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Circulating N-terminal pro-B-type natriuretic peptide in fetal anemia before and after treatment

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Background: N-terminal pro-B-type natriuretic peptide (nt-proBNP) is an established marker of heart failure in adult cardiology. We analyzed nt-proBNP in the circulation of fetuses with increased volume load secondary to anemia and investigated the effect of treatment on nt-proBNP concentration.

Methods: Fetuses undergoing intrauterine transfusion (IUT) were examined. nt-proBNP was measured before IUT and correlated with hemoglobin concentrations, ultrasonographic findings, and Doppler measurements of the peak systolic velocity of the middle cerebral artery (MCA-PSV).

Results: A total of 27 patients (7 with hydrops) and 78 controls were examined. nt-proBNP was markedly elevated in anemia ($P < 0.001$). Concentrations were highest in hydropic fetuses ($P < 0.03$); no differences were present in hemoglobin and MCA-PSV values between hydropic and nonhydropic cases. In fetuses undergoing multiple IUTs nt-proBNP normalized after the third IUT, whereas hemoglobin and MCA-PSV remained abnormal.

Conclusion: Levels of circulating nt-proBNP correlate well with the degree of myocardial workload in the hyperdynamic state of fetal anemia. We hypothesize that normalization of nt-proBNP after serial transfusions is an indicator of myocardial adjustment to chronic anemia. nt-proBNP measurement may be useful in the management of fetal anemia, particularly in cases at risk of hydrops and fetuses requiring multiple transfusions.

Anemia increases workload on the fetal heart; in order to maintain tissue oxygenation, the combined cardiac output is increased. The hyperdynamic circulation is characterized by a rise in myocardial stretching and filling pressures, reduced afterload, and cardiomegaly (1). Myocardial perfusion in particular is dependent on oxygen supply. Adaptive mechanisms allow a four- to fivefold increase in coronary perfusion and include increased coronary perfusion pressure, decreased coronary resistance, and autoregulation (2). If these adaptations fail to meet oxygen demand, myocardial ischemic changes occur, eventually resulting in decreased myocardial function and dilated cardiomyopathy. Although the precise mechanisms are debated, the development of hydrops in fetal anemia heralds deterioration of the fetal status and impending death.

In adult cardiology brain natriuretic peptide (BNP) and its inactive cleavage product n-terminal pro-B-type natriuretic peptide (nt-proBNP) are established markers of heart failure supporting diagnosis, risk stratification, and treatment response monitoring (3,4). Elevated BNP concentrations result from re-expression of the cardiac embryonic gene program and induce cardiac remodeling and fibrosis (5,6). The natriuretic peptide system is functional by mid-gestation (7).

We hypothesized that the hyperdynamic circulation present in fetal anemia may result in increased levels of circulating nt-proBNP. Therefore, nt-proBNP was measured before intrauterine transfusion (IUT) and correlated to hemoglobin concentrations and ultrasonographic and Doppler findings. Furthermore, the effect of treatment on nt-proBNP concentration was assessed in cases with multiple IUTs.

RESULTS

Patients

In total, 27 patients were recruited; hydrops was present in 7. Anemia was caused by the following conditions: Rhesus alloimunization ($n = 10$); parvovirus-B19 infection ($n = 8$); tumors with associated hemorrhage ($n = 5$); chronic fetomaternal hemorrhage ($n = 2$); and elliptocytosis and unknown (one case each). Cases with parvovirus-B19 infection were equally distributed in the nonhydropic and hydropic groups. Procedure-related complications and intrauterine or perinatal deaths did not occur. The median time between the first and second IUT was 6.0 d (range 1–21); the median number of IUTs per patient was 3.0 (range 1–7).

Anemia was classified as mild in six, moderate in four, and severe in 12 fetuses. One case of known Rhesus alloimmunization received the first IUT at 23 wk of gestation; hemoglobin and peak systolic velocity of the middle cerebral artery (MCA-PSV) were within normal range before the first transfusion. Another patient with normal hemoglobin concentration had signs of hyperdynamic cardiac dysfunction at 14 wk of gestation, when the first IUT was undertaken. Subsequently, chronic fetomaternal hemorrhage was diagnosed and five more IUTs were performed. Samples for hemoglobin or nt-proBNP measurement could not be collected before the first transfusion

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in two and three fetuses, respectively. Median gestational age (GA) of the study group was 23.0 wk (range 14–33). There was no difference in GA between cases and controls nor between cases with and without hydrops.

nt-proBNP

Nt-proBNP concentrations before treatment are detailed in **Table 1**. Plasma levels were higher in the anemic group and increased with disease severity. Hydropic fetuses had higher levels of circulating nt-proBNP as compared with nonhydropic cases (see **Figure 1**). On the basis of these findings, nt-proBNP <10,000 ng/l had a negative predictive value of 100% for identification of fetal hydrops, with a positive predictive value of 44.4%. Subgroup analysis revealed no difference in nt-proBNP levels of fetuses with parvovirus-B19-associated anemia.

Hemoglobin and MCA-PSV levels did not differ in hydropic and nonhydropic fetuses. Correlation between nt-proBNP and hemoglobin and between MCA-PSV and hemoglobin was good ($r = -0.637$ and $r = -0.626$, respectively); no correlation was present between nt-proBNP and MCA-PSV. Doppler flow velocity waveform indexes of the ductus venosus were within normal range for GA with the exception of two cases (both nonhydropic) showing increased pulsatility; indexes for umbilical artery and uterine artery were within normal range in all cases.

Cardiothoracic area ratio was increased in anemic fetuses before treatment (median 0.337, interquartile range 0.066); no difference was present between hydropic and nonhydropic cases. Correlation between cardiothoracic area ratio and nt-proBNP was moderate ($r = 0.477$); no correlation was detected between cardiothoracic area ratio and hemoglobin or MCV-PSV.

Changes in nt-proBNP, hemoglobin, and MCA-PSV levels during the course of treatment are detailed in **Table 2**. After the first IUT, nt-proBNP levels dropped significantly; concentrations in initially hydropic cases were comparable with the remaining group. Pre- and posttransfusion values for hemoglobin and MCA-PSV did not change significantly, and no correlation was present between any of the variables after the first IUT. nt-proBNP normalized after the third IUT ($P = 0.116$). In contrast, hemoglobin and MCA-PSV values remained abnormal (see **Table 2**).

Table 1. nt-proBNP plasma levels in various degrees of fetal anemia before treatment, and controls

	n	nt-proBNP (ng/l)
Controls	78	1,874 (1,092)
Anemia	22	15,167 (27,758)*
Mild	6	4,876 (6,735)*
Moderate	4	17,302 (48,662)**
Severe	12	18,152 (40,755)

Values are expressed as median (interquartile range). In five cases pretreatment measurements could not be obtained.

nt-proBNP, N-terminal pro-B-type natriuretic peptide.

*$P < 0.01$ as compared with controls; **$P < 0.02$ as compared with mild anemia.

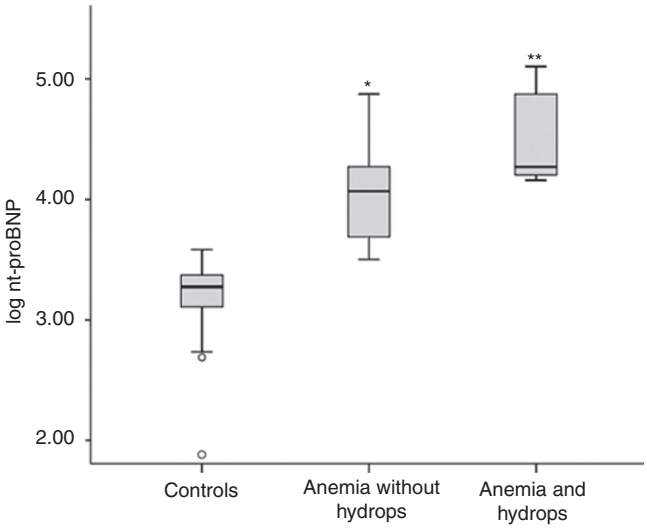

Figure 1. N-terminal pro-B-type natriuretic peptide (nt-proBNP) plasma levels in anemic fetuses with and without hydrops before treatment, and controls. Depicted values are log-transformed. ° Denotes outliers. *$P < 0.001$ as compared with controls; **$P < 0.03$ as compared with anemia without hydrops.

DISCUSSION

We found elevated levels of circulating nt-proBNP in fetuses with high cardiac output secondary to anemia. Concentrations correlated well with the degree of anemia. Peak nt-proBNP levels were present in cases with hydrops. Treatment resulted in a significant decrease and eventually normalization of nt-proBNP values despite persistently abnormal hemoglobin and MCA-PSV measurements.

As compared with postnatal life, nt-proBNP concentrations are higher in the human fetus (8). The difference is not because of maternal/placental transfer or differences in metabolic pathways (7,9) but may be a consequence of the reduced compliance of the immature fetal myocardium. Parallel to the decrease in left-ventricular afterload with advancing gestation and maturation of the fetal myocardium, plasma levels gradually decrease (8). Placental expression of atrial natriuretic peptide, but not BNP, has been proven and may be involved in vasodilatation of the feto-placental vascular bed (7).

The longer half-life and higher stability make nt-proBNP a more reliable parameter as compared with BNP (10). However, values are assay-specific with limited comparability (11,12). In addition, cross-reactivity with pro-BNP, the precursor form with six- to eightfold lower biological activity, and glycosylated forms of BNP has been demonstrated (13). This is of particular importance as patients with heart failure have evidence of increased levels of circulating pro-hormone (14,15). Further studies are needed to address this subject.

A role of nt-proBNP in cardiovascular dysfunction during fetal life has been demonstrated by investigations of cases with growth restriction secondary to uteroplacental dysfunction and fetuses with structural cardiac defects. Fetal growth restriction results in increased left-ventricular output and

Table 2. nt-proBNP, Hb, and MCA-PSV levels in the course of treatment of fetal anemia

	n	nt-proBNP (ng/l)	Hb (g/dl)	MoM Hb	MCA-PSV (cm/s)	MoM MCA-PSV
Before first IUT	27	15,167 (27,758)	5.9 (3.9)	0.49 (0.26)	55 (22)	1.75 (0.42)
Before second IUT	16	12,305 (15,993)*	8.9 (3.5)	0.69 (0.31)	65 (28)	1.54 (0.77)
Before third IUT	14	5,830 (7,400)	8.4 (4.0)	0.64 (0.37)	67 (37)	1.67 (0.51)
Before fourth IUT	8	2,528 (1,200)	8.9 (2.5)	0.70 (0.19)**	60 (25)	1.76 (0.71)†

Values are expressed as median (interquartile range).

GA, gestational age; Hb, hemoglobin; IUT, intrauterine transfusion; MoM, multiple of median; nt-proBNP, N-terminal pro-B-type natriuretic peptide; MCA-PSV, peak systolic velocity of the middle cerebral artery.

*$P < 0.05$ as compared with the concentration before treatment; **$P < 0.02$ as compared with MoM for GA; †$P < 0.05$ as compared with MoM for GA.

decreased right-ventricular output, followed by diastolic dysfunction and cardiac compromise (16); a significant correlation of nt-proBNP and early markers of cardiac dysfunction in fetal growth restriction was found (17–19). Cardiac malformations have the potential to alter loading conditions during fetal life; increased levels of circulating nt-proBNP have been detected in fetal and umbilical cord blood samples (19,20). Concentrations were highest in cases with left- or right-ventricular outflow tract obstructions with intact ventricular septum; the associated rise in ventricular wall tension was assumed to be a potent stimulus for nt-proBNP secretion (21).

Our study is the first to report nt-proBNP levels in volume load secondary to anemia during intrauterine life. We have no direct measurements of cardiac stress, strain, or performance but inferred myocardial behavior from studies cited above. The close correlation between hemoglobin and nt-proBNP values allows the conclusion that nt-proBNP is secreted in response to the increased cardiac output required to compensate for the reduced oxygen-carrying capacity of the blood.

The development of hydrops is associated with worse fetal outcome. Current concepts on the pathophysiology of hydrops in anemia include genuine myocardial failure, high cardiac output failure, reduced plasmatic colloid-oncotic pressure, increased capillary permeability, and obstruction of venous and subsequently lymphatic flow. We found significantly higher nt-proBNP concentrations in hydropic fetuses; these results support a myocardial cause of hydrops development in anemia. The increased total feto-placental blood volume present only in hydropic fetuses with anemia (22) may exceed the myocardial capacity, resulting in high cardiac output failure; on the other hand increased end-diastolic ventricular pressure and reduced coronary perfusion pressure may induce hypoxic myocardial dysfunction. Our data do not allow a differentiation between these mechanisms. Investigating parameters of myocardial injury may help to clarify this issue.

This study confirms previous results of ductus venosus blood flow indexes in fetal anemia (1,23). A rise in precordial vein pressure is likely to be a late finding, confirming the adaptability of the fetal myocardium to the hyperdynamic circulation. Parvovirus-B19-associated myocarditis may impair myocardial function (24). We could not detect an impact of infection on nt-proBNP concentrations.

The normalization of circulating nt-proBNP after three IUTs despite persistently abnormal hemoglobin levels is remarkable given that the oxygen dissociation curve is more unfavorable for adult red blood cells (RBCs), and consequently a higher cardiac output is required for tissue oxygenation. This finding may be an indicator of the myocardial adjustment to increased workload, illustrating the plasticity of the fetal heart. Adaptive cardiac mechanisms to chronic anemia have been investigated in animals. Chronically anemic sheep fetuses show increases in myocardial mass and vascularization as well as changes in the expression of various angiogenic, hypoxia-related, and glycolytic genes (25,26). Furthermore, cardiac remodeling induced by chronic intrauterine anemia results in increased contractile response and coronary conductance in adult animals (27,28). Rheological changes secondary to the presence of adult RBCs in the fetal circulation may be an alternative explanation for the normalization of nt-proBNP despite persistent anemia. As compared with fetal RBCs, adult RBCs are smaller with less rigid cell membranes; in addition, the proportion of nucleated RBCs is lower. These factors may alleviate cardiac workload even in the presence of increased cardiac output.

The number of cases is small, limiting the significance of our findings. However, cases were carefully selected and fetuses with conditions that potentially exert an effect on any of the variables under investigation were excluded. We therefore assume our results to be valid. In addition, hemoglobin levels in the control group were not available, so comparisons were performed with calculated reference values. As fetuses in the control group did not have any malformation with potential impact on hemoglobin concentration or MCA-PSV levels and because echocardiographic and Doppler investigations were normal, we are confident that a systematic error was avoided.

Although an invasive procedure measurement of nt-proBNP provides insight into myocardial function and may be a useful adjunct in the management of fetal anemia. Noninvasive tests such as myocardial performance indexes (Doppler and tissue Doppler) and those of myocardial velocity, strain, and strain rates (speckle tracing) can give clues to cardiac dysfunction, but their clinical relevance for fetal monitoring and surveillance requires further evaluation (29). In severely anemic fetuses, hemoglobin and MCA-PSV measurements are not able to predict hydrops (30). Likewise, the decreasing sensitivity of MCA-PSV to detect fetal anemia after a previous IUT precludes its application in the management of cases with multiple IUTs (31). In these situations, nt-proBNP may provide valuable information given its high negative predictive value.

In conclusion, nt-proBNP correlates well with the degree of myocardial workload in the hyperdynamic state of fetal anemia. The normalization of circulating nt-proBNP after serial IUTs despite persistent anemia may indicate myocardial adjustment to increased workload. Incorporation of nt-proBNP measurement may be a useful tool for the management of fetal anemia, particularly in cases at risk of hydrops and after multiple transfusions.

METHODS

Study Population

Women referred to our Center of Prenatal Medicine between May 2006 and February 2010 with a suspected diagnosis of fetal anemia were eligible. Cases with anemia confirmed by fetal blood sampling who underwent IUT into the extra-abdominal part of the umbilical vein were included. Hydrops was defined as fluid accumulation in at least two of the following fetal compartments: skin, serous cavities (pleural or pericardial effusion), ascites, or polyhydramnios. Serologic tests were obtained in cases with suspected infection. None of the fetuses had any structural malformation, and all were appropriate for GA (estimated fetal birth weight >10th percentile). GA was confirmed by first-trimester ultrasonographic examination.

Previously established reference values served as controls (8). They were taken from fetuses that underwent fetocide within the context of termination of advanced pregnancy or cordocentesis for fetal platelet analysis in cases with human platelet antigen alloimmunization. Due to ongoing recruitment, 78 samples were available. Fetuses in the control group presented none of the following conditions that potentially exert an influence on nt-proBNP concentration: cardiac, urogenital, thoracic, skeletal or gastrointestinal malformations; neuromuscular disorders; tumors; hydrops; infections; and fetal growth restriction (estimated fetal birth weight <10th percentile). Monochorionic twin pregnancies were also excluded. Doppler indexes for umbilical artery, ductus venosus, and MCA were within normal range, and no fetus had any evidence of cardiac dysfunction by echocardiography. Hemoglobin values were not available for the control group. The study was conducted in accordance with human subject research guidelines and the Declaration of Helsinki and was approved by the University Bonn Review Board. Informed consent was obtained from all subjects.

Ultrasonographic and Echocardiographic Evaluation

High-resolution ultrasound equipment was used in all cases. The high-pass-filter was set at 60 Hz, and the spatial peak temporal average power output was kept at <100 W/cm^2, applying only the fetal-use-adapted ultrasound machine settings. A detailed assessment of the fetal anatomy and cardiovascular status including echocardiography and Doppler examination was performed in subjects and controls. Doppler recordings of blood flow in the MCA and umbilical artery were obtained at an insonation angle between 0° and 10° to flow and <30° for ductus venosus and uterine arteries, using standard positioning of the sample volume; angle correction was not performed. At least five consecutive uniform Doppler velocity waveforms with the highest velocities and a narrow band of frequencies were recorded, and one cycle was analyzed. Indexes were calculated accordingly; abnormal values were defined as umbilical artery pulsatility index >90th percentile for GA or absent or reversed end-diastolic flow; ductus venosus pulsatility index for veins >90th percentile for GA or negative a-wave; uterine artery resistance index >90th percentile for GA or bilateral notching; MCA-PSV >1.28 times the median for GA. All examinations were performed before invasive intervention.

Diagnosis and Treatment Protocol for Fetal Anemia

Our center's management protocol for suspected anemia stipulates establishing a diagnosis by ultrasound and Doppler studies. GA-adjusted multiples of median (MoMs) for MCA-PSV are applied to assess the degree of anemia (30). On the basis of these findings, arrangements for IUT are undertaken. Packed red cells are prepared to a hematocrit level of 72 and a hemoglobin level of 23–24 mg/dl. The transfusion volume is calculated (30–40 ml/kg), aiming for maximum blood volume expansion of 30–40 ml/kg fetal estimated weight per IUT. Our policy is to limit the transfusion volume to 50 ml per IUT to avoid acute volume overload, particularly in hydropic fetuses. In cases with severe anemia we prepare for a second IUT 2 d later. Fetal blood sampling and IUT are performed in one procedure, without fetal sedation or muscle relaxation. The umbilical vein in the umbilical cord is punctured under ultrasound control, if possible near its placental insertion site. After the procedure the patient remains under observation for 24 h. Thereafter, a control ultrasound examination is performed and further management is decided, taking the underlying diagnosis and degree of anemia into consideration. Subsequent IUTs follow the same protocol.

Sample Collection and Processing

Specimens were collected during the IUT procedure, after collection of the full blood count sample, and before commencement of transfusion. One milliliter of fetal venous blood was withdrawn. nt-proBNP was measured with a commercially available chemiluminescence immunoassay on a Dimension Vista 1500 (Siemens Healthcare Diagnostics, Eschborn, Germany) according to the manufacturer's instructions. All samples were processed within 2 h. Inter- and intra-assay coefficients of variation were 3.5 and 2.3%, respectively. Fetal full blood count was analyzed on a KX21 hematology analyzer (Sysmex, Norderstedt, Germany).

Statistical Analysis

To adjust for the effect of GA, hemoglobin values were transformed into MoMs (30). Expected hemoglobin values were calculated with the following formula: $e^{(2.84-8.55/GA)}$. MoMs were then calculated by dividing the measured values by the expected values. Anemia was classified as mild (0.84–0.65 MoM), moderate (0.64–0.55 MoM), or severe (<0.55 MoM) (30). Values for MCA-PSV were MoM-transformed accordingly, using the following formula for expected MCA-PSV values: $e^{(2.31+0.046\ GA)}$. Anemia according to MCA-PSV values was classified as mild (1.29–1.49 MoM), moderate (1.50–1.54 MoM), and severe (≥1.55 MoM) (30). For normally distributed values, between-group comparisons of continuous variables were performed by independent samples *t*-test. Otherwise, Mann–Whitney-*U* or Kruksal–Wallis test was used. Within-group comparisons were performed by paired samples *t*-test and Wilcoxon signed-rank test was applied to the post-ante differences. For correlation analysis, Spearman's coefficient was calculated. Unless indicated otherwise, results are reported as median and interquartile range.

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