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Troponin T Levels in the Cord Blood of Healthy Term Neonates

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Summary

Troponin T (TnT) is recently being considered to be an important diagnostic marker of myocardial damage in adults, but this marker has not yet been used in neonates. The present study was designed to determine the normal level of cardiac TnT in the cord blood of healthy term neonates. Cardiac troponin T concentration in cord blood was measured in 15 healthy term neonates using commercial kit (Enzymun-Test System, Boehringer, Mannheim). TnT serum concentration was 0.05±0.04 μg/l in 10 of 15 babies whereas in the remaining 5 haemolysed samples its concentration was elevated (mean 0.19±0.07 μg/l). It is important to consider that incidental haemolysis of blood samples can mimic pathological elevation of TnT by interfering with the assay.

Key words

Neonate – Cord blood – Troponin T – Creatine kinase

Introduction

Myocardial ischaemia and infarction are relatively common events in some groups of neonates – for example in neonates after tocolytic therapy (Katz *et al.* 1989, Gemelli *et al.* 1990) or in neonates with perinatal asphyxia (Kilbride *et al.* 1980, Way *et al.* 1979, Donnelly *et al.* 1980). Several studies were performed to determine some clinical, electrocardiographic and biochemical criteria for confirmation of myocardial injury. Unfortunately, the diagnosis of neonatal myocardial ischaemia is quite difficult. Serum creatine kinase (or CK-MB) and lactate dehydrogenase isoenzyme activity, the important tests in the diagnosis of acute myocardial infarction in adults, are not specific for myocardial injury in neonates. Many pathological conditions associated with the delivery (central nervous system injury, subarachnoid haemorrhage, extensive ischaemic rhabdomyolysis, haemolysis) can also result in elevation and alteration in the pattern of these isoenzymes (Bucciarelli 1991).

The analysis of electrocardiograms in healthy and stressed neonates also causes many problems because the ECG of newborns undergoes striking changes during the first few days of life. The arbitrary grading system described by Jedeikin *et al.* (1983) is usually used as the electrocardiographic criteria of myocardial ischaemia in the newborns. The sensitivity of the ECG as a detector of myocardial ischaemic damage at 5 to 8 hours after the birth is only 40 % and the specificity is 81 % (Jedeikin *et al.* 1983).

Recently, the attention of cardiologists has been drawn to a new serological marker of myocardial injury – cardiac troponin T – but this method has not yet been used by pediatric cardiologists. The present study was designed to determine the normal levels of cardiac troponin T in cord blood of healthy term neonates.

Methods

Fifteen babies who were enrolled in our study satisfied the following criteria:

1) uncomplicated labour and delivery
2) gestational age of 38 to 42 weeks
3) normal newborn physical examination

Table 1
Clinical features in 15 neonates

No.	Sex	GA (weeks)	Length (cm)	Weight (g)	Apgar score 1st min	Apgar score 5th min
1	F	40	54	4 020	9	10
2	M	40	51	3 690	6	8
3	F	39	47	3 340	8	10
4	F	41	51	3 420	9	10
5	M	40	50	3 250	9	10
6	M	41	50	2 950	9	10
7	F	41	52	3 640	9	10
8	F	38	51	3 460	9	10
9	M	38	47	3 000	8	9
10	F	41	50	3 100	9	10
11	F	40	50	3 740	8	10
12	M	42	54	3 900	7	10
13	F	42	52	3 480	8	8
14	F	40	49	3 180	9	10
15	M	40	51	3 400	9	10

GA – gestational age

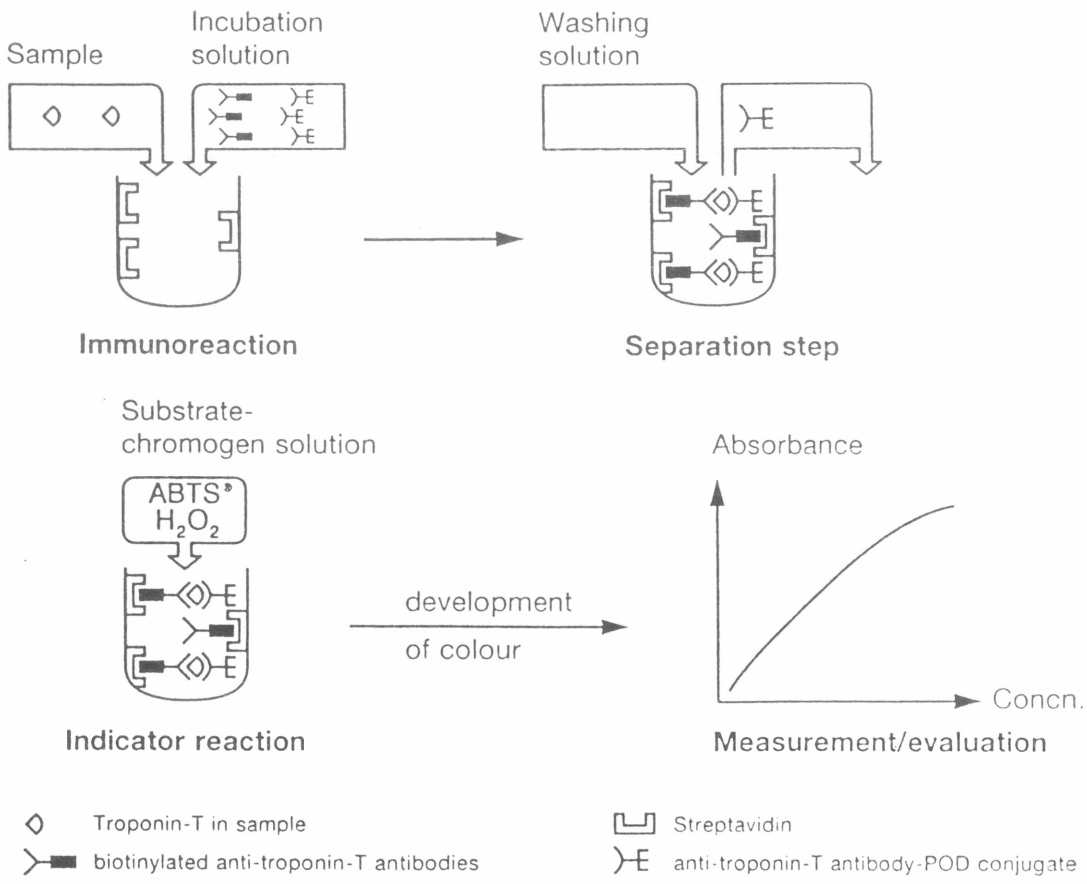

Fig. 1
Enzyme-imunological test for the quantitative determination of troponin T *in vitro* (Boehringer Mannheim, Germany). Test principle is based upon ELISA/1-step sandwich assay using streptavidin technology.

Clinical features of these newborn infants are summarized in Table 1. Mean gestational age was 40.2±1.2 weeks, mean birthweight was 3438±317 g, mean birthlength was 50.6±2.0 cm. The mean Apgar score was 8.4±0.9 at one min (range 6–9) and 9.7±0.7 at 5 min (range 8–10). Nine were female and six were male. Venous blood was obtained from these neonates at delivery (cord blood).

Laboratory analysis

In all babies the serum was immediately analyzed for glucose, electrolytes, AST, ALT, CK, CK-MB using routine biochemical methods. Serum levels of potassium were not measured in the haemolysed samples.

The serum samples for TnT measurements were stored frozen at −70 °C for a maximum of two months until analysis.

Troponin T Assay

An enzyme-linked immunoadsorbent assay (Boehringer Mannheim, Germany) developed by Katus *et al.* (1989) was used to determine the cardiac troponin T circulating in the plasma. The method is based on a single-step sandwich principle, with streptavidin-coated tubes as the solid phase and two monoclonal anti-human cardiac troponin T antibodies (see Fig. 1).

In this assay, an affinity-purified cardiospecific anti-troponin T fraction of polyclonal antibody is immobilized on polyvinylchloride test tubes. Troponin T standards or serum samples and peroxidase-labelled monoclonal anti-troponin T antibody are added to these antibody-coated test tubes. During the incubation period, the troponin T molecule is bound to indifferent epitopes by both the solid-phase polyclonal antibody fraction and by the liquid-phase monoclonal antibody-enzyme complex. After the non-bound peroxidase-labelled monoclonal antibodies were removed by washing, the antibody-enzyme complex adhering to the assay tubes corresponds to the amount of troponin T recognized by the polyclonal and monoclonal anti-troponin T antibodies. The amount of enzyme immobilized, as a direct measure of bound troponin T, is quantified in the spectrophotometer as peroxidase substrate conversion at a wavelength of 405 nm.

The assay is manufactured as a test kit consisting of seven components: antibody-coated test tubes, monoclonal antibody-enzyme complex, incubation buffer, troponin T standard, control sera, substrate buffer, and the diammonium salt of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS).

For troponin T measurements, the antibody-enzyme complex, troponin T standards, and ABTS substrate are dissolved in the buffer solution provided in the test kit and thoroughly mixed by vortex. Troponin T standard or unknown patient serum (200 µl) is manually added to the test tubes while 1 ml antibody-enzyme complex (45 IU) and all the remaining solutions are added by a batch enzyme-linked immunoadsorbent assay analyzer (Enzymun-Test System, Boehringer Mannheim, Germany). Solid-phase antibody, troponin T-containing solutions, and the antibody-enzyme complex are incubated at room temperature for 60 min. Thereafter, the tubes are emptied by suction and washed twice with tap water. To each test tube, 1 ml substrate solution is then added, and the substrate conversion is measured as color formation after 30 min of incubation at room temperature. All measurements were performed in duplicate.

The mean troponin T concentration was calculated from the duplicate samples using the calibration curve constructed from six calibration standards (ranging 0 to 15 µg/l), which consist of purified bovine cardiac troponin T in a human serum matrix.

Statistical analysis

The enzyme activities were found to have a normal distribution. Comparison between the mean values in the individual subgroups were evaluated using Student's test.

Results

Blood samples were divided into two groups. In the first group we assessed unhaemolysed serum (see Table 2), the second group consisted of five haemolysed samples (see Table 3).

Group 1

The TnT serum concentration in 10 unhaemolysed samples was 0.05±0.04 µg/l. The serum concentrations of glucose (4.98±1.57 mmol/l), sodium (139±3.0 mmol/l), potassium (4.84±0.55 mmol/l), chloride anions (106±4.0 mmol/l) and calcium (2.56±0.07 mmol/l) were in the physiological range. The activity of ALT was 0.27±0.07 µkat/l, AST 0.64±0.11 µkat/l, CK 4.67±1.21 µkat/l and CK-MB activity was 1.22±0.56 µkat/l. No correlation was found between troponin T serum concentration and the activity of creatine kinase or its isoenzyme CK-MB.

Table 2
Biochemical parameters in unhaemolysed samples of cord blood

Sample	TnT (μg/l)	CK (μkat/l)	CK-MB (μkat/l)	ALT (μkat/l)	AST (μkat/l)	Glucose (mmol/l)	Na (mmol/l)	K (mmol/l)	Cl (mmol/l)	Ca (mmol/l)
1	0.00	4.25	1.04	0.32	0.46	4.4	138	5.0	100	2.58
2	0.07	4.71	0.73	0.27	0.59	5.3	142	4.6	103	2.46
3	0.08	3.24	0.86	0.41	0.68	4.1	142	4.2	108	2.54
4	0.03	4.01	1.73	0.17	0.60	6.6	136	5.1	107	2.60
5	0.10	3.99	1.43	0.29	0.73	3.6	138	4.7	112	2.64
6	0.09	5.21	1.90	0.28	0.80	3.0	134	5.9	99	2.66
7	0.04	6.30	1.65	0.20	0.60	4.2	142	4.8	107	2.49
8	0.08	6.99	1.86	0.30	0.77	6.6	137	5.5	103	–
9	0.01	4.67	0.52	0.27	0.63	4.1	143	4.2	110	2.49
10	0.03	3.34	0.47	0.19	0.52	7.9	139	4.4	107	2.62
Mean	0.05	4.67	1.22	0.27	0.64	4.98	139	4.84	106	2.56
S.D.	0.04	1.21	0.56	0.07	0.11	1.57	3	0.55	4	0.07

Table 3
Biochemical parameters in haemolysed samples of cord blood

Sample	TnT (μg/l)	CK (μkat/l)	CK-MB (μkat/l)	ALT (μkat/l)	AST (μkat/l)	Glucose (mmol/l)	Na (mmol/l)	Cl (mmol/l)	Ca (mmol/l)
1	0.25	6.54	1.80	0.21	1.43	3.6	130	103	2.49
2	0.14	5.50	1.65	0.45	1.18	6.2	155	118	2.77
3	0.12	8.26	0.50	0.26	0.72	4.3	134	100	2.61
4	0.27	6.67	1.97	0.27	0.88	3.5	136	104	2.67
5	0.16	4.45	3.14	0.41	1.11	5.1	143	104	2.43
Mean	0.19	6.28	1.81	0.32	1.06	4.54	140	106	2.59
S.D.	0.07	1.42	0.94	0.10	0.27	1.13	10	7	0.13

Group 2

The TnT concentration in the remaining 5 haemolysed samples was elevated (0.19±0.07 μg/l). The concentration of serum glucose (4.54±1.13 mmol/l), sodium (140±10 mmol/l), chloride (106±7 mmol/l) and calcium (2.59±0.13 mmol/l) were also in the physiological range. The ALT activity was 0.32±0.10 μkat/l, the AST activity was 1.06±0.27 μkat/l. The CK activity was 6.28±1.42 μkat/l, the CK-MB activity was 1.81±0.94 μkat/l.

The TnT concentration was significantly ($P<0.001$) higher in the haemolysed samples compared to unhaemolysed samples.

Discussion

Troponin T is the tropomyosin-binding protein of the troponin regulatory complex (subunits I, C and T) located on the thin myofilament of the contractile apparatus. Three muscle specific isoforms of troponin T, which vary by as many as ten amino acids, can be differentiated by immunological techniques. These isoforms are found in slow and fast skeletal muscles and in the cardiac muscle of adults. That is why troponin T determination is recently considered to be useful in the assessment of cardiac injury in the presence of skeletal damage (Donnelly and Hillis 1993).

Several studies proved that 99 % of healthy adults had serum troponin T concentrations below 0.06 μg/l (Gerhardt *et al.* 1992, Mair *et al.* 1991, Collinson *et al.* 1993). Other reports attest the value of this new diagnostic marker under various conditions associated with myocardial damage and have stated that measurable amounts of troponin T are to be found in the blood only after degradation of the contractile proteins following severe ischaemia or cell necrosis (Katus *et al.* 1991, Zabel *et al.* 1993, Gerhardt *et al.* 1991). The peak concentration was observed during the first 24 hours after acute infarction, with values ranging between 9.6 – 2.7 μg/l (Mair *et al.* 1991).

The TnT serum concentration was 0.05 ± 0.04 μg/l in 10 unhaemolysed samples. Our data indicate that under physiological conditions almost no myocardial troponin T is present in cord blood. To our knowledge, this finding has not yet been reported.

Unfortunately, the specificity of TnT during ontogenesis has not been fully defined. Swynghedauw (1986) indicates that cardiac TnT which is suppressed in healthy adult skeletal muscle, can probably be expressed in fetal and neonatal skeletal muscles in humans and experimental animals. On the other hand, it is necessary to know that human myocardium and skeletal muscles can express more than one isoform of cardiac troponin T. In addition, ontogenetic development, adaptational changes and various diseases also alter troponin T expression. For example, one dominant isoform of cardiac troponin T – TnT1 – is expressed in normal adult myocardium but a second isoform TnT2 is upregulated in left ventricular failure (Anderson *et al.* 1991). Since troponin T regulates the myofibrillar response to calcium, the functional consequences of altered isoform expression appear to be of interest.

Using Western blots, four TnT isoforms were found in the fetal heart, two of which had the same electrophoretic mobilities as the adult cardiac isoforms TnT1 and TnT2. Moreover, Anderson *et al.* (1991) demonstrated that fetal skeletal muscles expressed two of the four fetal cardiac TnT isoforms, one of which corresponded to adult cardiac TnT1. These cardiac isoforms are expressed in low abundance in fetal skeletal muscles relative to seven fast skeletal muscle TnT isoforms.

Serum creatine kinase and its isoenzyme CK-MB have become important tests in the diagnosis of acute myocardial infarction in adults. A strong argument against the use of CK-MB as an index of myocardial damage in the newborn is that this isoenzyme is not cardiac-specific until 4 years of age (Bucciarelli 1991). Cao *et al.* (1971), Jedeikin *et al.* (1982) as well as our study indicate that total creatine kinase activity and CK-MB activity in the cord blood exceed the adult values.

It is well known that haemolysis elevates CK and CK-MB as well as AST activity (Masopust 1990). Our data indicate that incidental haemolysis of the blood samples could mimic pathological elevation of TnT because haemolysis interferes with the assay. We have found similar finding in adults (unpublished data).

In conclusion our study shows that cardiac TnT could become a suitable criterion in laboratory diagnosis of neonatal myocardial injury. According to the fact that developmental changes of structural proteins in the heart are somewhat complicated, it is necessary to define the specificity of TnT in newborns and evaluate the diagnostic performance of cardiac troponin T in various cardiac settings associated with myocardial ischaemia and damage in babies. Moreover, it should be considered that incidental haemolysis of the blood samples can interfere with the assay and could mimic the pathological elevation of TnT.

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Reprint Requests

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