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Original Paper

Plasma natriuretic peptide levels in fetuses with congenital heart defect and arrhythmia: a single-center prospective study

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ABSTRACT

Objectives: Diagnosing fetal heart failure remains challenging because it is difficult to know how well the fetal myocardium will perform as loading conditions change. In adult cardiology, natriuretic peptides (NPs) are established marker of heart failure. However, the number of studies investigating NP levels in fetuses is quite limited. The aim of this study was to evaluate the significance of plasma NP levels in the assessment of heart failure in fetuses with congenital heart defect (CHD) and arrhythmia.

Methods: This was a prospective observational study at a tertiary pediatric cardiac center. A total of 129 singletons with CHD, arrhythmia, or both and 127 controls from 2012 to 2015 were analyzed. Umbilical cord plasma atrial NP, brain NP and N-terminal pro-brain NP levels at birth were compared with ultrasonography findings indicating fetal heart failure such as a cardiovascular profile (CVP) score and morphological characteristics.

Results: Fetuses with CHD, arrhythmia, or both had higher NP levels than controls (P<0.01). NP levels of fetuses with CHD, arrhythmia, or both were inversely correlated with CVP score (P for trend <0.01). No differences were found in NP levels between fetuses with CHD or arrhythmia and a CVP score of ≥8 versus controls. Multivariate analysis showed that a CVP score of ≤5, tachy- or bradyarrhythmia at birth, preterm birth, and umbilical artery pH <7.15 are independently associated with high NP levels (P<0.01). Among fetuses with a CVP score of ≤7, abnormal venous Doppler sonography findings were significantly more common and more severe in fetuses with tachy- or bradyarrhythmia than those with CHDs, and fetuses with tachy- or bradyarrhythmia had higher NP levels than those with CHDs (P=0.01). Fetuses with right heart defect and moderate or severe tricuspid valve regurgitation had significantly higher NP levels than fetuses with other types of CHD (P<0.01).

Conclusions: Plasma NP levels in fetuses with CHD, arrhythmia, or both are correlated with

the severity of fetal heart failure. Elevated NP levels are mainly attributed to increases in central venous pressure secondary to arrhythmia or atrioventricular valve regurgitation due to a CHD, rather than the morphological abnormality itself.

Key words: arrhythmia; cardiovascular profile score; congenital heart defect; heart failure; natriuretic peptide; prenatal diagnosis

Introduction

Diagnosing fetal heart failure remains challenging because it is difficult to know how well the fetal myocardium will perform when loading conditions change[1]. Recently, the cardiovascular profile (CVP) score was found to be a superior marker for comprehensive and semi-quantitative assessment of fetal heart failure manifesting as fetal hydrops[2,3]. The role of the CVP score in the prognosis of fetuses with CHDs has been studied[4–6]. The American Heart Association statements mention that it may be useful in baseline and serial evaluations of fetuses at risk for or with myocardial dysfunction[7].

In adult cardiology, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) are established markers of heart failure[8–10]. However, few studies have investigated natriuretic peptide (NP) levels in fetuses with CHDs[11–14]. It has not been clearly established whether plasma NP levels in umbilical cord blood are indicators of fetal heart failure. In addition, to the best of our knowledge, there have been no studies investigating NP levels in fetuses with arrhythmias, although fetal tachy- or bradyarrhythmias are common causes of fetal hydrops[15–17].

The aim of the present study was to evaluate the significance of plasma NP levels in the assessment of fetal heart failure by comparing the pathophysiological status of fetuses with CHD and arrhythmia. We prospectively observed changes in CVP scores *in utero* and measured umbilical cord blood NP levels at birth.

Methods

A single-center prospective observational study was undertaken with approval from our institutional review board (M24-041) and written informed consent from the fetuses' parents. All singletons prenatally diagnosed with CHD, arrhythmia, or both at the National Cerebral and Cardiovascular Center between October 2012 and December 2015 were included in this

study. Exclusion criteria included a critical chromosomal anomaly such as trisomy 13 or 18, or a critical extracardiac anomaly that required surgical intervention during the neonatal period. Control subjects were normal fetuses with no complications such as CHD, extracardiac anomaly, and growth restriction that were recruited randomly. Subjects were excluded there they had maternal and obstetrical complications such as chronic hypertension, diabetes mellitus, preeclampsia, and gestational diabetes mellitus. Cases and controls with no available blood samples at birth were also excluded from analysis.

The CVP score was used to characterize fetal heart failure[7]. CVP scores of all cases were evaluated by the same person. The CVP score is based on a proposed composite scoring system to grade and serially follow the severity of fetal heart failure using 5 fetal echocardiographic parameters: fetal effusion, venous Doppler findings, heart size, cardiac function, and arterial Doppler findings. Heart failure severity is rated on a 10-point scale; points are deducted for abnormalities in each component marker[2–4]. A CVP score of ≥8 is considered to indicate no or mild heart failure, 6 or 7 moderate heart failure, and ≤5 severe heart failure[6]. Sixty-two CVP score data in the CHD group were presented in our previous study focused on CVP score as a predictor of acute intrapartum non-reassuring fetal status in infants with CHDs[18]. Umbilical cord blood NP data have not been previously published in any form.

All fetuses with CHD were diagnosed prenatally using fetal echocardiography with Voluson E8 ultrasound equipment (GE Medical Systems, Zipf, Austria). CHDs were morphologically categorized as having single ventricle or biventricular physiology, as in our previous study[18]. Our tertiary pediatric cardiac center has an established protocol for patients with a prenatal diagnosis of CHD or arrhythmia[18]. Patients are admitted to the hospital and assessed at least weekly with CVP and biophysical profile scores after 37 weeks of gestation or if they have a complication such as threatened labor or fetal growth restriction. Therefore,

all fetuses had a CVP score assessed within 1 week before birth.

All cases of fetal arrhythmia were diagnosed using fetal echocardiography and magnetocardiography (MC-6400, Hitachi High-Technologies Corporation, Tokyo, Japan). Fetal arrhythmias were categorized as tachyarrhythmia, bradyarrhythmia, or extrasystole. Fetal tachy- and bradyarrhythmias were defined by a ventricular rate of ≥180 bpm and <100 bpm, respectively. When fetal tachyarrhythmia was sustained for ≥50% of the time on monitoring prior to 37 weeks of gestation, fetal therapy was performed. Digoxin, sotalol, flecainide or a combination was used for supraventricular tachycardia and atrial flutter. Magnesium sulfate, propranolol, mexiletine, or a combination was used for ventricular tachycardia. When complete atrioventricular block was complicated by a fetal ventricular rate of <55 bpm with or without myocarditis before 34 weeks of gestation, fetal therapy using beta-sympathomimetics, steroids, or both was performed.

Umbilical vein (UV) blood samples were collected at the time of delivery into test tubes containing EDTA-2Na and aprotinin (final concentration: 1.5 mg/mL and 500 kallikrein inhibitor units/mL). Blood samples were chilled on ice. Plasma samples were prepared by centrifugation at 1500 × g for 15 min at 4 °C and immediately frozen at -80 °C until assays were performed. UV plasma ANP and BNP concentrations were measured using the AIA-PACK chemiluminescence immunoassay (TOSOH Corporation, Tokyo, Japan). An electrochemiluminescence immunoassay (Elecsys NT-proBNP II, Roche Diagnostics, Mannheim, Germany) was used to assess NT-proBNP concentrations in UV blood samples.

Statistical analysis was performed using Stata version 14.1 (StataCorp LP, College Station, TX, USA) and JMP 10 (SAS Institute, Cary, NC, USA). Data are presented as means ± standard deviation or numbers of patients. Student's t-test was used to compare continuous variable between groups. Categorical variables were evaluated using the chi-square test or Fisher's exact test as appropriate. Correlation between NP levels and CVP scores was

evaluated using the trend test. We also performed univariate and multivariate logistic regression of NP levels in fetuses with CHD, arrhythmia, or both. The best prediction model was selected by backward elimination with P=0.10 as the criterion for exclusion. Stepwise analysis was used to adjust for baseline variables. P<0.05 was considered significant in all analyses.

Results

Study cohort and baseline characteristics

A total of 143 fetuses with CHD, arrhythmia, or both and 137 control fetuses were prospectively enrolled in the present study (Figure 1). In the CHD and arrhythmia group, 4 cases of fetal demise, 3 cases of trisomy 18, and 7 cases with sampling failure were excluded, leaving 129 fetuses available for analysis. The 4 fetal demises were due to Ebstein's anomaly with circular shunt in 2 cases, dilated cardiomyopathy in 1 case, and double outlet right ventricle with severe fetal growth restriction in 1 case. Among the controls, 1 case of fetal hydronephrosis and 9 cases of sampling failure were excluded, leaving 127 fetuses available for analysis. Baseline perinatal characteristics are shown in Table 1. All controls had normal fetal growth and a CVP score of 10. In the control group, cesarean delivery was mainly performed due to previous cesarean delivery.

The types of CHD and arrhythmia among study participants are shown in Table 2. All diagnoses of CHD were confirmed soon after birth using echocardiography by pediatric cardiologists. Arrhythmias complicated by CHD were classified as arrhythmia group. One fetus with supraventricular tachycardia had a cardiac tumor, 2 fetuses with complete atrioventricular block and 2 fetuses with sinus bradycardia had left atrial isomerism, and 4 fetuses with atrial extrasystole had atrioventricular septal defect. Fetal therapy was performed in 22 fetuses with arrhythmia; supraventricular tachycardia or atrial flutter (n=15), ventricular

tachycardia (n=2) and complete atrioventricular block (n=5). At birth, 5 cases of tachyarrhythmia, 11 of bradyarrhythmia, and 8 of extrasystole were confirmed using electrocardiography.

NP levels and perinatal factors associated with CHD or arrhythmia

When examining the relationship between NP levels and CVP score of fetuses with CHD or arrhythmia versus controls, plasma ANP and BNP levels had an identical relationship with CVP score (Figure 2, Supplementary figure S1A and B). Therefore, we present data on UV NT-proBNP levels as representative of NP levels overall. Fetuses with CHD, arrhythmia, or both had a 3.2-fold higher UV NT-proBNP level than control fetuses (1935 pg/mL vs. 613 pg/mL, $P<0.01$). After dividing these fetuses into 3 groups by CVP score of ≥8 (n=107), 6 or 7 (n=13), and ≤5 (n=9), we found that UV NT-proBNP levels were inversely correlated with CVP score among cases (P for trend <0.01), while no differences were observed in UV NT-proBNP levels between fetuses with CHD or arrhythmia and a CVP score of ≥8 versus controls ($P=0.16$) (Figure 2).

To identify perinatal factors associated with high UV NT-proBNP levels, univariate and multivariate analyses were performed for fetuses with CHD, arrhythmia, or both (Table 3). Multivariate analysis showed that a CVP score of ≤5 (coefficient 3299.37, 95% confidence interval (CI) 1748.47–4850.27), tachy- or bradyarrhythmia at birth (coefficient 8719.68, 95% CI 7365.91–10073.45), preterm birth (coefficient 1281.68, 95% CI 327.08–2236.27), and umbilical artery (UA) pH <7.15 (coefficient 7903.22, 95% CI 5455.22–10351.21) were independently associated with high UV NT-proBNP levels ($P<0.01$). Similar results were obtained for UV plasma ANP and BNP levels. The main reasons for preterm birth in the CHD and arrhythmia group were progression of fetal heart failure or hydropic status (n=7), spontaneous labor (n=3), and abnormal fetal heart rate pattern (n=2). Of 12

preterm births, 10 cases underwent elective cesarean delivery. One fetus with Ebstein's anomaly with circular shunt (CVP score of 0) born at 34 weeks of gestation had UA pH of 7.11 and the other case had fetal premature ventricular contraction (CVP score of 8) with a UA pH of 7.14.

Comparison of CHD and arrhythmia and NP levels

Tachy- or bradyarrhythmia but not extrasystole was observed in all 10 fetuses with arrhythmia and a CVP score of ≤7 at birth. These fetuses had 2.9-fold higher UV NT-proBNP levels than fetuses with CHD and a CVP score of ≤7 (10900 pg/mL vs. 3757 pg/mL, $P<0.01$) (Figure 3). In contrast, no differences were observed in UV NT-proBNP levels between arrhythmia versus CHD in fetuses with a CVP score of ≥8 (756 pg/mL vs. 945 pg/mL, $P=0.49$). Among fetuses with a CVP score of ≤7, fetuses with CHD versus arrhythmia had similar last CVP scores (5.7 ± 2.1 vs. 5.4 ± 1.4, $P=0.74$). However, when comparing each parameter of the last CVP score individually, abnormal venous Doppler sonography findings were significantly more common and more severe in fetuses with arrhythmia than those with CHD ($P=0.01$) (Table 4).

NP levels and change in CVP score *in utero*

Sixteen fetuses with CHD, arrhythmia, or both had a decrease in CVP score from enrollment to birth (Supplementary Table 1). Fetuses with a decrease in CVP score *in utero* had significantly higher neonatal or infant mortality compared with fetuses without (17.6% vs. 0.9%, $P<0.01$). In the CHD and arrhythmia group, fetuses with a decrease in CVP score had moderate or severe atrioventricular valve regurgitation (8/16, 50.0%), or tachy- or bradyarrhythmia at birth (6/16, 37.5%); they had 6-fold higher UV NT-proBNP levels than those without (7099 pg/mL vs. 1163 pg/mL, $P<0.01$). Fetuses with right heart defect had

lower CVP scores than fetuses with other types of CHD (P=0.01). Notably, among fetuses with right heart defect, those with moderate or severe tricuspid valve regurgitation (TR) had 11.7-fold higher UV NT-proBNP levels than those without (6755 pg/mL vs. 579 pg/mL, P<0.01). However, in fetuses with CHD but without moderate or severe atrioventricular valve regurgitation, UV NT-proBNP levels were not significantly different by CHD category (P=0.43) Of 6 fetuses with hypoplastic left heart syndrome (HLHS), 3 had a highly restrictive foramen ovale with an abnormal pulmonary venous flow pattern. All fetuses with HLHS had no change in CVP score *in utero* and low UV NT-proBNP levels (median 920, range 331–1172 pg/mL). Results were similar for plasma ANP and BNP levels.

Discussion

Our study demonstrated that plasma NP levels in umbilical cord blood are correlated with the severity of heart failure in fetuses with CHD, arrhythmia, or both. Fetal tachy- or bradyarrhythmias and right heart defects with moderate or severe TR showed low CVP scores and high NP levels. Plasma concentrations of UV ANP, BNP, and NT-proBNP were associated with similar heart failure profiles in fetuses with CHD and arrhythmia.

Fetal tachy- or bradyarrhythmia at birth was strongly correlated with high NP levels. One major characteristic of the fetal circulation is the limited heart rate reserve. In our study, among fetuses with a CVP score of ≤7, abnormal venous Doppler sonography findings were significantly more common and more severe in fetuses with tachy- or bradyarrhythmia compared with those with CHD. Moreover, fetuses with tachy- or bradyarrhythmia had higher NP levels than fetuses with CHD. Abnormal venous Doppler sonography findings indicate elevation of central venous pressure[19]. The increase in wall stress will result in cardiac remodeling and hypertrophy, which increases myocardial oxygen consumption and aggravates myocardial dysfunction. To overcome the reduction in ventricular compliance,

end-diastolic filling pressure and hydrostatic central venous pressure will increase to maintain cardiac output, resulting in more NP release from the fetal heart[19–21]. Thus, we speculate that high NP levels may be associated with rapid progression to hydrops in fetuses with tachy- or bradyarrhythmia[15–17].

Right heart defects with moderate or severe TR were associated with lower CVP scores and higher NP levels than other types of CHDs. Merz *et al*. reported that fetuses with ventricular outflow tract obstruction and an intact interventricular septum have significantly higher NT-proBNP levels than fetuses with shunt lesions[13]. They speculated that high ventricular pressure was associated with elevated NP levels. In fact, fetuses with right heart defect but no or mild TR, which does not lead to high right ventricular pressure, had low NP levels in our study. However, all HLHS with restrictive foramen ovale had low NP levels in our study, even though these were presumed to have high left atrial and ventricular pressure. Taken together, we can safely presume that elevated NP levels can be mainly attributed to increases in central venous pressure secondary to atrioventricular valve regurgitation due to CHD, rather than the morphological abnormality itself.

Compared to controls, no differences were found in NP levels in fetuses with CHD, arrhythmia, or both and a CVP score of ≥8. This finding reflects the fact that fetuses with a high CVP score do not have heart failure *in utero*, even though they might have a complex CHD or arrhythmia. Given the physiological advantage of parallel circulation and bypassing of the pulmonary circulation, even fetuses with congenital single ventricle physiology could adapt during fetal circulation[22,23]. Therefore, mortality after birth cannot be predicted by CVP score and NP levels for some types of CHDs. For example, HLHS with highly restrictive foramen ovale is well known to have a poor prognosis soon after birth[7], but it was associated with high CVP scores and low NP levels in our study.

Preterm birth was independently correlated with high NP levels in our study. Earlier

studies have shown that gestational age is not an important determinant of fetal and newborn ANP levels[24,25]. Plasma ANP levels were higher in fetuses with hydrops than in controls[25]. Based on these reports, preterm birth caused by fetal heart failure or hydropic status may contribute to high NP levels. A previous study has shown that umbilical cord ANP levels were inversely related to UA pH[24]. Maternal hypertensive disorder and fetal acidemia during labor have been reported to stimulate fetal ANP production[26]. We found that UA pH <7.15 is independently correlated with high NP levels, which is consistent with these previous studies.

There were several limitations in the present study, including its single-center nature and the relatively small sample size. First, the most severe cases resulting in fetal demise were not included in the analysis, because umbilical cord blood samples were only available for live births. However, our institution is one of the largest tertiary pediatric cardiac centers in Japan, and a variety of complex CHDs and arrhythmias was included in the study cohort. In addition, all fetuses with CHD and arrhythmia were diagnosed prenatally with high accuracy and had a CVP score assessed within 1 week before birth. As a result, we demonstrated that CVP score could be used to assess heart failure for fetuses with CHD and fetal arrhythmia. Second, we were not able to investigate the relationship between NP levels and mortality after birth. Because of improvements in transplacental therapy for arrhythmias and neonatal management of severe complex CHDs, there were only 4 neonatal and infant deaths (3.2%) in the present study cohort, so multivariate analyses of mortality were not possible. Larger multicenter prospective studies involving CVP score and NP levels are required to better appreciate factors associated with mortality in fetuses with various types of CHDs and arrhythmias. Percutaneous umbilical blood sampling will be necessary to obtain real-time NP values that exclude the stress of labor. Since percutaneous umbilical blood sampling is an invasive procedure, we are planning to develop less invasive methods such as maternal blood biomarkers reflecting fetal heart failure.

In conclusion, plasma NP levels in fetuses with CHD, arrhythmia, or both are correlated with the severity of fetal heart failure. Fetal tachy- or bradyarrhythmias and right heart defects with significant TR show high NP levels. Elevated NP levels are mainly attributed to increases in central venous pressure secondary to arrhythmia or atrioventricular valve regurgitation due to CHD, rather than the morphological abnormality itself.

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Table 1. Perinatal characteristics (n=256)

	Controls (n=127)	Fetuses with CHD and Arrhythmia (n=129)	*P*
Maternal age, years	33.4 ± 4.5	31.8 ± 5.1	<0.01
Primipara status	48 (37.8)	68 (52.7)	0.02
Last cardiovascular profile score	10.0 ± 0	8.9 ± 0.1	<0.01
Last biophysical profile score	10.0 ± 0.1	9.5 ± 0.1	<0.01
Poly- or oligohydramnios	1 (0.8)	11 (8.5)	<0.01
Cesarean delivery	82 (64.6)	45 (34.9)	<0.01
Gestational age at birth, weeks	38.0 ± 1.3	38.1 ± 1.6	0.35
Preterm birth	8 (6.3)	12 (9.3)	0.37
Birth weight, g	2912 ± 353	2794 ± 481	0.03
SGA <10th percentile	0	30 (23.3)	<0.01
Male sex	67 (53.2)	69 (53.5)	0.96
Neonatal death within 1 month	0	2 (1.6)	0.50
Infant death from 1 to 3 months	0	2 (1.6)	0.50
Apgar score ≤7 at 5 minutes	0	9 (7.0)	<0.01
Umbilical artery pH <7.15	0	2 (1.6)	0.50
Ductal dependence	0	35 (27.1)	<0.01

Data are n (%) unless otherwise specified. Maternal age, cardiovascular profile score, biophysical profile score, gestational age at birth, and birth weight are shown as means ± SD. CHD, congenital heart defect; SGA, small for gestational age.

401 **Table 2.** Categories of CHD and arrhythmia (n=129)

CHDs (n=86)
Single ventricle physiology
Isomerism (n=15)
Right atrial isomerism (n=12)
Left atrial isomerism (n=3)
Hypoplastic left heart syndrome (n=6)
Right heart defect (n=20)
Ebstein's anomaly or tricuspid valve dysplasia (n=6)
Pulmonary atresia with an intact ventricle septum (n=4)
Tricuspid atresia (n=10)
Biventricular physiology
Cyanotic heart defect (n=28)
Transposition of the great arteries (n=7)
Double outlet right ventricle (n=5)
Tetralogy of Fallot (n=12)
Truncus arteriosus (n=4)
Acyanotic heart defect (n=17)
Coarctation of the aorta (n=9)
Atrioventricular septal defect (n=8)
Arrhythmias (n=43)*
Tachyarrhythmia (n=20)
Supraventricular tachycardia or atrial flutter (n=18)
Ventricular tachycardia (n=2)
Bradyarrhythmia (n=11)
Sinus bradycardia (n=5)
Second-degree atrioventricular block (n=1)
Complete atrioventricular block (n=5)

Extrasystole (n=12)

*A total of 8 fetuses had arrhythmia complicated by CHD; 1 fetus with supraventricular tachycardia had a cardiac tumor, 2 fetuses with complete atrioventricular block and 2 fetuses with sinus bradycardia had left atrial isomerism, and 4 fetuses with atrial extrasystole had atrioventricular septal defect.

CHD, congenital heart defect.

Table 3. Univariate and multivariate analyses of UV NT-proBNP levels in fetuses with CHD, arrhythmia, or both (n=129)

	Univariate			Multivariate*		
	Coefficient	**95% CI**	***P***	**Coefficient**	**95% CI**	***P***
CVP score of ≤5	9788.62	8289.47 – 11267.77	<0.01	3299.37	1748.47 – 4850.27	<0.01
Preterm birth	4686.71	3424.36 – 5949.06	<0.01	1281.68	327.08 – 2236.27	<0.01
Weight at birth	-1.21	-2.08 – 0.33	0.01			
UA pH <7.15	9674.64	5821.02 – 13528.25	<0.01	7903.22	5455.22 – 10351.21	<0.01
Tachy- or bradyarrhythmia at birth	10924.2	9567.57 – 12280.83	<0.01	8719.68	7365.91 – 10073.45	<0.01
Cesarean delivery	1013.44	298.72 – 1728.16	0.01			

*The best prediction model was selected using backward elimination with *P*=0.10 as the criterion for exclusion. Stepwise analysis was used to adjust for baseline variables.

CHD, congenital heart defect; CI, confidence interval; CVP score, cardiovascular profile score; NT-proBNP, N-terminal pro-brain natriuretic peptide; UA, umbilical arterial; UV, umbilical vein.

Table 4. Comparison of 5 echocardiographic parameters comprising the last CVP score in fetuses with CHD versus arrhythmia and suspected moderate or severe heart failure (CVP score of ≤7, n=22).

	CHD (n=12)	**Arrhythmia* (n=10)**	***P***
Last CVP score	5.7 ± 2.1	5.4 ± 1.4	0.74
1. Fetal effusion†			0.78
Absence of effusion	6 (50.0)	6 (60.0)	
Abdominal, pleural, or pericardial effusion (-1 pt)	4 (33.3)	2 (20.0)	
Skin edema (-2 pt)	2 (16.7)	2 (20.0)	
2. Venous Doppler finding†			0.01‡
Normal venous Doppler	4 (33.3)	1 (10.0)	
Reversed ductus venosus flow (-1 pt)	7 (58.3)	2 (20.0)	
Pulsatile flow in the umbilical vein (-2 pt)	1 (8.3)	7 (70.0)	
3. Heart size			0.53
CTAR <35%	0	1 (10.0)	
CTAR between 35% and 50% (-1 pt)	9 (75.0)	7 (70.0)	
CTAR >50% (-2 pt)	3 (25.0)	2 (20.0)	
4. Cardiac function			0.56
Normal cardiac function	1 (8.3)	2 (20.0)	
Holosystolic TR, or ventricular FS < 28% (-1 pt)	6 (50.0)	3 (30.0)	
Holosystolic MR or CAVVR, or monophasic inflow pattern (-2 pt)	5 (41.7)	5 (50.0)	
5. Arterial Doppler finding			0.24
Normal UA Doppler	9 (75.0)	10 (100)	
No end-diastolic UA flow (-1 pt)	2 (16.7)	0	

Reversed end-diastolic UA flow (-2 pt)	1 (8.3)	0

Data are n (%) unless otherwise specified.

*Tachy- or bradyarrhythmia but not extrasystole was observed in all 10 fetuses with arrhythmia and a CVP score of ≤7 at birth.

†Only the worst finding was counted for each echocardiographic parameter in the last CVP score. All fetuses with pulsatile flow in the umbilical vein had reversed ductus venosus flow. All fetuses with skin edema had abdominal, pleural, or pericardial effusion.

‡Abnormal venous Doppler sonography findings were significantly more common and more severe in fetuses with tachy- or bradyarrhythmia than those with CHD (P=0.01).

CAVVR, common atrioventricular valve regurgitation; CHD, congenital heart defect; CTAR, cardiothoracic area ratio; CVP score, cardiovascular profile score; FS, fractional shortening; MR, mitral valve regurgitation; TR, tricuspid valve regurgitation; UA, umbilical artery.

Figure Legends

Figure 1. Study flowchart

CHD, congenital heart defect.

Figure 2. UV NT-proBNP concentration and CVP score in fetuses with CHD, arrhythmia, or both

All controls had a CVP score of 10. All fetuses with CHD, arrhythmia, or both were divided into 3 groups according to the severity of fetal heart failure.

(i) Fetuses with a CVP score of 6 or 7 and a CVP score of ≤5 had higher UV NT-proBNP levels than controls, respectively (*P<0.01). No differences were observed in UV NT-proBNP levels between fetuses with CHD or arrhythmia and a CVP score of ≥8 versus controls (P=0.16).

(ii) Concentrations of UV NT-proBNP in fetuses with CHD, arrhythmia, or both were inversely correlated with CVP score (P for trend <0.01).

Boxes extend from the 25th to the 75th percentile. The middle horizontal line within each box indicates the median. Vrtical lines extend from the box to a distance of at most 1.5 times the interquartile range. Outliers are plotted separately.

CHD, congenital heart defect; CVP score, cardiovascular profile score; NT-proBNP, N-terminal pro-brain natriuretic peptide; UV, umbilical vein.

Figure 3. CHD, arrhythmias, and UV NT-proBNP concentrations

Tachy- or bradyarrhythmia but not extrasystole was observed in all 10 fetuses with arrhythmias and CVP score of ≤7 at birth. Among fetuses with a CVP score of ≤7, those with arrhythmia had higher UV NT-proBNP levels than fetuses with CHD (*P<0.01).

458 CHD, congenital heart defect; CVP score, cardiovascular profile score; NT-proBNP, N-
459 terminal pro-brain natriuretic peptide; UV, umbilical vein.

460 **Supplementary Figure Legends**

461

462 **Figure S1.** UV ANP and BNP concentration and CVP score in fetuses with CHD,

463 arrhythmia, or both

464 (i) Fetuses with a CVP score of 6 or 7 and with a CVP score of ≤5 had higher UV NP levels

465 than controls, respectively (*P<0.01). No differences were observed in UV NP levels

466 between fetuses with CHD or arrhythmia and a CVP score of ≥8 versus controls

467 (ii) Concentrations of UV ANP (S1A) and BNP (S1B) in fetuses with CHD, arrhythmia, or

468 both were inversely correlated with CVP score, respectively (P for trend <0.01).

469 ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHD, congenital heart defect;

470 CVP score, cardiovascular profile score; UV, umbilical vein.

Figure 1

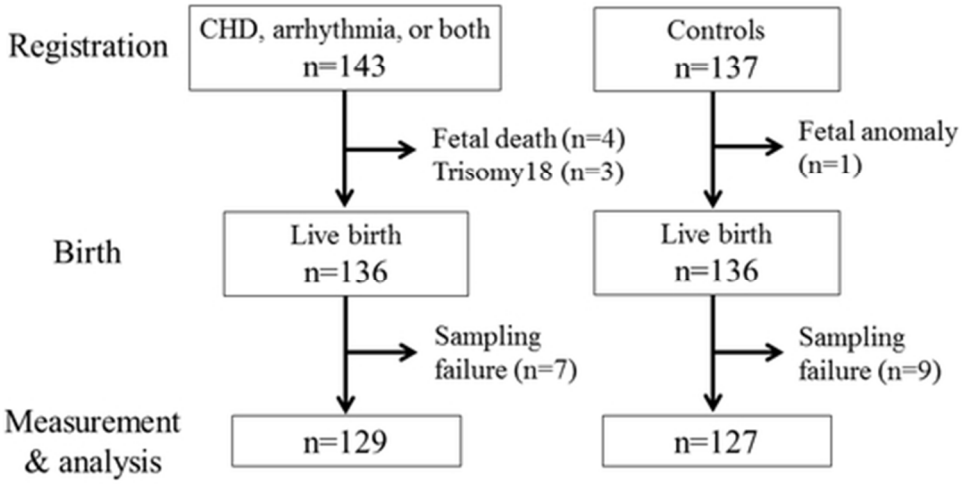

Figure 1

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Figure 2

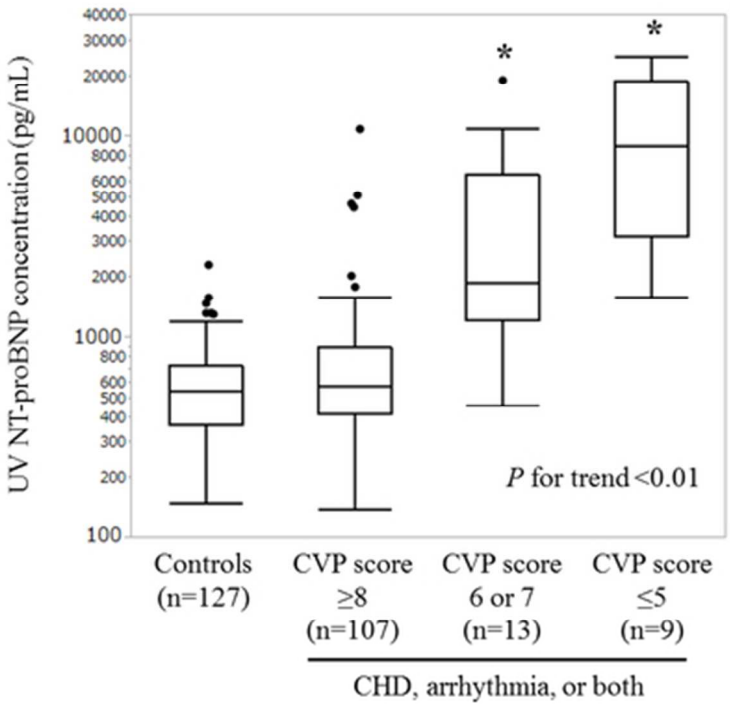

Figure 2

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Figure 3

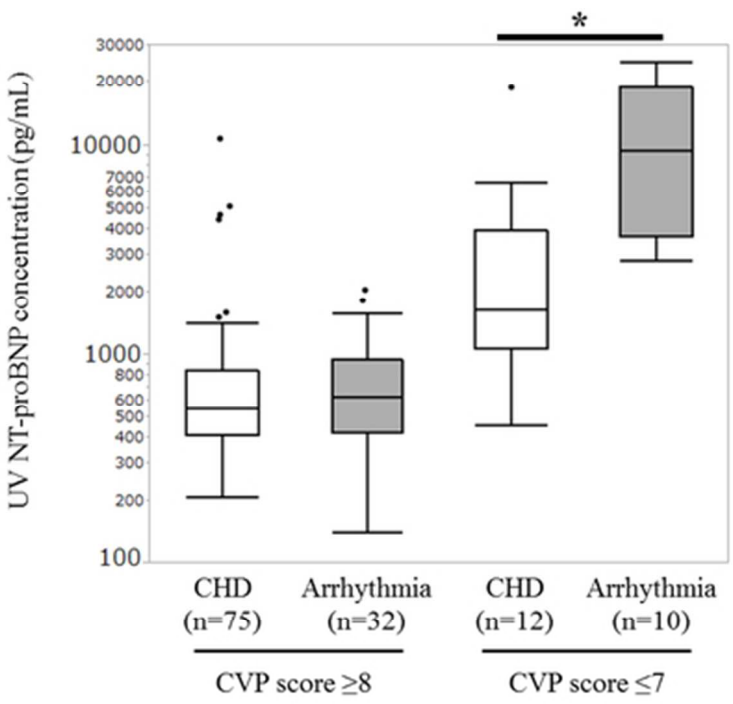

Figure 3

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Supplementary Table 1. CVP scores in fetuses with CHD, arrhythmia, or both (n=129)

	First CVP score	Last CVP score	Decrease in CVP score
CHD (n=86)			
Single ventricle physiology			
Isomerism (n=15)	9.2 ± 1.3	9.1 ± 1.2	3 (20.0)
Hypoplastic left heart syndrome (n=6)	8.8 ± 0.4	8.8 ± 0.4	0
Right heart defect (n=20)*	8.1 ± 2.4	7.9 ± 2.5‡	4 (20.0)
Biventricular physiology			
Cyanotic heart defect (n=28)	9.6 ± 0.6	9.5 ± 0.7	2 (7.1)
Acyanotic heart defect (n=17)	9.5 ± 1.2	9.4 ± 1.6	1 (5.9)
Arrhythmia (n=43)			
Tachyarrhythmia (n=20)	7.3 ± 1.7	8.9 ± 1.9	3 (14.3)
Bradyarrhythmia (n=11)†	7.9 ± 1.9	7.6 ± 2.6	3 (27.3)
Extrasystole (n=12)	8.9 ± 1.0	9.0 ± 1.0	0

CVP score, gestational week at diagnosis, and follow-up duration are shown as means ± SD.

Data are n (%) unless otherwise specified.

*One neonatal death was due to Ebstein's anomaly with circular shunt. †One neonatal death was due to complete atrioventricular block with left atrial isomerism. ‡Right heart defect vs. other categories of CHD (P<0.01).

CHD, congenital heart defect; CVP score, cardiovascular profile score.

Figure S1A

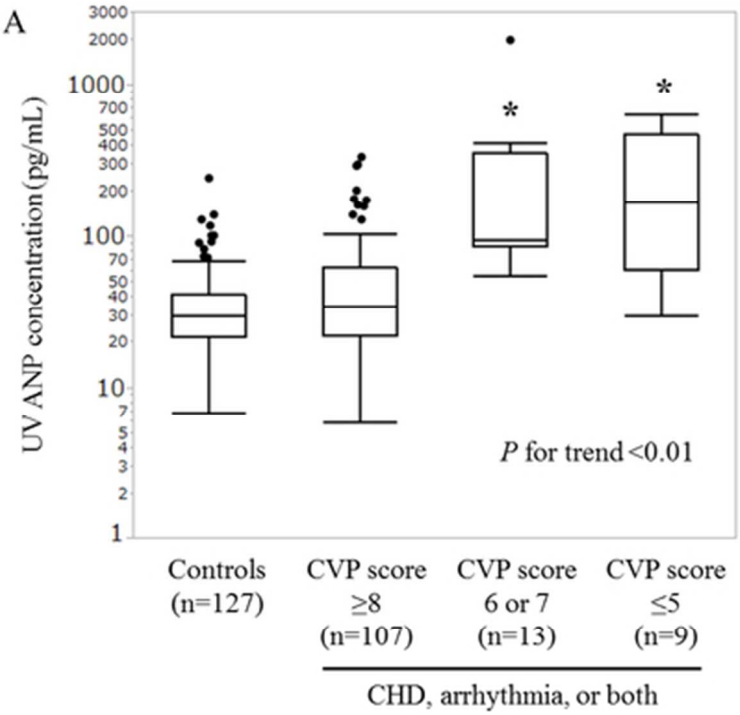

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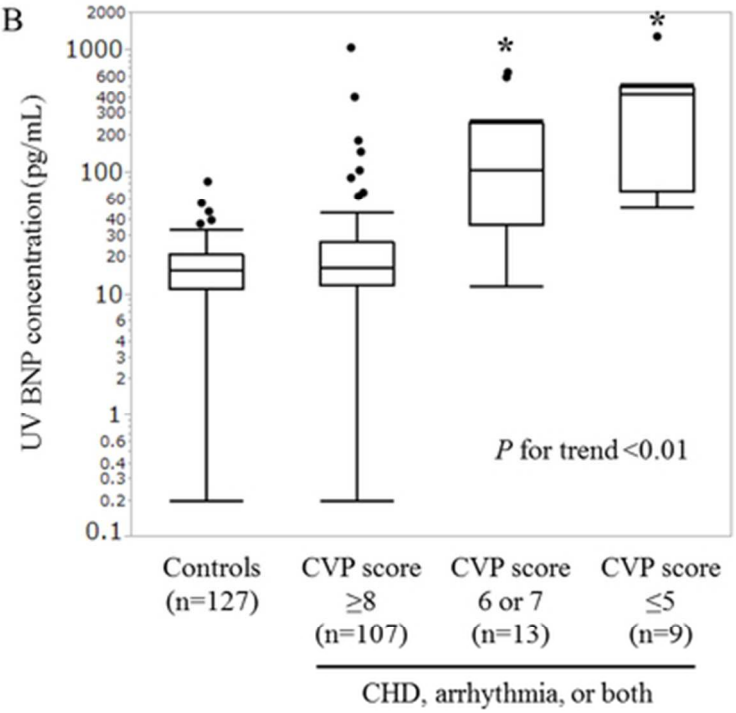
Figure S1B



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