influence of physical stress and transient hypoxia during vaginal birth could not be excluded (Costa *et al.* 2007, Cruz *et al.* 2006). This theory is supported by studies reporting an elevation of cardiac troponin T in healthy adults after strenuous physical exertion (Apple 2009, Hewing *et al.* 2015). Indeed, hscTnT values were significantly higher after vaginal birth when compared to surgical delivery in our study (**Figure 2**). However, neither physical stress nor transient perinatal hypoxia fully elucidates the elevated concentrations of hscTnT in healthy newborns and young infants. It is well known that elevated plasma concentrations of TnT decline to normal levels in successfully treated adults within 2 weeks (Skeik *et al.* 2008). In our study, elevated plasma levels of hscTnT in healthy neonates and infants return to adult physiological range substantially slower. This process persisted up to six months (**Figure 3**). When excluded extracardiac pathological causes such as sepsis, renal failure, severe anaemia or hyperbilirubinaemia, another hypothesis might be taken into account. Initiation of spontaneous breathing promotes changes in pulmonary circulatory system in newborns. The decreasing pulmonary arterial resistance leads to reduction of the right ventricle afterload and the dominance is shifted from the right to the left ventricle within first months of life. We speculate that this physiological process linked with subtle structural myocardial changes might release a small amount of troponins into the circulation. These are detectable by high-sensitive TnT assay during newborn period and early infancy.

Haemolysis is known to negatively interfere in hscTnT assays. Up to 45% of our samples contained some degree of haemolysis. This result is in agreement with a former study (Li *et al.* 2013) and it is of critical importance when interpreting hscTnT concentration. A haemoglobin concentration of 1.9 g/L

or higher might decrease the hscTnT level up to 20%. We found a significant reduction in the hscTnT concentration at haemoglobin concentrations ≥2.0g/l (haemolysis index ≥ 3). Consideration of preanalytical variables is essential, such as time interval from blood sampling to laboratory analysis. A significant decline in the hscTnT concentration over time has been reported (Li *et al.* 2013). The acceleration in haemolysis might be explained by the release of erythrocyte proteases, causing a disruption in the TnT-epitope used in the diagnostic kit.

Higher hscTnT concentrations in boys when compared to girls in our study are in contrast to results of previous study using the conventional troponin T assay (Baum *et al.* 2004). However, this small difference in hscTnT concentrations has no clinical relevance.

Strengths and limitations

Our study was conducted on a quite large number of neonates. Despite excluding up to 45% of samples due to haemolysis (haemolysis index ≥ 2), a distribution curve was constructed with sufficient statistical power. On the other hand, probands were not followed-up after study. A longitudinal study would require repeated blood sampling in healthy infants which is ethically questionable.

Conclusion

Substantially higher reference plasma concentrations of hscTnT in neonates and young infants when compared with the adult population require careful clinical interpretation. It is advised to consider many extracardiac factors as haemolysis and age that might affect the hscTnT levels in these children.

Acknowledgements:

The authors thank ROCHE for the provision of the reagents for the hscTnT assessment. This study was conducted within the research project PROGRES O-39.

Conflict of interest:

Elecsys Troponin I Ths STAT lab sets were provided by ROCHE without any financial ties to the investigators.

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Figure 1

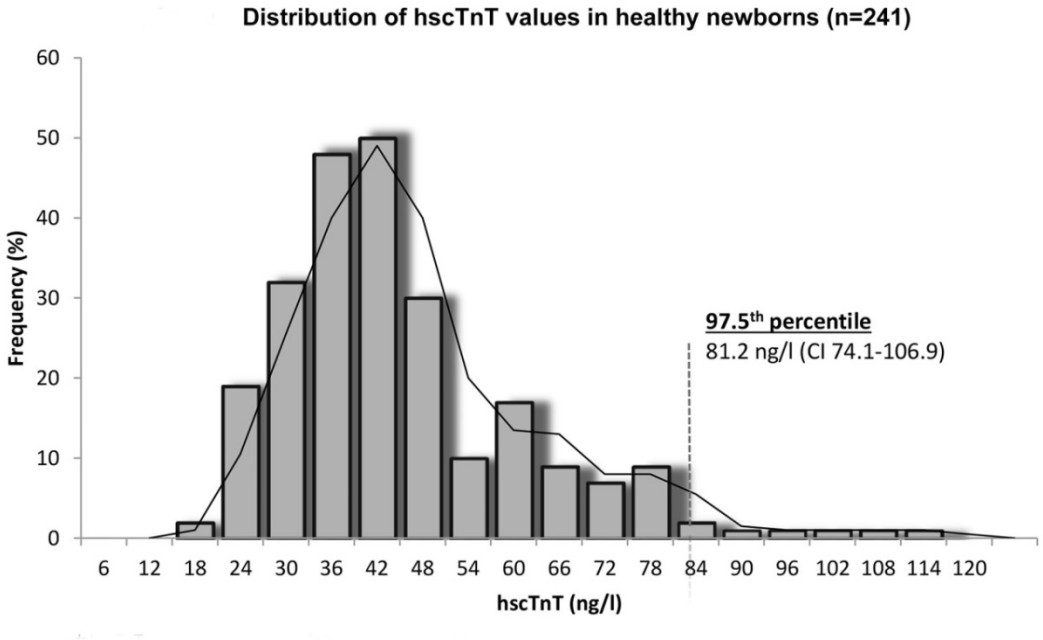

Figure 1 legend: **hscTnT** – high-sensitive troponin T; **CI** – confidential interval.

Figure 2

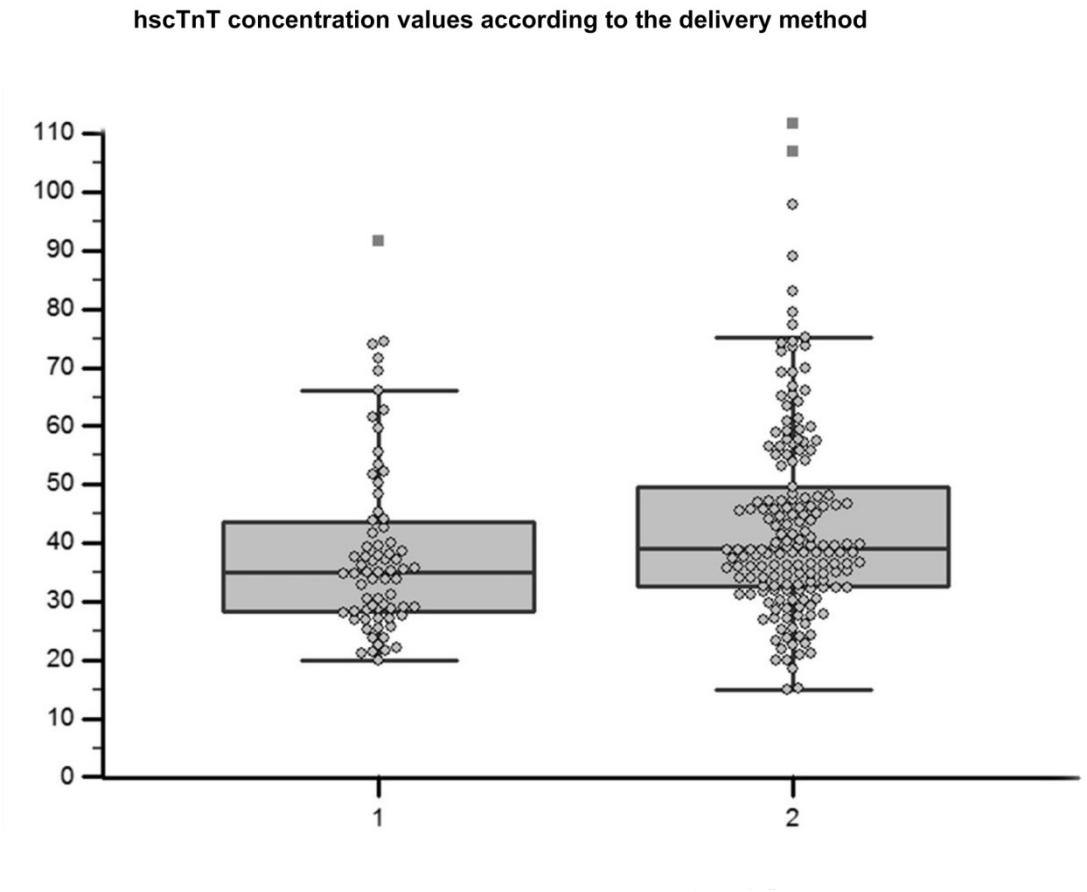

Figure 2 legend: Boxes indicate the inter-quartile range. Horizontal lines within boxes indicate medians. Whiskers extend to the highest or lowest value. 1. Caesarean section (n=70) 2. Vaginal delivery (n=171); p – *p*-value

Figure 3

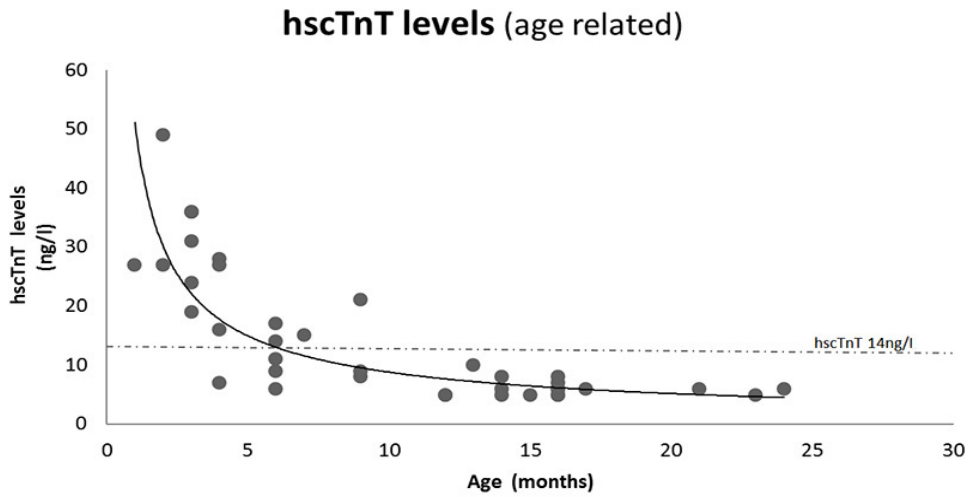

Figure 3 legend: Relationship of the hscTnT levels with the age of probands. 7

Table 1

	Girls	**Boys**	**p-value**
Probands	114	127	
Gestational age at birth (wks)	39.0(39;40)	39.0(39;40)	NS
Birth weight (kg)	3.310 (3.030;3.600)	3.510 (3.207;3.837)	0.001
Apgar score 1., 5. and 10. minute	10(10;10), 10(10;10), 10(10;10)	10(10;10), 10(10;10), 10(10;10)	NS
Umbilical cord blood acid-base analysis at delivery			
pH	7.33(7.29;7.33)	7.34(7.30;7.38)	NS
base excess	-2.7 (-5.1;-1.1)	-3.3 (-5.6;-1.3)	NS
hscTnT (ng/l)	**36.7 (29.2;47.1)**	**38.7 (33.0;52.8)**	0.05
hscTnT (ng/l) - 97.5th percentile	**71.55**	**93.67**	
Haemolysis index	0 (0;1)	0 (0;1)	NS

Table 1 legend: The values are expressed as median and inter-quartile range (25.-75.percentile) in the brackets; **hscTnT** – high-sensitivity troponin T**; p** - p-value; **haemolysis index** – scale from 0-5, grade 0 (0-0.5g/l), grade 1 (0.51-1.0g/l), grade 2 (1.01-2.0g/l), grade 3 (2.01-3.0g/l), grade 4 (3.01-4,0g/l), grade 5 (4.01g/l and more).