ORIGINAL ARTICLE

Rev Bras Cir Cardiovasc 2008; 23(3): 378-382

Evaluation of serum cardiac troponin I values in children less than 1 year of age

Avaliação dos valores séricos de troponina I cardíaca em crianças menores de 1 ano de idade

Antonio Carlos Arruda SOUTO[1], Werther Brunow de CARVALHO[2]

RBCCV 44205-1003

Abstract

Objective: **The objective is to verify the cardiac troponin I values in children less than 1 year of age without clinical cardiac dysfunction.**

Methods: **The cardiac troponin I values were determined in 99 children less than 1 year of age, including term infants without diseases related to cardiac dysfunction using the specific kit Opus T Troponin I (cTn) (Dade Behring Inc. - Newalk, DE 19714, USA).**

Results: All children have values of cardiac troponin I less than 0.1 ng/ml.

Conclusion: **We verified that the cardiac troponin I value is less than 0.1 ng/ml in children less than 1 year, including term infants without cardiac dysfunction, when analized by the kit Opus T Troponin I (cTn) test modules.**

Descriptors: **Child. Troponin I/blood. Reference values.**

Resumo

Objetivo: **Verificar os valores séricos para troponina I cardíaca em crianças abaixo de um ano de idade, sem disfunção cardíaca clínica.**

Métodos: **Os níveis séricos de troponina I cardíaca foram determinados em 99 crianças com idade abaixo de um ano, incluindo-se recém-nascidos a termo, sem doenças relacionadas a comprometimento da função cardíaca identificável clinicamente, por meio do kit específico Opus T Troponin I (cTn) (Dade Behring Inc. - Newalk, DE 19714, USA).**

Resultados: **A dosagem sérica de troponina I cardíaca apresentou, em todos os pacientes, valor menor que 0,1 ng/ml.**

Conclusão: **Verificamos que o valor da dosagem sérica de troponina I cardíaca é menor do que 0,1 ng/ml para pacientes pediátricos, sem disfunção cardíaca, desde recém-nascidos a termo até um ano de idade, quando realizada por meio do kit Opus T Troponin I (cTn) test modules.**

Descritores: **Criança. Troponina I/sangue. Valores de referência.**

INTRODUCTION

Discovered by Setsuro Ebashi [1], in 1963, the troponins constitute a complex of proteins which modulates the velocity and the strength of striated muscle contraction. The complex consists of three subunits: Troponin T (TnT), responsible for binding the complex to tropomyosin; Troponin C (TnC) is a calcium-binding protein and has a key role in muscle contraction; and Troponin I (TnI) inhibits muscle contraction [2].

TnI can be found in three isoforms with different protein structures determined by distinct and specific genes for

1. Master in Medicine; Head physician; Pediatric ICU; Hospital Padre Albino.
2. Full Professor; Pediatric Specialities Discipline, Pediatric Department; UNIFESP-EPM.

This study was carried out at the Federal University of São Paulo – Paulista Medical School, São Paulo, SP, Brazil.

Correspondence address:
Antonio Carlos Arruda Souto. Rua Avaí, 100 - Catanduva – SP –Brasil - CEP 15800-150. Brasil. Tel/Fax: 55 17 3523-1247.
E-mail: acasouto@terra.com.br

Article received on March 26th , 2008
Article accepted on July 28th, 2008

each isoform [3]. Two isoforms are present in the skeletal muscle, one in fast-twich fibers and other in slow-twich fibers. The third isoform, present in the myocardium, é called Cardiac Troponin I (cTnI) [4].

The structural difference between TnI isoforms allowed that Cummins et al. [5] to develop the first radioimmunoassay capable of identify the release of cTnI in the acute myocardial infarction (AMI) and to publish their results in 1987.

The improvement of the method [6] and the great number of studies investigating its usefulness in the diagnosis of myocardial injury [7] have made it to become one of the major biochemical markers of AMI [8].

In pediatrics, researches are focused on the use of this marker in the diagnosis of myocardial injury related to surgical repair of congenital heart defect. These studies demonstrate the application of the method in different situations, since the diagnosis of events following the performance of operative procedures to its use as a prognostic marker [9-12].

Also, cTnI values have been presented as an important method to assist in the management of other pediatric diseases, such as in myocarditis [13], sepsis [14], and in Kawasaki disease [15], diseases in which cardiac dysfunction is a common event.

For an adequate use of biochemical markers in daily clinical practice, it is necessary to define which reference values to use [16]. Some studies have emerged with this purpose, achieving results in pediatrics similar to those of adult researches [17], however, others have presented increased cTnI values in children under one year of age [18,19].

The definition in relation to the reference values for cTnI in pediatrics and the importance of this diagnosis method, by its characteristics and applicability, has motivated this research.

METHODS

This research was undertaken after being approved by the Research and Ethics Committee of the participant institutions. This is a non-randomized, prospective observational clinical trial to refine new diagnosis approaches.

Between January 15 and February 13 2003, 99 children under the age of one year were included in the study after a written informed consent was signed by their legal authorized representatives. cTnI values were then measured.

The children enrolled in the study have met the following inclusion criteria: age under 1 year, including full term infants, no previous diagnosis of heart diseases, or any clinical condition somehow related to cardiac dysfunction, hemodynamic stability, determined through physical examination without using drug therapy or mechanical support.

The patients were divided into two groups according to their age group. Group I consisted of newborns up to 28 days of life. Group II consisted of infants between 29 days of life and one year. The newborns in group I have cTnI values collected at the moment of nursery routine examinations, and children in group II, at the moment of preoperative ward examinations and for child care follow-up; the exams were ordered by practitioners not involved in the study.

The sample collection, storage, and assay followed the manufactures guidelines and the pertinent literature.

Samples were collected using conventional peripheral venipuncture techniques, preferably in the right cubital region, using a disposable plastic syringe and needles with no anticoagulant solution.

After sample collection, 2 mL of blood was poured into a glass tube and set out for serum separation by means of centrifugation at 3000 rpm for 10 minutes. Serum was stored in a glass tube at +3ºC. Samples remained stored at an average of 24 hours not surpassing a 72-hour period storage until cTnI dosage.

A specific kit, Opus T Troponin I (cTn) test modules (Dade Behring Inc. - Newalk, DE 19714, USA) and the device Opus plus® analyzer (Behring Diagnostics Inc. - Westwood, MA 02090, USA) were used to analyze the samples.

The assay is based on fluorogenic immunoassay principles linked to a double antibody sandwich enzyme (antibody-antigen-antibody), or linked to two sites with mouse monoclonal antitroponin I antibodies.

Analysis was performed automatically by the Opus plus analyzer.

Serum concentration is expressed in nanograms per milliliter (ng/mL).

Method assay range varies from 0.1 to 50.0 ng/mL.

Assay analytic sensitivity is 0.1 ng/mL. All values below are referred by the analyzer as < 0.1 ng/mL. The reference value for normalization established by the laboratory is lower than 0.5 ng/mL.

RESULTS

The study population consisted of 99 children undergoing cTnI dosage divided into two groups: group I consisted of 74 newborns, 35 males (3.18 ± 0.44 kg) and 39 females (3.14 ± 0.42 kg); group II consisting of 25 infants, 16 males (8.5 ± 2.4 kg) and 9 females (8.2 ± 1.0 kg).

Group I infants were sampled in their first day of life, except one who was sampled on the 13th day of life. Male and female infants' mean age (group II) at the moment of sampling was 7.6 ± 2.8 months and 8.2 ± 1.9 months, respectively.

The dosage of cTnI was found to be below the assay analytic sensitivity level (0.1 ng/mL) in all children and it was registered by the analyzer (Opus plus) as < 0.1 ng/mL.

DISCUSSION

The results of our study are compatible with the results of other studies which have observed normal values of cTnI in pediatric patients without myocardial injury [17]. The distribution of all values found within the reference range for normality (<0.1 ng/mL) is not an unexpected fact, once the literature data relates a high specificity to the method [20] and the study population did not present any clinical data suggesting the existence of myocardial injury, which was established by the study inclusion criteria.

Among the studies, we have found two publications aiming at defining the reference values in a pediatric population. The authors considered a new and exciting datum to have as a result a large percentage of patients in the first year of life presenting increased cTnI values [18,19].

The abovementioned authors suggest that the increased serum levels of cTnI is related with the apoptosis of myocardial cells. However, it is difficult to establish this relationship because apoptosis is a physiological phenomenon playing an important role in the development of several organs, being suppressed at the beginning of extrauterine life, and despite of being named as cell death, it occurs usually in isolated cells and it is not followed by an inflammatory process. The cells undergo herniation of cell membrane, volume reduction, condensation of nuclear chromatin and, finally, they are phagocytosed by macrophages and surrounding cells [21-23].

An important consideration to be made when different study results are compared is the influence of the methodology used. There is a great variability of results between two methods [24].

This variability is related to a series of processes that will change the cTnI presentation form after its release. Only 10% of the released cTnI remains in its free form; the other 90% build up complexes, especially with Troponin C; moreover, cTnI can be submitted to other biochemical processes, causing it to be oxidized or reduced. These several changes interfere with the reaction of cTnI with the specific antibodies present in different methods and, consequently, with its results [25].

The method used in our study, the Opus T Troponin I (cTn) test modules (Dade Behring Inc. - Newalk, DE 19714, USA) was chosen due to its commercial use and with which an important ability in daily usage has been developed; in addition, it was available at our institution.

The daily use of serum dosage of cTnI for AMI diagnosis in adult patients through this diagnostic method has yielded outcomes contrary to literature data regarding sensibility, specificity, and temporal course, turning this method into an important aid tool in the diagnosis and follow-up of these patients [20].

As the method analytical sensitivity was set at 0.1 ng/mL, our result did not allow the performance of statistical processes. Likewise, with the analyzer Opus Plus assigning values <0.1 ng/mL, our result was rather a qualitative variable than a quantitative one, not showing variation.

Levin [26] defined Statistics as "…a set of techniques to reduce quantitative data (i.e., a series of number) into a more convenient and easily communicable number of smaller descriptive terms".

Taking into consideration the characterization of our result and the definition proposed by Levin, we believe to be justifiable not to apply a statistical outlining to our study, however, this does not interfere in the assessment regarding its applicability, once the distribution of all values below 0.1 ng/mL allows concluding that all patients presented normal values, regardless its variation within this interval.

After assessing all data presented, it becomes difficult to justify the findings of increased cTnI in normal children; on the contrary, we believe these data cause our study result to be expected, and they justify the incisive fashion as this result turned to be, which has been repeated in newly published studies [27,28].

Despite the variability in the results found among different methods, the use of this myocardial injury biochemical marker has been playing an important role in patients' diagnosis and follow-up. It is important that the interpretation of the results in daily practice be carried out relatively to local laboratory standards and comparatively to other methods.

New studies are needed to investigate several aspects related to the dosage of serum cTnI and its applicability.

CONCLUSION

We concluded that the reference value (0.1 ng/mL) for serum cardiac troponin I dosage, when performed through Opus T Troponin I (cTn) test modules (Dade Behring Inc.-

Newalk, DE 19714, USA) for children without heart condition, from full term infants up to 1 year of age, is lower.

AKNOWLEDGEMENTS

This study was carried out at the Post-graduation Program, Federal University of São Paulo – Paulista Medical School – under the supervision of Dr. Werther Brunow de Carvalho.

I would like to thanks Dr. Domingo Marcolino Braile, Dr. Eduardo Juan Troster e Dr. Heitor Pons Leite, members of the examining board, by their impressive comments, which were important to write this article.

REFERENCES

1. Ebashi S. Third component participating in the superprecipitation of "natural actomyosin". Nature. 1963;200:1010.

2. Katus HA, Scheffold T, Remppis A, Zehlein J. Proteins of the troponin complex. Lab Med. 1992;23(5):311-7.

3. Wade R, Kedes L. Developmental regulation of contractile protein genes. Annu Rev Physiol. 1989;51:179-88.

4. Hunkeler NM, Kullman J, Murphy AM. Troponin I isoform expression in human heart. Circ Res. 1991;69(5):1409-14.

5. Cummins B, Auckland ML, Cummins P. Cardiac-specific toponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J. 1987;113(6):1333-44.

6. Heeschen C, Goldmann BU, Moeller RH, Hamm CW. Analytical performance and clinical application of a new rapid bedside assay for the detection of serum cardiac troponin I. Clin Chem. 1998;44(9):1925-30.

7. Panteghini M, Bonora R, Pagani F. Rapid and specific immunoassay for cardiac troponin I in the diagnosis of myocardial damage. Int J Clin Lab Res. 1997;27(1):60-4.

8. Myocardial infarction redefined: a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Eur Heart J. 2000;21(18):1502-13.

9. Salerno PR, Jatene FB, Figueiredo PE, Bosisio IJ, Jatene MB, Santos MA, et al. Comportamento da troponina I e CK-MB massa em crianças submetidas a correção cirúrgica das cardiopatias congênitas. Rev Bras Cir Cardiovasc. 2003;18(3):235-41.

10. Montgomery VL, Sullivan JE, Buchino JJ. Prognostic value of pre- and postoperative cardiac troponin I measurement in children having cardiac surgery. Pediatr Dev Pathol. 2000;3(1):53-60.

11. Hammer S, Loeff M, Reichenspurner H, Daebritz S, Tiete A, Kozlik-Feldmann R, et al. Effect of cardiopulmonary bypass on myocardial function, damage and inflammation after cardiac surgery in newborns and children. Thorac Cardiovasc Surg. 2001;49(6):349-54.

12. Kannankeril PJ, Pahl E, Wax DF. Usefulness of troponin I as a marker of myocardial injury after pediatric cardiac catheterization. Am J Cardiol. 2002;90(10):1128-32.

13. Briassoulis G, Papadopoulos G, Zavras N, Pailopoulos V, Hatzis T, Thanopoulos V. Cardiac troponin I in fulminant adenovirus myocarditis treated with a 24-hour infusion of high-dose intravenous immunoglobulin. Pediatr Cardiol. 2000;21(4):391-4.

14. Thiru Y, Pathan N, Bignall S, Habibi P, Levin M. A myocardial cytotoxic process is involved in the cardiac dysfunction of meningococcal septic shock. Crit Care Med. 2000;28(8):2979-83.

15. Checchia PA, Borensztajn J, Shulman ST. Circulating cardiac troponin I levels in Kawasaki disease. Pediatr Cardiol. 2001;22(2):102-6.

16. Sunderman FW Jr. Current concepts of "normal values", "reference values", and "discrimination values", in clinical chemistry. Clin Chem. 1975;21(13):1873-7.

17. Hirsch R, Landt Y, Porter S, Canter CE, Jaffe AS, Ladenson JH, et al. Cardiac troponin I in pediatrics: normal values and potential use in the assessment of cardiac injury. J Pediatr. 1997;130(6):872-7.

18. Soldin SJ, Murthy JN, Agarwalla PK, Ojeifo O, Chea J. Pediatric reference ranges for creatine kinase, CKMB, Troponin I, iron and cortisol. Clin Biochem. 1999;32(1):77-80.

19. Quivers ES, Murthy JN, Soldin SJ. The effect of gestacional age, birth weight, and disease on troponin I and creatine kinase MB in the first year of life. Clin Biochem. 1999;32(6):419-21.

20. Sanhai WR, Romero LF, Hickey G, Ruttle D, Christenson RH. Performance characteristics of a revised cardiac Troponin I assay for the Opus plus immunoassay system. Clin Biochem. 2001;34(7):579-82.

21. Poelmann RE, Molin D, Wisse LJ, Gittenberger-de Groot AC. Apoptosis in cardiac development. Cell Tissue Res. 2000;301(1):43-52.

22. Malamitsi-Puchner A, Sarandakou A, Tziotis J, Trikka P, Creatsas G. Evidence for a suppression of apoptosis in early postnatal life. Acta Obstet Gynecol Scand. 2001;80(11):994-7.

23. James TN. Normal and abnormal consequences of apoptosis in the human heart. From postnatal morphogenesis to paroxysmal arrhythmias. Circulation. 1994;90(1):556-73.

24. Wu AH, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. Clin Chem. 1998;44(6 Pt 1):1198-208.

25. Shi Q, Ling M, Zhang X, Zhang M, Kadijevic L, Liu S, et al. Degradation of cardiac troponin I in serum complicates comparisons of cardiac troponin I assays. Clin Chem. 1999;45(7):1018-25.

26. Levin J. Por que o pesquisador usa estatística?. In: Levin J, editor. Estatística aplicada a ciências humanas. 2ª ed. São Paulo:Harbra;1987. p.1-12.

27. Trevisanuto D, Pitton M, Altinier S, Zaninotto M, Plebani M, Zanardo V. Cardiac troponin I, cardiac troponin T and creatine kinase MB concentrations in umbilical cord blood of healthy term neonates. Acta Paediatr. 2003;92(12):1463-7.

28. Ramakrishnan KA, McCabe S, McNicholas J, Litherland P, Papachan VJ. Cardiac troponin T levels in infants less than 9 months of age undergoing cardiac suegery. Pediatr Crit Care Med. 2005;6(2):246.