Cardiac Biomarkers in Neonatology

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Cardiale biomarkers in de Neonatologie

(met een samenvatting in het Nederlands)

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ἄνδρα μοι ἔννεπε, μοῦσα, πολύτροπον, ὃς μάλα πολλὰ
πλάγχθη, ἐπεὶ Τροίης ἱερὸν πτολίεθρον ἔπερσεν·
(Homerus, Odyssee)

Voor mijn vader en moeder

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Chapter 1

General introduction

BIOMARKERS

Over 2000 years ago Indian physicians reported a sweet urine disease called "madhumeha" as a hereditary disease in Hindu patients. Ants and dogs were used to identify the sweetness of the urine and to differentiate between diabetes mellitus and diabetes insipidus. (1,2) The search for reliable biomarkers to identify disease is not new.

A biomarker is defined as a characteristic that can be measured objectively and can be evaluated as an indicator of normal biological processes, pathologic processes, or pharmacologic responses to therapeutic interventions. (3) Biomarkers can help in understanding disease processes; aid in (early) diagnosis and treatment; and assist in determining a prognosis. Apart from high sensitivity and specificity, an ideal biomarker should be cheap, easy to measure and to interpret, and would preferably be obtained through a non-invasive or minimally invasive technique. Plasma, urine, cerebrospinal fluid, and cells all carry an abundance of possible biomarkers. Presently, most used biomarkers are biological products, but genetic markers and even EEG, ultrasound, or MRI can be considered as biomarkers. (3,4)

CARDIAC BIOMARKERS

Cardiac biomarkers have been introduced in routine clinical practice in adult patients. More than 30 years ago the diagnosis of ischemic heart disease was made based on "classic biomarkers", clinical history, ECG, and changes in serum enzymes. (5) The MB isoenzyme of creatine kinase (CK-MB) was used as a marker of cardiac ischemia. (6) However, due to relatively low sensitivity and specificity, it has fallen out of favor. (7) During the past two decades the myofibrillar proteins cardiac troponin T and I (cTnT and cTnI), have emerged as sensitive and specific markers of cardiomyocyte injury. Introduction into clinical practice has improved diagnosis, risk stratification, and care for patients with acute coronary syndromes. (7,8) Troponin is currently regarded as the gold standard in diagnosing coronary ischemia. (9) Along with the cardiac troponins (cTn), the natriuretic peptides, B-type natriuretic peptide (BNP) and amino-terminal proBNP (NT-proBNP) have emerged as important markers for diagnosis and management of cardiac disease. (7) They are routinely used to diagnose heart failure. (10) The combination of natriuretic peptides and cTn provide clinicians with a powerful tool to differentiate between myocardial infarction, unstable angina pectoris, and non-cardiac causes of chest pain and respiratory problems. (11) Furthermore, they can be used to predict (adverse) outcome. (12,13)

PERINATAL TRANSITION

The perinatal period is a cardiovascular challenge for an infant. The rapid transition and subsequent adaption to the extrauterine life leave little room for error. Preterm birth, hypoxia, and perinatal disease can have a significant impact on this adaptation process. (14) Cardiocirculatory compromise, often found in newborn infants, can further affect the perinatal transition process. (15,16)

A wide variety of assessment to identify circulatory failure is available to the clinician. Various clinical and biochemical parameters can be used to identify the infant at risk. However, signs are often interpreted subjectively, and predictive values are limited. (17,18) Novel approaches with targeted or functional echocardiography and near infrared spectroscopy are useful in the hemodynamic monitoring of newborn infants and are making their way into standard care. (19-23)

In recent years, cardiac biomarkers have been introduced into Neonatology. Considerable research has been conducted, especially in the identification of cardiocirculatory compromise after perinatal asphyxia and in the diagnosis of a hemodynamically significant patent ductus arteriosus. (24-28) However, a clear role for cardiac biomarkers in routine clinical practice has not been established.

The aim of this thesis was to investigate the clinical role of cardiac biomarkers in neonatal disease and to study their relationship to disease burden and short- and long-term (neurodevelopmental) outcome.

OUTLINE OF THE THESIS

Chapter 2 provides a review of the current literature regarding cardiac biomarkers in neonatal disease and is the starting point for further investigation.

The term infant

Chapter 3 investigates hemodynamic adaptation in infants after perinatal asphyxia and the possible role for cardiac biomarkers. In addition, the influence of hypothermia treatment on these biomarkers is investigated.

Chapter 4 continues on the clinical value of cardiac biomarkers after perinatal asphyxia and investigates the relation to short- and long-term (neurodevelopmental) outcome.

In **Chapter 5**, BNP in the treatment of persistent pulmonary hypertension of the newborn is explored, especially concerning the occurrence of a rebound after weaning from treatment.

The preterm infant

Chapter 6 illustrates the role of the placenta and placental pathologic lesions in preterm infants with suspected cardiovascular compromise.

Chapter 7 further elucidates these findings by exploring BNP as a marker of fetal cardiocirculatory adaptation. Moreover, a connection with antenatal Doppler ultrasound measurements and perinatal compromise is investigated.

Finally, in **Chapter 8** the results and conclusions of the presented studies are summarized and discussed. Suggestions are made for clinical practice implications and the direction of future research.

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Chapter 2

Use of cardiac biomarkers in Neonatology

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ABSTRACT

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 peptides are elevated during atrial strain (atrial natriuretic peptide (ANP)) or ventricular strain (B-type natriuretic peptide (BNP)). They 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 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.

INTRODUCTION

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) These markers are used in clinical trials to provide insight into the extent of compromise and 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 outcome 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 an amino-acid ring-shaped structure, 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 a massive diuresis and natriuresis occurred. (7) This observation started research into the endocrine role of 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 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 for 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 cardiac biomarker. (11,12) When their application in adult medicine 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 primarily excreted by the kidney, its use depends on renal function. (14) BNP and NT-proBNP are good indicators of ventricular function and can be used in the diagnosis of diseases influencing this function, 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 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 caesarean section than in infants after spontaneous birth. In fact, NT-proBNP levels were higher after elective caesarean section than after a caesarian section carried out after active labor, suggesting that the decrease in NT-proBNP levels is probably attributable to labor rather than to the mode of delivery. (19) 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 the hemodynamic adaptation after birth. The fall of 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 ranges or control patients are 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 extra-uterine life further complicate the use of natriuretic peptides as biomarkers. However, an increase in use, an emphasis on the need to generate reference 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 (CK-MB) and the cardiac troponins I and T (cTnI and cTnT) are the ones that are most in use. CK-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 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 the result of perinatal cardiovascular remodeling in course of adaptation to extra uterine 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 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 ANP increase. (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) Elevated cardiac troponin levels are much less common in the perinatal period. Tocolytic therapy with ß-sympathomimetics was related to an elevation of cTnT in the neonate. (39) Elevated levels of cTnT were found in infants born to mothers who experienced preeclampsia, thereby associating maternal disease to 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 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 *el al.* showed that infants experiencing neonatal encephalopathy maintained elevated ANP levels when compared with healthy infants, suggesting compromise of cardiac function. (42) Plasma NT-proBNP levels were higher in neonates with hypoxic-ischemic induced encephalopathy complicated by myocardial ischemic injury. (43)

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. (44)

Troponins appear in the blood 2-4 h after perinatal asphyxia and consequent myocardial compromise, and remain detectable for up to 21 d. (45) 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. (46) 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 diagnosing tricuspid insufficiency, reported earlier as being more common in newborns who had experienced asphyxia. (47) 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. (48) 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. (49) In study by our group, no difference was found between cTnI levels in infants treated with hypothermia and those not treated with hypothermia infants. (44) This was also the finding of Shastri *et al.*, who compared cTnI levels at 48 h after birth. Possibly this is related to the relative differences in timing of the hypoxic incident in the prenatal period. (50)

The levels of cTnT and cTnI are related to the severity of perinatal hypoxia-ischemia. (50,51) In adult medicine, cardiac troponins are used to predict adverse outcome in patients admitted to the 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 outcome in newborn infants. (52,53) Boo *et al.* did find that cTnT levels were significantly higher in infants that did not go on to survive, thereby suggesting that cardiac troponins can be used to predict short-term outcome as well as and long-term prognosis. (54) Kanik *et al.* reported that survivors of neonatal encephalopathy had significant lower cTnI levels at days 1 and 3. (55)

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. Troponins are used as prognosticator after perinatal asphyxia; however, this remains 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 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 for the hsPDA identification, with different cut-off values. (**Table 1**) (5,20,56-61) Associations were found between natriuretic peptides and ductal size, magnitude of shunting, and left-atrial to aorta root ratio. (5,60) BNP levels were found to be correlated with hemodynamic alterations caused by ductal size, constriction, and dilatation. (13,20,61) However, because the high degree of variability, BNP measurements are not clinically useful for predicting changes in shunt magnitude. (62) 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.

Table 1. Natriuretic peptides in hemodynamically significant PDA identification

Gestational age	Included/hsPDA (n)	Proposed cut-off value	Day	Sensitivity	Specificity	Reference
<28 weeks	67/24	159 pmol/l (BNP)	2	83%	86%	(5)
<36 weeks	20/20	88 pmol/l (BNP)	>2	- [a]	- [a]	(20)
<34 weeks	29/14	20 pmol/l (BNP)	7 (median)	93%	73%	(57)
<34 weeks	66/23	320 pmol/l (BNP)	3	100%	95%	(61)
<34 weeks	49/18	1345 pmol/l (NT-proBNP)	3	100%	95%	(56)
<33 weeks	35/12	1202 pmol/l (NT-proBNP)	2	100%	91%	(58)
<33 weeks	56/20	2850 pmol/l (NT-proBNP)	3	90%	89%	(59)
<30 weeks	48/25	5000 pmol/l (NT-proBNP)	3	70%	87%	(60)

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

The difference between estimates of successful and unsuccessful closure was too small to be clinically relevant. (63) 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%). (64) After successful treatment of an hsPDA, either noninvasively or surgically, NT-proBNP and BNP levels decrease as expected. The unsolved 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 intra-ventricular hemorrhage (grade III/IV). The infants with an hsPDA at 48 h after birth who subsequently died or developed severe intra-ventricular hemorrhage were compared with those who did not die or develop severe intra-ventricular hemorrhage. Although no differences were found in echocardiographic hsPDA characteristics, the levels of cTnT and NT-proBNP were significantly higher in those infants at risk for an adverse outcome. (65) 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 hsPDA hemodynamic significance of the hsPDA at 48h, 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. (66) Studies

investigating the value of troponins as indicators of myocardial compromise showed no equivocal results. (67,68) It is therefore unlikely that the 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 a 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 outcome 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 changes in BNP levels are of more importance than elevated levels alone. (69) 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. (70,71) With BNP and NT-proBNP being suggested as a screening biomarkers, it is important to realize that congenital heart defects can also present themselves with PPHN and elevated BNP levels. (72) A finding of BNP elevation should therefore prompt additional cardiologic evaluation in high risk infants. Apart from identifying elevated pulmonary vascular resistance, BNP 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 (NO) a decrease in BNP was found after initiation of treatment. However, where there is a sharp increase during cessation or weaning of NO, one should suspect a rebound; our study showed that an increase in BNP levels preceded the onset of clinical signs of a "rebound" PPHN. (71)

The studies described here suggest a role for natriuretic peptides in the diagnosis and treatment of PPHN; however, the role of troponin is less clear. Torbicki *et al.* studied cTnT as an independent marker of increased mortality risk in adult patients with chronic pre-capillary pulmonary hypertension. The results suggested a relationship between right-ventricular dysfunction and cTnI levels. However, similar studies have not been carried out in infants. (73) Validation is needed before these cardiac

biomarkers can be use in clinical practice. Prospective studies are needed to determine whether natriuretic peptides can be used to predict outcome of patients with PPHN.

Bronchopulmonary dysplasia

In "new bronchopulmonary dysplasia" (BPD), pulmonary arterial hypertension (PAH) occurs in infants who are more severely affected. (74) 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. (75) 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. (76) 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 levels of BNP or NT-proBNP levels, 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. (77) To our knowledge, there have been no further studies to investigate the use of natriuretic peptides as biomarkers in BPD. Given the underlying pathophysiologic principle and the importance of having an easy screening tool for PAH in BPD, further investigation 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. (78) How inflammation and myocardial dysfunction during sepsis are related to BNP 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 aging from 2 wk to 18 y showed elevated BNP levels in septic shock. BNP levels at 12 h correlated well with disease severity and myocardial dysfunction. (79) 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. (80)

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 pas 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 of 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.

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

	BNP		Troponin		Clinical relevance
	Premature	Term	Premature	Term	
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, 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

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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Chapter 3

Cardiac biomarkers as indicators of hemodynamic adaptation during postasphyxial hypothermia treatment

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ABSTRACT

Background Little is known about the effects of hypothermia on the cardiovascular system in term newborns with neonatal encephalopathy.

Objectives To evaluate whether mild hypothermia for neonatal encephalopathy is cardioprotective as indicated by the cardiac biomarkers cardiac Troponin I (cTnI) and B-type natriuretic peptide (BNP).

Methods This was an observational cohort study of infants treated for perinatal asphyxia. In infants, mild total body hypothermia treatment of 33.5°C during 72 h was initiated (n=20). Samples of cTnI and BNP were collected before start of hypothermia, at 24 and 48 h after birth, and after rewarming (84 h). BNP and cTnI values were then compared with BNP and cTnI values of asphyxiated infants not treated with hypothermia (n=28).

Results No differences were found between the groups in clinical patient characteristics or inotropic support. The hypothermia-treated patients seemed to be clinically more affected (5 minute Apgar score, $p<0.05$; umbilical artery pH, $p=0.08$), but showed similar encephalopathy scores. Significantly lower values for BNP were found in hypothermia- compared to nonhypothermia-treated infants at 48 h and at normothermia after rewarming [144 pmol/L (95 – 286) vs. 75 pmol/L (45 – 143), 182 pmol/L (73 – 341) vs. 43 pmol/L (24 – 163)]. No differences were found for cTnI concentrations between both groups.

Conclusions The raised, but similar, cTnI values between hypothermia- and nonhypothermia- treated infants indicate similar myocardial damage in both groups. The lower BNP levels during hypothermia treatment suggest that hypothermia after perinatal asphyxia exerts a beneficial effect on cardiac function.

INTRODUCTION

Perinatal asphyxia is associated with high mortality and morbidity. Recent studies have shown improved outcome after mild hypothermia treatment for neonatal encephalopathy. (1) This makes hypothermia the only established treatment for neonatal encephalopathy at this moment. B-type natriuretic peptide (BNP) is an endogenous peptide hormone secreted by cardiac ventricles in response to increased wall stress and ventricular filling pressure. It causes vasodilatation and has a diuretic and natriuretic effect. BNP is used in infants to identify significant patent ductus arteriosus and to diagnose cardiovascular disease, such as persistent pulmonary hypertension of the newborn (PPHN). (2-4) BNP concentrations in plasma correspond well with echocardiographic findings of ventricular strain in children with heart failure. (5) Troponin is an inhibitory protein complex located on the actin filament in all striated muscle and consists of three subunits T, C, and I. Previous studies have shown elevated levels of cardiac Troponin-I (cTnI) in asphyxiated neonates. cTnI is thought to be an indicator of perinatal asphyxia-induced myocyte damage. (6) cTnI correlates strongly with clinical grade of encephalopathy. (7) Hypothermia is linked to reduced cardiac output, peripheral vasoconstriction, sinus bradycardia, cardiac arrhythmias, hypotension, platelet dysfunction, and increased blood viscosity. (8-11) A study in newborn pigs has shown a cardioprotective effect of hypothermia; whether this is also true in human infants is not clear. (12) Our study was done to investigate whether mild hypothermia exerts a beneficial effect on the heart after perinatal asphyxia using the cardiac biomarkers cTnI and BNP.

METHODS

Study Design

This study was carried out in the neonatal intensive care unit at the University Medical Centre Utrecht from December 2006 to October 2008. The study was started before the introduction of hypothermia to assess the cardiac function of infants after perinatal asphyxia and was continued when hypothermia treatment was introduced in February 2008. Those infants admitted before the introduction of hypothermia as a treatment served as nonhypothermia-treated group. Patients were eligible if they were born at or after 36 completed weeks of gestation. Criteria related to the identification of perinatal asphyxia and encephalopathy are shown in **table 1**. Hypothermia treatment was started within 6 h after birth. Infants with major congenital malformations or known chromosomal abnormalities were excluded from the study. Parental consent was obtained in all cases. This study was conducted following the guidelines of the local medical ethical committee.

Table 1. Study entry criteria

Gestational Age ≥ 36 weeks
AND
Perinatal asphyxia
• Apgar 5 min ≤5
or
• Resuscitation/ ventilation during 10 minutes after birth
or
• Umbilical artery pH <7.0 and "Base deficit" >16 mmol/L
or
• lactate >10.0 mmol/L <1 hour after birth
AND
Encephalopathy
• Clinical: Thompson score >7 between 1 and 3 hours after birth or
• aEEG: normal background with some seizure activity, moderately abnormal activity, suppressed acitivity or continuous seizure acitivity.
AND
Admission <6 hours after birth

aEEG – Amplitude-integrated electroencephalography

Patient characteristics and clinical data were collected prospectively. To assess the intensity of blood pressure support, a blood pressure support scoring system was used, depending on the intensity of the treatment necessary (score 0: no treatment; score 1: volume expansion and/or dopamine ≤5 µg/ kg/min; score 2: dopamine >5 ≤10 µg/kg/min; score 3: dopamine >10 µg/kg/min or dopamine + dobutamine ≤10 µg/kg/min; score 4: dopamine + dobutamine >10 µg/kg/min; score 5: additional adrenaline and/or corticosteroids). (13) The blood pressure score and heart rate was registered at the time of sampling for BNP and cTnI.

Blood was collected at admission (< 6 h), at 24 and 48 h after birth and at 84 h (normothermia).

Echocardiography was performed if cardiac abnormalities were suspected or if there was a clinical indication, such as suspected persistent pulmonary hypertension, therapy resistant hypotension, cardiac murmur, or persistent low oxygen saturations. PPHN was defined as hypoxemia with echocardiographic findings of elevated pulmonary artery pressure and/or right to left shunting through a patent foramen ovale or the patent ductus arteriosus. Clinical treatment was left to the discretion of the attending neonatologist, following standardized protocols.

Hypothermia patients were treated with total body cooling according to our hypothermia protocol using the Criticool™ device (MTRE, Milton Keynes, UK). Infants were wrapped into a body shaped 3 dimensional garment upon admission to our NICU. If infants were transported from another hospital, there was no active rewarming during transport. Mild hypothermia was started within 6 h after birth and maintained for 72 h. Patients were kept at 33.5 °C; the temperature was servo-controlled with

a thermometer probe rectally placed. When a period of 72 h was completed, rectal temperature was allowed to rise 0.5 °C/h until normothermia (36.5 °C) was achieved. This temperature (36.5 °C) was maintained during 24 h. After this period the garment was removed and normothermia was achieved according to standard infant protocol until discharge.

BNP and cTnI measurements

Blood samples were collected from an arterial catheter or by capillary samples (heel stick) into a standard collection vial with ethyl-enediamine tetra acetic acid for BNP and lithium heparin (Capijet®, VWR, West Chester, PA, USA) for cTnI. BNP and cTnI were analyzed directly after collection on a DxI 800 immunochemistry system (Beckman Coulter Diagnostics, Brea, CA, USA). The lower limit of detection for this assay is 5 pmol/L for BNP and 0.01 µg/L for cTnI. Maintenance and quality performance of the instrument were followed according to company instructions. Blood samples were simultaneously collected with other routine bloodwork.

Statistics

Descriptive data are presented as medians with absolute range; BNP and cTnI levels are presented as medians with interquartile range. There were no missing data at any of the mentioned time points. Comparison of the groups was done by nonparametric statistical analyses using the Mann-Whitney U test. Spearman's rank-sum test was used to correlate BNP to associated variables. The statistical analysis took multiple comparisons and repeated measures into account. The distribution of BNP and cTnI values was illustrated by box-and-whisker plots. A p value of <0.05 was considered significant. For the statistical analysis Predictive Analytics SoftWare statistics 17.0 (IBM® SPSS® Armonk, New York) was used.

RESULTS

Patient characteristics

In total, 48 infants were included in the study, 28 in the nonhypothermia- and 20 in the hypothermia-treated group. Patients' characteristics of both groups are shown in **table 2**. There were no significant differences between the groups in birth weight, gestational age, gender, or race. There were no significant differences in asphyxia-related criteria such as serum lactate levels, at admission between the groups. There were no significant differences between the groups in the total amount of resuscitation needed, inotropic support, or occurrence of PPHN. In hypothermia-treated infants, a lower Apgar score at 5 minutes [median 5 (range 0 – 8) vs. 3 (0 – 7); $p<0.05$]

Table 2. Characteristics of patients treated with hypothermia and without hypothermia

	Normothermia (n=28)	Hypothermia (n=20)	p
Birth weight (g)	3333 (2030 - 4360)	3902 (3010 - 5000)	0.10
Gestational age (wk)	40.5 (36.4 - 42.3)	40.2 (39 - 41.9)	0.54
Birth			
• Meconium-stained amniotic fluid (n)	14	11	1.00
• Caesarean section (n)	17	10	0.99
Hospital birth (%)	97	85	0.74
Male (%)	52	75	0.85
Apgar score at 5 min	5 (0 - 8)	3 (0 - 7)	0.03
Umbilical artery pH	6.99 (6.75 - 7.21)	6.82 (6.64 - 7.25)	0.08
Base deficit (mmol/l)	15.4 (7 - 25)	19.4 (10 - 24)	0.09
Lactate (mmol/l)	10.8 (2 - 33.7)	14.9 (4.8 - 28.7)	0.43
Mechanical ventilation (%)	79	86	0.74
PPHN*/ Nitric oxide (n)	6	5	0.96
Inotropic support score	2.6	2.8	0.36
Encephalopathy			
• Sarnat	2	2	0.19
• Thompson	10	11.5	0.09
Seizures (%)	52	75%	0.11
Mortality (n)	8	9	0.25

* PPHN – Persistent pulmonary hypertension of the newborn

and a trend towards a lower umbilical artery pH [median 6.99 (range 6.75 – 7.21) vs. 6.82 (6.64 – 7.24); p=0.08] was found. The level of encephalopathy on admission was similar between the groups. The median clinical encephalopathy grade (Sarnat classification) was 2 in both groups (range: 1 – 3); the median Thompson score was not significantly different between the hypothermia- and nonhypothermia-treated group [median 11.5 (8 – 19) vs. 10 (range 4 – 19); p=0.10]. (14) There were more infants with seizures in the hypothermia-treated group (52% vs. 75%; p=0.11), although this difference was not statistically significant. Mortality was similar between the two groups.

Pattern of BNP

The study showed increased BNP levels in both groups, nonhypothermia-treated vs. hypothermia-treated, after perinatal asphyxia as compared to normal reference range for term infants described in the literature (day 0-1, 67 pmol/L; day 4-6, 14 pmol/L) .(15) At admission and at 24 h after birth no significant differences were found between the nonhypothermia- versus the hypothermia-treated group [200 pmol/L (61 – 1072) vs. 285 pmol/L (24 – 908), 186 pmol/L (112 – 415) vs. 230 pmol/L (77 – 1022),

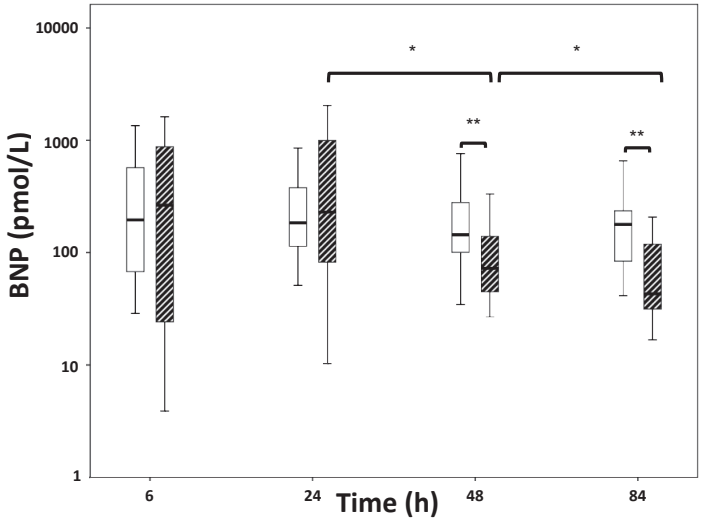

Figure 1. Box-whisker plot comparing hypothermia treated patients (dash) and non-hypothermia treated patients (blank) for BNP serum levels in a logaritmic scale at specified time points. Horizontal lines indicate median values, boxes 25th-75th percentile range, outer whiskers 5th-95th percentile range, *p<0.05 decrease in hypothermia treated infants at 24 h vs. 48 h and 48 h vs. 84 h, **p<0.05 hypothermia vs. non hypothermia treated infants at 48h and 84h.

respectively]. We found a significantly lower BNP level in the hypothermia-treated group as compared to the nonhypothermia-treated group at 48 h after birth and after rewarming [144 pmol/L (95 – 286) vs. 75 pmol/L (45 – 143), 182 pmol/L (73 – 341) vs. 43 pmol/L (24 – 163) , p<0.05, respectively]. Furthermore, in the hypothermia-treated group we found a significant (p<0.05) decrease in BNP levels after 24 h. The nonhypothermia-treated group showed relatively steady median BNP levels (**figure 1**). No significant correlations were found between BNP and possible confounders, such as changes in heart rate, PPHN, epinephrine use at resuscitation, or hypotension treatment.

Pattern of cTnI

cTnI levels in both groups were elevated compared to normal reference range for term infants described in the literature (day 0, 0.3 µg/L; day 3, 0.1 µg/L), as is expected in patients after perinatal asphyxia. (16) As shown in **figure 2**, we did not find a difference between the groups in cTnI serum levels at the various points of time. No significant correlations were found between cTnI and possible confounders.

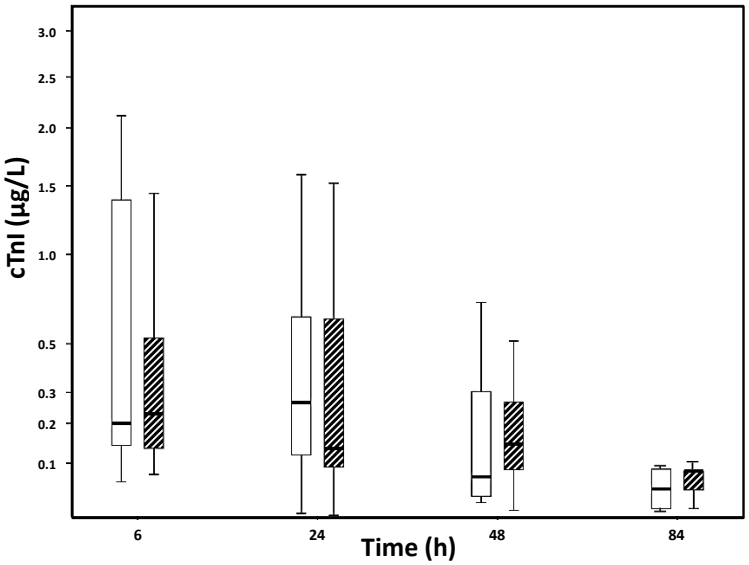

Figure 2. Box-whisker plot comparing hypothermia treated patients (dash) and non-hypothermia treated patients (blank) for cTnI serum levels in a semi-logaritmic scale at specified time points. Horizontal lines indicate median values, boxes 25th-75th percentile range, outer whiskers 5th-95th percentile range.

Echocardiography

During our study in 29 infants, echocardiographic measurements were performed simultaneously with BNP and cTnI measurements (10 in nonhypothermia-treated infants, 19 in hypothermia-treated infants). The measurements were taken at 24 h and included the presence of valve regurgitation (especially tricuspid valve regurgitation), cardiac function measurements with fractional shortening and stroke volume, the presence of pulmonary hypertension, patent ductus arteriosus, and internal dimensions of left and right ventricle. We found BNP correlated with echocardiographic measurements representing ventricular volume (Left ventricular internal dimension during diastole and systole (r= 0.57, p=0.006; r=0.55, p=0.008), end diastolic and end systolic volumes (r=0.56, p=0.007; r=0.56, p= 0.007). No correlations were found between cTnI and echocardiographic measurements.

DISCUSSION

In our study we found a significant decrease in BNP levels from 24 h after birth in hypothermia-treated infants. This was not found in nonhypothermia-treated infants after perinatal asphyxia. Furthermore, we found BNP levels to be lower in the hypothermia-treated groups at 48 and 84 h after birth. The similar but raised levels

of cTnI in hypothermia- and nonhypothermia-treated infants indicate similar hypoxia-induced myocardial damage in both groups. The decrease in BNP during hypothermia treatment suggests that hypothermia after perinatal asphyxia exerts a beneficial effect on cardiac function. To our knowledge this is the first clinical study comparing cardiac biomarkers between hypothermia- and nonhypothermia-treated infants after perinatal asphyxia.

BNP is released by the cardiac ventricles in response to physical stress. Stress can be ventricular wall expansion, pressure overload, or increased wall tension. BNP is involved in the regulation of systemic blood pressure by countering the effects of the renin-angiotensin system and other vasoconstricting neurohormonal systems through a cyclic guanosine monophosphate second messenger. BNP has therefore been established as an accurate indicator of cardiovascular disease in infants and children. (17) BNP levels are closely related to myocardial dysfunction as diagnosed by echocardiography. (18,19) We found BNP levels to correlate with echocardiographic parameters representing ventricular size and volume. However, echocardiographic evaluation was not a standard part of our study and the infants were not evaluated with every biomarker measurement. Therefore, it is possible that infants with more circulatory instability were evaluated by echocardiography and the correlation is only valid for those infants. As heart rate is lower in infants treated with hypothermia, it is possible that this poses less stress on the heart resulting in lower BNP levels. (9) In our study we could not find a correlation between changes in heart rate and BNP levels. Moreover, the differences between BNP levels were also found at normothermia when heart rate differences are no longer present.

Gebauer *et al.* described the cardiac performance using Doppler echocardiography during mild hypothermia after perinatal asphyxia and rewarming. They found that cardiac output was reduced during hypothermia. Tissue perfusion and oxygenation seemed to remain sufficient, as lactate levels did not increase during hypothermia treatment. (8) The cardiac adaptation to lower cardiac output and higher peripheral vascular resistance might explain the initial rise of BNP levels of in hypothermia-treated patients, but no difference was found between the hypothermia- and nonhypothermia-treated group at 24 h. Thus, the initial increase in BNP is more likely related to the cardiac adaptation after perinatal asphyxia. As echocardiography was not a standardized part of our study, we cannot relate the reduction in cardiac output and increased vascular resistance to BNP levels. Previous studies have shown, that BNP levels are very accurate in representing cardiac function. (2,5) It is possible that cardiac function, after an initial adaptation, recovers faster in hypothermia-treated patients because of the lower metabolic demand of the body during hypothermia.

Reynolds *et al.* reported elevated levels of BNP in infants with PPHN. (2) Falling BNP levels during hypothermia treatment may reflect improving pulmonary pressure, rather than a protective effect of the treatment. Furthermore, PPHN has been reported

as a possible complication during hypothermia treatment. (11) However, we have not found a difference between the groups in PPHN occurrence, neither clinical nor subclinical as diagnosed by echocardiography.

The use of cTnI after perinatal asphyxia as indicator of myocardial damage was debated in several articles, as slow skeletal muscle Troponin I is the predominant form during fetal development. (20) However, Bodor *et al.* showed that cTnI is exclusive to the myocardium and therefore elevated levels of cTnI must be of cardiac origin. (21) High levels of cTnI were found in this study in perinatal asphyxiated patients, as was found by others. (6) The similar cTnI levels at 6 h suggest that the severity of hypoxia-induced cardiac myocyte damage was comparable in both groups. From the present study, however, it cannot be concluded that hypothermia has no effect on ongoing cardiac injury after asphyxia. Roka *et al.* suggested that systemic hypothermia may protect against cell necrosis and tissue dysfunction of the internal organs after neonatal asphyxia. (22) In an animal study, lower levels of cTnI were found in hypothermia-treated newborn pigs. (12) In real life, unlike in the laboratory, insults are not clearly defined. Injury may begin hours before birth and can involve repeated and prolonged periods of hypoxia. Cardiac injury may already be evolving at the time of birth, leading to an absent or relatively shorter period of possible intervention. (23)

The nonhypothermia-treated patients in this study were included before mild hypothermia was available as an established treatment modality for perinatal asphyxia. It is possible that during the course of time, apart from the introduction of hypothermia, clinical management of infants changed. To our knowledge, the treatment of nonhypothermia patients was comparable to the hypothermia-treated group, particularly with regards to fluid management. The use of cTnI and BNP is part of the standard clinical care in perinatal asphyxia patients and of course a double blinded study is impossible to perform. This is a limitation of this study.

We conclude that a significant decline and lower BNP levels in hypothermia-treated infants suggest a protective effect of hypothermia on cardiac function. Lower BNP levels suggest less myocardial strain during mild hypothermia and the early decline suggests less impairment of cardiac function after perinatal asphyxia. Whether myocardial damage, as represented by cTnI, is affected or reduced by hypothermia treatment cannot be proven in this study. Future investigations should be directed towards further elucidating the effect of hypothermia on cardiac damage and function after perinatal asphyxia and the relation of cardiac damage and function to long-term outcome.

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Chapter 4

Prognostic value of cardiac biomarkers in infants with neonatal encephalopathy

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Submitted

ABSTRACT

Objective To investigate the value of cardiac biomarkers in the association short- and long-term (neurodevelopmental) outcome in hypothermia-treated infants with neonatal encephalopathy.

Methods Term infants (n=64) with neonatal encephalopathy, treated with hypothermia between January 2008 and December 2010, were included. B-type natriuretic peptide (BNP) and cardiac troponin I (cTnI) were measured on admission and at 24 and 48h after birth. In 50 infants cranial MRI was available for analysis (median 5 days [range 2 – 7]). BNP and cTnI were correlated to clinical characteristics, severity of encephalopathy (Thompson score) on admission, hypoxic-ischemic changes on cranial MRI as indicated by lower apparent diffusion coefficient (ADC) measurements, and long-term follow-up. Follow up was done using the BSITD-III scores at 24 months of age.

Results In surviving infants, BNP and cTnI values were related to days admitted on the NICU (r 0.46, $p<0.05$) and respiratory support (r 0.49, $p<0.001$). In infants who died significantly higher cTnI levels were found (median 0.17µg/L vs. 0.28 µg/L, $p<0.05$). Furthermore, cTnI correlated with the level of encephalopathy on admission (r 0.45, $p<0.001$). In surviving infants, elevated BNP levels were related to lower cognitive composite scores on the BSITD-III at 24 months of age (r 0.51, $p<0.001$). Furthermore, elevated BNP levels were related to a lower ADC in the white matter (r 0.36, $p<0.05$) and thalamus (r 0.37, $p<0.05$).

Conclusion Early cardiac biomarker levels are associated with short- and long-term outcome in infants treated with hypothermia for neonatal encephalopathy.

INTRODUCTION

Neonatal encephalopathy is an important cause of mortality and morbidity in the newborn period. (1) Many biomarkers have been studied in an effort to predict the neurodevelopmental outcome of these infants. (2) Mild hypothermia treatment has shown to improve outcome after neonatal encephalopathy. (3,4) However, hypothermia might influence the usefulness of known biomarkers. (5,6) The current biomarkers, such as umbilical pH levels, amplitude-integrated electroencephalogram (aEEG) and MRI, generally relate directly to the hypoxic-ischemic period and reflect the actual neurological state or visualize the neurological damage. (7-9) As neonatal encephalopathy is associated with multi-organ failure, biomarkers of hypoxic tissue or cellular damage are also used to predict outcome. (5,10) Cardiocirculatory dysfunction is common after perinatal asphyxia, which can aggravate the already compromised cerebral perfusion. (11,12)

Natriuretic peptides and troponins are established cardiac biomarkers and are suggested to be useful in the treatment of neonatal disease. B-type natriuretic peptide (BNP) is secreted by the cardiac ventricles as a result of increased wall stress or ventricular filling pressure. (13) BNP has been shown to predict the outcome of critically ill adult patients. (14) Cardiac Troponin I (cTnI) is part of the inhibitory protein complex located on the actin filament of cardiac muscle. Moreover, cTnI is elevated in asphyxiated neonates and is used as an indicator of hypoxia-induced myocyte damage. (15) cTnI has been proposed as a marker of poor outcome after neonatal encephalopathy. (16-19)

The aim of this study was to investigate whether the cardiac biomarkers BNP and cTnI in infants with neonatal encephalopathy, who underwent hypothermia treatment, can be used to predict short- and long-term outcome.

METHODS

In this cohort study, all infants with signs of perinatal asphyxia and neonatal encephalopathy, who were admitted to the neonatal intensive care unit (NICU) at the University Medical Centre Utrecht in the Netherlands from January 2008 until December 2010, were included. Criteria related to the identification of neonatal encephalopathy and initiation of hypothermia treatment are shown in **table 1**. The Thompson score was used to select infants for hypothermia, and the Sarnat score was used to assess the severity of encephalopathy. (20,21) Patients were eligible if they were born at or after 36 completed weeks of gestation. Infants with major congenital malformations, known chromosomal abnormalities or known causes of psychomotor delay apart from

Table 1. Clinical encephalopathy and hypothermia criteria

Perinatal asphyxia
• Apgar 5 min ≤5
or
• Resuscitation/ ventilation during 10 minutes after birth
or
• Umbilical artery pH<7.0 and "Base deficit" >16 mmol/L
or
• Lactate >10.0 mmol/L < 1 h after birth
Encephalopathy
• Clinical: Thompson score[20] >7 between 1 and 3 h after birth *or*
• aEEG: seizure activity, flat tracing (FT; very low voltage, mainly inactive [isoelectric] tracing with activity below 5μV; continuous extremely low voltage (CLV; continuous background pattern of very low voltage [approximately or below 5μV]; burst suppression (BS; discontinuous background pattern: periods of very low voltage [inactivity] intermixed with burst of higher amplitude

aEEG – Amplitude-integrated electroencephlography

neonatal encephalopathy were excluded from the present study. Patient characteristics and clinical data were obtained from an electronic patient database. The number of days on the NICU, days on ventilation and days on inotropic support were analyzed only for the surviving infants. To determine the intensity of blood pressure support, a blood pressure support scoring system was used, depending on the intensity of the treatment required (score 0: no treatment; score 1: volume expansion and/or dopamine ≤5 μg/ kg/min; score 2: dopamine >5 ≤10 μg/kg/min; score 3: dopamine >10 μg/kg/min or dopamine + dobutamine ≤10 μg/kg/min; score 4: dopamine + dobutamine >10 μg/kg/min; score 5: additional adrenaline and/or corticosteroids). (22) Echocardiography was performed if cardiac abnormalities were suspected or if there was a clinical indication such as suspected persistent pulmonary hypertension (PPHN), therapy-resistant hypotension, cardiac murmur, or persistent low oxygen saturations. PPHN was defined as hypoxemia with echocardiographic findings of elevated pulmonary artery pressure with right to left shunting through a patent foramen ovale or the patent ductus arteriosus. This study was exempt from medical ethics approval. Parental consent was obtained for the anonymous use of patient data. Blood samples were collected as part of routine clinical care from an umbilical or peripherally inserted arterial line.

Hypothermia

All patients were treated with total body cooling according to our hypothermia protocol using the CritiCool™ device (MTRE, Yavne, Israel). After admission to the NICU, infants were wrapped into a body shaped three-dimensional garments. If infants were transported from another hospital, there was no active rewarming during transport. Mild hypothermia was started within 6 hours after birth and continued for 72 hours.

Patients were kept at 33.5°C; the temperature was servo-controlled with a rectally placed thermometer probe. When the period of 72 hours was completed, rectal temperature was allowed to rise 0.2°C per half hour until normothermia (36.5°C) was achieved. This temperature (36.5°C) was maintained during 24 hours. After this period, normothermia was achieved according to standard infant treatment until discharge.

MR imaging

MR imaging was done using a 1.5T or 3T unit (Gyroscan ACS-NT or Achieva; Philips Medical Systems, Best, the Netherlands), according to the protocol described by Alderliesten *et al.* (9) MR imaging included sagittal T1- and transverse T2- and inversion recovery–weighted sequences. An echo-planar imaging technique was used for diffusion-weighted imaging (DWI) (repetition time msec/echo time msec = 3800–5200/89), with a 180 × 180-mm field of view, 4-mm-thick sections, 0-mm section gap, and *b* factors of 0 and 1000 (1.5T) or 800 (3.0T) sec/mm^2.

ADC measurement

Trace apparent diffusion coefficient (ADC) maps were generated on the basis of DW images acquired over the three orthogonal axes (trace ADC = [X + Y + Z]/3). Four regions of interest (ROIs) were manually selected: the left and right basal ganglia (BG) and the left and right medial thalamus (as previously described). (9,23) Furthermore, four ROIs in the white matter (WM) (frontal left and right and occipital left and right) were selected and two ROIs in the cerebellum (left and right). (**Figure 1**) ROIs were placed on the section of the ADC map using T2 and the b0 image of the DW images for anatomic reference. ROIs were oval, with a pixel area of 27–55 mm^2 (mean, 36 mm^2) in the BG, 27–80 mm^2 (mean, 50 mm^2) in the medial thalamus, 25 - 55 mm^2

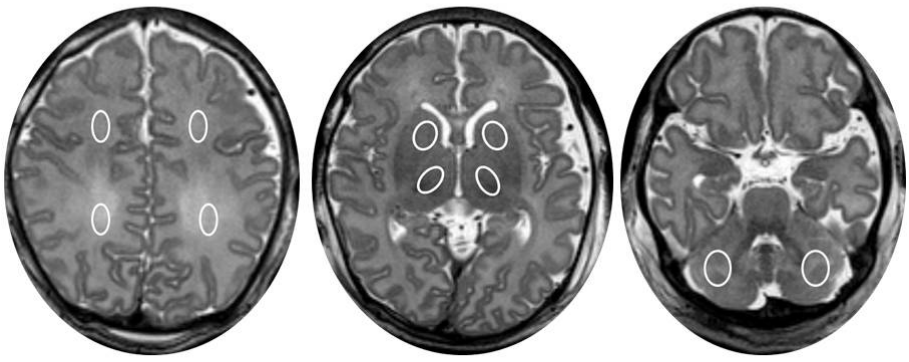


Figure 1. Transverse MR images, regions of interest are placed in the basal ganglia, thalamus, frontal and occipital white matter and cerebellum.

(mean 35 mm^2) in the frontal WM, 27 – 70 mm^2 (mean, 39 mm^2) in the occipital WM and 31 – 73 mm^2 (mean, 50 mm^2) in the cerebellum. Contact with cerebral spinal fluid or grey matter was avoided. All measurements were performed retrospectively on an MR imaging operator console by using Philips Medical Systems proprietary software, without reference to clinical outcome.

BNP and cTnI measurements

BNP and cTnI are routine daily measurements in infants after perinatal asphyxia in our NICU. Blood samples were collected from an arterial catheter into a standard collection vial with ethylenediamine tetra-acetic acid for BNP and lithium heparin for cTnI (Capijet®, VWR, West Chester, PA, USA). BNP and cTnI were analyzed directly after collection on a DxI 800 immunochemistry system (Beckman Coulter Diagnostics, Brea, CA, USA). The lower limit of detection for this assay is 5 pmol/L for BNP and 0.01 µg/L for cTnI. The detection limit value was assigned to undetectable samples. The local reference values for adult patients for BNP are <30 pmol/L: between 30 and 120 pmol/L: suggestive cardiac failure, and >120 pmol/L: probable cardiac failure. The local clinically used cutoff value for cTnI is <0.06 µg/L. Maintenance and quality performance of the instrument were followed according to company instructions. Blood samples were simultaneously collected with other routine blood samples.

Follow-up

At two years of age (mean 24.0±0.33 months), the children were assessed with the Bayley Scales of Infant and Toddler Development third edition (BSITD-III) by a developmental specialist (ICvH). (24) Only the cognitive and fine and gross motor subtests were used and not the language subtest due to the limited time the child was able to concentrate in one session. Both scaled scores (mean 10; standard deviation (SD) 3) of the three subtests as well as the cognitive and total motor composite scores were calculated. Mean composite scores in a normative population are 100 ± a SD of 15. The children were classified as normal, mildly delayed or severely delayed in development using the test normative values. If their scaled or composite scores were outside minus 2 SD (i.e. <4 or <70 respectively) of the expected score for their age, they were considered severely delayed, if the scores were between minus 1 and 2 SD (i.e. 4 and 7 or 70 and 85 respectively), they were considered mildly delayed. Parents were asked to fill out a Child Behavior Checklist (CBCL) questionnaire. (25) The total CBCL problem score is a global index, a measure for emotional and behavioral problems. There are two main dimensions: internalizing and externalizing behavior. A T-score of 50 is the mean for the norm population with an SD of 10. A higher score constitutes more behavioral problems. A T-score between 60 and 70 is in the borderline clinical range, a T-score of >70 is in the clinical range.

Statistics

Descriptive data are presented as median with an absolute range or mean with SD, where applicable. BNP and cTnI levels are presented as medians with an interquartile range. When no significant differences between right and left were found, the left ADC measurement was used. For all ADC measurements SD were calculated. X^2 -test was used for normal data. Kolmogorov-Smirnov was used to test for the normality of the distribution and Log transformation was performed to correct for skewness. If data remained skewed after transformation, non-parametric tests (Wilcoxon signed-rank test or independent-samples Mann-Whitney U test, where appropriate) were used. Spearman's rho was used for correlations. Linear or logistic regression analysis was used to test for related variables. A receiver operating characteristic (ROC) curve was used to calculate the optimal sensitivity and specificity for the BNP and cTnI levels related to a specified outcome measure. A p value <0.05 was considered significant. For the statistical analysis SPSS 20 (IBM corporation, Armonk, New York, USA) was used.

RESULTS

In the studied period, 227 infants after (mild) perinatal asphyxia were admitted to our hospital. Infants, who were not treated with hypothermia (n=156; 69%), because of absence of neonatal encephalopathy or admission after 6 hours after birth, were excluded from the study. Seventy-one infants (31%) were treated with hypothermia, but for several reasons seven of them were excluded (preterm birth before 36 weeks of gestation [n=2], trisomy 21 [n=1], Scimitar syndrome [n=1], *COL4A1* mutation [n=1], mitochondrial disorder [n=1], and alveolar capillary dysplasia [n=1] (**Figure 2**)). Of the remaining 64 evaluated infants, 20 died (31%) as a result of neurological deterioration or multi-organ failure followed by redirection of care. Clinical characteristics of the study population are shown in **Table 2**. In 54 infants, a cranial MRI was performed as part of routine clinical practice. The MRI was performed at a median of 5 days after birth (range 2 – 17 days). In all but four infants, the MRI was performed within the first week after birth. These four infants were excluded from the MRI analysis. (26) Forty-four infants were available for follow-up. All infants presented with signs of neonatal encephalopathy either clinical, as measured by the Thompson score (median 10 [range 4-20]). Most infants (32/64, 50%) presented with a burst suppression background pattern. Background patterns ranged from continuous normal voltage to a flat trace. In 43 infants, seizure discharges were recognized on aEEG. Fifty out of 64 (78%) infants were treated with phenobarbital for suspected, proven seizures or for sedation. Due to persistent seizure activity, treatment with midazolam was needed in

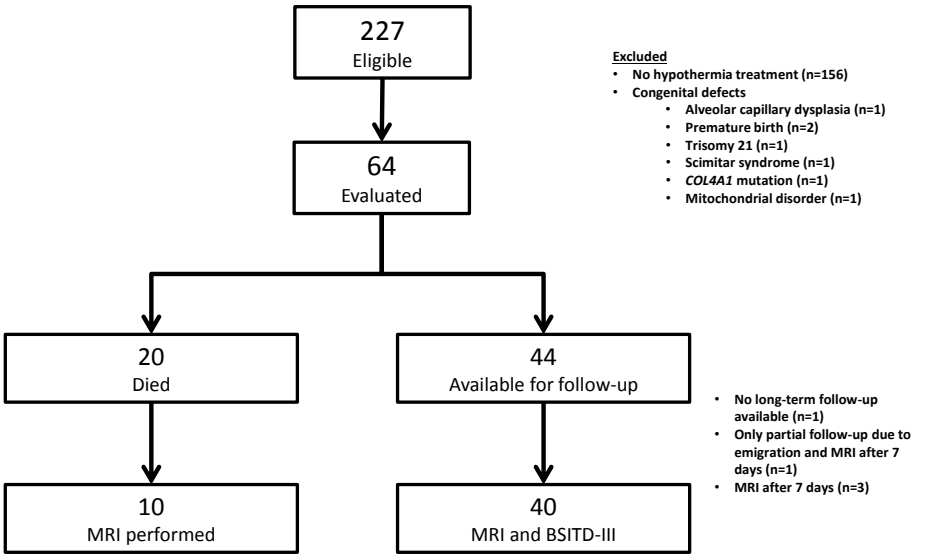


Figure 2. Flow diagram of the eligible infants in this study

30 infants (47%), and with lidocaine in 20 infants (31%) according to the neonatal seizure treatment protocol. No circulatory complications were seen after administration of lidocaine. All children were treated with morphine (0.25mg/kg/day) during hypothermia treatment for comfort and pain relief. If this was inadequate, midazolam was started (0.05mg/kg/hour).

Cardiovascular data

BNP and cTnI levels on admission, 24 hours, and 48 hours after birth showed positive correlation ($p<0.05$ at all time points) with hypotension scores calculated at the time of sampling. Furthermore, BNP and cTnI levels at 24 hours correlated with the presence of PPHN (BNP $r=0.37$, $p<0.01$ and cTnI $r= 0.38$, $p<0.01$). BNP and cTnI were significantly elevated at 24 hours in infants with PPHN (median BNP 124 pmol/L [31 pmol/L – 2748 pmol/L] vs. 608 pmol/L [23 pmol/L – 4957 pmol/L] and median cTnI: 0.23 µg/L [0.03 µg/L – 6.44 µg/L] vs. 0.95 µg/L [0.02 µg/L – 1.97 µg/L]) and at 48 hours (median BNP 64 pmol/L [12 pmol/L – 1700 pmol/L] vs. 153 pmol/L [38 pmol/L – 2304 pmol/L] and median cTnI: 0.07 µg/L [0.02 µg/L – 4.02 µg/L] vs. 0.14 µg/L [0.04 µg/L – 4.32 µg/L]), as can be seen in **Figures 3a and b**. BNP was not related to the duration of resuscitation, the use of cardiac massage, or adrenalin administration during resuscitation. However, cTnI levels on admission, 24 hours, and 48 hours after birth were significantly related to cardiac massage (admission $r=0.37$, $p<0.01$; 24 hours $r=0.35$, $p<0.01$, and 48 hours $r=0.32$, $p<0.05$) and to the use of adrenalin (admission $r=0.31$, $p<0.05$; 24 hours $r=0.29$, $p<0.05$, and 48 hours $r=0.27$, $p=0.08$).

Table 2. Clinical characteristics of the study population

	Hypothermia treated infants (n=64)
Gestational age (w)	40.2 (37.3 – 42.6)
Birth weight (g)	3590 (2150 – 5000)
Caesarean section	37 (58)
Sex (male/female)	40/25 (62/38)
Primipara	36 (56)
Maternal age at birth (y)	32.2 (22.5 – 39.7)
Meconium stained amniotic fluid	27 (42)
Days on ventilator	6 (0 – 14)
Days on inotropic support	5 (0 – 12)
PPHN*	21 (33)
Inhaled Nitric oxide	17 (33)
Apgar score 1 minute	2 (0 – 9)
Apgar score 5 minutes	3 (0 – 10)
Umbilical artery pH	6.94 (6.53 – 7.34)
Base excess (mmol/L)	–18 (–3 – –31.8)
Serum lactate (mmol/L)	14.4 (2.1 – 28.7)
Time to hypothermia (h)	4 (1.5 – 6)
Clinical encephalopathy score	
• Thompson[20]	10 (4 – 20)
• Sarnat[21]	2 (1 – 3)
Seizures	44 (69)
MR imaging	54 (84)
• 1.5T	38 (70)
• 3T	16 (30)
Days to MR imaging	5 (2-17)
Mortality	20 (31)

Desciptive data is presented as median with range or n (%). *PPHN – Persistent pulmonary hypertension of the newborn.

Short-term outcome

BNP was related to high-frequency oscillation ventilation and the use of nitrous oxide, but this relation disappeared when correcting for PPHN. In surviving infants, BNP at 24 hours showed a significant correlation with days on respiratory support and days of inotropic support ($r=0.49$, $p<0.001$, and $r=0.37$, $p<0.05$, respectively). BNP (any time point, maximum concentration or area under the curve [AUC]) was not related to survival ($p>0.05$ at any time point). (**Figure 4a**) In surviving infants, cTnI on admission, 24 hours, and 48 hours were strongly correlated to the number of days requiring respiratory support ($r=0.44$, $p<0.01$; $r=0.47$, $p<0.01$, and $r=0.57$, $p<0.001$,

respectively). Furthermore, cTnI at 48 hours was significantly related to the number of days on the NICU (r=0.46, p<0.05). Troponin was related to the Thompson score prior to initiation of hypothermia and the Sarnat score at 24 hours (r 0.42, p<0.001 and r 0.45, p<0.0001) and thus to the severity of neonatal encephalopathy. Furthermore, cTnI on admission and at 24 hours was related to lower Apgar scores at 5 (p<0.01) and 10 minutes (p<0.05) and higher serum lactate levels (p<0.01). cTnI on admission and at 24 hours were related to mortality, cTnI levels being significantly higher in infants who died (cTnI admission survivor vs. death: median 0.17µg/L [0.02 µg/L – 1.25 µg/L] vs. 0.28 µg/L [0.1 µg/L – 2.44 µg/L,p <0.05], cTnI 24 hours survivor vs. death: median 0.1 µg/L [0.02 µg/L – 1.51 µg/L] vs. 0.17 µg/L [0.03 µg/L - 6.44 µg/L, p<0.05],

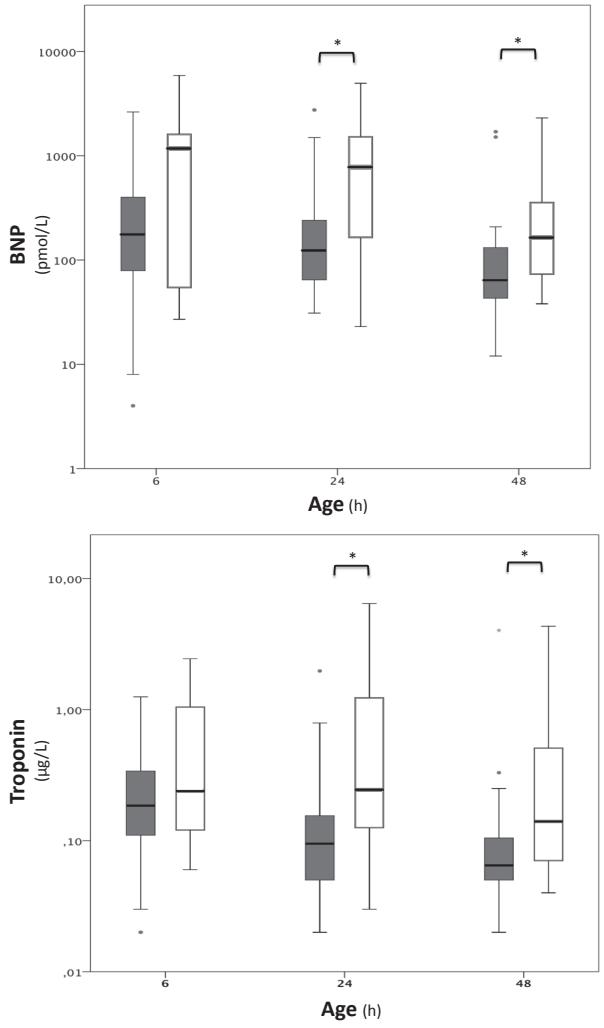


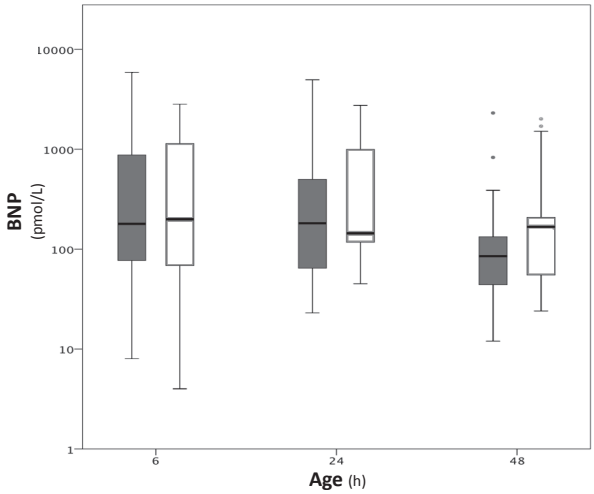
Figure 3a. Box-whisker plot comparing hypothermia-treated patients without (grey) and with (white) persistent pulmonary hypertension (PPHN) for B type natriuretic peptide (BNP), serum levels in pmol/L on a logarithmic scale at specified time points in hours (h) after birth. Horizontal bold lines indicate median values, the solid boxes show the 25th-75th percentile range, outer whiskers 5th-95th percentile range, the points represent outliers (1.5-3 inter quartile range [IQR] from the box). *p<0.05 BNP is elevated in PPHN infants vs. infants without PPHN at 24h and 48h. There was no infant mortality before 48h.

3b. Box-whisker plot for cardiac Troponin-I (cTnI) serum levels in µg/L. *p<0.05 cTnI is elevated in PPHN infants vs. infants without PPHN at 24h and 48h. There was no infant mortality before 48h.

see also **Figure 4b**). Finally, in the infants who did not survive, cTnI at 24 and 48 hours showed a high correlation with earlier death, possibly related to more multi-organ failure (24h r –0.49, p<0.05 and 48h r= –0.72, p<0.01). A cTnI level on admission of >0.18 µg/L had a sensitivity of 75% and a specificity of 53% to predict mortality (AUC 0.70, p<0.05). A cTnI level at 24 hours of >0.15 µg/L had a sensitivity of 70% and a specificity of 77% to predict mortality (AUC 0.74, p<0.01). (**Figure 5**) No relationship was found between cTnI and the duration of inotropic support.

MRI

No differences in ADC values were found between left and right ROIs for BG and WM measurements. For thalamic (Z –4.9; p<0.001) and cerebellar (Z –3.1; p<0.01) measurements, there was a significant difference, with the right side being higher. In



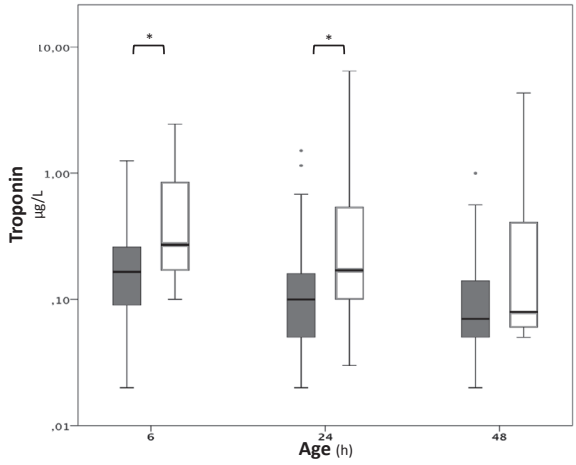


Figure 4a. Box-whisker plot comparing hypothermia-treated patients who survived (grey) and who died (white) after perinatal asphyxia for B type natriuretic peptide (BNP), serum levels in pmol/L on a logarithmic scale at specified time points in hours (h) after birth. Horizontal bold lines indicate median values, the solid boxes show the 25th-75th percentile range, outer whiskers 5th-95th percentile range, the points represent outliers (1.5-3 inter quartile range [IQR] from the box). Comparisons at all specified time points showed that BNP was not related to survival.

4b. Box-whisker plot for cardiac Troponin-I (cTnI) serum levels in µg/L. cTnI is elevated in non survivors vs. survivors on admission (6 h) and at 24h. *p<0.05.

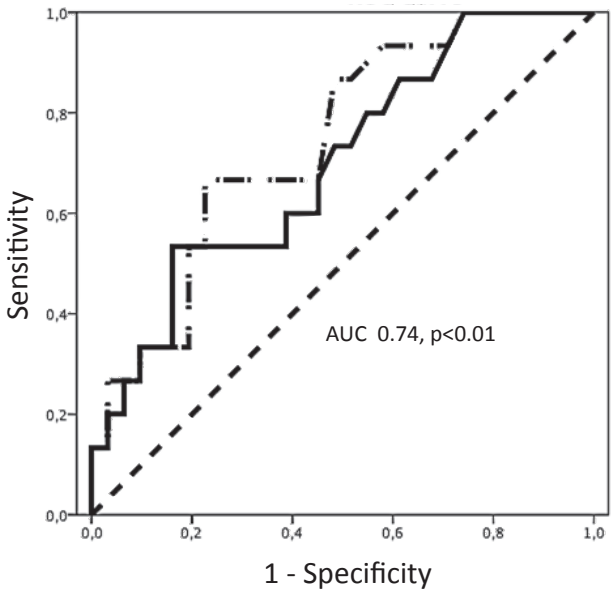


Figure 5. Reciever operating characteristic (ROC) curve representing the sensitivity and 1- specificity of cardiac Troponin-I (cTnI) at admission to the NICU (continuous line) and cTnI at 24 hours (upper interrupted line) after birth of hypothermia treated infants after perinatal asphyxia. The area under the curve (AUC) for cTnI at 24 hours was 0.74, which was statistically significant (p<0.01).

WM, there was a significant difference between frontal and occipital measurements, with occipital values being higher (p<0.001). ADC values were significantly lower in all the measured ROIs in infants who died (p<0.001 for BG, thalamus and WM; p<0.05 for cerebellum).

To evaluate the relationship between cardiac biomarkers and ischemic cerebral lesions, BNP and cTnI were related to ADC measurements in the different ROIs. Significant correlations were found between lower ADC measurements in the left and right thalamus and higher BNP levels at 48 hours (r 0.37, p<0.05). Alderliesten *et al.* suggested that ADC measurements were related to adverse outcome (BG 936 x 10^{-6} mm^2 /sec, thalamus 876 x 10^{-6} mm^2 /sec). (9) BNP levels were significantly higher in those infants with value below this cut-off level (BG: BNP admission 196 vs. 964 pmol/L, BNP 48h 81 vs. 184 pmol/L, p<0.05; thalamus: BNP admission 196 vs. 964 pmol/L, BNP 48h 78 vs. 201 pmol/L, p<0.05). Moreover, higher BNP levels on admission were significantly related to low ADC measurements in the frontal (r 0.41, p<0.01) and occipital WM (r 0.36, p<0.05). This result was most prominent in those infants who died (frontal: r 0.85, p<0.01; occipital: r 0.75, p<0.01). In the whole group a BNP level of more than 585 pmol/L showed a sensitivity of 100% and a specificity of 70% in predicting an ADC measurement in the lowest 10% (frontal WM: <1007 x 10^{-6} mm^2

/sec, occipital WM: <1231 x 10^{-6} mm^2 /sec) in this selected population (AUC 0.901, p<0.01).

Long-term outcome

In total 42/44 (95%) surviving children were tested with the BSITD-III. One infant moved out of the country but had a normal developmental quotient on an earlier test with the Griffiths Mental Developmental Scales at 18 months. (27) One infant was lost to follow-up after three months. The infants tested with the BSITD-III had a median motor composite score (MCS) of 105 (range 80-145) and the median cognitive composite score (CCS) of 112 (range 76-133). The follow-up characteristics are presented in **Table 3**. In two infants an abnormal development was found (cerebral palsy), although both tested within the normal limits of the BSITD-III score. Behavioral problems were reported in the CBCL. However, no correlation with cardiac biomarkers was found. In none of the infants significant hearing loss, as measured by automated auditory brainstem response or visual impairment was found. In 3 infants visual tests were performed because of suspected visual impairment, but no impairment related to the neonatal encephalopathy was found (no abnormalities [n=1] and hypermetropia [n=2]). BNP at 24 and 48 hours showed a good correlation with the CCS, with a higher BNP level correlating to a lower score on the CCS (24h r 0.51, p<0.001 and 48h r 0.45, p<0.01). This correlation was also found for the fine motor scaled score and BNP at 24 hours (r 0.41, p<0.05), but no relationship was found for the gross motor scaled score and total MCS. However, a significant correlation was found between cTnI at 48 hours and a lower total MCS (r 0.39, p<0.05). Furthermore, an association was found between the onset of walking and cTnI on admission and at 48 hours (r 0.36, p<0.05 and r 0.38, p<0.05) with a higher cTnI correlating to later walking. A significant relationship (r^2 0.35, p<0.001) was found between CCS and BNP at 24

Table 3. Follow-up data study population

Any follow-up available	43 (98)
• BSITD-III[24] 24 months	42 (95)
BSITD-III 24 months	
• Age (m)	24 (23 – 24.8)
• Motor composite score	112 (76 – 133)
• Cognitive composite score	105 (80 – 145)
Child behavioral checklist[25] (T score)	47 (28 – 69)
Walking at chronological age (m)	13.5 (11 – 22.5)
Head circumference (cm)	48 (44.7 – 52)

Desciptive data are presented as medians with ranges or n (%). BSITD-III – Bayley scales of infant and toddler development 3rd edition.

hours with a higher BNP correlating to a lower CCS. When the relation was subdivided into infants with and without PPHN, the association in both infant groups remained significant (PPHN, r^2 0.43, $p<0.05$ vs. no PPHN, r^2 0.22, $p<0.05$) but the relationship in the non-PPHN infants was less strong (**figures 6a and b**).

Established parameters

In the present cohort, the predictive value of the classic markers of perinatal asphyxia (umbilical artery pH and Base Excess, Apgar score at 1, 5 and 10 minutes) was associated with short- and long-term outcome. In this population of hypothermia treated infants, we found no relationship between mortality and umbilical artery pH and Base Excess, serum lactate levels or 1-minute Apgar scores. There was a relation between lower Apgar scores at 5 or 10 minutes and adverse outcome (5 min, r 0.36, $p<0.01$ and 10 minutes, r 0.41, $p<0.01$). No association was found between these markers

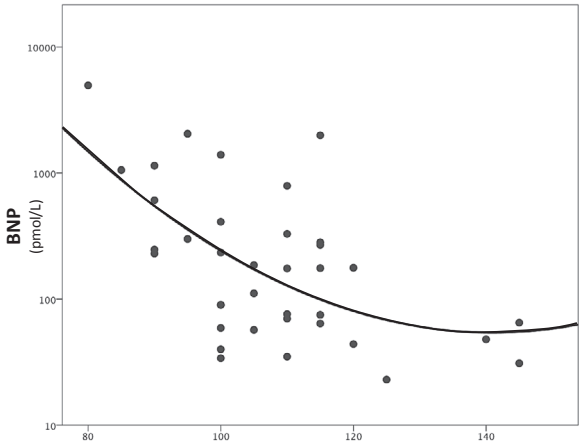
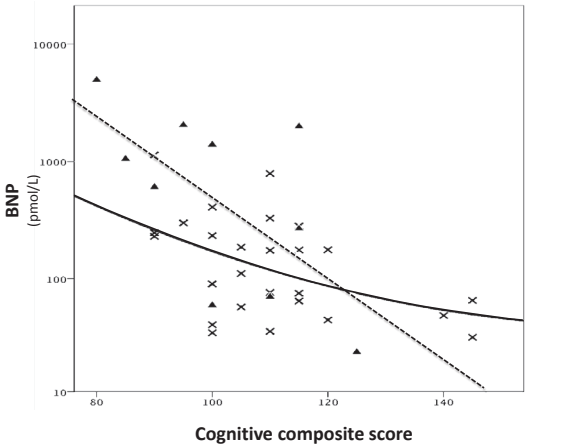


Figure 6a. The relation between B -type natriuretic peptide (BNP) in a logarithmic scale at 24 hours after birth and the cognitive composite score (CCS) of the Bayley Scales of Infant and Toddler Development-III[24] in hypothermia treated infants after perinatal asphyxia. The line shows the best fit quadratic relation (r^2 0.35, $p<0.001$).



6b. The relation between BNP in a logarithmic scale at 24 hours after birth and the CCS in hypothermia treated infants after perinatal asphyxia. The *stiped line* shows the linear relation of BNP and CCS in infants who were treated for persistent pulmonary hypertension (▲) (r^2 0.41, $p<0.001$), the *continuous line* shows the best fit quadratic relation of BNP and CCS in non-PPHN infants (x).

and long-term outcome (MCS or CCS). The Thompson scores on admission and Sarnat score at 24 hours (i.e. clinical encephalopathy) were significantly correlated with mortality (Sarnat, r 0.64, p<0.001 and Thompson, r 0.53, p<0.001), but no significant correlation with long-term outcome in the surviving infants was found.

DISCUSSION

BNP and cTnI are biomarkers of ventricular strain and myocardial damage. (13) Because multi-organ failure and cardiocirculatory compromise are often present after perinatal asphyxia, there is a possible role for cardiac biomarkers in predicting short- and long-term outcome. To the best of our knowledge this is the first study investigating a possible relation between cardiac biomarkers, MRI and long-term outcome in term infants with neonatal encephalopathy. We found that cTnI and BNP are associated with outcome. BNP levels were related to disease burden as indicated by days of NICU admission, days on a ventilator, and need for cardiovascular support. A large portion was explained in the relation between BNP and PPHN. This was previously reported by our group and Reynolds *et al*. (28,29) cTnI levels were related to mortality and days on the NICU. Although the sensitivity and specificity were relatively low and not able to provide a clear cut-off level for mortality, correlations between cTnI and survival are strong. Interesting is the relationship between long-term outcome and early BNP levels. Unexpectedly, a linear relation was found between BNP and the CCS. This relationship was partly explained by the relation between BNP and PPHN and emphasized by the relation between BNP and ischemic cerebral lesions as described by lower ADC measurements in the thalamus and WM. However, some infants without PPHN also had elevated BNP levels and had a worse score on the BSITD-III. One can speculate that these infants were most affected by multi-organ failure and therefore had a less favorable outcome. Thus, BNP could be a marker to identify the more seriously ill infants with the highest risks of an adverse outcome. Infants with high BNP and cTnI levels deserve careful observation and long-term neurodevelopmental follow-up.

A potential limitation of this study is that only hypothermia treated infants were included, and no control group was used. However, infants with encephalopathy are at particular risk for an adverse neurodevelopmental outcome and thus might benefit the most from an early predictive marker. (20,21) Furthermore, by including only a homogeneous group like hypothermia treated infants, extrapolating the results to other infants might be possible. Some reference ranges for cardiac biomarkers of the age group studied are available. (13) The BNP and cTnI values found in this study are well above these reference values. (30,31) A second limitation is that no routine echocardiographic data were used in this study. Further investigation of the

cardiovascular adaptation after perinatal asphyxia and during hypothermia treatment would be interesting. In this study, only limited echocardiographic data were available, which did not provide an adequate sample. (32) Moreover, a pediatric cardiologist or expertise with functional echocardiography is not available in all NICU's. A third limitation is that the number of infants who survived with an adverse outcome is limited. Furthermore, the BSITD-III has not been validated for the Dutch population. This could have influenced our findings. However, a linear relation between BNP and outcome was found in the surviving infants, which remains interesting. Finally, it is important to state that this was a retrospective study and data should be interpreted with care. Although in our unit sampling for BNP and cTnI is routine clinical practice in infants with neonatal encephalopathy, some samples were missing.

To the best of our knowledge, no previous studies evaluated the use of BNP in evaluating long-term outcome after perinatal asphyxia. El-Khuffash *et al.* assessed NT-proBNP levels and outcome. In two of their studies in preterm infants, a relation was found between natriuretic peptides and outcome. (33,34) The elevated levels of (NT-pro)BNP most probably reflect the infants' total illness (PPHN, hypotension, inotropic support) and are therefore related to outcome, which was also found in this study. In adult patients, BNP correlates with outcome in different studies and settings. This was amongst others found in an unselected intensive care population. (14) However, the heart is a more prominent part of the disease in adult patients. Their age and possibly degenerative disease have affected the cardiac function and limited the regenerative possibilities. In infants, the heart can be affected by hypoxia causing cardiocirculatory failure needing inotropic support. We speculate that this situation is usually temporary with limited structural damage affecting long-term outcome. In this study BNP mirrored the infant illness, i.e. days on a ventilator, days on the NICU, and PPHN, and showed a relation to the amount structural cerebral damage, possibly a better marker for long-term outcome.

Troponin is a marker for myocardial damage. Several studies have shown high levels of cTnI in infants after perinatal asphyxia. (15,35) Trevisanuto *et al.*, as opposed to this study, did not find a relationship between cTnI and traditional markers of perinatal asphyxia, Apgar score, umbilical pH, and bicarbonate levels. (15) Shastri *et al.* also found that cTnI concentrations at 36 hours correlated with the clinical grade of neonatal encephalopathy. Besides, they also found a relation with the duration of inotropic support. (30) Our research supports this finding. Türker *et al.* showed that cTnI can be used as an early predictor of death. (18) In cord blood samples, the cTnI levels were significantly higher in the non-surviving infants. They also showed that cTnI levels were more elevated in those infants with severe neonatal encephalopathy. In the present study, we found a correlation between cTnI levels and the severity of neonatal encephalopathy. In a study by Türker *et al.*, no hypothermia treatment was used. It

is possible that this might have influenced our results. Kanik *et al.* and Agrawal *et al.* suggested cTnI as a predictor of mortality. However, as was found in the present study, the sensitivity and specificity of cTnI in the prediction of mortality were limited. (17,36) Lui *et al.* also reported a cut-off level of 0.15 µg/L of cTnI in hypothermia treated infant. They stated relatively comparable sensitivity (65%) and specificity (82%) for adverse outcome. They also described a negative correlation between cTnI at 24 hours and a motor developmental score; the present study supports this finding, but only for cTnI at 48 hours. (37)

Are there better markers for predicting outcome in these infants? Several biochemical markers have been suggested. (10,16) As neonatal encephalopathy is primarily affecting the brain, brain related biomarkers provide the best sensitivity and specificity for poor outcome. However, these markers also have their limitations. (2,5,38) They require continuous measurement, are influenced by medication (aEEG, near infrared spectroscopy), require infant transportation or specific interpretation (MRI), or have a limited time course interval of use (ADC measurement on MRI). No, or scarce randomized studies are available, especially in relation to mortality. (39) Hypothermia treatment possibly influences the reliability of these markers, especially in the first hours after birth. (5,7)

In conclusion, the cardiac biomarkers BNP and cTnI are associated with (neurodevelopmental) outcome in infants treated with hypothermia for neonatal encephalopathy. BNP is related to the clinical characteristics of the infant in the first days after birth and is indicative of the severity of illness. If the infant survives, higher BNP levels are associated with a worse neurodevelopmental outcome, especially in infants with PPHN. Troponin is related to short-term outcome and mortality. Although cTnI levels are more elevated in those who do not survive after perinatal asphyxia, the sensitivity and specificity in this study are limited. A prospective follow-up study with structured echocardiography and (long-term) hemodynamic follow-up would be of additional value to establish the importance of these cardiac markers after neonatal encephalopathy.

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Chapter 5

B-type natriuretic peptide as biomarker for rebound during persistent pulmonary hypertension treatment in newborn infants

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ABSTRACT

Objective To investigate whether serum B-type natriuretic peptide (BNP) is a useful biomarker in evaluating the course of persistent pulmonary hypertension of the newborn (PPHN) and effectiveness of treatment.

Methods Prospective follow-up study of infants with clinical and echocardiographic signs of PPHN, who were treated with inhaled nitric oxide (iNO). Of 24 patients with PPHN, who were treated, serum BNP levels were determined longitudinally in 21. BNP levels were compared between infants with (n=6) and without rebound PPHN (n=15).

Results BNP levels in all PPHN infants were not significantly different at the initial start of iNO. BNP levels decreased in both groups during iNO treatment. In the infants in whom rebound PPHN developed after weaning from iNO, a significantly higher increase was found in BNP (283 pmol/L to 1232 pmol/L) compared with that in infants without rebound (98 pmol/L to 159 pmol/L). This occurred before the onset of clinical deterioration. BNP again decreased significantly after iNO treatment was restarted.

Conclusions BNP, a biomarker of cardiac ventricular strain, proved to be useful in evaluating the efficacy of PPHN treatment, and moreover, BNP helps to predict a rebound of PPHN.

INTRODUCTION

Persistent pulmonary hypertension of the newborn (PPHN) occurs when pulmonary vascular resistance fails to decrease after birth. (1) When the respiratory distress is complicated by oxygenation problems and low saturations, PPHN should be suspected. Because most diseases of the newborn start with respiratory problems, PPHN is often difficult to diagnose. (2)

B-type natriuretic peptide (BNP) is an endogenous peptide hormone, secreted by cardiac ventricles in response to an increased wall stress and ventricular filling pressure. BNP causes vasodilatation and has a diuretic and natriuretic effect. It has been used in infants and children to provide information on ventricular function and to diagnose significant cardiovascular disease. (3,4) BNP concentrations in plasma correspond well with echocardiographic findings of ventricular strain. (5,6) Reynolds *et al.* suggested BNP as an early indicator of PPNH in the presence of respiratory illness in the newborn in the absence of congenital heart disease. (4)

When PPHN is suspected, echocardiographic evaluation is necessary to exclude congenital heart disease (CHD) and to confirm signs of elevated pulmonary pressure. (7) Treatment is directed to improvement of oxygenation (surfactant replacement), pulmonary vascular dilatation (inhaled nitric-oxide [iNO]) and to maintain adequate blood pressure (fluid replacement, inotropic support). (1,8) The decision how long iNO treatment should be continued is often difficult. After exposure to iNO, the pulmonary circulation can be sensitized for vasoconstriction, possibly due to suppression of endogenous NO production. Even when careful weaning is used, some infants will have a rebound of PPHN. (9) The objective of this study was to investigate whether BNP is a useful biomarker to evaluate the course of PPHN and the effectiveness of treatment.

METHODS

In a prospective follow-up study, all infants with clinical and echocardiographic signs of PPHN who were treated with iNO and admitted to the University Medical Center Utrecht Neonatal Intensive Care Unit (NICU) from January 2009 until December 2009, were included in the study. Exclusion criteria were major congenital malformations including cardiac malformations, chromosomal abnormalities, and the need for extracorporal membrane oxygenation (ECMO), because then referral to another NICU was necessary. Because of the observational nature of the study approval from the medical ethics commission was not required. Parental consent for the anonymous processing of patient data was obtained in all cases. BNP blood samples were collected at admission and then daily during iNO treatment and at least once after completion of iNO

treatment. In case of signs of rebound and reinstitution of iNO, daily determinations were continued. Echocardiography was routinely performed by an experienced pediatric cardiologist on suspicion of PPHN to confirm the diagnosis and to exclude CHD.

PPHN was defined as hypoxemia with echocardiographic findings of elevated pulmonary artery pressure, right to left shunting through a patent foramen ovale or the patent ductus arteriosus, or both. Rebound was defined as the reoccurrence of clinical signs of PPHN after decreasing or discontinuing the iNO treatment. Clinical signs were hypoxemia, decrease of "post-ductal" saturation, and lower partial pressure of oxygen levels, as a result of right-to-left shunting, which prompted the attending clinician to reinstitute iNO treatment and increase the fraction of inspired oxygen (FiO2). This rebound was confirmed by using echocardiography with known measures of increased tricuspid regurgitation as an estimate of elevated right ventricular pressure and by identifying the presence of right-to-left shunting at that moment.

The demographic data collected were gestational age at birth, birth weight, sex, prenatal and perinatal history, and associated illness. When each blood sample for BNP was taken, blood gas analysis was performed. The oxygenation index (OI) and alveolar/arterial oxygen gradient (AaDO2) as indicators of disease severity were calculated. (10,11) The iNO requirement at that time point was registered.

To assess the intensity of blood pressure support, a blood pressure support scoring system was used, depending on the intensity of the treatment necessary (score 0, no treatment; score 1, volume expansion and/or dopamine $\leq$5 µg/ kg/min; score 2, dopamine >5 $\leq$10 µg/kg/min; score 3, dopamine >10 µg/kg/min or dopamine + dobutamine $\leq$10 µg/kg/min; score 4, dopamine + dobutamine >10 µg/kg/min; score 5, additional adrenaline and/or corticosteroids). (12)

BNP measurements are standard clinical practice in our department and were done daily or more if required. Blood samples were collected from an arterial catheter or by capillary samples (heel stick) into a standard collection vial with ethyl-enediamine tetra acetic acid (Capijet®, VWR, West Chester, PA, USA). BNP was analyzed on a DxI 800 immunochemistry system (Beckman Coulter Diagnostics, Brea, CA, USA).

Statistics

Descriptive data are presented as median and ranges. Comparison of BNP levels over time was done by non-parametric statistical analysis using the Mann-Whitney *U* test because the dataset had a non-parametric distribution. Spearman rank-sum test was used to correlate BNP to associated variables. A *P* value of <0.05 was considered significant. For the statistical analysis SPSS 15.0 (SPSS, Chicago, Illinois) was used.

RESULTS

Twenty-four patients were admitted to the NICU for PPHN treatment with iNO. Three patients were referred for extracorporal membrane oxygenation treatment and were subsequentially excluded from the study. Of the 21 infants included in this study, 6 had rebound as defined in the Methods section, and 15 did not. The etiology of the PPHN in the rebound group was meconium aspiration syndrome (n=2), meconium aspiration syndrome and perinatal asphyxia (n=1), and perinatal asphyxia (n=3). In the non-rebound group, the etiology was sepsis (n=5), meconium aspiration syndrome (n=5), sepsis and perinatal asphyxia (n=1), perinatal asphyxia (n=1), "dry lung" after premature rupture of membranes (n=1), pneumothorax (n=1), and pulmonary hypoplasia (n=1).

The patient characteristics of the groups are shown in **Table 1**. No significant differences were found between the rebound and non-rebound group for birth weight, gestational age, gender, number of days iNO treatment, Apgar score at 1 minute, Apgar score 5 minutes, or inotropic support.

Table 1. Characteristics of patients treated for persistent pulmonary hypertension of the newborn

	Rebound (n=6)	No rebound (n=15)	P
Birth weight (g)	3390 (2100-4920)	3436 (1475-4790)	0.74
Gestational age (w)	39 0/7 (32-41 3/7)	39 2/7 (30-42 1/7)	0.51
Male (%)	67	60	0.94
1-min Apgar score (median)	5	5	0.97
5-min Apgar score (median)	6	7	0.64
Total iNO (days)	9 (5-14)	5 (1-11)	0.11
Inotropic support	4.2 (2-5)	4.2 (2-5)	0.78

The first BNP level was obtained just before the start of iNO treatment. The mean levels for the 21 infants were 361 ± 499pmol/L (median, 155; range, 9 – 1838 pmol/L). In the rebound PPHN group the BNP levels were initially higher (median, 289 pmol/L; range 9 – 1838 pmol/L) than in the non-rebound group (median, 147 pmol/L; range, 54-649 pmol/L); however, this difference was not statistically significant. After the initiation of iNO treatment, BNP levels decreased, as is shown in **Figure 1**, which shows a non-chronological time interval, because treatment duration was not identical for all patients. At the specified points during PPHN treatment, a BNP measurement was performed in all patients. After weaning and subsequent cessation of iNO treatment, a non-significant rise in BNP was seen in the non-rebound group from a median of

98 pmol/L (range, 44 – 144 pmol/L) to 159 pmol/L (range, 7 – 448 pmol/L; p=0.07). The rebound group showed a much greater rise in BNP plasma levels, from a median of 283 pmol/L (range, 49 – 341 pmol/L) to 1232 pmol/L (range, 430 – 2339 pmol/L; p=0.004). This was also significantly different when compared to the non-rebound group (p<0.001). The 6 infants who showed rebound manifested the clinical signs of rebound at a median time of 31hours (range, 7-56h) from the time of BNP measurement.

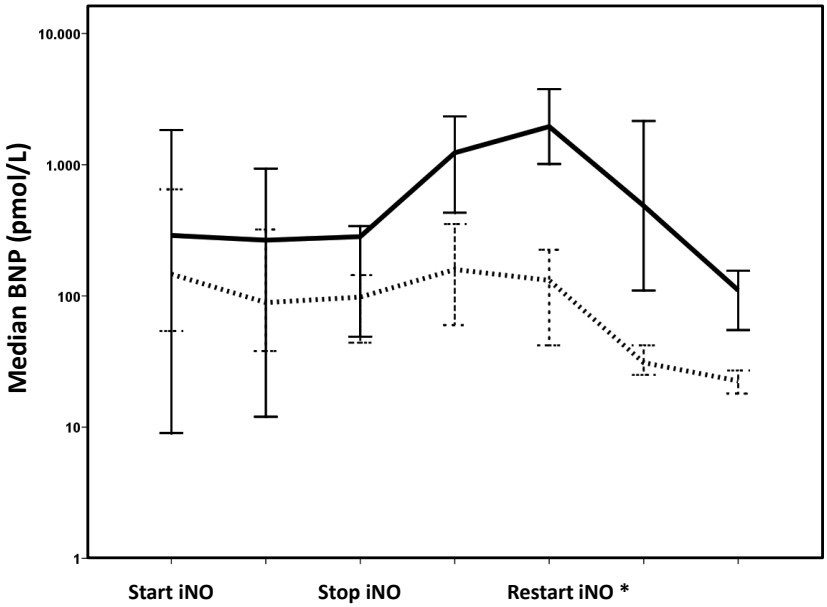

Figure 1. Line plot representing median changes in BNP (pmol/L) during PPHN treatment. The *continuous line* represents the patients with rebound PPHN; the *interrupted line* represents the PPHN patients without rebound. The *errorbars* represent the range. * *Only in rebound patients*

The OI and AaDO2 can be used to determine severity of PPHN and thus represent the current clinical condition of the patient. **Figure 2** shows the pattern of OI and AaDO2 corresponding to the BNP measurements. No correlation was found between the OI or the AaDO2 and BNP at the specified points. The measured AaDO2 and calculated OI values did not rise in conjunction with a significant increase in BNP, but did rise when the clinical signs of rebound PPHN became apparent. The increase in FiO2 can be seen in the AaDO2 increase after iNO reinstitution, with a median of 197 (range, 50-349) increasing to 365 (range, 88-484). So before the onset of clinical symptoms of PPHN, BNP was elevated in the patients who subsequently appeared to have had a rebound of PPHN.

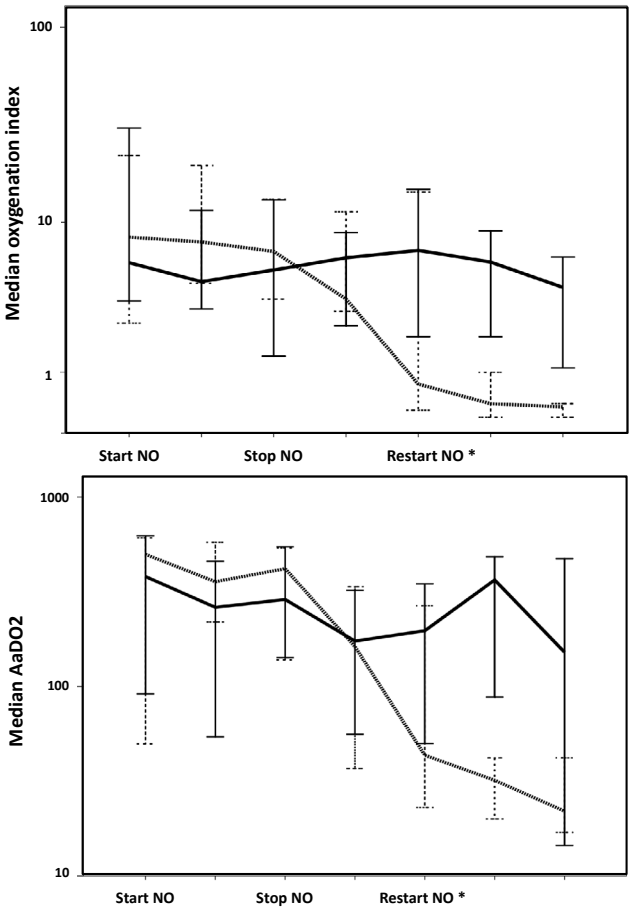

Figure 2. Clinical characteristics during PPNN treatment.

2a. Line plot representing the median changes in OI during the course of PPHN treatment.

2b. line plot representing the median changes in AaDO2 during the course of PPHN treatment. The *continuous line* represents the patients with rebound PPHN; the *interrupted line* represents the PPHN patients without rebound. The *errorbars* represent the BNP range. * *Only in rebound patients*

To exemplify the course of BNP related to these measures of disease severity, the treatment course of an infant with rebound PPHN is shown in **figure 3**. At 78 hours of life, iNO treatment is gradually weaned. A clear rise in BNP is seen in the absence of clinical deterioration (AaDO2 and OI remain stable). At 126 hours of life, when BNP is still increased, the condition of the patient suddenly deteriorates. The changes in AaDo2 (310 to 350) and OI (6.5 to 14.9) indicate an increase in ventilation and FiO2 and reinstitution of iNO.

The gradient of tricuspid regurgitation (TR) in maximum jet velocity was measured during echocardiography. Results are shown in **figure 4**. A significant correlation was found between BNP and TR (p=0.001; r=0.665; n=21). The correlations were comparable for rebound and non-rebound patients. The point at which the measurements were performed did not influence the correlation.

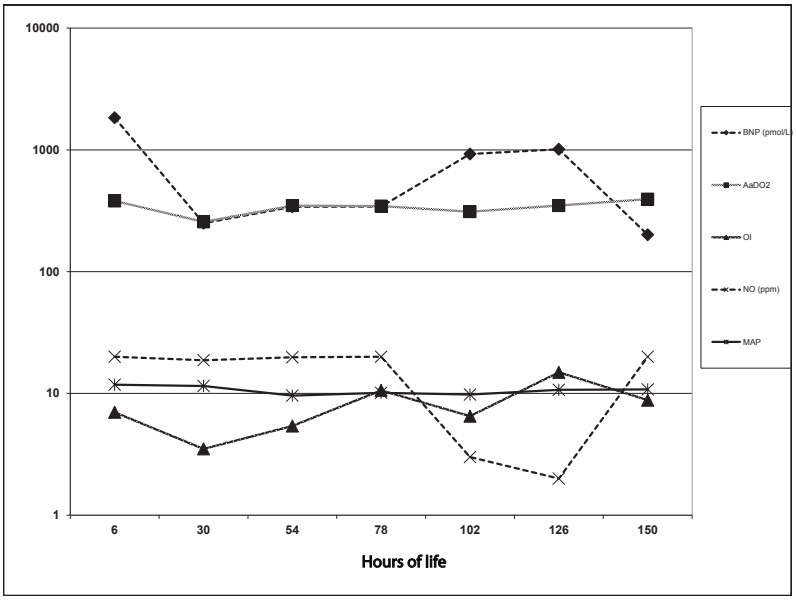

Figure 3. Example of BNP levels, AaDO2, OI, amount of iNO receiving, and Mean Airway pressure versus hours of life for a selected patient.

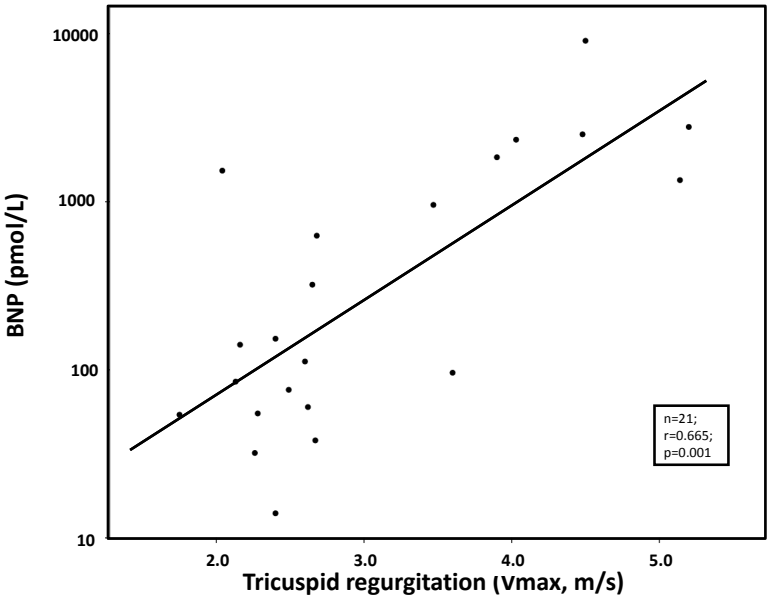

Figure 4. Scatterplot representing BNP measurements versus maximum velocity of TR jet. (n=21; r=0.665; p=0.001)

DISCUSSION

BNP is released by the cardiac ventricles in response to physical stress. Stress can be defined as ventricular wall expansion, pressure overload or increased wall tension. BNP is involved in the regulation of systemic blood pressure by countering the effects of the renin-angiotensin system and other vasoconstricting neurohormonal systems through a cyclic guanosine monophosphate second messenger. It has been established as an accurate indicator of cardiovascular disease in infants and children. (3,13) Myocardial dysfunction as diagnosed by echocardiography is closely related to BNP levels. (5,14-16) In a study done by Ikemoto *et al.* a strong correlation was found between BNP levels and pulmonary artery pressure in premature infants. (17) Reynolds *et al.* were the first to describe elevated levels of BNP in patients with PPHN. (4) Their study, as did this study, found a significant correlation between BNP levels and the gradient of tricuspid regurgitation or TR velocity as an indicator of elevated pulmonary arterial pressure. It was suggested that elevated BNP levels cannot be used as an indicator of disease severity, because a weak relation with the OI and no correlation between AaDO2 and BNP was found, which is confirmed by this study. BNP levels increased before the onset of clinical symptoms. AaDO2 and OI are accurate indicators of disease severity at the time of measurement, but are unable to predict PPHN treatment failure. (4)

BNP has been used as a biomarker in the diagnosis and management of pulmonary hypertension (PH) in pediatric patients. (18-20) Although PPHN is different from PH in pediatric patients, elevated pulmonary pressure is found in both. BNP levels are elevated in patients with PH and have been shown to have a prognostic value. (21) In PH, changes in BNP levels are suggested to be of more importance than elevated levels alone. (19) The present study clarifies that this is also the case in PPHN.

Apart from being a biomarker for disease severity and follow-up, it is suggested that BNP has biologic properties enabling pulmonary vascular relaxation. In a study done by Klinger *et al.*, BNP was effective in blunting PH in rats exposed to 3 weeks of hypoxia. (22) Thus, the elevation of BNP in PPHN patients also suggests a therapeutic role for BNP. Research has been done to evaluate the possible use of BNP as a therapeutic agent in iNO-resistant PH in infants, but until now no trials in infants have been reported. (16,23)

BNP could be used to support the diagnosis PPHN. This study supports the use of BNP as a method to assess disease progression; however, initial echocardiographic evaluation remains necessary to rule out possible CHD. When CHD has been excluded, BNP can serve as a biomarker in the follow-up of treatment of PPHN. (4,24)

In this study, clinical treatment of PPHN was not blinded for BNP results. We found that elevation of BNP did not influence the medical decision making, because BNP

levels were well elevated before a change in treatment was initiated. Treatment was based on clearly defined clinical signs of treatment failure, as described in the Methods section, and treatment was only initiated after clinical signs were present, as can be seen by a sharp increase of AaDO2 levels after iNO reinstitution.

BNP, a biomarker of cardiac ventricular strain, may be useful in evaluating the course and treatment of PPHN and can serve as a predictor of rebound PPHN. When during weaning or after cessation of iNO a significant rise in BNP occurs, intervention should be directed to restart treatment or slowing down the weaning process. Future studies are warranted to identify the possible use of BNP as a therapeutic agent in PPHN treatment.

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Chapter 6

Placental pathologic patterns in very preterm infants with suspected cardiovascular compromise

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In preparation

ABSTRACT

Objective To investigate whether elevated BNP levels after birth, as a marker of intrauterine cardiovascular adaptation, are related to placental pathology.

Methods Preterm infants (<32 weeks) were retrospectively evaluated. Placental pathology reports were systematically analyzed and lesions or pathologic processes were related to BNP levels at admission to the NICU.

Results The placentas of 133 infants were available for analysis. Chorioamnionitis was reported in 44 (33%), ischemic changes in 73 (55%), placental infarction in 41 (31%), and increased villous maturation in 43 (32%) of the placental pathology reports. Higher levels of BNP were found with more pathologic processes reported (r^2 0.15, $p<0.001$). A higher BNP at birth showed a high correlation with hypoxia as reported by nucleated red blood cell elevation, especially when infarctions were reported. However, this association was only found in male infants (r^2 0.46, $p<0.001$). In females, an association was only found with nucleated red blood cells (r^2 0.35, $p<0.01$). In placentas with reported chorioamnionitis, no elevated levels of BNP were found.

Conclusion Placentas of preterm infants frequently showed pathologic changes. Signs of placental dysfunction, such as placental infarction, lower weight, or presence of nucleated red blood cells, were associated increased BNP levels, which was more prominent in the male infant.

INTRODUCTION

Preterm birth is a serious and frequent occurring complication of pregnancy. It is associated with severe morbidity and mortality. (1) Intrauterine pathologic processes can compromise postnatal cardiac function. (2) This impaired cardiovascular function may complicate the neonatal period and can persist into early childhood and even adulthood. (3,4)

B-type natriuretic peptide (BNP), an endogenous peptide hormone secreted by the cardiac ventricles in a response to wall stress and increased filling pressure, has been related to antenatal cardiovascular adaptation. (5) The placenta can provide a window to intrauterine life and careful analysis is of great importance. (6,7) Placental pathologic patterns have been associated with illness severity, periventricular hemorrhagic infarction, cerebral palsy, and long-term neurodevelopmental outcome in very preterm infants. (8-11) However, routine placental examination is not standard in many hospitals and often requires a research setting. (12) Even when placental pathology is performed, to the clinician only the pathology report is available. (13)

In this study, we investigated the relation between placental pathology in very preterm infants and signs of perinatal cardiovascular adaptation, expressed by BNP. We hypothesized that significant lesions are common in very preterm infants and associated with postnatal BNP elevation.

METHODS

In a retrospective cohort study, all infants born between October 2009 and October 2010 with a gestational age of less than 32 weeks and admitted to the neonatal intensive care unit (NICU) of the University Medical Center Utrecht were included. Infants without placental pathologic reports were excluded from the study.

This study was conducted following the guidelines of the local medical ethics committee; parental consent was obtained for the anonymous use of patient data. Blood samples were taken as part of a prospective study investigating BNP for the identification of a patent ductus arteriosus, for which approval from the medical ethics committee of the UMC Utrecht was obtained.

Maternal and infant clinical characteristics were obtained from an electronic database. (MOSOS, BMA, Houten, the Netherlands; Metavision, *i*MD*soft*, Needham, MA, USA) The collected maternal variables were maternal age, prenatal and perinatal medication (including antihypertensive drugs), premature rupture of membranes, group B streptococcal status, method of delivery, and hypertensive disorders such as pregnancy induced hypertension, preeclampsia (PE) or Hemolysis Elevated liver

proteins and Low platelet count syndrome (HELLP). PE was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy as a de novo rise in systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in the second half of pregnancy and proteinuria ≥300 mg/24h. (14) HELLP syndrome was diagnosed based on the following laboratory abnormalities: AST >50 U/L or ALT >50 U/L, LDH >600 U/L, platelet count <100 x10^9/L, and evidence of hemolysis. (15) The course of antenatal corticosteroids was considered completed if two doses of betamethasone were given at least 24 h before delivery. Signs of fetal distress such as fetal bradycardia or variable decelerations on cardiotocogram were noted. The infant characteristics collected were gestational age gender, birth weight, Apgar scores, umbilical artery pH, serum lactate, thrombocyte count (at admission and at 24 hours after birth), BNP at admission to the NICU (within 6 hours after birth), and nucleated red blood cells in peripheral blood (NRBC). Intrauterine growth restriction (IUGR) was defined as growth below the 10^{th} percentile for that gestational age. (16)

Placental pathology

Placental pathologic reports were retrieved from an electronic database (EZIS, ChipSoft, Amsterdam, the Netherlands). Placental pathologic examination is routine in our hospital following preterm birth. The lesions or processes in the placenta were identified and are presented in **table 1**. (12) The identified processes were grouped in lesions associated with hypoxia (increased villous maturation, infarction, ischemic changes, small placental size), inflammation (chorioamnionitis, funiculitis, vilitis of unknown origin, vasculitis), and other reported lesions or calculations (fetal thrombotic vasculopathy, umbilical cord coiling).

Table 1. Reported placental lesions and the relation with B-type natriuretic peptide

		n (%)	r	p
Hypoxia	Small placental size	42 (32)	0.25	p <0.01
	Ischemic changes	73 (55)	0.25	p <0.01
	Increased villous maturation	43 (32)	0.18	p <0.05
	Placental infarction	41 (31)	0.27	p <0.01
Inflammation	Chorioamnionitis	44 (33)	-	p =0.08
	Funiculitis	23 (17)	-	p =0.60
	Vilitis of unknown origin	11 (8.3)	-	p =0.12
	Vasculitis	2 (1.5)	-	p =0.15
Other	Fetal thrombotic vasculopathy	6 (4.5)	-	p =0.78
	Umbilical cord coiling	-	-	p =0.54

Descriptive data are presented as n (%). A single placenta can have more than one reported lesion.

BNP samples

A BNP sample was drawn at admission to the NICU (within 6 h after birth). Blood samples were collected from an arterial catheter into a standard collection vial with ethylenediamine tetra-acetic acid (Capijet®, VWR, West Chester, PA, USA). BNP was analyzed directly after collection on a DxI 800 immunochemistry system (Beckman Coulter Diagnostics, Brea, CA, USA). The lower limit of detection for this assay is 5 pmol/L. The detection limit value was assigned to undetectable samples. The local reference values for adult patients for BNP are <30 pmol/L, normal value; between 30 and 120 pmol/L, suggestive cardiac failure; >120 pmol/L, probable cardiac failure. Maintenance and quality performance of the instrument were followed according to company instructions. Blood samples were simultaneously collected with other routine blood samples.

Statistical analysis

Descriptive data are presented as means with standard deviations or medians with absolute range, where appropriate. Pearson's Chi-squared test was used for nominal and ordinal categories. Student's t-test was used to compare continuous variables. Kolmogorov-Smirnov was used to test for the normality of the distribution and Log transformation was done to correct for skewness. If data remained skewed after transformation, the Mann–Whitney U test was used. Spearman rho was used for correlations. Linear regression analysis was used to test for related variables. A p value <0.05 was considered significant. For the statistical analysis SPSS 21 (IBM corporation, Armonk, New York, USA) was used.

RESULTS

In total, 187 infants were eligible for inclusion. Twenty-three infants were excluded, as was specified in **figure 1**. Of the remaining 164, thirty infants were born in another hospital, where no placental examination was performed. In 134 infants a placental pathological examination was available. Due to antenatal laser coagulation, one of the placentas was not fully assessable and was excluded from the evaluation. Maternal and infant characteristics are reported in **table 2**. The median time of BNP sampling was 1.3 h after birth (range, 0.5 – 4.5 h). No significant correlation was found between time of sampling and the level of BNP . The median BNP level was 77 pmol/L (range, 4 – 1373 pmol/L); the interquartile range was 36.0 to 150 pmol/L. No significant difference in BNP was found between the group with placental pathologic examination and the group without (56 pmol/L (range, 5 – 281 pmol/L); NS). No significant difference was found for BNP levels in male and female infants (median, 79 vs. 78 pmol/L; NS).

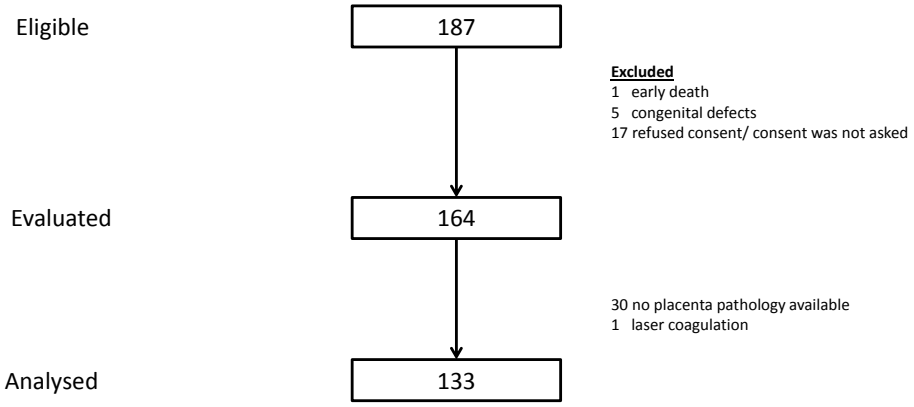

Figure 1. Flow diagram of the eligible infants in this study

Placental lesions

Pathologic changes were frequently reported. Ischemic changes in the placenta were described most frequent, in 73 (55%) of the examined placentas. Furthermore, as is described in **table 1**, signs of increased villous maturation 43 (32%), placental infarction 41(31%), and signs of chorioamnionitis 44 (33%) were reported. No significant difference between singleton and multiple gestation was found for the different pathologic findings. However, lesions grouped as hypoxia (increased villous maturation, infarction, ischemic changes, small placental size) were more common in singleton pregnancies, especially in singleton males (Chi-square, $p<0.01$). Furthermore, these lesions were associated with lower infant birth weight (r 0.25, $p <0.01$) and more signs of perinatal hypoxia, such as the presence of NRBC (r 0.28, $p<0.01$), thrombocytopenia (r 0.39, $p<0.01$), increased serum lactate (r 0.24, $p<0.01$), and lower umbilical artery pH (r 0.34, $p<0.01$). The reported lesions were closely related to PE (r 0.57, $p<0.01$) and HELLP (0.27, $p<0.05$). Chorioamnionitis (CA) was associated with a lower Caesarian section rate (Chi-square, $p<0.001$). Funiculitis was found more often in male infants (Chi-square, $p<0.05$).

B-type natriuretic peptide

Lesions, grouped as hypoxia, were related to elevated levels of BNP. Higher levels of BNP were found with more pathologic processes reported (r^2 0.15, $p<0.001$), as is shown in **figure 2**. In a univariate analysis all reported subtypes of hypoxia (increased villous maturation, infarction, small placental size and ischemic changes showed a significant relation with BNP. (**Table 2**) After correction for gestational age, birth weight, and signs of perinatal hypoxia multivariate analysis showed that placental infarction and the presence of NRBC were the most prominent factors for BNP elevation (r^2 0.35, <0.01). However, when correcting for gender, there was a male predominance in the

relation between hypoxia lesions (placental infarction, small placental size) and BNP levels. Where in females hypoxia was the determining factor (r^2 0.35, $p<0.01$) with little added value of placental pathology, in males, placental infarction added significantly to the regression model (r^2 0.46, $p<0.001$). When a cut-off point of 150 pmol/L was chosen (p75 for this population and significantly elevated according to adult reference ranges) the male predominance was emphasized in the relation between placental infarction, small placental size and elevated BNP levels. (**Table 3**) No relation between BNP levels and PE or HELLP was found (r 0.15, $p>0.05$; r 0.01, $p>0.05$).

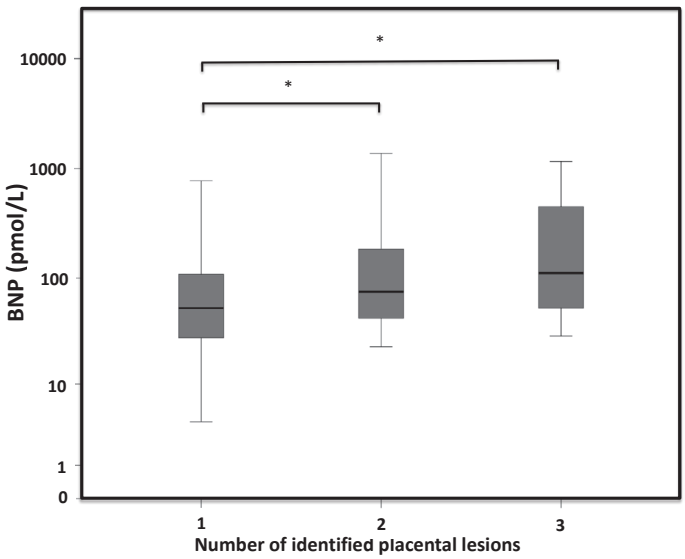

Figure 2. Box and whisker plot for BNP serum levels on a logarithmic scale related to an increasing number of placenta pathologic findings (placental infarction, signs of ischemia, increased villous maturation). 1 = one of the findings was found, 2 = 2 out of 3, 3 = all of the possible signs were found. * $p<0.05$

Table 2. Characteristics of the study population

Gestational age (w)	29.5 (24.3-31.9)
Birth weight (g)	1195 (540-2200)
Male	65 (48.5)
Multiple gestation	35 (26)
Corticosteroids	102 (76)
Inborn	131 (97.8)
Caesarian section	86 (64.2)
Maternal illness	
• Preeclampsia	34 (25.4)
• HELLP	12 (9)
• Chorioamnionitis (clin)	18 (13.4)
BNP (pmol/L)	76 (4-1375)
Thrombocytes at admission	219 (48-443)
NRBC	1.54 (0.12-45.2)
Apgar score 1 minute	7 (0-10)
Apgar score 5 minutes	8 (2-10)

Descriptive data are presented as medians with ranges or n (%). HELLP = Hemolysis, elevated liver proteins and low platelet count; clin = clinical diagnosis; BNP = B-type natriuretic peptide; NRBC = Nucleated red blood cells

Table 3. Incidence of placental lesions related to elevated levels of B-type natriuretic peptide.

	BNP<150 pmol/L n=99	**BNP>150 pmol/L N=34**	**p**
Funiculitis	20 (20)	3 (9)	NS
Chorioamnionitis	41 (41)	3 (9)	<0.01 male/female
Vilitis	10 (10)	1 (3)	NS
Vasculitis	2 (2)	0 (0)	NS
Infarction	23 (23)	18 (53)	male: <0.01 female: NS
Fetal trombosis	4 (4)	2 (6)	NS
Size <p10	26 (26)	16 (47)	male: <0.01 female: NS

Descriptive data is presented as n (%). Abbreviations: BNP = B-type natriuretic peptide, NS = not significant.

DISCUSSION

In this study, we evaluated the placental pathology reports of very preterm infants. Pathologic lesions in the placenta of the very preterm infant were frequently reported. In infants with elevated BNP levels after birth, the placenta often showed signs of ischemia, infarctions, increased villous maturation, and small size. If more lesions were present, BNP level was more elevated. Furthermore, a male predominance was found in the relation between placental infarction and elevated BNP levels. Chorioamnionitis

showed no correlation with BNP levels. To our knowledge this study is the first to show a correlation between elevated BNP levels and placental pathology, especially the relation with signs of ischemia.

Placental lesions have been associated with significant morbidity and mortality. (13) Although antenatal Doppler imaging can provide information on fetal cardiovascular adaptation, placental insufficiency can go undetected. (5,17) Placental pathologic examination will provide evidence of ischemic changes and can assist in the prediction of short- and long-term outcome. (11,18) However, the careful placental examination takes time, and the reports are, unfortunately, not always available to the pediatrician caring for a newborn infant. Early BNP measurement showed a close correlation with placental lesions and can, directly after birth, suggest the presence of these lesions, later proven by further pathologic examination.

In this study, only placental pathologic reports were used, instead of a complete review of pathologic histological findings. There is a possibility that significant findings could have been underreported. (19-21) A full histological study would have provided a more detailed view and description. However, we suggest that our detailed and protocolled pathological reports, mostly reviewed by an experienced single pathologist, provided enough information to come to relevant conclusions.

Because placental examination was not performed, most of the out-born infants could not be included in the study. Sudden onset of labor or serious fetal distress prevented antenatal transfer to our hospital. Although it is possible that the results of these infants would have altered our results, the BNP levels of the excluded infants did not significantly differ from the included infants, making this less likely. It is possible that physiological perinatal cardiovascular adaptation could have had an effect on BNP levels. (22) However, this cannot fully explain the differences found.

It is known that placental dysfunction is more common in pregnancies with male infants. Male sex is an independent risk factor for adverse pregnancy outcome. The placenta of the male fetus is suggested to be more prone to severe dysfunction as diagnosed by Doppler ultrasound. (23,24) In this study, we found a relation between placental lesions and elevated BNP levels in male infants, suggesting an important role for placental dysfunction in BNP elevation in a male fetus.

In a study done by Vinnars *et al.* the severity of clinical manifestations of PE was correlated to the amount of placental infarction. In mild and severe PE they found that a large proportion of placentas had histological signs of ischemia. Accelerated villous maturation was found in 53.3% of the mild and in 71.6% of the mothers with severe preeclampsia. (25) Our research supports a strong correlation between placental lesions and PE. However, in our study PE showed no correlation with BNP. This could suggest that changes in the placenta related to preeclampsia do not directly lead to fetal cardiovascular adaptation. Nevertheless, if the placental function is compromised

and leads to relative hypoxia, the fetal cardiovascular system might be comprised expressed by increased BNP. This is in line with an earlier study reported by our group. We found that elevated levels of BNP were more frequently found in small for gestational age infants and that signs of perinatal hypoxia were related to increased BNP levels. (5) The strong relation between BNP, NRBC and placental infarctions in this study underlines this.

Harteman *et al.* found, in infants with an atypical presentation of periventricular hemorrhagic infarction, that fetal thrombosis, a small placenta (<p10), and placental infarction were more common. The early antenatal presentation possibly agrees with our finding of an adapting and possible compromised cardiovascular system which could lead to a cerebral vascular incident. (8)

Roescher *et al.* related placental pathology to illness severity in preterm infants in the first 24 hours after birth. They found a relation between illness severity and elevated nucleated red blood cells. (9) Although our study did not investigate the newborn period, we did find a relation between NRBC, placental infarction and elevated BNP levels at birth. Fetal cardiovascular adaptation possibly predisposes these infants to more extra-uterine problems.

Elevated BNP levels in the early neonatal period are associated with the incidence of placental pathologic lesions, especially in the male infant, and hypoxia. If BNP can be used to identify those preterm infants at risk for an adverse outcome needs further study.

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Chapter 7

Early detection of prenatal cardiocirculatory compromise in small for gestational age infants

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ABSTRACT

Background Impairment of gas and substrate exchange through the placenta leads to fetal hypoxia and growth restriction. Oxygenation of vital organs is maintained with preferential perfusion at the expense of less vital organs, challenging the fetal cardiovascular system.

Objectives To identify cardiovascular compromise in preterm small for gestational age (SGA) infants using the cardiac biomarker B-type natriuretic peptide (BNP), which indicates the workload of the myocardium.

Methods In this retrospective case-control study, 26 SGA infants born at less than 32 weeks of gestation from October 2009 to October 2010 were matched for gestational age and month of birth with 26 appropriate for gestational age (AGA) infants. Antenatal Doppler ultrasound was used to identify fetal hemodynamic changes by determination of the pulsatility index (PI) of the middle cerebral artery (MCA-PI), umbilical artery (UA-PI) and ductus venosus (DV-PIV). These indices were compared with BNP levels obtained within 6 h after birth.

Results Antenatal PIs of MCA, UA and DV were significantly related to elevated BNP levels after birth in SGA infants, but not in AGA infants (SGA: MCA-PI= r^2 0.23, $p<0.05$; UA-PI= r^2 0.46, $p<0.01$; DV-PIV= r^2 0.31, $p<0.05$). Furthermore, signs of perinatal (chronic) asphyxia coincided with elevated levels of BNP. SGA was related to more postnatal cardiocirculatory complications. No significant relations between postnatal cardiac ultrasound measurements, placenta size and BNP were found.

Conclusion BNP levels were elevated early after birth in SGA infants and corresponded positively with Doppler indices of circulatory compromise. This suggests an increased workload of the myocardium.

INTRODUCTION

Impairment of gas and substrate exchange through the placenta leads to fetal hypoxia and growth restriction. Oxygenation to vital organs is maintained to some extent by an altered systemic vascular resistance and tachycardia with preferential perfusion at the expense of less vital organ systems such as the intestines and kidneys. (1,2) This intrauterine adaptation of the fetal circulation due to growth restriction persists after birth and may have long lasting effects on blood pressure and myocardial function. (3-5)

B-type natriuretic peptide (BNP), an endogenous peptide hormone, is secreted by cardiac ventricles in response to an increased wall stress and ventricular filling pressure. BNP concentrations in plasma correspond well with echocardiographic findings of ventricular strain. (6) Circulating levels of BNP and N-terminal-proBNP (NT-proBNP) are high at birth and decline immediately postpartum, likely due to transitional changes in the neonatal circulation. BNP does not cross the placenta or reflect maternal values at birth. (7) Elevated levels of BNP are found after perinatal asphyxia and in umbilical cord blood samples of growth restricted infants. (8-10)

The objective of this study was to investigate if BNP levels determined in early neonatal life were related to the extent of changes of the fetal and neonatal cardiovascular system.

METHODS

In a retrospective case control study, all infants born at less than 32 weeks of gestation and admitted to the neonatal intensive care unit of the University Medical Center Utrecht from October 2009 to November 2010 were included in the study. Only those infants who had Doppler-ultrasound assessments of the umbilical artery (UA), middle cerebral artery (MCA) and ductus venosus (DV) were included in the study. Small for gestational age (SGA) infants were matched to appropriate for gestational age (AGA) infants in such a way that for each SGA infant the next born AGA infant with similar gestational age was selected. SGA was defined as a birth weight 2 SD below the mean for the gestational age according to the Dutch growth charts of Kloosterman. (11) This study was part of a prospective follow-up study investigating the usefulness of cardiac ultrasound, near-infrared spectroscopy and BNP in preterm infants with suspected hemodynamically significant patent ductus arteriosus (hsPDA). Information on the clinical characteristics, gestational age, birth weight, gender, antenatal corticosteroids, pre- and perinatal history, associated illness and perinatal medication use of mother and infant was collected from an electronic database. Hemodynamic

data during the first 24 h after birth were recorded. Fetal distress was defined as abnormalities in fetal diagnostic test (cardiotocogram abnormalities, fetal scalp blood sample) leading to a prompt therapeutic intervention. Acute asphyxia was defined as signs of fetal distress with postnatal complications, i.e. Apgar score of less than 5 after 5 minutes and/or meconium stained amniotic fluid and/or umbilical pH of less than 7.00 and/or serum lactate above 10 mmol/l. Chronic asphyxia was defined as signs of fetal hypoxia, such as abnormal fetal heart rate tracings during the antenatal period, intrauterine growth retardation, oligohydramnios, abnormal Doppler findings, elevated levels of nucleated red blood cells (NRBC) and/or thrombocytopenia. (1,12,13) Neonatal morbidity was defined as presence of any of the following complications: respiratory distress syndrome, bronchopulmonary dysplasia, hsPDA, periventricular-intraventricular hemorrhage, cystic periventricular leukomalacia, necrotizing enterocolitis, or proven early-onset sepsis. The diagnosis of PDA was based on clinical indices and confirmed by echocardiographic investigation (left atrium/aorta ratio: >1.4; internal ductal diameter: >1.4 mm; left pulmonary artery end diastolic flow: >0.2 m/s). (14) Early-onset sepsis was defined as a positive result on one or more blood cultures of drawn within the first 72 h of life, together with clinical signs or symptoms suggestive of infection. (15)

This study was conducted following the guidelines of the local ethics committee, and parental consent was obtained for the anonymous use of patient data. This study was exempt from ethics committee approval; blood samples were taken as part of the prospective study for which approval from the medical ethics committee was obtained.

Antenatal Investigation

Parameters of antenatal investigation (biometry and Doppler ultrasound assessments of the UA, MCA and DV) were retrieved from an electronic ultrasound database (MOSOS-U version 11.00, BMA, Houten, the Netherlands). Pulsatility index (PI) of the UA (UA-PI), MCA (MCA-PI) and the pulsatility index for veins of the DV (DV-PIV) were determined in at least 3 reproducible waveforms. Doppler findings were considered abnormal if the mean PI calculated over 3-5 cycles was more than 2 SD above or below the mean, with or without absent or reversed end-diastolic flow. (2,16) Intrauterine growth restriction was defined as antenatal growth below the 10^{th} percentile for gestational age. (17)

Placental Pathology

Placental pathology was performed if the infant was born in our hospital. A senior pathologist with expertise in the perinatal field routinely examined the placenta. The placentas were weighted after trimming the cord and membranes. Placental weight

was related to gestational age. All macroscopically detected focal changes in the placentas were investigated. Histologically, infarction was recognized as an area of ischemic necrosis of the villi. The extent of infarction was related to the total volume of the placenta.

BNP Measurements

BNP measurements were done on admission within 6 h after birth. Blood samples (0.6 ml per sample) were collected from an indwelling arterial or venous catheter into a standard collection vial with ethylenediaminetetraaceticacid (Capijet®, VWR, West Chester, PA, USA). BNP was directly analyzed on a DxI 800 immunochemistry system (Beckman Coulter Diagnostics, Brea, CA, USA). The lower limit of detection for this assay is 5 pmol/l for BNP. Maintenance and quality performance of the instrument were followed according to company instructions. Blood samples were simultaneously collected with routine blood sampling.

Echocardiography

All infants were routinely screened by an experienced pediatric cardiologist on day 2. Cardiac anatomy and the hemodynamic significance of a PDA were investigated. (e.g. left atrium/aorta ratio, PDA diameter and velocity pattern, velocity pattern in abdominal arteries, velocity pattern in left pulmonary artery). Furthermore, the internal and wall dimensions of the right and left ventricle and interventricular septum were determined.

Statistical analysis

Descriptive data are presented as medians and ranges. Missing data were corrected for in the analysis. Correlations between variables were tested with Fischer's exact test. A nonparametric independent samples test, the Mann-Whitney U test, was used if the distribution was skewed. Linear regression was used to identify those factors associated with BNP levels. Multivariate analysis was performed to identify the most important factors after univariate analysis. A p value of <0.05 was considered significant. SPSS statistics 20 (IBM corporation, Armonk, New York) was used for statistical analysis.

RESULTS

In total, 187 infants were eligible for inclusion in this study. Twenty-three infants were excluded from the study: in 17 infants parental consent was refused or not obtained, 1 infant died shortly after admission and 5 infants were excluded because of

major congenital abnormalities. After exclusion for missing data and the availability of ultrasound data a total of 70 infants (AGA and SGA) were available for matching. Of these, 26 SGA infants were matched with 26 AGA infants. For the other 18 infants (6 AGA, 12 SGA), no accurate match could be made. Figure 1 summarizes the inclusion path. Maternal and patient characteristics are shown in **table 1**. No significant differences were found between the groups concerning patient maternal characteristics or complications affecting the newborn apart from birth weight. There was a trend towards more bronchopulmonary dysplasia in the SGA group, but this was not significant ($p = 0.08$). Heart rate and blood pressure in the first 24 h after birth did not differ between groups. We did, however, find that more SGA infants needed circulatory support with dopamine, suggesting a more sustained hypotension. BNP levels in the SGA group were significantly higher as compared to AGA infants (122 vs. 54 pmol/l; $p<0.05$). More signs of perinatal chronic and acute hypoxia were present in the SGA infants. We found lower platelet counts in SGA infants, especially on day 2 after birth ($108x10^9$/l vs. 212 $x10^9$/l; $p<0.0001$) and higher levels of NRBC (3.2 $x10^9$/l vs. 1.4 $x10^9$/l; $p<0.05$). Furthermore, significantly higher levels of lactate were found in the SGA infants (7.3 vs. 4.2 mmol/l; $p<0.05$). Five minute Apgar scores (8 vs. 8; $p=0.19$) and UA pH levels (7.25 vs. 7.30; $p=0.08$) were not significantly different. The SGA infants showed significantly lower MCA-PI values (1.31 vs. 1.70; $p<0.01$) and higher UA-PI levels (1.55 vs. 1.14; $p<0.01$;**table 2**) No significant differences were found

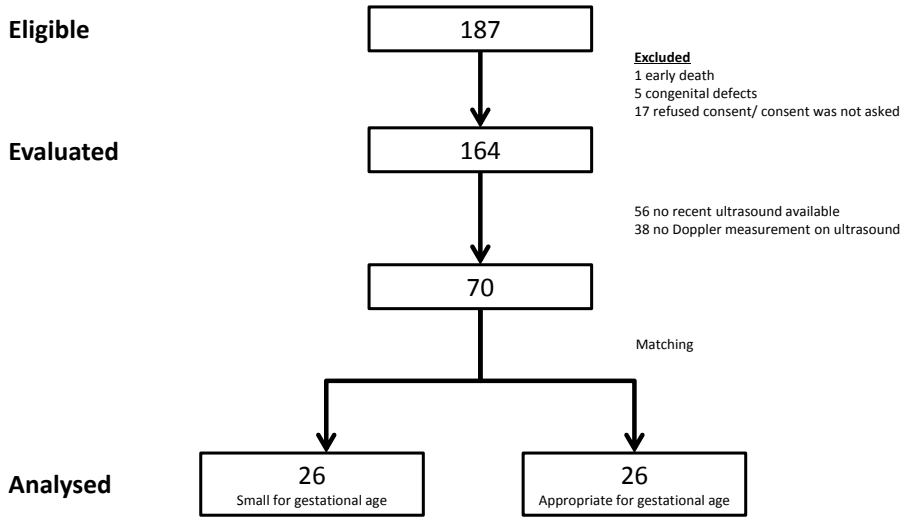

Figure 1. Flow diagram representing the study group
Flow diagram shows the eligible infants for this study. From a total of 70 infants, 26 SGA infants were matched with 26 AGA infants for gestational age and period of birth. For 18 infants no accurate match could be made.

between the groups for internal and wall dimensions of right and left ventricle and interventricular septum, only the left ventricular end diastolic diameter was found to be lower in the group, which correlated with the smaller size of the infant.

Table 1. Characteristics of the study groups

	SGA infants (n=26)	AGA infants (n=26)	p
Gestational age, weeks	29.4 (25.3-31.0)	29.6 (24.7-31.6)	0.43
Birth weight, g	857 (540-1400)	1335 (550-2200)	<0.001
Male/ female	11/15	13/13	0.78
Antenatal steroids	26 (100)	23 (88)	0.24
Caesarian section	22 (85)	21 (81)	1.0
Maternal characteristics			
• Preeclampsia	11 (42)	7 (27)	0.38
• HELLP [a]	3 (12)	2 (8)	1.0
• Labetalol IV	2 (8)	0	0.49
• Maternal age	29 (20-43)	30 (20-41)	0.36
• Chorioamnionitis	3 (12)	7 (27)	0.29
Infant characteristics			
• 5 min Apgar score	8 (5-10)	8 (6-10)	0.19
• UA pH	7.25 (6.95-7.38)	7.30 (7.22-7.36)	0.08
• Surfactant	10 (38)	7 (27)	0.55
• BPD [b]	8 (31)	2 (8)	0.08
• hsPDA [c]	4 (15)	3 (12)	1.0
• Any PIVH [d]	3 (12)	4 (15)	1.0
Circulatory characteristics			
• Dopamine	8 (31)	2 (8)	<0.05
• BNP pmol/l	122 (23-1162)	54 (8-533)	<0.05

Descriptive data are presented as medians and ranges or n (%). [a] HELLP – Hemolysis, elevated liver proteins and low platelet count; [b] BPD – bronchopulmonary dysplasia; [c]hsPDA – hemodynamically significant patent ductus arteriosus; [d] PIVH – periventricular/ intraventricular hemorrhage

B-type Natriuretic Peptide

In SGA infants, BNP levels were related to low thrombocyte counts and NRBC. Furthermore, a significant relation was found between BNP and Doppler imaging of the MCA, UA and DV. Most prominent was the relation with UA-PI (r^2 0.46, $p<0.001$) and MCA-PI (r^2 0.23, $p<0.05$), further emphasized by their ratio (r^2 0.52, $p<0.001$;**table 3**). After multivariate analysis, elevated levels of NRBC in peripheral blood and the UA-PI/ MCA-PI ratio remained the most prominent factors in BNP elevation in SGA infants

Table 2. Antenatal Doppler imaging and placenta

	SGA infants (n=26)	AGA infants (n=26)	p
Doppler imaging			
MCA-PI [a]	1.31 (0.94-2.05)	1.70 (0.86-2.12)	<0.01
MCA-V_{max} [b] ,cm/s	42 (32-64)	41 (23-69)	0.84
UA-PI [c]	1.55 (0.50-2.70)	1.14 (0.70-1.92)	<0.01
DV-PIV [d]	0.66 (0.31-1.17)	0.64 (0.39-0.80)	0.76
Placenta			
• Umbilical cord coil, coils/cm	0.15 (0.03-0.53)	0.13 (0.03-0.48)	0.17
• Infarction >5%	12 (46)	8 (31)	0.26
• Growth restriction	15 (58)	5 (19)	<0.01

Descriptive data are presented as medians and ranges or n (%). [a] MCA-PI – pulsatility index of the middle cerebral artery, [b] MCA-V_{max} – Maximum flow velocity of the middle cerebral artery, [c] UA-PI – pulsatility index of the umbilical artery, [d] DV-PIV – pulsatility index of veins of the ductus venosus

Table 3. Variables associated with elevated BNP levels (univariate and multivariate analysis)

Univariate analysis	r^2 (p value)		
	total group	SGA	AGA
Birth weight	0.17 (<0.01)	0.23 (<0.05)	0.001 (0.87)
Gestational age	0.09 (0.50)	0.01 (0.73)	0.00 (0.95)
NRBC	0.33 (<0.001)	0.29 (<0.01)	0.06 (0.23)
5-minute Apgar score	0.16 (<0.01)	0.20 (<0.05)	0.04 (0.32)
UA pH	0.23 (<0.01)	0.19 (0.12)	0.07 (0.25)
Platelet count [a]	0.31 (<0.001)	0.26 (<0.01)	0.21 (<0.05)
Platelet count [b]	0.21 (<0.01)	0.12 (0.08)	0.26 (<0.01)
Base excess	0.25 (<0.01)	0.32 (<0.05)	0.09 (0.19)
Lactate	0.36 (<0.001)	0.48 (<0.001)	0.00 (0.99)
Corticosteroids (days before birth)	0.07 (0.07)	0.13 (0.08)	0.02 (0.56)
Placental size	0.15 (<0.01)	0.14 (0.07)	0.03 (0.45)
Growth restriction (weeks)	0.24 (<0.001)	0.21 (<0.05)	0.02 (0.49)
MCA-PI	0.21 (<0.05)	0.23 (<0.05)	0.10 (0.38)
UA-PI	0.37 (<0.001)	0.46 (<0.001)	0.00 (0.93)
DV-PIV	0.29 (<0.01)	0.31 (<0.01)	0.22 (0.35)
Ratio UA-PI/MCA-PI	0.50 (<0.001)	0.52 (<0.001)	0.25 (0.17)
Dopamine <24h after birth	0.16 (<0.01)	0.14 (0.06)	0.03 (0.43)
PDA	0.44 (0.14)	0.00 (0.95)	0.66 (<0.001)
Multivariate analysis			
Ratio UA/ MCA -PI *and* NRBC		0.63 (<0.001)	
PDA			0.66 (<0.001)

[a] On admission to the NICU. [b] On day 2 of admission.

(r^2 0.63, p<0.001;**table 3**). No such relations were found for AGA infants. In AGA infants, elevated levels of BNP were found in those infants with a low thrombocyte count on admission and on day 2 after birth. Furthermore, elevated levels were found in those infants who would go on to develop hsPDA (r^2 0.66, p<0.001).

DISCUSSION

This is the first study investigating the use of BNP to reveal the extent of prenatal cardiovascular alterations in very preterm SGA infants. The present study showed that perinatal cardiovascular alterations were common in very preterm SGA infants when compared to their AGA counterparts. It was shown that BNP levels at birth were elevated in those infants with significant prenatal changes in fetal hemodynamics, as indicated by a strong relationship between prenatal Doppler ultrasound and elevated BNP levels after birth. Infants with elevated BNP levels were more likely to need postnatal resuscitation, as indicated by lower 5 min Apgar scores, and postnatal cardiovascular support, as indicated by increased use of dopamine within 24 h after birth. Hypoxia and fetal cardiovascular alterations appeared to be the most determining factors in the elevation of BNP levels at birth in preterm infants.

The placenta is characterized by a low pressure perfusion system. If placental function is compromised, vascular resistance increases, leading to more strain on the fetal cardiovascular system as indicated by an increased UA-PI. (18,19) A raised BNP is expected, as was found in the SGA infants, when the myocardium is exposed to an increased workload. (9) If this condition continues, the fetus is prone to perinatal hypoxic-ischemic incidents as was suggested by the relationship between BNP and parameters indicating chronic and acute asphyxia in this study. (1) Antenatal stress and low Apgar scores have been associated with elevated BNP and NT-proBNP levels. (20)

A limitation of this study is that only a relatively small number of infants was included. However, significant results were found. A second limitation may be that matched controls were used, which limits the possibility of extrapolating our findings to the general population; however, we have shown that our findings are most prominent in the high-risk SGA population. Furthermore, only limited echocardiographic information was available, newer techniques such as tissue Doppler imaging or Speckle tracking could have been used to further investigate the postnatal cardiac function.

It is possible that physiologic perinatal cardiovascular adaptation would have had an effect on BNP levels. (21) However, this cannot fully explain the difference between AGA and SGA infants or the relation with prenatal Doppler findings. We assume that postnatal BNP level difference is accentuated by the prenatal alterations of hemodynamics of SGA infants, thus indicating their postnatal maladaptation due to prenatal compromise. Our study suggests that fetal growth restriction influences BNP levels after birth. Furthermore, a ‘second hit’ such as perinatal asphyxia can aggravate the cardiovascular compromise.

Several studies have shown that intrauterine growth restriction is associated with fetal cardiovascular dysfunction, as diagnosed by echocardiography and elevated BNP levels. (8,9,18) Girsen *et al.* investigated cardiovascular hemodynamics and UA NT-proBNP in SGA infants. They reported a positive correlation between UA-PI and NT-proBNP, suggesting that an increase in placental vascular resistance leads to a rise in cardiac afterload, which is most prominent in the right ventricle. (9) Our study proposes that this finding is also true for the very preterm SGA infants with early growth restriction. In the present study we found an association between MCA-PI and BNP levels suggesting that brain sparing is prominent in very preterm SGA infants. In contrast, Iacovidou *et al.* reported lower levels of NT-proBNP in SGA infants. They suggest that active placental transport and blood flow redistribution might play a role in their findings. Our study showed opposite findings, but in the preterm SGA infant. It is possible that early growth restriction and chronic hypoxia might have a more prominent impact on cardiovascular function, which might explain the difference of our findings as compared to those of Iacovidou *et al.* (8,22) A study by Baschat *et al.* suggested worsening of fetal venous flow as an indicator of further clinical deterioration. (23) In our study, a significant relationship was found between BNP and DV-PIV, but in the multivariate analysis this was not the most prominent determinant of BNP increase. It is, however, possible that because antenatal Doppler imaging is used as a screening method for intervention, most infants are born before placental dysfunction becomes severe enough to cause significant postnatal cardiovascular compromise. BNP may be able to identify those with prenatal cardiovascular compromise where routine Doppler ultrasound was not performed.

Rakza *et al.* described an earlier presentation of hsPDA in SGA infants. They found a higher rate of hsPDA in SGA infants. In this study no difference in PDA occurrence was found. (24) We did find, as did a study by Lee *et al*, early BNP elevation to be related to a hsPDA in the AGA group. Although this is an interesting find, it is beyond the scope of this paper. (25)

In conclusion, BNP is elevated in very preterm SGA infants with evidence of chronic or acute hypoxia in the perinatal period. Since we were not able to firmly show postnatal cardiac dysfunction, it remains to be investigated if elevated BNP levels are also indicative for postnatal cardiovascular compromise. Whether our results in SGA infants are related to long-term (neurodevelopmental) outcome needs further investigation.

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Chapter 8

Discussion and future perspectives

IS THERE A ROLE FOR CARDIAC BIOMARKERS IN NEONATOLOGY?

Cardiac biomarkers are part of routine clinical care in adult patients. In the diagnosis of myocardial infarction and heart failure, troponins and natriuretic peptides are considered "the gold standard". (1,2) In recent years, cardiac biomarkers were introduced into Neonatology. The aim of this thesis was to investigate the clinical role of these biomarkers in postnatal adaptation and neonatal complications and to establish a link with short- and long-term outcome. As the research developed, it became apparent that the newborn couldn't be investigated without information about the fetal and perinatal period. (**Introduction**)

Cardiac biomarkers are used to identify cardiac dysfunction and failure in term and preterm infants. Natriuretic peptide levels are elevated in atrial (atrial natriuretic peptide [ANP]) or ventricular strain (B-type natriuretic peptide [BNP]). (3) These markers correspond with cardiac function. (4) BNP or the inactive N-terminal fragment of BNP (NT-proBNP) is presently preferred, due to a longer half-life and better relation to cardiac function. (3,5) Cardiac troponins (cTn) are used to assess cardiomyocyte compromise. Affected cardiomyocytes release troponin into the bloodstream, resulting in elevated levels. In **chapter 2**, the current role of these cardiac biomarkers was discussed as a starting point for further research. Natriuretic peptides and cardiac troponins are suggested as potential biomarkers in the diagnosis and treatment of neonatal disease complicated by circulatory compromise. However, some limitations are addressed. First, precise reference ranges are scarce. Furthermore, several assays are used to measure natriuretic peptide and troponin levels, making comparison between different studies difficult. (6-8) Third, normal physiologic adaptation, before and after birth, can affect cardiac biomarker levels. (8-10) These changes can influence study results in pathophysiologic investigations. Research findings will be more accessible to clinicians when changes in cardiac biomarkers, rather than absolute values, are reported. Cardiocirculatory compromise and cardiac dysfunction are common in newborn infants. Several pathophysiologic processes are identified where knowledge about cardiac biomarkers could benefit diagnosis, treatment, and prognosis.

TERM INFANT

Perinatal asphyxia

Perinatal asphyxia (and subsequent encephalopathy) remains one of the principal contributors to mortality and morbidity in term infants. (11) Cardiovascular compromise can contribute to an adverse outcome after perinatal asphyxia. (12,13) Few studies have investigated the role of natriuretic peptides in identifying significant cardiovas-

cular compromise after perinatal asphyxia. (14,15) Hypoxia results in cardiomyocyte damage, which causes a subsequent increase of cTn in serum. (16) Several authors have reported elevated levels of cTn (T and I) after perinatal asphyxia and suggested a relation with (echocardiographic signs of) myocardial injury and compromised function. Furthermore, associations with short-term outcome were described. (17-22)

Perinatal asphyxia and hypothermia

The introduction of hypothermia as a causative treatment for neonatal encephalopathy has improved outcome. (23) In **chapter 3**, the influence of hypothermia on cardiovascular function after perinatal asphyxia was evaluated. Hypothermia influences circulation after hypothermia with bradycardia and reduced cardiac output. (24) Animal studies have suggested a cardioprotective effect. (25) The aim was to investigate the cardioprotective effect in newborn infants after perinatal asphyxia. When compared to a historical non-hypothermia treated group, BNP levels were significantly lower at 48h after birth and after rewarming at 72h (144 pmol/L vs. 74 pmol/L and 182 pmol/L vs. 43 pmol/L). In both groups, elevated levels of cTnI were found. However, no significant differences were found. The lower levels of BNP indicated a beneficial effect on cardiac function in the hypothermia group.

This study adds to the knowledge that perinatal asphyxia can cause significant cardiocirculatory compromise and that the cardiac biomarkers can be used to identify it. For cTnI, a decline can be seen in the days after the hypoxic incident (**chapter 2, figure 2**). This decline suggests a pre- or perinatal event and a subsequent clearance of cTnI from the circulation. The hypoxic episode in infants may occur hours before birth and can involve repeated and prolonged periods of hypoxia. How hypothermia influences later adaptation to postnatal life and recovery to the hypoxic-ischemic incident event could not be determined with cTnI in this study. (21)

With hypothermia as an established therapy to reduce brain damage after perinatal asphyxia, a randomized study looking specifically at the cardioprotective effect of hypothermia would be unethical. (23) Nevertheless, multi-organ failure with cardiocirculatory compromise remains a significant complication after perinatal asphyxia and requires careful monitoring. Cardiac biomarkers can contribute to the assessment of the cardiovascular system of asphyxiated newborn, possibly in conjunction with clinical investigation, such as near-infrared spectroscopy and functional echocardiography. (26,27) Furthermore, the assessment of cardiac biomarkers after perinatal asphyxia could provide information on short- and long-term outcome.

Perinatal asphyxia and outcome

Predicting outcome after perinatal asphyxia remains a challenge. Many biomarkers have been studied in an effort to establish an early prognosis, especially for long-term

neurodevelopment. (28) The introduction of hypothermia has influenced the usefulness of previously established biomarkers. (29,30) Cardiocirculatory dysfunction might compromise cerebral circulation, influencing neurodevelopmental outcome. (13) The hypothesis was that cardiac biomarkers could aid in predicting short- and long-term outcome in hypothermia-treated infants. (**Chapter 4**) In 64 infants with neonatal encephalopathy, cardiac biomarkers BNP and cTnI were related to short- and long-term outcome. In surviving infants, early (< 48h after birth) BNP levels were associated with short-term outcome. This included days on the NICU (r 0.46, $p<0.01$), days on respiratory support (r 0.49, $p<0.001$), and a need for cardiovascular support (r 0.37, $p<0.05$). This correlation was partly explained by the significantly higher levels of BNP in infants with persistent pulmonary hypertension (PPHN) (median, 124 pmol/L vs. 608 pmol/L). This relation was discussed further in **chapter 5**. Additionally, early cTnI levels were related to the number of days on respiratory support (r 0.44, $p<0.05$) and the stay on the NICU, both in surviving infants (r 0.46, $p<0.05$). Troponin was significantly higher in infants who died. A cTnI level of >0.15 µgr/L at 24h had a limited sensitivity (70%) and specificity (77%) in predicting mortality.

BNP was related to long-term outcome in the surviving infants. A correlation with the cognitive composite score (CCS) of the Bayley scale of infant and toddler development (BSITD-III) was found. (31) A higher BNP level at 24h (r 0.51, $p<0.001$) and 48h (r 0.45, $p<0.01$) was related to a lower CCS. As stated earlier, BNP is influenced by PPHN, but even after correcting for PPHN, the association remained statistically significant. Furthermore, a significant association was found between cTnI and motor composite score (MCS) (r 0.39, $p<0.05$) and the onset of walking (r 0.36, $p<0.05$). Additionally, brain injury assessed by cerebral MR imaging was investigated. With diffusion-weighted imaging, apparent diffusion coefficient (ADC) values were obtained. Lower ADC values are a marker for cytotoxic edema, indicating ischemia in the measured area and have been related to an adverse outcome. (32) Higher BNP levels were related to lower ADC measurements in the basal ganglia (r 0.37, $p<0.05$) and thalami (r 0.37, $p<0.05$). Furthermore, higher BNP levels correlated with lower ADC measurements in the white matter.

The reported correlations suggest an association between cardiovascular compromise, neurodevelopmental outcome, and mortality. However, the reported findings most likely show an association rather than a cause-effect relationship. Cardiac biomarkers could serve as a tool to investigate the level of multi-organ failure rather than an independent marker of long-term outcome. Although the reported results are not sensitive or specific enough to provide a definite prognosis, cardiac biomarkers can aid in clinical treatment.

Persistent pulmonary hypertension

Persistent pulmonary hypertension occurs when pulmonary circulation fails to undergo normal physiological transition after birth. PPHN can be idiopathic , secondary to neonatal pulmonary condition or resulting from perinatal asphyxia. Several pulmonary conditions are described, such as congenital diaphragmatic hernia, respiratory distress syndrome, pneumonia, meconium aspiration syndrome (MAS), and transient tachypnea of the newborn. (33,34) When PPHN is suspected, echocardiographic evaluation is necessary to exclude congenital heart disease (CHD) and to confirm high pulmonary pressure. (35) BNP has been suggested as an early indicator of PPHN in the absence of CHD. (36) In **chapter 5**, we investigated whether BNP would be a useful biomarker to evaluate the course of PPNH and the effectiveness of treatment. In 21 infants, the development of BNP during a treatment with inhaled nitrous oxide (iNO) was evaluated. BNP was elevated at the start of therapy. Interestingly, in the group who would go on to develop a rebound of PPHN, BNP levels were higher, although this was not statistically significant (median, 289 pmol/L vs. 147 pmol/L). Furthermore, a significant correlation between BNP measurements and the maximum velocity of the tricuspid valve regurgitation jet, an important estimation of pulmonary hypertension, was found (r 0.67, p <0.001). This association illustrates the relationship between BNP and echocardiographic findings. An increase in BNP levels was found after weaning from or cessation of the iNO treatment, which suggests an increase in cardiac workload. However, this increase was significantly higher in infants who developed a rebound PPHN (median, 283 pmol/L to 1232 pmol/L vs. 98 pmol/L to 159 pmol/L). Interestingly, this increase preceded the onset of clinical symptoms and thus predicted the onset of a clinical rebound. A rebound PPHN will prolong the need for iNO treatment and possibly the time spent on the NICU, however the number of days on the iNO was not significantly different between the groups in our study. No definite reference ranges or sensitivity and specificity are mentioned in our results. A well-defined cut-off for rebound could be calculated with high sensitivity and specificity. However, as reference ranges can differ between different arrays and methods of detection, the significant rise in BNP was emphasized as the most important marker and not an absolute number. To be of additional value in the treatment, BNP must be measured repetitively and longitudinally.

After this study, BNP has been introduced as a marker of rebound of PPHN in infants weaning from iNO. In several infants who showed a sharp increase in BNP during weaning, the treatment was intensified to prevent a clinical rebound. BNP can support clinical decision making in PPHN treatment.

In conclusion, the investigated cardiac biomarkers, BNP and cTnI, provide additional information on the circulatory status of the infant and can aid in diagnosis, treatment,

and prognosis in perinatal asphyxia and PPHN. Especially in the treatment of PPHN, BNP can be a valuable diagnostic tool.

PRETERM INFANT

The second part of this thesis is focused on the preterm infant. Preterm birth is a serious complication of pregnancy and associated with severe morbidity and mortality. (37) Although less well-specified as a clinical entity, perinatal asphyxia is common in preterm infants. Placental dysfunction can lead to (chronic) hypoxemia which can affect cardiac function. (38) Furthermore, it predisposes the preterm infant to a greater risk of further perinatal hypoxemia. Few studies are available investigating cTn in the preterm infant. (38,39) The primary focus of our study was to investigate the perinatal cardiovascular adaptation and not myocardial damage; therefore, no data on cTn in these infants was generated.

Perinatal adaptation

Placental pathology

The placenta can provide a window to the intrauterine life. Intrauterine pathologic processes can compromise postnatal cardiac function. (40) Impaired cardiovascular function may complicate the neonatal period, especially in preterm infants. Placental pathologic patterns have been associated with several diseases affecting the infant after birth. In **chapter 6**, the relationship between placental pathologic findings in very preterm infants and signs of perinatal cardiovascular adaptation were studied. Of 133 infants, the pathologic reports were retrospectively reviewed and related to BNP levels after birth. Ischemic changes in the placenta, such as increased villous maturation, infarctions, and small placental size were associated with higher BNP levels after birth (r^2 0.15, $p<0.01$). Placental infarction and the presence of nucleated red blood cells (NRBC) showed the closest correlation to BNP elevation (r^2 0.35, $p<0.01$). However, when correcting for gender, the association between placental infarction and BNP elevation was found only in male infants . In females, the addition of the placental pathology to NRBC did not change the relationship (r^2 0.35, $p<0.01$). In males, placental infarction significantly improved the correlation (r^2 0.46, $p<0.001$). The relation between BNP and NRBC is discussed more in detail in **chapter 7**.

This study emphasized the importance of placental pathology after preterm birth. Many interesting pathologic findings would go unnoticed if the pathologic examination was not performed. Furthermore, an association between signs of intrauterine hypoxia (NRBC) and elevated levels of BNP after birth was found. In male infants,

placental infarctions added to the correlation. Why this was true for males could not be established. It has been suggested that the male placenta is more prone to severe dysfunction. (41,42) However, this does not entirely explain the similar BNP levels between male and female infants in this study. Possibly, the placenta is more affected by the underlying pathologic process in males than in females. To prove that the placenta of male infants is affected differently, more research is needed.

Small for gestational age

Infants with intrauterine growth retardation (IUGR) are those most affected by placental dysfunction. An altered vascular resistance and tachycardia maintain oxygenation and perfusion of vital organ systems at the expense of less vital organ systems. (43,44) Based on the findings in **chapter 6**, where hypoxia was related to BNP elevation, one would expect higher BNP levels in infants with IUGR. In **chapter 7**, we studied whether the relation of elevated BNP levels with adaptations of the fetal and neonatal cardiovascular system. In a matched retrospective case-control study with 52 infants, we investigated the relation between postnatal BNP levels and antenatal Doppler ultrasound-determined blood flow velocities and their ratios of the umbilical artery (UA), the middle cerebral artery (MCA), and the venous duct (DV). Doppler ultrasound can be used to identify compromise of the fetal circulation. (45) Furthermore, several clinical characteristics of the appropriate for gestational age (AGA) and the small for gestational age infant (SGA) were investigated. BNP levels were higher in SGA infants (122 pmol/L vs. 54 pmol/L). In the SGA infant, clinical signs of prenatal and perinatal hypoxia were related to elevated BNP levels after birth (NRBC, serum lactate, Apgar score, thrombocytopenia, umbilical pH). More interestingly, a relationship was found between fetal cardiocirculatory adaptation, as diagnosed by antenatal Doppler ultrasound (UA pulsatility index/ MCA pulsatility index ratio, r^2 0.50, $p<0.001$) and elevated BNP levels after birth. In AGA infants, a correlation was found with thrombocytopenia and, remarkably, a patent ductus arteriosus, which is discussed later.

Patent Ductus Arteriosus

A hemodynamically significant patent ductus arteriosus (hsPDA) remains a diagnostic and therapeutic challenge. (46,47) Although no clear benefit has been shown for routine closure, some infants will have significant left atrial and ventricular overload. A specific marker of ventricular strain could provide information on hemodynamic significance and may help to identify those infants who will benefit from treatment. Natriuretic peptides have been suggested as markers of hemodynamic significance of the PDA. (48-57) In **chapter 2**, after a literature review, a table with proposed cut-off levels for BNP and NT-proBNP in the identification of an hsPDA is presented. The wide range of cut-off values signify the difficulty of incorporating natriuretic peptides to

treatment protocols without local reference ranges. In **chapter 7**, a new challenge was identified, especially for early identification of an hsPDA. As discussed earlier, higher levels of BNP were found in SGA infants and in infants with marked fetal cardiocirculatory adaptation. It would be difficult to differentiate between high BNP levels as a result of adaptation and an hsPDA. Interestingly, in AGA infants, elevated levels were found in those infants who would go on to develop hsPDA (r^2 0.66, $p<0.001$). This study was done in a small and matched group of preterm infants. Therefore, the clinical significance is limited and needs further study. (58) Further investigation is necessary to determine if natriuretic peptides can add to current treatment protocols and improve outcome.

(Persistent) Pulmonary Hypertension

PPHN is not limited to term infants. In **chapter 5**, the majority of the infants was term or near-term. Only two preterm infants were included. In these infants, the same BNP pattern was found as in the term and near-term infants. Treatment with iNO in preterm infants is controversial. (59) However, iNO can be used as a last resort in severe respiratory failure. No significant benefit has been observed in mortality or morbidity apart from short-term improvement of oxygenation. (60) It is unlikely that the role of BNP in the treatment of PPHN in preterm infants will be further elucidated. Nevertheless, the very limited evidence provided by our study suggests a potential role in treatment guidance.

Pulmonary hypertension and ventricular dysfunction can be complications of bronchopulmonary dysplasia. (61,62) A possible role of natriuretic peptides is suggested in **chapter 2**. (63) Given the underlying pathophysiological principle, natriuretic peptides might make an easy screening tool, but further research is needed.

In conclusion, in the preterm infant, elevated levels of BNP are found in those infants with significant or suspected cardiovascular compromise. This compromise can be of prenatal or perinatal origin and is accentuated by hypoxia. BNP can aid in the identification of infants with significant compromise, especially when this has not been established earlier with antenatal Doppler imaging. However, post-natal consequences of an elevated BNP level at birth need further investigation.

CONCLUSIONS

In this thesis, the role for cardiac biomarkers in Neonatology was investigated. Several clinically relevant results were reported. In term and preterm infants, hypoxia and subsequent adaptation play an important role in cardiac biomarker elevation. The elevated natriuretic peptides are indicative of abnormal function; elevated troponins

are suggestive for cardiomyocyte damage. This methodology makes these biomarkers of additional value in the treatment of newborn infants, separate or as a combination. In the **introduction**, the characteristics of the perfect biomarker were described. It can be concluded that cardiac biomarkers are close to perfection. They are relatively inexpensive to determine, have a high reproducibility, and can be measured in most hospitals. As an indicator of a possible rebound of PPHN, BNP has a high sensitivity and specificity. However, the interpretation and implementation need further investigation.

CLINICAL PERSPECTIVES

- **BNP and cTn can be used in term infants to identify significant cardiovascular compromise after perinatal asphyxia.**
- **Elevated levels of BNP and cTn in term infants have been associated with poor outcome and MRI abnormalities after perinatal asphyxia.**
- **In the treatment of PPHN, BNP can be used to predict a rebound after weaning from or cessation of inhaled nitric oxide.**
- **Elevated levels of BNP are found in preterm infants with signs of fetal hypoxia, also expressed by placental infarctions in male infants.**
- **Elevated levels of BNP are found in SGA preterm infants, suggesting a compromised cardiovascular adaptation.**

FUTURE DIRECTIONS

Research on cardiac biomarkers and their clinical implications has intensified in the last decade. This thesis, as well as literature, suggests several possible indications. First step before cardiac biomarkers are introduced into routine clinical care is the establishment of unambiguous reference ranges adapted to the gestational age of the infant. These ranges should be described in every study reporting on biochemical markers with references to the method of detection and storage. For clinical implementation, fast detection and accurate reference ranges are a must. Until then, we must rely on changes instead of absolute numbers. To make rapid and bedside analyses possible, the role for point-of-care measurement can be evaluated. In hospitals where biomarker measurements are not routine, this could be of even more benefit. Furthermore, point-of-care analysis would decrease the amount of blood needed and could make the cardiac biomarkers more easily accessible. This easy access could open doors to more careful monitoring of the infants' circulation, hopefully in conjunction with

echocardiographic and cardiac output measurements. PPHN and perinatal asphyxia remain significant cardiovascular challenges to the clinician treating these infants. Further correlation between (functional) echocardiography and cardiac biomarkers could have a synergetic effect and improve the outcome of these infants. In clinical trials where intervention can cause or prevent cardiovascular compromise, cardiac biomarkers can be incorporated in the study.

The precise relation between cTn and the magnitude myocardial damage in infants is still unclear. Relating postmortem investigation of the myocardium to the cTn measurements, for example, after perinatal asphyxia, could address this.

Troponins were not studied in preterm infants. Possibly, cTn could have provided evidence of myocardial compromise, especially in the SGA infant. This relation can be a focus of further studies. In the presented studies, only limited references were made to the outcome of the preterm infant and the relation with cardiac biomarkers. Many of the problems affecting the preterm infant, such as bronchopulmonary dysplasia or developmental delay, can have an origin in fetal or perinatal hypoxia. If elevated BNP levels can be a marker for this needs further investigation.

Randomized trails are needed to firmly establish the benefits of cardiac biomarkers in the treatment of newborn infants. BNP in the treatment of PPHN is the first logical step.

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Hoofdstuk 9

Summary in Dutch
Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Biomarkers

Biomarkers zijn meetbare organische stoffen die gevormd worden in of bij biologische processen. Biomarkers kunnen worden gebruikt om ziektes op te sporen. Zij kunnen iets zeggen over de kans op een ziekte, hoe het verloop van de ziekte is en wat de prognose zal zijn. Biomarkers kunnen worden bepaald uit bloed, hersenvocht en urine, maar ook cellen bevatten vele (potentiele) biomarkers. Een goede biomarker moet zekerheid geven over diagnose of prognose en moet eenvoudig te bepalen en te interpreteren zijn.

Cardiale biomarkers

Cardiale biomarkers worden veel gebruikt in de geneeskunde bij volwassenen. Momenteel wordt bijvoorbeeld cardiaal troponine gebruikt om een hartinfarct vast te stellen. Het gebruik van deze biomarker heeft het eenvoudiger gemaakt om een diagnose te stellen.

In dit proefschrift worden twee cardiale biomarkers gebruikt, cardiaal troponine-I (cTnI) en B-type natriuretisch peptide (BNP). Troponine is een eiwit dat onderdeel is van het knijpsysteem van het hart. De cardiale versie komt niet in andere cellen van het lichaam voor. Troponine zit in de cel vast en wordt in het bloed nauwelijks gevonden. Bij schade aan het hart, zoals bij een infarct, komt troponine vrij in het bloed en kan vervolgens door bloedonderzoek worden gemeten. Troponine is aldus een maat voor de schade aan de hartspier.

BNP is een eiwit dat door het hart zelf wordt aangemaakt als het hart onder druk komt te staan. Deze aanmaak ontstaat indien te veel bloed moet worden rondgepompt of het hart het bloed niet meer weg krijgt, door hoge tegendruk of afsluiting van bloedvaten. BNP zorgt ervoor dat de bloedvaten wijder worden om de tegendruk te verlagen. Daarnaast zorgt BNP dat de nieren meer urine gaan produceren om de hoeveelheid vocht die het hart moet rondpompen te verminderen. BNP kan worden gebruikt om hartfalen vast te stellen. Kortom: door gebruik te maken van deze twee eiwitten is iets zeggen over beschadigingen aan en de werking van het hart.

Rondom de geboorte

Bij de geboorte verandert er veel voor een baby, vooral rondom hart en longen. Voor de geboorte krijgt de baby zuurstof en voedingsstoffen vanuit de moederkoek. Het bloed stroomt vanaf de moederkoek naar de rechterkant van het hart. Omdat de longen niet nodig zijn vóór de geboorte worden die omzeild met twee afslagen, de ductus van Botalli (een verbinding tussen de grote lichaamsslagader en de longslagader) en het foramen ovale (een verbinding tussen de twee boezems van het hart).

De bloedstroom wordt door een hoge weerstand in de longen tegengehouden. Als de baby na de geboorte begint te huilen gaan de longen open. De bloedvaten in de longen worden wijder, waardoor het bloed makkelijker door de longen kan stromen. Het bloed zal van de rechterkant van het hart naar de longen stromen en de afslagen passeren. Daarna stroomt zuurstofrijk bloed vanuit de longen terug naar het hart en wordt door de linkerkant van het hart naar de rest van het lichaam gepompt. De afslagen zullen zich vervolgens sluiten. Hiermee heeft de bloedstroom van de baby zich aangepast aan het leven buiten de baarmoeder.

Deze aanpassing kan door ziekte zoals een infectie, zuurstoftekort, meconium in vruchtwater en longen alsmede door vroeggeboorte worden beïnvloed. De werking van het hart en de bloedvoorziening naar de organen kan dan minder zijn dan gewenst. Op tal van manieren kan een indruk worden verkregen van de bloedsomloop van een pasgeborene, zoals door bloeddrukmeting, meten van de hoeveelheid zuurstof in bloed en hersenen en met een echo van het hart. De biomarkers troponine en BNP kunnen verder helpen bij het krijgen van een indruk van de bloedsomloop van een pasgeborene en bij het achterhalen wat niet goed gaat. Deze biomarkers worden in de Neonatologie al wel gebruikt, tot nu toe is niet duidelijk of ze helpen in de behandeling en wat de beste toepassing zou zijn. Doel van dit proefschrift was om dit nader te onderzoeken. Daarnaast werd onderzocht of deze biomarkers iets over de prognose van zieke pasgeboren baby's zouden kunnen zeggen.

Dit proefschrift

In **hoofdstuk 2** wordt een overzicht gegeven van troponine, BNP en enkele ziektebeelden. Hierbij wordt beschreven wat tot op heden is onderzocht en hoe bruikbaar deze eiwitten zijn in de behandeling van de beschreven ziektes. De ziektes ontstaan vaak al voor de geboorte. Ook kunnen ze samenhangen met zuurstoftekort rondom de geboorte (perinatale asfyxie). Zij kunnen de baby treffen in de eerste dagen (niet sluiten van de ductus van Botalli) of zelfs weken na de geboorte (longbeschadiging of bronchopulmonale dysplasie).

In **hoofdstuk 3** worden de cardiale biomarkers beschreven in de behandeling na zuurstoftekort rondom de geboorte bij op tijd geboren baby's. De hersenen en andere organen van een pasgeborene kunnen beschadigd raken door zuurstoftekort, met mogelijk ernstige gevolgen voor later. Sinds kort wordt koelingstherapie gebruikt om de hersenen te beschermen na een periode van zuurstoftekort. Deze therapie lijkt bij baby's goed te werken. Het is onduidelijk of deze koelingstherapie (hypothermie) ook de werking van het hart beïnvloedt. Mogelijk geeft de koelingstherapie dezelfde bescherming aan het hart als aan de hersenen.

In dit hoofdstuk zijn kinderen die wel en kinderen die niet met koeling werden behandeld met elkaar vergeleken. Het beloop van de biomarkers werd gevolgd. De

onderzochte eiwitten waren duidelijk verhoogd ten opzichte van gezonde baby'sin beide groepen. De verhoging van het troponine was vergelijkbaar tussen de gekoelde en niet-gekoelde groep en daalde langzaam in de periode na de geboorte. Voor het BNP werden lagere waarden gevonden bij kinderen die met koeling werden behandeld. Hieruit kan worden geconcludeerd dat behandeling met koeling een voordelig effect zou kunnen hebben op werking van het hart.

In **hoofdstuk 4** wordt verder gekeken naar de gekoelde kinderen. Bij deze kinderen zijn biomarkers gemeten om naar de werking en schade van het hart te kijken. Daar zuurstoftekort het hele lichaam treft, is het mogelijk dat de hersenbeschadiging en de beschadiging van het hart vergelijkbaar zijn. De biomarkers zouden dan iets kunnen zeggen over de hersenbeschadiging en daarmee over de uiteindelijke prognose van de baby. Als het hart beschadigd is, is dit van invloed op de bloedvoorziening naar de hersenen, met als gevolg dat de hersenbeschadiging erger wordt. Om dit te onderzoeken is het beloop van de biomarkers na de geboorte vergeleken met verschillende andere observaties en metingen tijdens de opname op de NICU. Verder is gekeken of een relatie bestaat tussen de gemeten biomarkers en beschadiging van de hersenen zoals te zien is op een MRI scan. Tenslotte is onderzocht of de biomarkers iets konden zeggen over de ontwikkeling van de baby in de eerste twee jaar na de geboorte. Uit het onderzoek kwam naar voren dat de biomarkers verhoogd waren bij kinderen die zieker waren, langer beademing nodig hadden en langer opgenomen waren. Tevens bleek een relatie te bestaan tussen afwijkingen van de hersenen (te zien op een MRI) en verhoogde BNP waarden. Mogelijk hangt deze vondst samen met de laatste bevinding, te weten dat verhoogd BNP samen lijkt te gaan met een lagere ontwikkelingsscore op de leeftijd van twee jaar. De onderzochte biomarkers zouden iets kunnen zeggen over hoe ernstig de baby is aangedaan na zuurstoftekort rondom de geboorte.

Zuurstoftekort kan ervoor zorgen dat de normale aanpassing aan het leven buiten de baarmoeder niet goed verloopt. Het lichaam houdt dan vast aan de situatie van voor de geboorte waarbij de longen worden omzeild en waarbij hoge weerstand in de longen blijft bestaan, persisterende pulmonale hypertensie van de pasgeborene (PPHN). Wanneer het bloed moeilijk door de longen kan, blijft het bloed door de ductus van Botalli en het foramen ovale stromen en kan het geen zuurstof opnemen. De organen houden dan een zuurstoftekort. De rechterkant van het hart, die het bloed door de longen moet pompen, komt in de problemen door de hoge weerstand van de longen. In reactie daarop gaat het hart BNP aanmaken. BNP kan gebruikt worden als maat voor de vaatweerstand van de longen in dit ziektebeeld.

In **hoofdstuk 5** wordt onderzocht of BNP bruikbaar is om de behandeling van PPHN te volgen. Bij PPHN is de belangrijkste behandeling de weerstand in de longen te laten dalen door zuurstof te geven of door de baby te beademen. Soms is het nodig stikstof

monoxide (NO) via de longen te geven. Dit medicijn zorgt ervoor dat de bloedvaten in de longen wijder worden en het bloed makkelijker kan passeren. Het BNP zou moeten gaan dalen zodra het bloed makkelijker door de longen kan stromen. Indien tijdens de behandeling de PPHN terugkeert, komt het hart weer in het gedrang en gaat het opnieuw BNP aanmaken. BNP zou een maat kunnen zijn voor het succes van de behandeling. Wanneer kinderen bij het stoppen van de NO een stijging van het BNP lieten zien, was dat een voorbode voor verslechtering van de toestand van het kind. BNP geeft kortom al eerder aan dat er problemen zijn, dan dat de baby het ook minder gaat doen. Hiermee wordt aangetoond dat BNP goed bruikbaar is in de behandeling van kinderen met PPHN.

In het tweede deel van dit proefschrift wordt gekeken naar de prematuur. De prematuur verschilt wezenlijk van de op tijd geboren baby in aanpassing na de geboorte en ziektebeelden. De oorzaak van veel problemen is bij beiden evenwel vergelijkbaar, namelijk zuurstoftekort.

Prematuur geboren worden heeft vaak een oorzaak -en daarmee al een ontstaansgeschiedenis- voor de geboorte. De moederkoek voorziet de ongeboren baby van zuurstof en voedingsstoffen. Wanneer de werking van de moederkoek tijdens de zwangerschap minder wordt kan dit een bedreiging vormen voor de baby. In eerst instantie zal de baby zich aanpassen en de voedingsstoffen verdelen naar de belangrijkste organen, zoals de hersenen. De baby zal proberen meer zuurstof op te nemen door meer rode bloedcellen te gaan aanmaken. Naarmate de werking van de moederkoek verslechtert, zal het hart van de baby het steeds moeilijker krijgen. Dit kan zich uiten in een toename van BNP.

In **hoofdstuk 6** is naar de bron gekeken, de moederkoek zelf. Van iedere prematuur geborene in het WKZ wordt de moederkoek onderzocht. Bij dat onderzoek wordt gekeken naar infarcten, tekenen van infectie of ontsteking en andere afwijkingen die een verklaring zouden kunnen geven voor de vroeggeboorte. In de onderzochte moederkoeken werden veel afwijkingen gezien. Afwijkingen die passen bij een minder werkende moederkoek werden vaker gevonden bij kinderen met een hoog BNP. Een relatie met infarcten van de moederkoek werd alleen bij jongens gevonden. Deze bevindingen passen bij het idee dat het hart zich aanpast aan zuurstof- en voedingstekorten tijdens de zwangerschap en dat BNP mogelijk is te gebruiken achteraf problemen gedurende de zwangerschap vast te stellen. Om dit nader te onderzoeken wordt in **hoofdstuk 7** gekeken naar de te vroeg geboren baby's die het meest te lijden hebben gehad voor de geboorte, de kinderen met een groeiachterstand. Deze kinderen werden vergeleken met prematuren met een normaal geboortegewicht. Tijdens de zwangerschap is het mogelijk om met echo-Doppler onderzoek de bloeddoorstroming van de navelstreng en van de bloedvaten in de hersenen te meten. Als het hart zich moet aanpassen aan zuurstoftekort zal dit zichtbaar worden door een

verandering in de bloedstroom in deze vaten. Indien ernstige afwijkingen worden gevonden kan dit zelfs een reden zijn om een kindje vroeger geboren te laten worden. Meer Doppler-afwijkingen zijn een aanwijzing voor hogere belasting van het hart en mogelijk leidt deze belasting tot een hogere BNP-waarde bij geboorte. In dit onderzoek werd gevonden dat de groeivertraagde kinderen ook daadwerkelijk een hoger BNP hadden. Wanneer de bloeddoorstroming van de navelstreng en hersenvaten meer was aangedaan werd een hoger BNP gevonden. Ook bij zuurstoftekort rondom de geboorte -een teken dat er nog weinig reserve was- werden hogere BNP-waarden gezien. Deze relaties werden alleen gezien bij de kinderen met een groeiachterstand. BNP zou aldus als maat gebruikt kunnen worden om direct na de geboorte te kunnen zien of een baby het voor de geboorte moeilijk heeft gehad.

In **hoofdstuk 8** wordt het proefschrift samengevat en van overwegingen voorzien. Als aanvulling wordt nog kort ingegaan op het gebruik van BNP in de diagnose en behandeling van een niet sluitende ductus van Botalli. Zijdelings komt dit probleem van de te vroeg geboren baby in dit proefschrift wel aan de orde, maar het wordt niet als onderzoek beschreven.

In dit proefschrift worden, zoals gezegd, de mogelijkheden voor het gebruik van cardiale biomarkers bij op tijd geboren en te vroeg geboren zuigelingen beschreven. Er lijken mogelijkheden te zijn voor het klinisch gebruik van de biomarkers troponine en BNP. Vooral op het gebied van zuurstoftekort en bloedsomloopaanpassingen rondom de geboorte kunnen deze biomarkers bijdragen aan een verbeterde behandeling van de pasgeborene. Zowel bij het stellen van een diagnose, het volgen van het ziekteproces als de langetermijnprognose zijn deze biomarkers van toegevoegde waarde. In de toekomst kan verder gekeken worden welke stappen nog genomen moeten worden om een brede klinische toepassing mogelijk te maken.

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LIST OF ABBREVIATIONS

AaDO2	Alveolar/arterial oxygen gradient
ADC	Apparent diffusion coefficient
AGA	Appropriate for gestational age
ANP	Atrial natriuretic peptide
BG	Basal ganglia
BNP	B-type natriuretic peptide
BPD	Bronchopulmonary dysplasia
BSITD-III	Bayley scales of infant and toddler development third edition
CA	Chorioamnionitis
CBCL	Child behavior checklist
CCS	Cognitive composite score
CHD	Congenital heart disease
CHF	Congestive heart failure
CK-MB	MB-isoenzyme of creatine kinase
cTn	Cardiac Troponin
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
DV	Ductus venosus
DWI	Diffusion weighted image
ECMO	Extra-corporal membrane oxygenation
iNO	Inhaled nitric oxide
IRDS	Infant respiratory distress syndrome
IUGR	Intrauterine growth retardation
HELLP	Hemolysis elevated liver proteins and low platelet count
hsPDA	Hemodynamically significant patent ductus arteriosus
MAP	Mean airway pressure
MCA	Middle cerebral artery
MCS	Motor composite score
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NRBC	Nucleated red blood cells
NS	Not significant
NT-pro BNP	N-terminal ormino-terminal pro BNP
OI	Oxygenation index
PAH	Pulmonary arterial hypertension
PH	Pulmonary hypertension
PI	Pulsatility index

PDA	Patent ductus arteriosus
PE	Preeclampsia
PPHN	Persistent pulmonary hypertension of the newborn
SGA	Small for gestational age
TR	Tricuspid valve regurgitation
UA	Umbilical artery
Vmax	Maximum velocity
WM	White matter

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DANKWOORD

Een proefschrift vormt het eindpunt van een reis, een reis met hindernissen, een reis van leren, kennis vergaren en vele ontmoetingen. Mensen die begeleiden, bekritiseren, rode pen of highlights toevoegen aan manuscripten, maar bovenal ondersteunen om ervoor te zorgen dat de wetenschap een stapje verder komt. Een proefschrift komt niet tot stand zonder de hulp van velen aan wie ik dank verschuldigd ben.

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Curriculum vitae

Daniel Vijlbrief was born on the 30th of December 1975 in Rotterdam. He is the eldest of three sons of Kees Vijlbrief en Jenneke van der Schaft. He went to secondary school at the Erasmiaans Gymnasium in Rotterdam. After graduation in 1994, he studied at Millersville University, Millersville, Pennsylvania through the NACEE/ Fulbright program. Subsequently, he started Medical School at the Utrecht University in 1995. During his study, he developed an interest in Neonatology after a research project investigating the risk factors of necrotizing enterocolitis (under supervision of dr. H. Brouwers and dr. T. Krediet) and an elective internship at the neonatal Medium Care. After graduation in 2002, he began his training in Pediatrics at the Wilhelmina Children's Hospital in Utrecht (under supervision of prof. dr. J.L.L. Kimpen and dr. J. Frenkel) and the Elisabeth Hospital in Tilburg (under supervision of dr. P.J. van Dijken). After finishing his pediatric training in 2008, he started a fellowship in Neonatology at the Isala clinics in Zwolle (under supervision of dr. D. van Zoeren-Grobben), which he finished in 2012 at the Wilhelmina Children's hospital in Utrecht (under supervision of prof. dr. F. van Bel). He currently works as a member of staff of the department of Neonatology at the Wilhelmina Children's Hospital.

Daniel is married to Linda Smit. They have two beautiful children, Maurits (2011) and Quirine (2013).

Daniel Vijlbrief werd op 30 december 1975 geboren in Rotterdam. Hij is de oudste van drie zonen van Kees Vijlbrief en Jenneke van der Schaft. In 1994 behaalde hij zijn Gymnasium diploma aan het Erasmiaans Gymnasium in Rotterdam. Met het NACEE/ Fulbright programma studeerde hij hierna een jaar in Amerika aan de Millersville University in Pennsylvania. Vervolgens begon hij in 1995 met de studie geneeskunde aan de Universiteit van Utrecht. Zijn aandacht werd gegrepen door de Neonatologie na een onderzoeksproject. Hierin werden de risicofactoren voor het ontwikkelen van een necrotiserende enterocolitis onderzocht (supervisie dr. Brouwers en dr. T. Krediet). Hierna volgde een keuzecoschap op de medium care afdeling van de Neonatologie. In 2004, na een ANOIS periode in het Wilhelmina Kinderziekenhuis, begon hij aan de opleiding tot Kinderarts in ditzelfde ziekenhuis (Supervisie prof. dr. J.L.L. Kimpen en dr. J. Frenkel). Zijn perifere stage liep hij in het Elisabeth ziekenhuis in Tilburg (Supervisie dr. P.J. van Dijken). Na zijn opleiding in 2008 begon hij aan een fellowship Neonatologie in de Isala klinieken in Zwolle (Supervisie dr. D. van Zoeren-Grobben) en later in het Wilhelmina Kinderziekenhuis (Supervisie prof. dr. F. van Bel). Hij is thans werkzaam als neonatoloog in het Wilhelmina Kinderziekenhuis.

Daniel is getrouwd met Linda Smit. Zij hebben twee prachtige kinderen, Maurits (2011) en Quirine (2013).