N-terminal pro-B-type natriuretic peptide: a potential marker of fetal heart failure in hemolytic disease

Katarzyna Luterek [1], Iwona Szymusik [1], Robert Bartkowiak [1], Lukasz Koltowski [2], Krzysztof J. Filipiak [2], Miroslaw Wielgos [1]

1 1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Poland
Head of the Department: Prof. Miroslaw Wielgos, MD., PhD.
2 1st Department of Cardiology, Medical University of Warsaw, Poland
Head of the Department: Prof. Grzegorz Opolski, MD, PhD

Correspondence to: Prof. Miroslaw Wielgos, MD., PhD.
1st Department of Obstetrics and Gynecology, Medical University of Warsaw
Plac Starynkiewicza 1/3, 02-015 Warsaw, Poland.
tel: +48 22 5021430; fax: +48 22 5022157; e-mail: miroslaw.wielgos@wum.edu.pl

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Abstract

OBJECTIVES: The hemolysis of red blood cells due to isoimmunisation results in fetal anaemia and hypoxia leading to fetal heart failure. An assessment of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a routine practice in adult heart failure. No studies on this marker have been reported in fetal heart failure. The aim of the current study was to investigate changes in fetal NT-proBNP serum concentrations before and after intrauterine transfusions and to assess its value as a marker of fetal heart failure.

DESIGN: Therapy of Rh immunisations was performed in 14 fetuses with 61 intrauterine transfusions. Hemoglobin concentration, red blood cells count, hematocrit and NT-proBNP concentrations were assessed in samples taken before and after each transfusion.

RESULTS: An increase in the concentrations of blood parameters was strongly correlated with a decrease in the concentration of NT-proBNP. NT-proBNP concentrations were the greatest with the smallest Hb (4.0–5.9 g/dl), hematocrit and red blood cell (RBC) concentrations, respectively. An increase in Hb concentration by 1mg/dl and the RBC count by 1M/μl resulted in a corresponding decrease in NT-proBNP concentration by 659.0 and 2 007.1 pg/ml, respectively. The NT-proBNP concentrations decreased significantly following 52 transfusions.

CONCLUSIONS: The fetal serum concentration of NT-proBNP appears to be a satisfactory marker for heart failure in fetuses inflicted with severe anaemia caused by hemolytic disease. Intrauterine therapy decreases the severity of anaemia and reduces the fetal heart failure index. There appears to be a strong inverse correlation between the pre-transfusion NT-proBNP concentration and morphological parameters.

ORIGINAL ARTICLE

INTRODUCTION

Fetal hemolytic disease (FHD) which results from feto-maternal immunizations still represents a serious issue in modern perinatal care (Illanes & Soothill 2010). It leads to immune fetal hydrops, which always has to be differentiated from nonimmune cases of such a condition (Florjanski *et al.* 2009). Due to recent advances in ultrasound imaging, it is now possible to precisely evaluate the severity of hemolytic disease and to successfully treat this condition. A massive hemolysis of red blood cells (RBC) due to isoimmunisation, has been reported to result in progressive fetal anaemia and subsequent intrauterine hypoxia (Neal 2001). Severe fetal anaemia can result in various physiological hemodynamic changes, such as tachycardia and an increase in fetal cardiac output. Both of these hemodynamic changes can, in turn contribute to an increased velocity in blood flow in the major vessels. Consequently, the above mentioned hemodynamic disorders can lead to fetal heart failure (Oberhoffer *et al.* 1999). Nevertheless, there is no established biochemical marker of fetal heart failure that could be used for perinatal diagnostics.

An assessment of cardiac biomarkers has become routine practice in adult patients who suffer from heart failure. More specifically, the serum concentrations of the N-terminal pro-B-type natriuretic peptide (NT-proBNP) and/or B-type natriuretic peptide (BNP) are routinely determined in accordance to heart failure guidelines (Dickstein *et al.* 2008). In children, BNP and/or NT-proBNP have also been investigated as markers of heart failure (Sugimoto *et al.* 2010). However, to the best of our knowledge, broad and in-depth studies which examine the clinical use of BNP and/or NT-proBNP in fetal heart failure have never been undertaken. In addition, in contrast to physiologically active BNP, NT-proBNP is biologically inactive degradation product of BNP. NT-proBNP is comparatively more stable and displays a longer half-life, which makes its use as a diagnostic marker for fetal heart failure comparatively easier and more practical (Ishii 2008).

The aim of the current study was to investigate the changes in fetal NT-proBNP serum concentrations prior to and following intrauterine transfusions. These results were then used to assess the clinical possibility of using this peptide as a biomarker of fetal heart failure.

MATERIALS AND METHODS

Twenty fetuses with hemolytic disease, diagnosed and treated at the 1st Department of Obstetrics and Gynaecology, Medical University of Warsaw, Poland, in the years 2003–2006, were enrolled in the study. The study was conducted according to the principles of the Declaration of Helsinki and approved by the local ethics committee.

Prenatal therapy of Rh(D) immunizations was performed in 14 fetuses with a total number of 61 intrauterine transfusions. At the initiation of the study, six fetuses underwent only diagnostic cord punctures, which are currently not performed as separate procedures (Mari *et al.* 2000).

The majority of performed transfusions (63.9%) took place between 28–34 weeks of gestation. According to current guidelines, the management of pregnancy complicated by feto-maternal immunizations depends on Doppler ultrasound measurements of the middle cerebral artery peak systolic velocity (MCA PSV). A value greater than 1.5 multiples of median (MoM) is considered a positive predictive value for fetal anaemia and this has become a new criterion for invasive intrauterine diagnostic and therapeutic procedures (Brennand & Cameron 2008).

All intrauterine procedures were performed under ultrasound guidance, with the use of a 5MHz convex duplex scanner probe (Viking®, BK, Denmark). The site of the fetal vessel puncture depended on the location of placenta. Two different approaches were used for puncturing fetal vessels: transplacental approach if the placenta was located on the anterior uterine wall; or transamniotic approach in cases where the placenta was positioned on the posterior uterine wall or in the fundus of the uterus. A total blood sample volume of 1–2 ml was collected. Fetal blood type, morphological parameters (hemoglobin concentration (Hb), RBC count and hematocrit (Ht)) and NT-proBNP serum concentration were assessed for each sampl e. Following blood sample collection, the intrauterine transfusion was performed. The amount of packed RBC which were transfused depended on the presented clinical situation i.e.: gestational age and the severity of fetal anaemia which was calculated by the Astraia® software (Munich, Germany). Following the completion of each transfusion, an additional fetal blood sample was collected and tested for post-transfusion morphological parameters as well as NT-proBNP serum concentration.

In order to determine the serum concentration of NT-proBNP, blood samples were collected and deposited into EDTA-containing tubes. Each tube was immediately centrifuged at 3 000 rpm for 10 minutes. The plasma was stored at –20 °C for further analysis. NT-proBNP concentration (pg/ml) was determined by means of an electroluminescence immunoassay (Elecsys 1010®, Roche Diagnostics, USA).

Statistical analysis of the results was performed with the use of both parametric and non-parametric tests for dependent and independent variables. Data was subjected to correlation and regression analysis, namely: partial correlation; multivariable correlation and regression analysis. In addition, the influence of variables on the concentrations of NT-proBNP was systematically determined.

RESULTS

A total of 61 intrauterine transfusions were performed on 14 fetuses in the cohort study group. A minimum of 2 and a maximum of 9 cord punctures per fetus were performed (Table 1). The remaining six fetuses underwent diagnostic cordocentesis only.

Following 56 transfusions, the Hb concentration increased by an average of 2.7±1.1 g/dl (min. 0.73 g/dl, max. 5.98 g/dl). This value equated to 30.6% of the average Hb concentration before the transfusion (8.83±2.14 g/dl). Similarly, there was an increase in the Ht value by 8.08±3.58% (min. 2.1%, max. 18.2%, an increase of 31.6% from the baseline), and in the RBC value by 0.985±0.439 M/µl (min. 0.3 M/µl, max. 2.53 M/µl, an increase of 36% from the baseline). The increase in all three morphological parameters was statistically significant ($p<0.001$).

A relatively large variation was observed in the NT-proBNP measurements both before and after the blood transfusions as demonstrated by the variability index of 85% and 80.4%, respectively (Table 2). The NT-proBNP concentration was large (>3 000 pg/ml) in 40% of the patients before the transfusion and in 41.7% after the transfusion.

There is a strong correlation between the values of all four morphological variables before and after the intrauterine therapy (Table 3). For Hb, Ht and RBC the correlation coefficient values (r_{xy}) range between 0.822 and 0.866. This indicates that the three morphological parameters are indicative of the same information about the nature of the studied phenomenon (anaemia). Since all three parameters are functionally related, it may be justifiable to use only one of these characteristics as an index of anaemia in fetal hemolytic disease. For NT-proBNP the value for r_{xy} was determined to be 0.928. This indicates an almost perfect correlation (Table 3). There is a negative correlation between morphological parameters (Ht, RBC, Hb) and NT-proBNP concentration. This indicates that an increase in concentrations of morphological parameters is associated with a decrease in the concentration of NT-proBNP. The observed values of correlation coefficients between NT-proBNP concentrations and morphological parameters are indicative of a relatively strong correlation. Correlation matrices of the analysed parameters before and after the transfusion are shown in Table 4.

The regression charts of NT-proBNP concentrations related to morphological parameters before the transfusion prove that the relationships are linear (Figure 1). Empirical lines of regression based on the mean values of NT-proBNP concentrations and various morphological characteristics are statistically similar to theoretical lines of regression, as determined by Snedecor's F distribution.

The NT-proBNP concentrations (calculated before transfusions) were the greatest (mean: 9379.2 pg/ml) with the lowest Hb concentrations (4.0–5.9 g/dl). The regression analysis shows that the increase in Hb by 1 mg/dl is associated with a decrease in NT-proBNP concentration by 926.1 pg/ml. This declining trend in the NT-proBNP concentrations was also observed with the increasing values of Ht and RBC. The increase in Ht by 1% resulted in a corresponding decrease in the con-

Tab. 1. The number (A) and time (B) of intrauterine transfusions performed in the study group.

A. Transfusions performed			B. The time of transfusion		
The number of transfusions (n)	Number of foetuses	Total number of transfusions	Weeks of gestation	n	%
2	3	6	22;23	2	3.3
3	2	6	24;25	4	6.6
4	4	16	26;27	6	9.8
5	1	5	28;29	8	13.1
6	2	12	30;31	14	22.9
7	1	7	32;33	17	27.9
9	1	9	34;35	7	11.5
Total	**14**	**61**	36;37	3	4.9
			Total	**61**	**100.0**

Tab. 2. Analysed parameters related to blood transfusion (n=56).

Characteristics	Before transfusion		After transfusion	
	$\bar{x}$ ± SD[a,b]	V (%)[c]	$\bar{x}$ ± SD[a,b]	V (%)[c]
Hb (g/dl)	8.83±2.14	24.3	11.52±2.12	18.4
Ht (%)	25.53±6.41	25.1	33.61±6.41	19.1
RBC (M/µl)	2.74±0.74	26.8	3.72±0.74	19.8
NT-proBNP (pg/ml)	3 778.5±3210.8	85.0	3239.6±2603.2	80.4

[a] $\bar{x}$ denotes the arithmetic mean value
[b] SD denotes the standard deviation
[c] V denotes the variability index

Tab. 3. Correlation of values of analyzed morphological characteristics before and after intrauterine transfusions*.

Value	Correlation index (r_{xy})	Regression equation y = a + bx
Hb	0.866	y = 3.9446 + 0.8587x
Ht	0.843	y = 12.0573 + 0.8442x
RBC	0.822	y = 1.4574 + 0.8273x
NT pro BNP	0.928	y = 395.2477 + 0.7528x

*x denotes values before transfusion; y denotes values after transfusion; a is the free expression of the equation; b denotes the index of regression

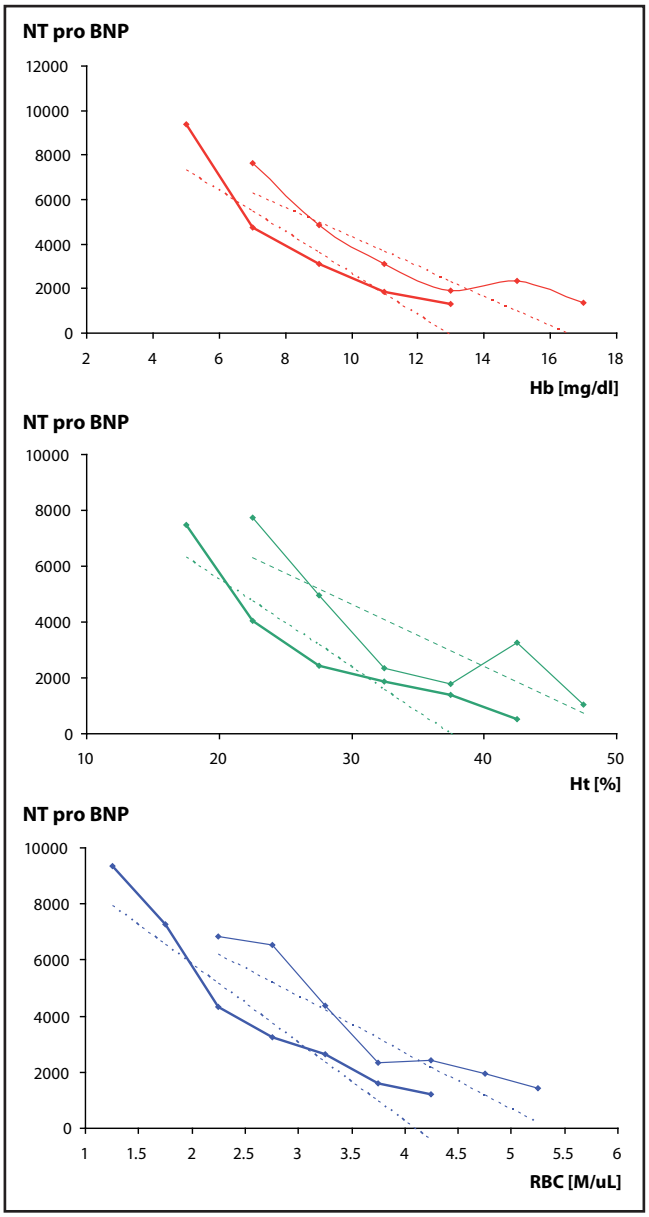

Fig. 1. The regression of NT-proBNP concentrations related to morphological parameters before and after blood transfusions (Ht – haematocrit, Hb – haemoglobin, RBC, red blood cell).

Tab. 4. Correlation matrices of morphological parameters and NT-proBNP before and after the administration of blood transfusions.*

Before transfusion			
Ht	0.989		
RBC	0.891	0.900	
NT-proBNP	–0.618	–0.631	–0.636
Parameter	Hb	Ht	RBC
After transfusion			
Ht	0.964		
RBC	0.913	0.948	
NT-proBNP	–0.537	–0.546	–0.569
Parameter	Hb	Ht	RBC

*All correlation coefficients are statistically significant ($p<0.001$); Hb=haemoglobin, Ht=haematocrit, RBC=red blood cell.
Colour key:
denotes almost perfect correlation $r_{xy} \geq 0.9$;
denotes very strong correlation $0.7 \leq r_{xy} < 0.9$;
denotes strong correlation $0.5 \leq r_{xy} < 0.7$

Tab. 5. The mean values of NT-proBNP serum concentrations in subgroups of fetuses subjected to diagnostic puncture and intrauterine therapy.

	I - Cordocentesis only (n = 6)		**II - Intrauterine therapy [a] (n = 6)**		**Differences (I vs II)**
	$\bar{x} \pm SD^{a,b}$	**V %**	$\bar{x} \pm SD^{a,b}$	**V %**	
NT pro BNP	731.8±618.1	84.5	5 231.7±3 803.6	72.7	$p<0.001$ [b]

a) before first transfusion; b) Mann-Whitney test $\bar{x}$ - the mean value; SD - standard deviation; V % - variability index

centration of NT-proBNP of ~316.4 pg/ml, whereas an increase in RBC by 1M/μl was accompanied by a decrease in the NT-proBNP concentration by 2785.5 pg/ml.

In the post-transfusion measurements the correlation coefficients assessing the relationship between NT-proBNP and other morphological parameters of anaemia are also negative. However, the value of the correlation coefficient is less following the transfusion than before the procedure. This implies that morphological characteristics that have been improved by means of blood transfusions have a smaller, albeit still clinically significant, impact on NT-proBNP concentrations. An increase in Hb by 1mg/dl and RBC by 1M/ul resulted in a corresponding decrease in the concentration of NT-proBNP by 659.0 and 2 007.1 pg/ml, respectively. The increasing concentrations of Hb, RBC and Ht did not always result in a linear decrease in NT-proBNP concentrations. For example, large values for morphological parameters such as Ht were sometimes associated with large concentrations of NT-proBNP.

In all cases the serum concentration of NT-proBNP was evaluated. The mean NT-proBNP plasma concentration in fetuses prior to intrauterine therapy was 3 778.5±3 210.8 pg/ml. Following intravascular transfusion, the plasma concentration of the peptide decreased to an average value of 3 239.6±2 603.2 pg/ml. This represents a statistically significant ($p<0.001$) decrease in the serum concentration of NT-proBNP. This decline in the concentration of NT-proBNP is equivalent to a value of 538.9±1 251.8 pg/ml and corresponds to a 14.3% decrease in the peptide serum concentration. An increase in the serum concentration of NT-proBNP following a transfusion, compared to before a treatment, was observed in only 4 out of 52 procedures (Table 2).

Six fetuses were subjected only to diagnostic cordocentesis. The average NT-proBNP serum concentration in these fetuses was 7 times less than in the transfused group and this value was statistically significant ($p<0.001$) (Table 5).

DISCUSSION

In the present study we report, for the first time to our knowledge, that the NT-proBNP is a plausible biochemical marker of severe heart failure in fetuses. The heart failure caused by severe anaemia and hemolytic disease seems to be a suitable model to study the changes in NT-proBNP concentrations.

One of the limitations of the current study is that echocardiography was not used to evaluate fetal cardiac function but rather clinical morphological parameters were used as such indicators. In addition, another limitation of the study is the lack of known normal values of NT-proBNP in the fetus. However, such values are difficult to gauge due to ethical considerations and empirical differences in methodology between different studies. Some data is reported in the literature. The level of natriuretic peptide (BNP) in the fetal circulation is greater than that found in adults (Mannarino *et al.* 2010). In addition, fetal ventricles express greater levels of BNP compared to adult ventricles (Das *et al.* 2009). Schwachtgen *et al.* reported that NT-proBNP concentrations in the cord blood samples in healthy neonates ranged between 281 and 2 595 pg/ml, with an average value of 818 pg/ml (Schwachtgen *et al.* 2005). These values were determined with the same Roche diagnostic system which was used in this study. Importantly, in that study measurements were performed postnatally. Such differences in the study design are noteworthy since the role of BNP and its active receptors in the human uterus during pregnancy is not fully understood (Itoh *et al.* 1994).

A number of other studies have examined the plasma concentration of NT-proBNP in fetal cord blood, neonates and children. Habli *et al.* (2010) evaluated the plausibility of NT-proBNP as a biomarker for cardiomyopathy in twin-twin transfusion syndrome. Using a fetoscopic technique to collect amniotic fluid samples, those authors showed that NT-proBNP levels can be used as a screening biomarker for the development of cardiomyopathy in that particular syndrome. In a different study, Merz *et al.* (2010) examined the range of fetal NT-proBNP concentration during normal pregnancies between 20 and 34 weeks of gestation. They concluded that the average NT-proBNP concentration was 1998 pg/ml and that a significant decline in this value occurred with advancing gestational age. Similar findings were recorded by Seong *et al.* (2010) who also reported that the the mode of delivery, gestational age as well as a number of other parameters can influence the level of NT-proBNP in umbilical cord blood. Importantly, Lechner *et al.* (2009) investigated the NT-proBNP levels in the umbilical cord blood of 60 neonates who were prenatally diagnosed with congenital heart defect (CHD). They observed that neonates affected with CHD displayed elevated pro-BNP levels compared to levels observed in healthy neonates. Fortunato *et al.* (2006) measured the concentration of NT-proBNP in the umbilical cord of healthy neonates. They reported that the upper limit for healthy neonatal NT-proBNP concentration was ~1 690 pg/ml. Since the levels of the measured peptide have been shown to decline with gestational age as well as improved cardiac function, this value somewhat agrees with the values obtained in the current study. Hammerer-Lercher *et al.* (2005) also examined NT-proBNP concentrations in postpartum healthy neonates and found it to be 1 052.0±181.5 pg/ml. This again fits in with the results of the current study.

The intrauterine transfusion is, from a pathophysiological perspective, an adequate model to study the influence of rapid changes in volume load on the NT-proBNP serum concentrations. To date, there is limited data on BNP levels in umbilical cord blood measured at the time of delivery (Chu *et al.* 2009). In contrast to relatively high values of NT-proBNP concentrations which were reported in this study in fetuses with heart failure, it is known that in healthy term neonates cord blood BNP concentrations are comparatively low. On the other hand, BNP values are large and express great variability immediately following delivery because of the physiological adjustment to postnatal circulation (Mannarino *et al.* 2009). It is also known that the natriuretic peptide system appears to respond greatly to volume stimuli and regulates both blood pressure as well as the salt and water balance in the developing embryo (Cameron & Ellmers 2003; Walther *et al.* 2001).The other limitation of the current study is the lack of an estimation of maternal NT-proBNP serum concentrations. However these concentrations have been reported not to influence the NT-proBNP values in the fetus.

Ferreira *et al.* (2009) published a similar study in which plasma concentrations of BNP in anaemic fetuses influenced by Rh alloimmunization. In that study it was found that the serum concentration of BNP is increased when dilation of the heart exists. In addition, the peptide concentration was shown to correlate with the cardiofemoral index as was determined in ultrasonography measurements. Importantly, the authors of that study did not determine NT-proBNP levels.

The present study determined NT-proBNP serum concentrations in fetuses affected by heart failure due to isoimmunisation. Knowledge relating to NT-proBNP concentrations in fetuses is important both to gynaecologists as well as to paediatric cardiologists. In addition, studies such as this one could potentially increase our cardiology-related knowledge concerning the pathophysiological mechanisms of NT-proBNP release from cardiomyocytes.

CONCLUSIONS

Intrauterine therapy of fetal hemolytic disease decreases the severity of anaemia and influences the fetal heart failure index. There appears to be a strong inverse correlation between pre-transfusion NT-proBNP concentrations and morphological parameters. An improvement in the latter results in a systematic decrease in the studied peptide concentrations. NT-proBNP serum concentration appears to be a plausible biochemical marker of heart failure in fetuses which are affected by severe anaemia caused by hemolytic disease. This marker can be used not only as an indicator of fetal heart failure but also as a tool, alongside basic morphological parameters, to gauge successful intrauterine therapy.

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