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Review

Use of cardiac biomarkers in neonatology

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Cardiac biomarkers are used to identify cardiac disease in term and preterm infants. This review discusses the roles of natriuretic peptides and cardiac troponins. Natriuretic peptide levels are elevated during atrial strain (atrial natriuretic peptide (ANP)) or ventricular strain (B-type natriuretic peptide (BNP)). These markers correspond well with cardiac function and can be used to identify cardiac disease. Cardiac troponins are used to assess cardiomyocyte compromise. Affected cardiomyocytes release troponin into the bloodstream, resulting in elevated levels of cardiac troponin. Cardiac biomarkers are being increasingly incorporated into clinical trials as indicators of myocardial strain. Furthermore, cardiac biomarkers can possibly be used to guide therapy and improve outcome. Natriuretic peptides and cardiac troponins are potential tools in the diagnosis and treatment of neonatal disease that is complicated by circulatory compromise. However, clear reference ranges need to be set and validation needs to be carried out in a population of interest.

Cardiac biomarkers provide a view into the structure and functioning of the heart in newborn infants. Several of these biomarkers are already in clinical use, and others are under investigation. The most commonly used cardiac biomarkers are the natriuretic peptides and troponins. The former relate to cardiac stress and ventricular strain, whereas the latter signal cardiomyocyte compromise (1,2). The markers are used in clinical trials to provide insight into the extent of compromise in the functioning of the newborn's heart (3).

The aim of this review is to discuss the clinical indications of the most relevant cardiac biomarkers. The use of these biomarkers in the prediction of short- and long-term outcomes will also be discussed (4,5). Finally, we evaluate the status regarding the use of cardiac biomarkers in determining treatment protocols and the use of these biomarkers in future clinical trials. This review focuses on the use of biomarkers in newborns; congenital heart defects and surgery are discussed only marginally.

CARDIAC BIOMARKERS

Natriuretic Peptides

Since electron microscopy first revealed secretory granules in atrial cells containing atrial natriuretic peptide (ANP), four natriuretic peptides have been described; natriuretic peptides A, B, C, and D (6). All natriuretic peptides have aminoacid ring-shaped structures, but they differ in their modes of action. The function of ANP was discovered after atrial extracts were infused into rats. De Bolt *et al.* found that massive diuresis and natriuresis occurred (7). This observation started research into the role of endocrines in the heart, and eventually led to the discovery of other natriuretic peptides, primarily B-type natriuretic peptide (BNP). Natriuretic peptide C and Dendroaspis natriuretic peptide have also been identified, but their clinical value has yet to be evaluated (8,9). In the past two decades, much research has been done to clarify the functions of natriuretic peptides. ANP is released from the atrial myocardium in response to stretching of the atrial wall. BNP is released from the ventricular myocardium in response to stretching of the ventricular wall (10). These properties make natriuretic peptides attractive in the identification of congestive heart failure. Given that the serum half-life of BNP is longer than that of ANP, and that the former more accurately reflects cardiac function, BNP or the inactive N-terminal fragment of BNP (NT-proBNP) is generally preferred as a cardiac biomarker (11,12). When their application in adult medicine was found to show promise, research began to be directed toward the potential use of natriuretic peptides in neonatal medicine. Hölmstrom *et al.* found a strong correlation between BNP and NT-proBNP; therefore both are considered to be useable depending on local availability (13). Because NT-proBNP is excreted primarily by the kidney, its use depends on renal function (14). BNP and NT-proBNP are good indicators of ventricular functioning and can be used in the diagnosis of relevant diseases, thereby reducing the need for echocardiography (2).

Normal values. Efforts have been made to provide normative values for term and preterm infants (6,15). BNP and NT-proBNP concentrations rise at birth in normal healthy infants, level off at 3–4 d, and then fall steadily to stable but low levels in infancy (12,16). In several studies, the method of delivery of the newborn did not influence natriuretic peptide levels (17,18). However, Fortunato *et al.* reported that NT-proBNP was significantly higher in infants delivered by elective cesarean section than in infants delivered after spontaneous birth. In fact, NT-proBNP levels were higher after elective cesarean section than after a cesarean section carried out during active labor, suggesting that the decrease in NT-proBNP levels is probably attributable to labor rather than to the mode of delivery (19).

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After birth, the right ventricle of the neonate is exposed to high pulmonary pressure. The high levels of BNP provide vasodilatation and diuresis, thereby playing a crucial role in hemodynamic adaptation after birth. The fall in pulmonary pressure due to lung expansion, and the onset of diuresis with renal maturation explain the subsequent fall in BNP levels. Variability between different assays and local practices makes it difficult to carry out comparisons among studies involving natriuretic peptides (6). It is recommended that reference values relating to control patients be used in order to make evaluation possible. Although this may be feasible for research studies, it is very impractical in a clinical setting. The changes involved while adapting to extrauterine life further complicate the use of natriuretic peptides as biomarkers. However, an increase in use, an emphasis on the need to generate normal ranges, standardization of methods of measurement, and an increase in commercial availability, possibly in combination with a "point-of-care" facility, will help to overcome these hurdles (20). In future studies that address the clinical use of natriuretic peptides in neonatology, authors should emphasize the changes in natriuretic peptide levels rather than specific levels *per se*. This approach would make the biomarkers more accessible for clinical use.

Cardiac Troponins

For detection of compromised myocardial functioning, many biomarkers have been proposed. Creatine kinase-MB and the cardiac troponins I and T (cTnI and cTnT) are the ones that are most in use. Creatine kinase-MB levels are elevated in newborns after perinatal and neonatal hypoxia–ischemia, but this elevation is not specific enough to be of clinical value (21,22). Troponin is an inhibitory protein complex located on the actin filament in all striated muscles, and consists of three subunits T, C, and I. There is conclusive preclinical and clinical evidence in adult medicine to show that cardiac troponin is reliable in assessing cardiomyocyte injury (23). cTnT and cTnI are used to detect myocardial compromise in the newborn, although the use of cTnI as a marker in newborns is debated. Although some issues have been resolved, further research is necessary before cTnI can be used in clinical practice (24–30).

Normal values. The levels of cTnT in healthy term and preterm infants rise during the first few days of life, peaking on day 3. The reason for this rise is unclear. Several hypotheses have been proposed. It could be that the perinatal period causes minimal myocardial compromise; alternatively, the rise may be a result of perinatal cardiovascular remodeling in the course of adapting to extrauterine life, possibly influenced by respiratory compromise (31,32). No relationship was found between elevated troponin levels and the method of delivery (33,34). Reference values are available, but, especially in the case of cTnI, these are related to the type of assay used (31). As discussed earlier, this is an important limitation for routine clinical use.

Clinical Indications

Growth restriction and antenatal stress. Placental dysfunction leading to growth restriction and preeclampsia is a common and serious complication during pregnancy. Placental dysfunction leads to hypoxemia and nutritional deficiency with inevitable effects on myocytes and cardiac function. Several studies found elevated cord blood BNP or NT-proBNP to be associated with antenatal stress and intrauterine growth restriction. Elevated BNP levels are found in full-term and preterm infants experiencing placental dysfunction *in utero*. Cardiac dysfunction was related to the progression of fetal compromise (35,36). No additional effect of maternal preeclampsia on peripartum cardiac dysfunction was found (37). Prepartum evaluation of the pulsatility in fetal systemic veins significantly correlated to the cardiac secretion of ANP. Fetal myocardial compromise resulted in a distribution of cardiac output toward the left ventricle and a rise in the right-ventricular afterload, explaining the increase in ANP (38). Seong *et al.*, in a study to evaluate the relationship between NT-proBNP levels and the method of delivery, found that infants with low Apgar scores and low umbilical cord blood pH appeared to have increased levels of NT-proBNP (18). Elevation in cardiac troponin levels is much less common in the perinatal period. Tocolytic therapy with β-sympathomimetics was related to an elevation in cTnT in the neonate (39). Elevated levels of cTnT were found in infants born to mothers who experienced preeclampsia, thereby associating maternal disease with neonatal myocyte compromise (40). Makikallio *et al.* found that cTnT levels were elevated in infants born after severe placental insufficiency, although other studies failed to show an increase in cTnI levels during intrauterine growth restriction (35,41).

Elevation in the levels of natriuretic peptides and troponins in the immediate postnatal period needs further study. It remains to be investigated whether these are a result of cardiac compromise or of postnatal adaptation. Furthermore, it remains to be investigated whether these findings add to information that is already clinically available and whether they are clinically discernible from perinatal complications such as perinatal asphyxia. Studies considering the use of cardiac markers in the immediate postnatal period must take into account elevated levels, as they might lead to misinterpretation.

Perinatal asphyxia. There are a limited number of studies of the role of natriuretic peptides in identifying cardiac compromise after perinatal asphyxia. A study by Carbonell *et al.* showed that infants experiencing neonatal encephalopathy maintained elevated ANP levels as compared with healthy infants, suggesting compromise of cardiac function. Plasma NT-proBNP levels were higher in neonates with hypoxia–ischemia-induced encephalopathy complicated by myocardial ischemic injury (42).

We conducted a study to compare the roles of cardiac biomarkers in infants with neonatal encephalopathy receiving hypothermia treatment after a possible ischemic event and those not receiving such treatment. BNP levels decreased significantly during hypothermia treatment and were significantly lower at 48 h after birth and after rewarming in these infants as compared with infants who did not receive hypothermia treatment. This suggests cardiac adaptation during

treatment with hypothermia and possibly a protective effect on cardiac function (43).

Troponins appear in the blood 2–4 h after perinatal asphyxia and consequent myocardial compromise, and remain detectable for up to 21 d (44). Much research has been done to evaluate the predictive value of cord blood cardiac troponins (1,22). Möller *et al.* showed that cTnT had a high positive predictive value in the postnatal diagnosis of perinatal asphyxia (45). Szymankiewicz *et al.* determined cTnT levels at 12 and 24 h after birth in infants who had experienced asphyxia and those who had not. The authors of that study found cTnT to be the most useful tool for assessing myocardial injury. In their study, echocardiography appeared to be of less value, apart from its help in the frequent diagnosis of tricuspid insufficiency, reported earlier as being more common in newborns who had experienced asphyxia (46). However, Costa *et al.* did report such a relationship between higher cTnT levels and echocardiographic signs of myocardial compromise in infants who had experienced asphyxia. In newborns with echocardiographic signs of myocardial compromise (diminished left-ventricular output and stroke volume), cTnT levels were found to be more elevated (47). The effect of hypothermia on cardiac function has not been established, although the results of studies investigating the role of troponin in animals suggest that hypothermia treatment may have a protective effect (48). In a study by our group, no difference was found between cTnI levels in infants treated with hypothermia and those not treated with hypothermia (43). This was also the finding of Shastri *et al.*, who compared cTnI levels at 48 h after birth. Possibly this finding is related to the relative differences in the timing of the hypoxic incident in the prenatal period (49).

The levels of cTnT and cTnI are related to the severity of perinatal hypoxia–ischemia (49,50). In adult medicine, cardiac troponin levels are used to predict adverse outcomes in patients admitted to intensive care, even in those not presenting with myocardial injury. However, unlike in the adult population, circulatory failure is not a common cause of adverse outcomes in newborn infants (51,52). Boo *et al.* did find that cTnT levels were significantly higher in infants who did not go on to survive, thereby suggesting that cardiac troponins can be used to predict short-term outcomes as well as long-term prognosis (53). Kanik *et al.* reported that survivors of neonatal encephalopathy had significantly lower cTnI levels at days 1 and 3 (54).

Cardiac troponins are used as markers of myocardial ischemia-induced injury. There is only limited research evidence identifying these biomarkers as indicators of clinically relevant postnatal circulatory compromise. It is possible that, even when biochemical signs of myocardial damage are present, the damage in most infants is not severe enough to be of clinical significance. Troponin levels are used as prognosticators after perinatal asphyxia; however, this remains only a surrogate marker of cerebral damage.

Patent ductus arteriosus. Given the pathophysiologic basis of a hemodynamically significant patent ductus arteriosus (hsPDA), natriuretic peptides, with their physiologic role, are candidates to be used as biomarkers in the identification of and treatment of this condition. hsPDA causes left-atrial and, subsequently, left-ventricular overload, leading to increased production of BNP and NT-proBNP. Several studies have proposed that natriuretic peptides may have a role as an additional diagnostic tool in hsPDA identification, with various cut-off values (**Table 1**) (5,20,55–60). Associations were found between natriuretic peptide levels and each of the following: ductal size, magnitude of shunting, and left-atrial to aorta root ratio (5,59). BNP levels were found to be correlated with hemodynamic alterations caused by ductal size, constriction, and dilatation (13,20,60). However, because of the high degree of variability, BNP measurements are not clinically useful for predicting changes in shunt magnitude (61). It would be of greater interest if natriuretic peptide levels could be useful in predicting responsiveness to treatment. In a study performed by Hammerman *et al.*, NT-proBNP was not sensitive enough to predict ductal responsiveness to therapy. The difference between estimates of successful and unsuccessful closure was too small to be clinically relevant (62). Hsu *et al.* used BNP to predict the responsiveness of hsPDA to indomethacine. It was found that elevated levels of BNP (>522 pmol/l) predicted nonresponsiveness with high sensitivity (88%) and high specificity (87%) (63). After

Table 1. Natriuretic peptides in identification of hemodynamically significant PDA (hsPDA)

Gestational age	Included/hsPDA (*n*)	Proposed cut-off value	Day	Sensitivity	Specificity	Reference
<28 Wk	67/24	159 pmol/l (BNP)	2	83%	86%	5
<36 Wk	20/20	88 pmol/l (BNP)	>2	—[a]	—[a]	20
<34 Wk	29/14	20 pmol/l (BNP)	7 (median)	93%	73%	55
<34 Wk	66/23	320 pmol/l (BNP)	3	100%	95%	59
<34 Wk	49/18	1,345 pmol/l (NT-proBNP)	3	100%	95%	54
<33 Wk	35/12	1,202 pmol/l (NT-proBNP)	2	100%	91%	56
<33 Wk	56/20	2,850 pmol/l (NT-proBNP)	3	90%	89%	57
<30 Wk	48/25	5,000 pmol/l (NT-proBNP)	3	70%	87%	58

BNP, B-type natriuretic peptide; hsPDA, hemodynamically significant patent ductus arteriosus; NT-proBNP, N-terminal fragment of BNP.

[a]In the absence of control patients, no sensitivity and specificity could be calculated for hsPDA.

successful treatment of an hsPDA, either noninvasively or surgically, NT-proBNP and BNP levels decrease as expected. The unresolved question is whether, during treatment with indomethacine, there is any additional value in using natriuretic peptides as markers over and above repeated echocardiographic evaluation. Attridge *et al.* found that, when BNP was used as an indicator, the number of indomethacine doses during the first course of treatment was reduced; however, the total number of indomethacine doses remained unaltered (3). Two studies by El-Khuffash *et al.* evaluated NT-proBNP levels in preterm infants with hsPDA as a predictor of long-term outcome. In these two studies, NT-proBNP and cTnT were used as markers to identify infants with hsPDA at risk of death before discharge and those at risk of severe intraventricular hemorrhage (grade III/IV). The infants with an hsPDA at 48 h after birth who subsequently died or developed severe intraventricular hemorrhage were compared with those who did not die or develop severe intraventricular hemorrhage. Although no differences were found in echocardiographic hsPDA characteristics, the levels of cTnT and NT-proBNP were significantly higher in infants at risk for an adverse outcome (64). In a follow-up study the same authors showed that NT-proBNP, in conjunction with cTnT and an hsPDA scoring system based on six echocardiographic criteria related to the hemodynamic significance of the hsPDA at 48 h, could be used for the identification of infants at greatest risk of poor neurodevelopmental outcome (4).

An hsPDA can lead to stealing of coronary arterial blood flow, potentially leading to ischemia of the myocardium and elevation of cardiac troponin levels (65). Studies investigating the value of troponins as indicators of myocardial compromise showed no equivocal results (66,67).It is therefore unlikely that myocardial compromise as signaled by troponin levels is of clinical significance (4).

The identification of an hsPDA in the clinical setting can be difficult, often calling for repeated echocardiographic measurements. Biological markers may facilitate the diagnosis. Elevated levels of BNP and NT-proBNP at day 3 can be used to identify an hsPDA. However, to validate the role of natriuretic peptides as markers in hsPDA treatment, a large prospective study is needed. Further investigation is required to determine whether biomarker-guided treatment protocols will improve short- and long-term outcomes for hsPDA as compared with current diagnostic protocols.

Persistent pulmonary hypertension. Persistent pulmonary hypertension of the neonate (PPHN) is a severe disease seen mostly in term infants. Because PPHN is usually associated with conditions affecting pulmonary function (sepsis, meconium aspiration, asphyxia), it is often difficult to arrive at an early diagnosis of PPHN, especially when echocardiographic evaluation is not available. BNP is used as a biomarker in the diagnosis and management of pulmonary hypertension (PH) in pediatric patients, and has been shown to have prognostic value. It has been suggested that, in PH, changes in BNP levels are of more importance than elevated levels alone (68). BNP levels have also been used to identify PPHN; Reynolds *et al.* showed that an initial BNP level of >159 pmol/l has a specificity of 90% and a sensitivity of 100% in predicting PPHN. They reported (as our group also did in its study) a strong relationship between BNP and echocardiographic signs of increased pulmonary vascular resistance (69,70). With BNP and NT-proBNP being suggested as screening biomarkers, it is important to realize that congenital heart defects can also present themselves with PPHN and elevated BNP levels (71). A finding of BNP elevation should therefore prompt additional cardiologic evaluation in high-risk infants. Apart from identifying elevated pulmonary vascular resistance, BNP levels can be used to monitor the response to treatment. We investigated the use of BNP as a biomarker in the treatment of PPHN. In infants treated with nitric oxide, a decrease in BNP was found after initiation of treatment. However, where there is a sharp increase during cessation or weaning of nitric oxide, one should suspect a rebound; our study showed that an increase in BNP levels preceded the onset of clinical signs of a "rebound" PPHN (70).

The studies described here suggest a role for natriuretic peptides in the diagnosis and treatment of PPHN; however, the role of troponins is less clear. Torbicki *et al.* studied cTnT as an independent marker of increased mortality risk in adult patients with chronic precapillary PH. The results suggested a relationship between right-ventricular dysfunction and cTnI levels. However, similar studies have not been carried out in infants (72). Validation is needed before these cardiac biomarkers can be used in clinical practice. Prospective studies are needed to determine whether natriuretic peptides can be used to predict outcomes in patients with PPHN.

Bronchopulmonary dysplasia. In "new bronchopulmonary dysplasia" (BPD), pulmonary arterial hypertension (PAH) occurs in infants who are more severely affected (73). Given that a reduction in arterial development coincides with the occurrence of BPD and PAH, it can be assumed that there is a reduction in the development of the pulmonary arteries in these infants (74). Right-ventricular changes occur as a result of changes in pulmonary structure and function. In addition, systemic hypertension and the use of steroids to treat BPD may lead to left-ventricular dysfunction, further exacerbating PAH (75). Echocardiography and cardiac catheterization are labor- and time-intensive, and their interpretation depends on the experience of the investigator. There is therefore a need for a reliable and easy screening method. Ventricular dysfunction as a result of PAH in a patient with BPD may lead to increases in the levels of BNP or NT-proBNP, thereby making them possible screening tools under these conditions. An observational study found that NT-proBNP levels showed elevated values corresponding to the severity of BPD; however, no evidence of ventricular dysfunction or pulmonary hypertension was found in the infants studied (76). To our knowledge, there have been no subsequent studies to investigate the use of natriuretic peptides as biomarkers in BPD. Given the underlying pathophysiologic principle and

Table 2. Conditions affecting newborn infants and the effect on cardiac biomarkers troponin (cTnI and cTnT) and BNP (and NT-proBNP)

	BNP		Troponin		
Condition	Premature	Term	Premature	Term	Clinical relevance
IRDS	⇑		⇑		Not known
PDA	⇑		⇑		Diagnosis and possibly therapy
Sepsis/NEC	⇑	=/⇑	=/⇑	=/⇑	Not known
PPHN	⇑	⇑			Diagnosis and possibly therapy
Asphyxia		⇑	⇑	⇑	Long-term outcome
Growth retardation	⇑		=/⇑		Not known
Preeclampsia	=/⇑		⇑		Not known
BPD	⇑	⇑			Not known

⇑, elevated levels are found; =/⇑, unequivocal reports; blank, no evidence available; cTnI, cardiac troponin I; cTnT, cardiac troponin T; BNP, B-type natriuretic peptide; BPD, bronchopulmonary dysplasia; IRDS, infant respiratory distress syndrome; NEC, necrotizing enterocolitis; NT-proBNP, N-terminal fragment of BNP; PDA, patent ductus arteriosus; PPHN persistent pulmonary hypertension of the newborn.

the importance of having an easy screening tool for PAH in BPD, further research is needed.

Sepsis. Investigations have shown that BNP levels are elevated in adult patients with severe sepsis and shock. The levels appeared to be comparable to those found in adults with acute heart failure (77). How inflammation and myocardial dysfunction during sepsis are related to BNP levels has yet to be determined, although cardiac dysfunction is common in patients with severe sepsis or septic shock. A study by Domico *et al.* in children ranging in age from 2 wk to 18 y showed elevated BNP levels in septic shock. BNP levels at 12 h after birth correlated well with disease severity and myocardial dysfunction (78). Clark *et al.* found that the cTnT levels were significantly higher in sick infants than in healthy ones. The use of inotropic support and oxygen requirement were independently associated with higher cTnT levels (79).

The validation of cardiac biological markers for sepsis in newborn infants is a complex task, especially in preterm infants in whom different causes for impaired cardiac function often coincide. Currently, there is no evidence to promote the routine use of cardiac biomarkers for this indication.

Future directions. Research on cardiac biomarkers and their clinical implications has intensified significantly in the past few years. Several possible clinical implications have been suggested, and these are summarized in **Table 2**. A first step before cardiac biomarkers can be introduced into routine care is the establishment of unambiguous normal ranges. Although efforts in this direction have been made, the variability in the assays used and in local conditions make comparison of study results difficult. However, as earlier stated, the increase in the use of cardiac biomarkers, the emphasis on the need to generate normal ranges, standardization of methods, and increased commercial availability, possibly in combination with a "point-of-care" facility, should help to overcome this problem. Studies have suggested that natriuretic peptides can be used as biomarkers in the identification and treatment of hsPDA and PPHN, and that troponins can find similar use in the identification of cardiac compromise in infants after perinatal asphyxia and neonatal encephalopathy. Another important step should be to validate these biomarkers in the populations of interest, where current evidence is most promising. This will determine whether therapy guided by these biomarkers will improve treatment outcomes.

CONCLUSIONS

The levels of natriuretic peptides and troponins are currently used in clinical practice to assess changes in cardiac function and cardiomyocyte compromise. This review shows that perinatal adaptations as well as diseases commonly affecting newborn infants influence the levels of these biomarkers. Currently there is insufficient evidence to promote the routine clinical use of these cardiac biomarkers in newborns. We therefore conclude that, although natriuretic peptides and cardiac troponins provide the possibility to better evaluate cardiocirculatory compromise in infants, the establishment of reliable normal ranges in the early newborn period and the validation of these biomarkers in a population of interest are the necessary first steps before routine clinical use can be advocated.

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