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ORIGINAL ARTICLE

Cardiac troponin T and N-terminal-pro-B type natriuretic peptide reflect myocardial function in preterm infants

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Background: Cardiovascular compromise is increasingly recognized in preterm infants. Currently, echocardiography is the best tool to assess myocardial contractility and guide management. Elevated levels of cardiac troponin T (cTnT) and N-terminal-pro-B type natriuretic peptide (NTpBNP) are associated with poor myocardial contractility and low cardiac output in adults.

Objective: To examine the range of cTnT and NTpBNP in preterm infants, to correlate NTpBNP and cTnT with echocardiographic markers of cardiac function and to assess the influence of antenatal and postnatal factors on these biochemical markers.

Study Design: Plasma concentrations of cTnT and NTpBNP were measured in infants <1500 g at 12 h of age. These were correlated with simultaneous echocardiographic measures of myocardial function and output. Antenatal data, infant demographics and postnatal characteristics were prospectively recorded.

Result: A total of 80 infant had samples taken and echocardiography performed. Their median (interquartile range) cTnT and NTpBNP values were 0.20 $\mu g\,l^{-1}$ (0.11 to 0.40) and 1273 $pmol\,l^{-1}$ (664 to 2798), respectively. There was a significant inverse correlation between cTnT and echocardiographic markers of myocardial function and stroke volume. NTpBNP significantly correlated with left atrial to aortic root ratio (LA:Ao). There was a weaker but significant negative correlation between NTpBNP and left ventricular (LV) function. The assays were not influenced by gestation, birth weight, gender or mode of delivery.

Conclusion: cTnT and NTpBNP are correlated with echocardiographic measures of cardiac performance in preterm infants. Measurement of levels in the first hours of life may provide useful information regarding myocardial function and volume loading.
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Introduction

The impact of cardiovascular compromise is becoming increasingly recognized in both term and preterm infants. Respiratory distress syndrome and perinatal asphyxia are associated with low cardiac output and poor myocardial contractility.[1] Echocardiography is currently the best diagnostic tool available to assess cardiovascular function.[2]

B-type natriuretic peptide (BNP) and N-terminal-Pro-BNP (NTpBNP) are released by the stressed myocardium in response to volume and pressure loading.[3] In neonates, BNP is a good marker of ductal significance, with levels rising in the presence of a patent ductus arteriosus (PDA) and falling with subsequent treatment.[4,5] BNP may also be used to distinguish neonates with persistent pulmonary hypertension from those with parenchymal lung disease.[6] NTpBNP is an inactive by-product of BNP, which is more stable, and has a longer half-life. Reference ranges for NTpBNP in term neonates have been published, and its potential role in PDA diagnosis was recently demonstrated.[7]

Troponin T is the largest subunit in the troponin complex, which attaches to tropomyosin and actin to facilitate cross-linking with the myosin thick filament leading to contraction during excitation.[8] Serum cardiac troponin T (cTnT) is a marker of cardiac injury and mortality in adults and is a good marker of myocardial injury in perinatally asphyxiated neonates.[9] cTnT levels are higher in premature infants with respiratory distress syndrome (RDS).[10]

Biochemical markers of impaired function and ischemic damage may serve as an important screening tool in centers where echocardiography resources are limited. We hypothesized that NTpBNP and cTnT are good markers of myocardial function in preterm infants. We aimed to (a) determine the range of values of both markers in preterm infants at 12 h of age, (b) correlate NTpBNP and cTnT with echocardiographic markers of cardiac function and (c) assess the influence of antenatal and postnatal factors.

Methods

All preterm neonates with birth weights between 500 and 1500 g born at the National Maternity Hospital, Ireland during July 2006 and June 2007 were eligible. The study was approved by the Institutional Ethics Committee and informed consent was requested from parents. Infants with major congenital abnormalities and cardiac lesions other than PDA were excluded. Clinical details including maternal preeclampsia (PET), histological chorioamnionitis, antenatal steroid administration, Apgar score at 5 min, RDS and ventilation days were recorded. RDS was defined as clinical signs of respiratory distress and a rising PCO_2 on an arterial blood gas sample in the presence of a confirmatory chest X-ray.[11] A complete course of antenatal steroids was defined as betamethasone 12.5 mg given twice, 12 h apart and a single dose was termed partial antenatal steroid treatment. Echocardiographic examination with simultaneous NTpBNP and troponin T measurements were carried out at 12 h of age by a single echocardiographer (AK).

Biochemical parameters

NTpBNP and cTnT measurements were taken immediately following the echocardiogram. A sample of 1 ml of blood was collected in a lithium heparin bottle and centrifuged and the plasma frozen at $-20^{\circ}C$ for later batch analysis. The Roche test is a non-competitive chemiluminescent technology for the determination of NTpBNP in human serum and plasma. This is a two-site (sandwich) assay incorporating a polyclonal NTpBNP-specific antibody and a polyclonal NTpBNP-specific antibody labeled with a ruthenium complex. The variance is 4.6% ($5.6\,pmol\,l^{-1}$) and 1.9% ($107.5\,pmol\,l^{-1}$), respectively, for low- and high-concentration patient samples, and the respective day-to-day variance is 5.5% ($6.4\,pmol\,l^{-1}$) and 2.6% ($113.6\,pmol\,l^{-1}$).[12]

The cTnT assay, an electrochemiluminescent sandwich enzyme-linked immunosorbant assay, has a lower limit of detection of $0.01\,pg\,l^{-1}$, with minimal cross reactivity with cardiac troponin I (0.002%) and skeletal troponin T (0.001%). This third generation assay is unaffected by bilirubin levels up to exchange values, sample hemolysis or renal insufficiency. The repeatability coefficient for a paired sample is 10% and the variability coefficient for precision analysis is 6.4%. (Anon 1999, Troponin T STAT data sheet, Roche diagnostics; www.roche.com).

Echocardiographic measurements

We used the Siemens Acuson Sequoia Ultrasound machine and a 10v4 cardiology multifrequency probe. Studies were performed by a single echocardiographer using standard neonatal windows including apical, parasternal, subcostal and high parasternal windows. The scans were recorded on the machine's internal hard drive for later measurements. Two dimensional, M-mode imaging, pulse and color Doppler information were recorded. The following echocardiographic variables were determined in each study:

(a) Left ventricular (LV) mean velocity of circumferential fibre shortening (mVcfs): this is a preload independent measure of LV function that takes into account afterload conditions. It is determined by the following method:[13]

$$mVcfs = (LVEDC - LVESC) \div (LVEDC \times Etc)$$

where LVEDC = LV end-diastolic circumference; LVESC = LV end-systolic circumference and ETc = LV ejection time corrected for heart rate ($ET/\sqrt{RR\ interval}$).

(b) LV and right ventricular (RV) stroke volumes and outputs: measurements of these parameters are established and published elsewhere.[1,14]

(c) Shortening fraction: measured using M-mode echocardiography.[15]

(d) Ductal diameter: absolute diameter in millimeters from the high parasternal view. A ductal diameter $\geqslant 1.5$ mm was considered significant.[16]

(e) Left atrial to aortic root ratio (LA:Ao): A large left atrium compared to a relatively constant aortic root size gives an indication of the degree of pulmonary venous return due to a large left to right shunt at ductal level. A larger shunt will increase the atrial diameter increasing LA:Ao. We used a cut-off ratio of $\geqslant 1.5$ to determine significance.[16]

Statistical analysis

The echocardiographic and plasma NTpBNP/cTnT measurements were non-parametric continuous variables and therefore expressed as medians (interquartile ranges). Medians were compared using the Mann–Whitney *U* test. Correlations were tested using Spearman's correlation coefficient. We considered a *P*-value of <0.05 as significant.

Results

Eighty preterm infants underwent echocardiographic examinations paired with simultaneous NTpBNP and cTnT determinations at 12 h of life. Their median gestation was 28 weeks (26.1to 29.5) and median birth weight 1.06 kg (0.87 to 1.22). Their characteristics are shown in Table 1. The median NTpBNP value for the cohort was $1273\,pmol\,l^{-1}$ (664 to 2798) and median cTnT was $0.20\,\mu g\,l^{-1}$ (0.11 to 0.40). Table 2 displays the median and range of the echocardiographic parameters of the group. At 12 h of life all infants had a PDA with left to right shunting.

The influence of antenatal and postnatal factors on NTpBNP and cTnT levels is illustrated in Table 3. Infants with RDS had significantly higher NTpBNP (1391 versus $815\,pmol\,l^{-1}$, $P = 0.04$) and cTnT (0.30 versus $0.09\,\mu g\,l^{-1}$, $P = 0.008$). Infants born to mothers with preeclampsia had significantly lower cTnT levels (0.11 versus $0.24\,\mu g\,l^{-1}$, $P = 0.02$). However, 15 out 16 infants in the PET group (94%) received a complete course of antenatal steroids compared to 35 out of 64 infants without PET

Table 1 Characteristics of the cohort

	Cohort (n = 80)
Gestation (weeks)	28.0 [26.14–29.53]
Birth weight (kg)	1.06 [0.87–1.22]
Apgars at 1 min	5 [4–8]
Apgars at 5 min	8 [6–9]
Male	45 (56)
Antepartum hemorrhage	23 (29)
Preeclampsia	16 (20)
AREDF	19 (24)
Prolonged ROM	26 (33)
Chorioamnionitis	16 (20)
Antenatal steroids	
None	12 (15)
Incomplete	18 (23)
Complete	50 (62)
Caesarean section	49 (58)
RDS	72 (90)
Inotropes	23 (29)

Abbreviations: ARDEF, absent or reversed end diastolic flow. ROM: rupture of membranes; RDS, respiratory distress syndrome.
Values are represented as median [interquartile range] or absolute value (%).

Table 2 Biochemical assays and echocardiographic markers in the cohort

	Median	*Interquartile range*	*Min–Max*
cTnT ($\mu g\,l^{-1}$)	0.20	0.11–0.40	0.04–1.52
NTpBNP ($pmol\,l^{-1}$)	1273	664–2798	98–10 700
mVcfs (circumference/s)	1.09	0.88–1.42	0.11–3.12
FS (%)	32	26–39	11–53
Left ventricle			
Stroke volume (ml)	1.10	0.84–1.50	0.38–2.8
Output ($ml\,kg^{-1}\,min^{-1}$)	166	118–211	54–349
Right ventricle			
Stroke volume (ml)	1.17	0.81–1.89	0.26–3.31
Output ($ml\,kg^{-1}\,min^{-1}$)	175	117–238	42–430
PDA diameter (mm)	1.78	1.42–2.47	0.5–4.1
LA:Ao ratio	1.60	1.26–1.79	0.56–3.12

Abbreviations: cTnT, cardiac troponin T; FS, shortening fraction; LA:Ao ratio, left atrial to aortic root ratio; Min–Max, minimum to maximum value; mVcfs, mean circumferential fiber shortening velocity; NTpBNP, N-terminal-pro-B type natriuretic peptide; PDA, patent ductus arteriosus.

(55%, $P = 0.01$). This association of lower cTnT with PET is lost when adjusted for antenatal steroids. The assays were unaffected by gender, prolonged rupture of membranes, histological chorioamnionitis or the mode of delivery. When RDS was controlled for, we found no correlation between gestation, birth weight and NTpBNP (partial correlation coefficient −0.27, $P = 0.87$, and −0.20, $P = 0.20$, respectively) and cTnT levels (partial correlation coefficient −0.19, $P = 0.24$, and −0.11, $P = 0.50$, respectively) Infants with lower 5 min Apgar scores had a significantly higher cTnT levels (partial correlation coefficient −0.36, $P = 0.02$). An increased level of cTnT seen in infants on inotropes did not reach statistical significance (0.30 versus $0.19\,\mu g\,l^{-1}$, $P = 0.19$).

Table 3 Influence of antenatal and post natal factors on NTpBNP and cTnT levels at 12 h

Grouping variable	*Group*	*NTpBNP ($pmol\,l^{-1}$)*		*cTnT ($\mu g\,l^{-1}$)*	
		Median	P-*value*	*Median*	P-*value*
Sex	Boy	1273	0.32	0.30	0.10
	Girl	1454		0.19	
Preeclampsia	No	1222	0.34	0.24	0.02
	Yes	1586		0.11	
PROM	No	1177	0.18	0.19	0.52
	Yes	1408		0.24	
Chorioamnionitis	No	1199	0.07	0.20	0.68
	Yes	2248		0.22	
Antenatal steroids	None	1586	0.42	0.52	0.11
	Partial	840		0.31	
	Full	1462		0.19	
Delivery	SVD	1275	0.60	0.28	0.28
	CS	1222		0.19	
Inotrope use	No	1368	0.83	0.19	0.19
	Yes	1269		0.30	
RDS	No	815	0.04	0.09	0.008
	Yes	1391		0.24	

Abbreviations: CS, caesarean section; PROM, prolonged rupture of membranes; RDS, respiratory distress syndrome; SVD, spontaneous vaginal delivery.
Mann–Whitney *U* test was used to compare medians.

There was a significant negative correlation between cTnT and echocardiographic markers of LV function including mVcfs, shortening fraction LV and RV stroke volumes. However, there was no correlation with LV and RV outputs. There was no correlation between PDA diameter or LA:Ao and cTnT. NTpBNP significantly correlated with LA:Ao but not with ductal diameter. There was a weaker but significant negative correlation between NTpBNP and LV mVcfs and shortening fraction but not with output (Table 4).

Discussion

We demonstrated that NTpBNP and cTnT are potentially useful markers of myocardial function in preterm infants. cTnT and NTpBNP were unaffected by gestation, birth weight, sex, chorioamnionitis and mode of delivery. This is consistent with the results of other groups[17–20] and suggests that assays of cTnT and

Table 4 Correlation of cTnT and NTpBNP with echocardiographic markers of function and PDA significance

	NTpBNP		*cTnT*	
	Spearman's correlation	P	*Spearman's correlation*	P
mVcfs	−0.32	0.019	−0.37	0.008
FS	−0.26	0.033	−0.38	0.006
RV stroke volume	−0.08	0.54	−0.34	0.013
RV output	0.08	0.57	−0.16	0.26
LV stroke volume	−0.14	0.25	−0.43	0.001
LV output	0.07	0.58	−0.21	0.14
PDA diameter	−0.14	0.24	−0.12	0.41
LA:Ao	0.33	0.006	−0.08	0.58

Abbreviations: cTnT, cardiac troponin T; FS, shortening fraction; LA:Ao, left atrial to aortic root ratio; LV, left ventricle; mVcfs: mean velocity circumferential fiber shortening; NTpBNP, N-terminal-pro-B type natriuretic peptide; PDA, patent ductus arteriosus; RV, right ventricle.

NTpBNP can be used as markers of myocardial dysfunction. However, the ranges of gestational age and birth weight in our cohort are narrow and may explain the lack of effect of gestational age or birth weight. In addition, the marked increase of levels seen in infants with RDS may have obscured the effects of gestational age on the levels of the biomarkers.

Cardiac troponin T

Our study included 72 infants (90%) with RDS, cTnT was higher in these infants compared to those without RDS. Poor myocardial function is common in these preterm infants,[21] and may be mediated by hypoxia and/or ischemia.[1] Infants requiring inotropes had higher cTnT compared to those who were normotensive but this did not reach statistical significance possibly due to the relatively small number of subjects. Clark SJ *et al*.[18,22] showed a similar effect of inotropes on cTnT levels. Whether the rise in cTnT in hypotensive infants is a direct result of the inotropes or cardiovascular compromise occurring before delivery is unclear. It is unlikely that inotropes are directly responsible for the cTnT release and therefore myocardial injury occurring before or during delivery may have caused the rise. A study measuring cTnT levels before and after inotrope use may clarify this issue.

cTnT significantly correlated with mVcfs, shortening fraction, and LV and RV stroke volumes. This direct correlation is previously unreported and may provide means of indirect assessment of myocardial function in preterm infants when echocardiography is not readily available. The higher cTnT levels in infants with RDS may therefore reflect the previously reported association between RDS and poor myocardial performance.[1] There was no relationship between cTnT and PDA diameter or LA:Ao suggesting that cTnT is less influenced by volume loading of the heart.

N-terminal-pro-natriuretic peptide

NTpBNP levels were not influenced by gestation, birth weight, gender, mode of delivery or antenatal factors. Reference ranges for NTpBNP in extremely low birth weight infants are scarce. The median NTpBNP level in our cohort is higher than normal values for term infants which range from 80 to 360 pmol l^{-1}.[3] The reason for this variation particularly in preterm infants may be a result of the presence of poor myocardial contractility and low cardiac output in this population rather than prematurity *per se*. RDS is common in this population. The associated higher NTpBNP levels highlight the presence of myocardial volume loading associated with RDS.

NTpBNP correlated weakly with echocardiographic markers of contractility and output but correlated significantly with LA:Ao, making it a better marker than cTnT of ventricular volume loading. We recently demonstrated higher NTpBNP levels in preterm infants with a PDA on day 3 of life.[7]

Conclusion

The relationship between cTnT, NTpBNP and echocardiographic markers of myocardial function are not reported previously. Measurement of levels in the preterm population warrants further research. They are not readily influenced by antenatal factors and therefore, their use during first hours following birth may provide useful information regarding myocardial function and volume loading, potentially guiding initial management. cTnT may be useful as a marker of myocardial contractility and therefore reflect the degree of myocardial compromise. NTpBNP may be useful in assessing ventricular volume overload and RDS severity. Further research is needed to establish reference ranges for both cTnT and NTpBNP in the preterm population and examine the influence of neonatal sepsis and hypotension on their levels.

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Conflict of interest

None.

References

1 Evans N, Kluckow M. Early determinants of right and left ventricular output in ventilated preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1996; **74**(2): F88–F94.

2 Kluckow M, Seri I, Evans N. Functional echocardiography: an emerging clinical tool for the neonatologist. *J Pediatr* 2007; **150**(2): 125–130.

3 El-Khuffash A, Molloy EJ. Are B-type natriuretic peptide (BNP) and N-terminal-pro-BNP useful in neonates? *Arch Dis Child Fetal Neonatal Ed* 2007; **92**(4): F320–F324.

4 Flynn PA, da Graca RL, Auld PA, Nesin M, Kleinman CS. The use of a bedside assay for plasma B-type natriuretic peptide as a biomarker in the management of patent ductus arteriosus in premature neonates. *J Pediatr* 2005; **147**(1): 38–42.
5 Choi BM, Lee KH, Eun BL, Yoo KH, Hong YS, Son CS *et al.* Utility of rapid B-type natriuretic peptide assay for diagnosis of symptomatic patent ductus arteriosus in preterm infants. *Pediatrics* 2005; **115**(3): e255–e261.
6 Reynolds EW, Ellington JG, Vranicar M, Bada HS. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. *Pediatrics* 2004; **114**(5): 1297–1304.
7 El-Khuffash AF, Amoruso M, Culliton M, Molloy EJ. N-terminal pro-B-type natriuretic peptide as a marker of ductal haemodynamic significance in preterm infants: a prospective observational study. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**(5): F421–F422.
8 Gomes AV, Potter JD, Szczesna-Cordary D. The role of troponins in muscle contraction. *IUBMB Life* 2002; **54**(6): 323–333.
9 Costa S, Zecca E, De RG, De LD, Barbato G, Pardeo M *et al.* Is serum troponin T a useful marker of myocardial damage in newborn infants with perinatal asphyxia? *Acta Paediatr* 2007; **96**(2): 181–184.
10 Clark SJ, Newland P, Yoxall CW, Subhedar NV. Sequential cardiac troponin T following delivery and its relationship with myocardial performance in neonates with respiratory distress syndrome. *Eur J Pediatr* 2006; **165**(2): 87–93.
11 Moss TJ. Respiratory consequences of preterm birth. *Clin Exp Pharmacol Physiol* 2006; **33**(3): 280–284.
12 Mueller T, Gegenhuber A, Poelz W, Haltmayer M. Comparison of the Biomedica NT-proBNP enzyme immunoassay and the Roche NT-proBNP chemiluminescence immunoassay: implications for the prediction of symptomatic and asymptomatic structural heart disease. *Clin Chem* 2003; **49**(6 Part 1): 976–979.
13 Osborn DA, Evans N, Kluckow M. Left ventricular contractility in extremely premature infants in the first day and response to inotropes. *Pediatr Res* 2007; **61**(3): 335–340.
14 Alverson DC, Eldridge MW, Johnson JD, Aldrich M, Angelus P, Berman Jr W. Noninvasive measurement of cardiac output in healthy preterm and term newborn infants. *Am J Perinatol* 1984; **1**(2): 148–151.
15 Lee LA, Kimball TR, Daniels SR, Khoury P, Meyer RA. Left ventricular mechanics in the preterm infant and their effect on the measurement of cardiac performance. *J Pediatr* 1992; **120**(1): 114–119.
16 El HM, Vaksmann G, Rakza T, Kongolo G, Storme L. Severity of the ductal shunt: a comparison of different markers. *Arch Dis Child Fetal Neonatal Ed* 2005; **90**(5): F419–F422.
17 Hammerer-Lercher A, Mair J, Tews G, Puschendorf B, Sommer R. N-terminal pro-B-type natriuretic peptide concentrations are markedly higher in the umbilical cord blood of newborns than in their mothers. *Clin Chem* 2005; **51**(5): 913–915.
18 Clark SJ, Newland P, Yoxall CW, Subhedar NV. Concentrations of cardiac troponin T in neonates with and without respiratory distress. *Arch Dis Child Fetal Neonatal Ed* 2004; **89**(4): F348–F352.
19 Clark SJ, Newland P, Yoxall CW, Subhedar NV. Cardiac troponin T in cord blood. *Arch Dis Child Fetal Neonatal Ed* 2001; **84**(1): F34–F37.
20 Bar-Oz B, Lev-Sagie A, Arad I, Salpeter L, Nir A. N-terminal pro-B-type natriuretic peptide concentrations in mothers just before delivery, in cord blood, and in newborns. *Clin Chem* 2005; **51**(5): 926–927.
21 Gill AB, Weindling AM. Echocardiographic assessment of cardiac function in shocked very low birthweight infants. *Arch Dis Child* 1993; **68**(1 Spec No): 17–21.
22 Clark SJ, Newland P, Yoxall CW, Subhedar NV. Sequential cardiac troponin T following delivery and its relationship with myocardial performance in neonates with respiratory distress syndrome. *Eur J Pediatr* 2006; **165**(2): 87–93.