

premature rupture of the membranes. *Am J Obstet Gynecol* 2002;187:1125–30.

3. Mannello F, Luchetti F, Canonico B, Papa S. Effect of anticoagulants and cell separation media as preanalytical determinants on zymographic analysis of plasma matrix metalloproteinases [Letter]. *Clin Chem* 2003;49:1956–7.
4. Galewska Z, Romanowicz L, Bankowski E, Jaworski S. Preeclampsia-associated decrease of potential collagenolytic and gelatinolytic activities in the wall of the umbilical cord vein. *Int J Biochem Cell Biol* 2002;34:24–32.
5. Mannello F, Sebastiani M. Zymographic analysis and measurement of matrix metalloproteinase-2 and -9 in nipple aspirate fluids [Technical Brief]. *Clin Chem* 2003;49:1546–50.

**Ferdinando Mannello**<sup>1\*</sup>

**Francesca Luchetti**<sup>2</sup>

**Barbara Canonico**<sup>3</sup>

**Elisabetta Falcieri**<sup>2,4</sup>

**Stefano Papa**<sup>2,3</sup>

<sup>1</sup> *Istituto Istologia e  
Analisi Laboratorio*

<sup>2</sup> *Istituto Scienze Morfologiche*

<sup>3</sup> *Centro Citometria e Citomorfologia  
Università Studi "Carlo Bo"  
Urbino (PU), Italy*

<sup>4</sup> *Istituti Ortopedici Rizzoli-Consiglio  
Nazionale delle Ricerche (ITOI-CNR)  
Bologna, Italy*

\*Address correspondence to this author at: Istituto Istologia e Analisi Laboratorio, Via E. Zeppi, snc, Università Studi "Carlo Bo", 61029 Urbino (PU), Italy. Fax 39-0722-322370; e-mail f.mannello@uniurb.it.

DOI: 10.1373/clinchem.2004.036061

## Plasma Concentrations of Cardiac Troponin I in Newborn Infants

To the Editor:

Ischemia and myocardial necrosis occur in 25–51% of newborn infants with perinatal asphyxia and are often associated with other adverse conditions specific to the neonatal period (1). Biochemical markers are more sensitive and specific than imaging techniques in the diagnosis of myocardial necrosis. Cardiac troponin I (cTnI) has high tissue specificity and sensitivity and is therefore suitable for use in diagnosing even microscopic lesions (2).

There is little information about

cardiac biochemical markers in newborns, and no reference intervals have been established by the NCCLS standard procedures (3). The European Society of Cardiology and the American College of Cardiology suggest that cTnI and cardiac troponin T (cTnT) concentrations above the 99th percentile in a reference group be used as evidence of myocardial necrosis in adults (2). The present study was carried out to measure cTnI concentrations in plasma from healthy newborns to explore the possibility of a variation in reference values according to gender, age of the newborn, and plasma bilirubin and to suggest an upper limit for the reference interval, which is essential for the interpretation of these measurements in sick infants.

We consecutively enrolled 206 apparently healthy infants for whom bilirubin measurements had been requested because of diagnosis of jaundice. cTnI was measured in lithium-heparin-anticoagulated plasma samples on the ACS:180 immunoassay system, an automated system based on a two-sandwich immunoassay method and direct chemiluminescent measurement. The day-to-day imprecision (CV) for this assay, as performed in the core laboratory, for cTnI concentrations of 1.1, 16.1, and 34.1  $\mu\text{g/L}$  was 8.3%, 9.4%, and 8.7%, respectively. Data from the manufacturer indicate a CV of 13% at 0.1  $\mu\text{g/L}$  (4).

Data analysis was carried out with the SPSS program. The Student *t*-test was used to compare means. The reference interval was calculated from the percentiles of the empirical sampling distribution (nonparametric method), and the Dixon test (5) was used to identify outliers. The reproducibility of the method was estimated by calculating the mean and SD of the differences between 29 repeat measurements and their intraclass correlation coefficient. Statistical significance was established at  $P < 0.05$ .

The mean (SD) maternal age was 31 (5.1) years, and gestational age was 38.7 (1.2) weeks. One hundred and six infants (51.5%) were female. Patient selection adhered strictly to

the study criteria: apparently healthy, with no problems that required surgical intervention, and no cardiac, neurologic, or other pathologies except jaundice. There were no adverse events associated with labor or delivery. The mean (SD) birth weight of newborn infants was 3200 (494.5) g, and the Apgar score was 9.1 (0.3) at 5 min. The mean (SD) age of the infants when blood was drawn was 2.6 (1.3) days. We identified no outlying values among any of the variables analyzed. The mean (SD) cTnI value was 0.28 (0.42)  $\mu\text{g/L}$ , the 99th percentile value was 2.8  $\mu\text{g/L}$ , and the highest cTnI value was 3.0  $\mu\text{g/L}$  (Table 1). The mean (SD) cTnI value for newborns younger than 48 h ( $n = 85$ ) was significantly lower [0.22 (0.24)  $\mu\text{g/L}$ ] than that for infants 48 h or older [ $n = 121$ ; 0.37 (0.59)  $\mu\text{g/L}$ ;  $P = 0.03$ ]. We found no relationship between cTnI values and gestational age, plasma bilirubin, or gender. For 29 individual samples run in duplicate, the mean (SE) difference was 0.008 (0.132)  $\mu\text{g/L}$  (95% confidence interval,  $-0.28$  to 0.26). The intraclass correlation coefficient for these same 29 repeat samples was 0.938 (SE = 0.02; 95% confidence interval, 0.894–0.982).

According to the manufacturer, the mean measured cTnI concentration in serum samples obtained from 99% of 158 apparently healthy adults was  $\leq 0.07 \mu\text{g/L}$ . The manufacturer does not supply reference values for

**Table 1. Plasma cTnI concentrations in 206 healthy newborns.**

Measurement	Value, $\mu\text{g/L}$	95% CI, $\mu\text{g/L}$
Mean	0.28	0.22–0.34
SD	0.42	
Minimum value	<0.01	
Maximum value	3.00	
Percentile		
2.5th	0.01	0.00–0.03
10th	0.01	0.00–0.04
25th	0.05	0.03–0.07
50th	0.16	0.13–0.19
75th	0.35	0.28–0.44
90th	0.63	0.50–0.72
95th	0.93	0.66–1.80
97.5th	1.77	0.89–2.88
99th	2.80	1.49–3.00

cTnI in plasma and states that the evaluation of heparinized plasma samples showed a median observed value that was 14% lower than in serum. They recommend that reference values be established to evaluate cTnI in serum and plasma and affirm that the ACS:180 immunoassay has a high specificity and does not show any cross-reactivity with cTnT, serum TnI, tropomyosin, troponin C, myosin, myoglobin, or creatine kinase-MB. Generally, cTnI is undetectable in healthy individuals, and there is no cross-reactivity with human skeletal muscle TnI (6). Therefore, cTnI can be considered an adequate evaluation tool for the detection of a myocardial lesion during the perinatal and neonatal periods.

Reed et al. (7) suggested that to achieve a 99% confidence limit, a minimum of 198 reference values would be necessary. We were unable to identify any previous study on cTnI reference intervals established in the NCCLS standard procedures that included such a large sample of newborn infants.

In human fetal hearts, the predominant TnI (>70%) is the slow, skeletal isoform, which is undetectable at 9 months of extra-uterine life. However, cTnI is not produced in any kind of skeletal muscle, irrespective of the stage of development of the fetus or disease stimulus (8).

No conclusion has yet been reached regarding why apparently healthy newborns present with higher cTnI values than those found in seemingly healthy adults. This study is the first to describe lower concentrations of cTnI in newborns

before 48 h of life than those found at 48 h or later. We do not know whether the higher values represent responses to the physiologic processes that occur during the adaptation of fetal circulation to extra-uterine life or whether the plasmatic elimination of cardiac troponin is different in newborns.

Although there were limitations to our study and the study would indeed be stronger if more than one evaluation method had been used, these findings confirm previous reports of higher cTnI concentrations in newborns compared with adults, in whom cTnI was measured by a different analyzer. However, the well-known standardization problems of cTnI assays hamper the comparison of absolute results from different assays. The ACS:180 method is now the second most commonly used method from that manufacturer. Larger studies are needed to identify the sensitivity and specificity of this promising early marker for identification of infants with myocardial ischemia secondary to severe perinatal asphyxia or in association with hypoxemic respiratory failure.

#### References

1. Barberi I, Calabró MP, Cordaro S, Gitto E, Sottile A, Prudente D, et al. Myocardial ischaemia in neonates with perinatal asphyxia: electrocardiographic, echocardiographic and enzymatic correlations. *Eur J Pediatr* 1999;158:742–7.
2. Alpert JS, Thygesen K, Antman E, Bassand J. Myocardial infarction redefined—a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction [Erratum in: *J Am Coll Cardiol* 2001;37:973]. *J Am Coll Cardiol* 2000;36:959–69.
3. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory; approved guideline, 2nd ed. NCCLS document C28–A2, Vol. 20, No. 13 [Replaces C28-A, Vol. 15, No. 4]. Wayne PA: NCCLS, 2000.
4. Morrow DA, Rifai N, Tanasijevic MJ, Wybenga DR, de Lemos JA, Antman EM. Clinical efficacy of three assays for cardiac troponin I for risk stratification in acute coronary syndromes: a Thrombolysis In Myocardial Infarction (TIMI) IIB substudy. *Clin Chem* 2000;46:453–60.
5. Dixon WJ. Processing data for outliers. *Biometrics* 1953;9:74–89.
6. Wright RS, Williams BA, Cramner H, Gallahue F, Willmore T, Lewis L, et al. Elevation of cardiac troponin I are associated with increased short-term mortality in noncardiac critically ill emergency department patients. *Am J Cardiol* 2002;90:634–6.
7. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. *Clin Chem* 1971;17:275–84.
8. Apple FS. Cardiac troponin I. In: Wu AHB, ed. *Cardiac markers*. Totowa, NJ: Humana Press Inc., 1998:229–43.

**Katiaci Araújo<sup>1\*</sup>**  
**José da Silva<sup>2</sup>**  
**Adriana Sañudo<sup>3</sup>**  
**Benjamin Kopelman<sup>4</sup>**

*Departments of <sup>1</sup> Neonatology  
 and <sup>2</sup> Laboratory Medicine  
 Hospital Aliança  
 Salvador, Bahia, Brazil*

*Departments of  
<sup>3</sup> Statistical and Preventive Medicine  
 and <sup>4</sup> Pediatrics  
 Federal University of São Paulo  
 School of Medicine  
 São Paulo, Brazil*

\*Address correspondence to this author at: Rua Santa Helena, 158, Apt. 1302 Pituba, Salvador, Bahia, CEP 41927-430, Brazil. Fax 55-71-240-9193; e-mail katiaci@lab-qualitech.com.br or katiaci@hospitalalianca.com.br.

DOI: 10.1373/clinchem.2004.033472