Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses

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OBJECTIVE: The purpose of this study was to assess cardiac function and cell damage in intrauterine growth-restricted (IUGR) fetuses across clinical Doppler stages of deterioration.

STUDY DESIGN: One hundred twenty appropriate-for-gestational-age and 81 IUGR fetuses were classified in stages 1/2/3 according umbilical artery present/absent/reversed end-diastolic blood flow, respectively. Cardiac function was assessed by modified-myocardial performance index, early-to-late diastolic filling ratios, cardiac output, and cord blood B-type natriuretic peptide; myocardial cell damage was assessed by heart fatty acid–binding protein, troponin-I, and high-sensitivity C-reactive protein.

RESULTS: Modified-myocardial performance index, blood B-type natriuretic peptide, and early-to-late diastolic filling ratios were increased in a stage-dependent manner in IUGR fetuses, compared with appropriate-for-gestational-age fetuses. Heart fatty acid–binding protein levels were higher in IUGR fetuses at stage 3, compared with control fetuses. Cardiac output, troponin-I, and high-sensitivity C-reactive protein did not increase in IUGR fetuses at any stage.

CONCLUSION: IUGR fetuses showed signs of cardiac dysfunction from early stages. Cardiac dysfunction deteriorates further with the progression of fetal compromise, together with the appearance of biochemical signs of cell damage.

Key words: cardiac function, Doppler ultrasound, heart fatty acid–binding protein, intrauterine growth restriction, myocardial damage

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Severe intrauterine growth restriction (IUGR) because of placental insufficiency affects 1% of pregnancies and contributes to 30% of total perinatal loss and severe morbidity.[1] The heart is a central organ in the fetal adaptive mechanisms to placental insufficiency and hypoxia. Elevated fetal levels of atrial and B-type natriuretic peptides and significant differences in echocardiographic parameters have been reported in small-for-date babies.[2-6] Monitoring of cardiac function is proposed as an adjunct to current methods to predict adverse outcome and death in IUGR[7]; however, suitable parameters remain to be established. Furthermore, fetal cardiac dysfunction might have important consequences in fetal programming of postnatal cardiac disease later in adulthood. Epidemiologic studies and animal models have established that low-birthweight babies have an increased risk of cardiovascular disease later in life.[8,9]

Clinically, IUGR fetuses are stratified in stages of severity, according to the sequential deterioration of fetoplacental Doppler patterns.[1,10] However, the onset and progression of fetal cardiac dysfunction across stages of severity in IUGR have not been established. Recently, Girsen et al[6] evaluated cord blood atrial and B-type natriuretic peptides in fetuses at different stages of Doppler deterioration and suggested that subclinical cardiac dysfunction might constitute an early event in the course of the disease.

Aside from cardiac function, it is unknown whether myocardial cell damage occurs at any stage of fetal deterioration.

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TABLE 1
Baseline characteristics of the study populations

	AGA		IUGR[a]		
Variable	**Term**	**Preterm**	**Stage 1**	**Stage 2**	**Stage 3**
Patients (n)	80	40	26	28	27
Clinical characteristics					
White (%)	96	71	88	81	84
Smoker (%)	12	27	27	44[b]	53[b]
Preeclampsia (%)	0	0	61[b,c]	76[b,c]	43[b,c]
Current Doppler data[d]					
Umbilical artery pulsatility index	−0.3 (1.3)	0 (2)	4.2 (1.7)[b,c]	8.4 (3.9)[b,c]	14.5 (19.2)[b,c]
Middle cerebral artery pulsatility index	0.1 (1.3)	−0.1 (1.2)	−2.1 (1.4)[b,c]	−2.1 (1)[b,c]	−2.4 (1.2)[b,c]
Cerebroplacental ratio	0 (1.2)	0 (1.2)	−2.6 (0.7)[b,c]	−3.2 (0.9)[b,c]	−3.3 (0.6)[b,c]
Ductus venosus pulsatility index	0 (0.9)	0.2 (0.5)	1 (1.9)[b,c]	1.6 (2.3)[b,c]	4.3 (4.4)[b,c]

[a] IUGR fetuses are classified according to the last ultrasound before delivery or death.

[b] $P < .01$, compared with term AGA.

[c] $P < .01$, compared with preterm AGA.

[d] Values are median (interquartile range).

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Cord blood troponin levels are within normal values in most fetuses with severe IUGR, which suggests that cell necrosis may be uncommon.[3,11,12] However, in adults with heart failure, biomarkers of myocardial cell damage (such as the recently described heart-fatty acid binding protein [H-FABP]) have been demonstrated to be more sensitive than troponins.[13-15] Additionally, high sensitivity C-reactive protein (hsCPR), a marker of tissue injury and inflammation, is being associated increasingly with chronic cardiac disease and damage in adults.[16,17] These cardiovascular biomarkers have not been evaluated in relation with fetal cardiac function in IUGR.

Here we document the evolution of cardiac dysfunction and cell damage across clinical stages of severity in IUGR by conducting a longitudinal study that integrated echocardiographic evidence with biomarkers of cardiac dysfunction and myocardial damage in a cohort of 81 fetuses with well-documented severe fetal growth restriction.

Materials and Methods

Study populations

Eligible cases were singleton pregnancies that were selected from women who attended the Maternal-Fetal Medicine Department at Hospital Clinic and at Harris Birthright Research Centre for Fetal Medicine. The study protocol was approved by the Ethics Committee at each participating institution, and patients provided written informed consent.

IUGR was defined as an estimated fetal weight below the 10th percentile according to local reference curves[18] together with a Doppler pulsatility index in the umbilical artery of >2 standard deviations.[19] For the purposes of this study only, those IUGR infants who died or were delivered between 24 and 34 weeks of gestation were included. IUGR fetuses were longitudinally followed-up with serial ultrasound scans. At each examination fetuses were classified in stages of severity according to the end-diastolic flow (EDF) in the umbilical artery as: stage 1 was defined by presence of EDF; stage 2 was absence of EDF; and stage 3 reversed EDF. Ultrasound scans were performed on a weekly basis for fetuses in stage 1 and every 48-72 hours from stage 2 onwards. If the IUGR fetus remained in the same severity stage as defined earlier during several examinations, the median of each cardiac parameter that was obtained at that stage was considered to be the representative value. Maternal and cord blood samples were collected at delivery for biochemical marker analysis. Exclusion criteria were birthweight >10th percentile, evidence of fetal infection, or structural/chromosomal abnormalities. Echocardiographic or biochemical data were not available to the managing clinicians. *Adverse perinatal outcome* was defined by the presence of perinatal death,[10] bronchopulmonary dysplasia,[20] hyaline membrane disease, neonatal intraventricular hemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis, or retinopathy grade 3 or 4.

Two different populations of appropriate-for-gestational age (AGA) fetuses were selected as control subjects. Ultrasonographic measurements were performed in 80 normal grown fetuses who were delivered at term (term AGA) matched for gestational age at enrolment (±2 weeks) with cases. In addition, maternal and cord blood biomarkers were measured at delivery not only in 30 of these control subjects who were delivered at term but also in 40 AGA fetuses who were delivered preterm without signs of infection (preterm AGA) and matched for gestational age at delivery (±2 weeks) with cases.

TABLE 2
Perinatal outcomes of the study populations

Populations	Term AGA	Preterm AGA	IUGR-s1	IUGR-s2	IUGR-s3
N (patients)	*80*	*40*	*26*	*28*	*27*
Cesarean section (%)	16	35[a]	96[a,b]	100[a,b]	100[a,b]
Gestational age at delivery (weeks)	39 (1)	30 (6)[a]	32 (3)[a]	30 (4)[a]	28 (3)[a,b]
Birth weight (g)	3110 (475)	1605 (1087)[a]	1160 (400)[a,b]	980 (360)[a,b]	600 (352)[a,b]
Birth weight percentile	45 (38)	50 (54)[a]	0 (0.5)[a,b]	0 (0.1)[a,b]	0 (0.1)[a,b]
5-min Apgar	10 (0)	10 (1)	10 (1)	9 (2)[a]	8 (2)[a,b]
Umbilical artery pH	7.30 (0.07)	7.27 (0.08)	7.27 (0.13)	7.21 (0.07)[a]	7.16 (0.1)[a,b]
Intrauterine death	0% (0/80)	0% (0/40)	0% (0/26)	7% (2/28)	33% (9/27)[a,b]
Neonatal death	0% (0/80)	3% (1/40)	4% (1/26)	0% (0/28)	26% (7/27)[a,b]
Perinatal death	0% (0/80)	3% (1/40)	4% (1/26)	7% (2/28)	59% (16/27)[a,b]
Adverse perinatal outcome	0% (0/80)	32% (13/40)[a]	36% (10/26)[a]	42% (12/28)[a]	78% (21/27)[a,b]

IUGR fetuses are classified according to the last ultrasound before delivery or death.
Values are median (interquartile range) or proportions. Adverse perinatal outcome defined by the presence of perinatal death, bronchopulmonary dysplasia, hyaline membrane disease, neonatal intraventricular haemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis or retinopathy grade 3 or 4.
AGA, appropriate for gestational age; *IUGR*, intrauterine growth restriction; *s1*, stage 1; *s2*, stage 2; *s3*, stage 3; *PI*, pulsatility index.

[a] $P < 0.01$ compared to term AGA.

[b] $P < 0.01$ compared to preterm AGA.

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Fetal echocardiography

Echocardiographic measurements included left myocardial performance index (MPI), early and late diastolic filling (E/A) ratios and cardiac output. We used a modified MPI (Mod-MPI) that was adapted to fetal assessment, which results in a substantial improvement in interobserver variability.[21] Mitral and tricuspid peak velocities of E/A ratios were estimated in an apical 4-chamber view, with the Doppler sample volume just below the atrioventricular valves.[22] Left and right cardiac outputs were calculated as $\pi \times$ (aortic or pulmonary valve diameter)$^2 \times$ (aortic or pulmonary artery systolic time-velocity integral) $\times$ heart rate. The combined cardiac output was calculated as the sum of both.[3,23]

Because of their correlation with gestational age, all individual ultrasonographic data were normalized by conversion of the measurements into *Z*-scores (standard deviation from the gestational age mean), with the exception of cardiac output, which was normalized by estimated fetal weight.[19,22-25]

Biomarkers in fetal and maternal blood

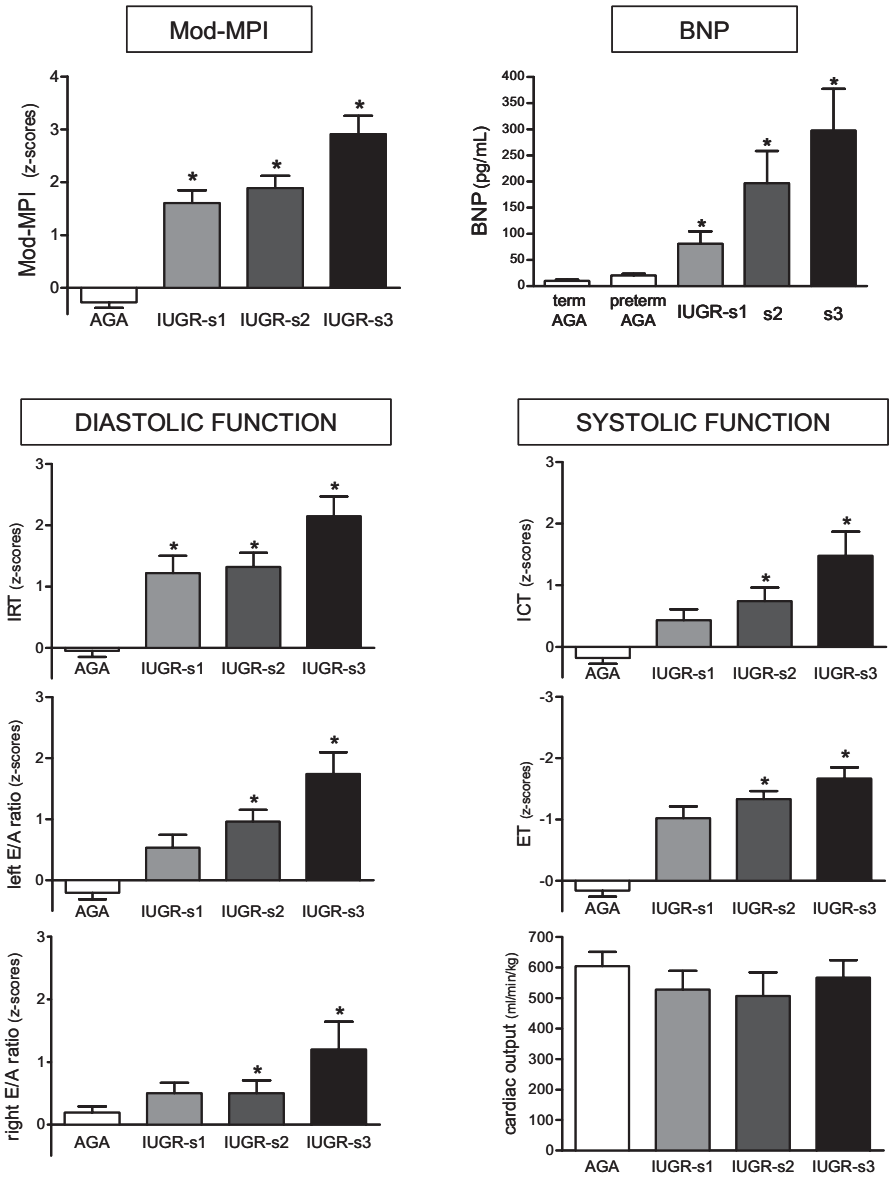
Fetal umbilical EDTA-treated blood was obtained from the umbilical vein after cord clamp at delivery. Maternal samples were drawn from the cubital vein at the time of fetal sampling. All samples were processed within 1 hour. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 4°C; samples were stored immediately at −80°C until assay.

Cord blood levels of B-type natriuretic peptide (BNP) were measured with an immunoassay system (ADVIA Centaur BNP; Siemens Healthcare Diagnostics, Deerfield, IL), as described previously.[26] Plasma concentrations of H-FABP were measured with a commercially available assay (Hycult Biotechnology, Uden, The Netherlands).[27] Troponin I and hsCPR levels were measured with commercially available assays with Centaur CP (Siemens Healthcare Diagnostics) troponin I assay and ADVIA 2400 Chemistry system (Siemens Healthcare Diagnostics), respectively.

Data analysis

Data were analyzed with the SPSS statistical software (version 13.0; SPSS Inc, Chicago, IL). Results were expressed as median ± interquartile range. Comparisons of perinatal outcome among groups were performed with analysis of variance based on log-transformed data that were adjusted with Bonferroni's post-hoc test. Comparisons of cardiac parameters among groups were performed with the use of regression (robust variance) to take into account repeated measurements per subject, with stage as a categoric variable. In addition, the Cuzick nonparametric test for trend across ordered groups was used to assess the existence to progressively different values within severity stages of IUGR.[28] Finally, the association of variables with perinatal death was assessed with the use of the last scan before delivery and cord blood biomarker levels at delivery by *T*-test. Differences were considered significant with probability values of $<.01$.

FIGURE 1
Cardiac function parameters in AGA and IUGR fetuses at different stages of severity



Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with AGA. *ET*, ejection time; *ICT*, isovolumetric contraction time; *IRT*, isovolumetric relaxation time; *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

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Results

Characteristics of the study populations

Clinical and perinatal data are shown in Tables 1 and 2. IUGR fetuses from severity stages 2 and 3 showed lower 5-minute Apgar scores and umbilical artery pH values and higher rates of adverse perinatal outcome, compared with AGA fetuses.

Fetal echocardiography

A total of 62, 65, and 47 ultrasound explorations were performed in IUGR-stages 1, 2, and 3, respectively. Values of echocardiographic parameters in term AGA and in IUGR fetuses are shown in Figure 1. Mod-MPI was significantly higher in stage 1 and showed a progressive increase through further stages of deterioration. All time periods that were used for calculation of the MPI were significantly different in IUGR fetuses. E/A values in both atrioventricular valves were significantly higher from stage 2 onwards. Cardiac output values were similar in control subjects and in IUGR fetuses at all severity stages. The statistical analysis for trend showed a significant tendency to different results with increasing stages of IUGR for all parameters with the exception of cardiac output (probability value of tendency: Mod-MPI, isovolumetric contraction, ejection time, relaxation time, left E/A, and right E/A, $P < .001$; cardiac output, $P = .375$).

Biomarkers in fetal blood

Maternal and cord blood samples were collected in 59 IUGR, 40 preterm AGA, and 30 term AGA fetuses. Data on cord blood biomarker levels are shown in Figures 1 and 2. BNP levels were significantly higher in fetuses at stage 1 and increased further across the stages of severity. H-FABP values were significantly increased in IUGR fetuses at stage 3 together with a significant linear increment across severity stages. Troponin I values were similar in AGA and in IUGR fetuses. Hs-CPR levels were significantly lower in IUGR fetuses at stages 2-3, compared with preterm AGA. Moreover, all biomarkers with the exception of troponin I showed a significant tendency to different results with increasing stages of IUGR (probability value tendency: BNP, H-FABP, and hsCPR, $P < .001$; troponin I, $P = .154$)

Biomarkers in maternal blood

All biomarkers showed similar maternal plasma levels among AGA and IUGR at any severity stage (BNP, $P = .717$; H-FABP, $P = .88$; troponin I, $P = .09$; hsCPR, $P = .673$). No significant correlation was measured between maternal and cord blood levels of any biomarker, with the exception of hsCPR (BNP, $R^2 = -0.008$, $P = .754$; H-FABP, $R^2 = 0.012$, $P = .129$; troponin I, $R^2 = -0.014$, $P = .910$; hsCPR, $R^2 = .261$, $P = .025$).

Association with perinatal death

Mod-MPI and E/A ratios and cord blood BNP, H-FABP and troponin I were significantly increased in IUGR fetuses who

died, in comparison with survivors (Tables 3 and 4).

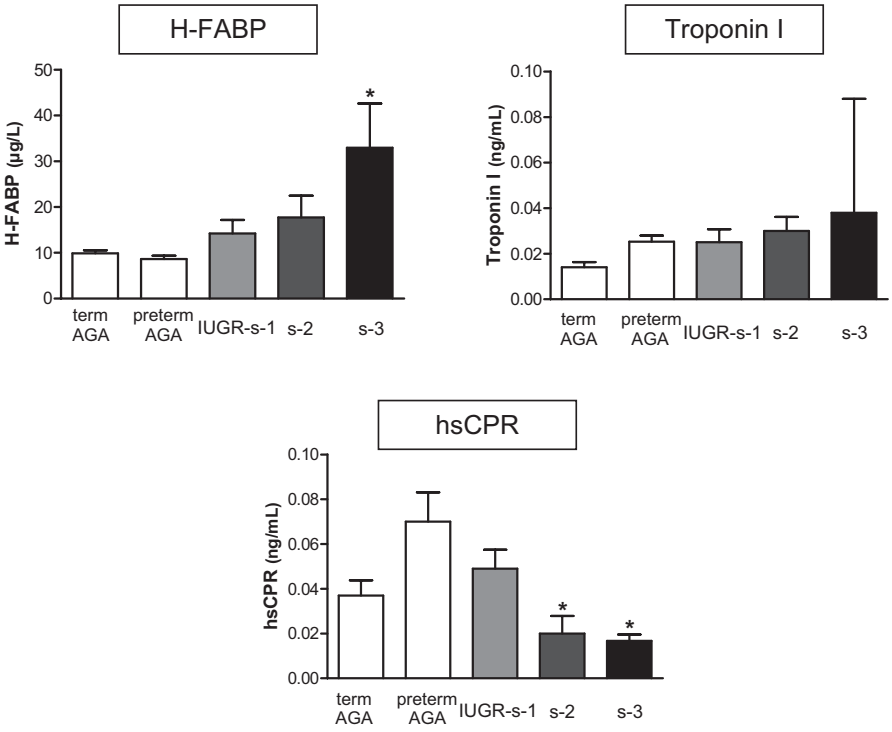
Comment

This study documents the evolution of cardiac dysfunction and cell damage in fetal growth restriction in relation with Doppler stages of severity that are used widely in clinical practice. It provides evidence that subclinical cardiac dysfunction is an early and progressive event in severe IUGR. Echocardiographic parameters and cord blood levels of BNP indicate that cardiac dysfunction increased progressively across the stages of fetal compromise. Advanced fetal Doppler deterioration was associated with a significant increase in H-FABP levels, which supports the existence of myocardial cell damage, but to such a small extent that it could not be detected by cord blood levels of troponin I.

Echocardiographic parameters that indicate subclinical cardiac dysfunction increased through the Doppler stages of fetal deterioration that were defined in this study. MPI has been reported previously to be increased in IUGR fetuses.[5,29] In this study we further described that Mod-MPI was elevated in fetuses at initial stages of hemodynamic compromise and progressed further with fetal deterioration. The progressive increment in Mod-MPI values was at the expense of all time periods that were involved in the calculation of this index, which suggests the existence of both systolic and diastolic subclinical dysfunction. E/A ratio evaluates ventricular filling during the diastole; in adults, decreased values are considered a sign of diastolic dysfunction. Contrary to adults, fetuses with overt heart failure have been reported to have increased E/A ratios.[30] In IUGR, earlier studies described unchanged or reduced E/A values,[2-4,31] but most recent studies found the ratios to be increased significantly with respect to control subjects.[3,6] We also observed a considerable increase in E/A values in fetuses with IUGR in both atrioventricular valves, which was present from stage 2 and increased further with fetal Doppler deterioration. Finally, this study confirmed previous observations that indicated that cardiac output is maintained within normal values, even in most severe clinical stages of IUGR.[6,23]

The progressive increase of BNP cord blood levels in IUGR fetuses was consistent with that observed for echocardiographic parameters. In adults, BNP is the gold standard biomarker for heart failure[32]; serum levels are elevated in early stages of subclinical diastolic dysfunction and increase in proportion to severity.[32] Our data in human fetuses with IUGR are in agreement with those of Girsen et al[6] who demonstrated that N-

FIGURE 2
Myocardial cell damage markers in AGA and IUGR fetuses at different stages of severity



Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with preterm AGA. *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

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TABLE 3
Echocardiographic parameters and perinatal deaths in IUGR fetuses

Variable	Survivors (n = 62)	Perinatal deaths (n = 19)	*P* value
Mod-MPI	1.7 (3)	2.5 (2)	.027
Isovolumetric contraction time	0 (2.2)	0.2 (2.7)	.063
Ejection time	−1.1 (1.4)	−2.1 (1.6)	.130
Isovolumetric relaxation time	1 (3.1)	2.1 (3.4)	.067
Left E/A	0.8 (2.2)	2.4 (2.2)	.045
Right E/A	1 (2.1)	2.3 (5.2)	.002
Cardiac output (mL/min/kg)	750 (274)	816 (248)	.484

Values are median (interquartile range). Doppler values are expressed in *Z*-scores, with the exception of cardiac outputs normalized by fetal weight.

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TABLE 4
Cord blood cardiac biomarkers and perinatal death in IUGR fetuses

Variable	Survivors (n = 51)	Perinatal deaths (n = 8)	*P* value
Cardiac function			
BNP (pg/mL)	64 (127)	350 (456)	.029
Myocardial cell damage			
H-FABP (μg/L)	11 (11)	23 (103)	<.001
troponin I (ng/mL)	0.02 (0.02)	0.07 (1.14)	.002
hsCPR (ng/mL)	0.02 (0.04)	0.01 (0.02)	.176

Values are median (interquartile range).

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terminal peptide of proBNP was elevated in fetuses with growth restriction and increased across fetoplacental Doppler stages of fetal compromise. In another study, Leipala et al[33] showed increased levels of BNP in preterm IUGR neonates during the first days of life, which points to a persistence of cardiac dysfunction postnatally.

The integrated evaluation of myocardial cell and tissue damage biomarkers offers new information for the understanding of fetal cardiac disease in IUGR. H-FABP cord blood levels showed a significant linear increase across stages and were increased significantly in fetuses with reverse flow in the umbilical artery, which suggests the presence of myocardial cell damage in advanced stages of fetal deterioration. H-FABP is a novel biochemical marker with a relatively small size that confers a high sensitivity to detect myocardial cell damage.[15] It has proved not only to be an excellent marker for the early detection of cardiac injury in acute coronary syndromes but also showed to be sensitive enough for the detection of chronic minor myocardial injury in heart failure.[13,14] On the other hand, troponin is a well-established marker for myocardial infarction. However, 94% of cardiac troponin is bound to myofibrillar structures, and it has first to dissociate from its matrix before it is released. This explains a time delay for raised levels in plasma and a lower sensitivity to subtle cell damage, compared with H-FABP.[15] Consistent with this notion, the observed elevation of H-FABP in this study occurred in the absence of significant changes in mean troponin levels. The latter finding is in line with previous studies that measured troponin in IUGR.[3,11,12] It must be noted that, despite of the absence of overall differences, this and previous studies have observed individual cases of severe fetal growth restriction with high cord blood troponin values,[3,11,12] which suggests that extended cell damage may occur in very advanced stages of the disease. In this study, fetuses who died had significantly higher levels of troponin, compared with survivors.

Finally, we could not demonstrate any increase in hsCPR cord blood levels in severe IUGR fetuses, compared with preterm AGA. On the contrary, there was a significant decrease of cord blood hsCPR in IUGR stages 2 and 3, compared with preterm AGA, but not with term AGA fetuses. These differences could be explained by an abnormal increase in hsCPR levels in preterm AGA because of the accidental inclusion of $\geq$1 cases with subclinical prenatal infection. Alternatively, it could reflect a true decrease in hsCPR levels in most severe forms of IUGR that we cannot explain. HsCPR is a well-established acute marker for tissue injury, and the lack of elevation in severe IUGR fetuses is consistent with the results that have been observed for troponin. On the other hand, hsCPR has also been described as a marker of chronic inflammation that leads to cardiovascular disease.[16,17] Thus, our data do not support the implication of this pathophysiologic mechanism in fetal cardiac disease that is related to growth restriction. The results in hcPCR are in disagreement with a recent study that described an increase in hsCPR levels in near-term small-for-gestational age fetuses.[34] Although the population is not comparable with that of the present study in terms of gestational age or in severity, we cannot find an explanation for the differences between both studies.

This study has several limitations. First, there was an elapsed period between the last ultrasound measurement and cord blood sampling. Although this did not exceed 48 hours, we acknowledge that, in a small number of cases, the severity stratification based on ultrasound parameters might have changed at the time of blood sampling. Second, sufficient cord blood for analysis could not be retrieved in approximately 20% of cases because of the inherent difficulty in obtaining samples from extremely preterm and small IUGR fetuses. Furthermore, cord blood was not available in all intrauterine deaths. This may have biased the results by attenuating the differences on the levels of biomarkers between control subjects and IUGR fetuses. Third, most biomarkers that were evaluated in this study are not fully cardiospecific. This may raise concern for the interpretation of H-FABP data. This protein is expressed abundantly in cardiomyocytes, but to a lesser extent also in skeletal muscle, distal tubular cells of the kidney, specific parts of the brain, lactating mammary glands, and placenta.[14,35] Although we postulate that cardiac damage is the most plausible reason for the observed increase in this study, we acknowledge that this concept cannot be demonstrated completely. Although the placenta can also produce H-FABP, reduced maternal levels in IUGR pregnancies support that placental production is unlikely to account for the observed increases in fetal blood. Future studies in animal models might help to clarify the origin of H-FABP in growth-restricted fetuses. Finally, it is unknown whether the association with maternal preeclamptic symptoms has any effect on fetal cardiac function. This potential confounding effect is being investigated in further studies.

The association between the risk of adverse outcome or perinatal death and the presence of abnormal echocardiographic parameters has been reported previously.[7,10] In this study, we confirm previous reports and further describe that those fetuses who died in utero or postnatally had significantly increased levels of H-FABP and troponin. Ultrasonographic assessment of cardiac function could be integrated into clinical practice to improve the short-term prediction of fetal death. This information is essential to guide clinical decisions of fetuses with severe growth restriction, but no single Doppler ultrasound parameter has been demonstrated to have sufficient predictive value.[7,10,36] In the present study, MPI and E/A were higher in fetuses who died, but their predictive value for perinatal death in IUGR remains to be evaluated. In a preliminary study, a composite cardiovascular score that integrated MPI with other Doppler indices improved the short-term prediction of fetal death, compared with ductus venosus Doppler alone.[37]

In summary, this study provides evidence that cardiac dysfunction is an early event in fetal growth restriction and that its magnitude increases in proportion to the severity of the fetal condition. Thus, subclinical cardiac dysfunction is present in fetuses with IUGR and mild degrees of Doppler deterioration, which currently is considered to have an overall good long-term outcome.[1,7,10] The data further suggest that advanced stages of fetal deterioration are associated with myocardial cell damage. It is tempting to speculate that the degree and duration of cardiac dysfunction and damage might be associated with distinct effects on fetal programming of cardiovascular function and disease in adulthood. The impact in long-term cardiovascular outcome of the changes here described remains to be established in long-term follow-up studies. ■

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Expanded Methods

Delivery indications and perinatal outcome definitions

The indications for delivery included decompensation of maternal disease (severe preeclampsia), decelerative fetal heart rate pattern, and deterioration of fetal venous indices (absent or reversal of atrial flow in the ductus venosus). When delivery was indicated for fetuses with gestational age of <26 weeks of gestation or with estimated weights of <500 g, patients where counseled regarding the high mortality rate and adverse sequelae, and the option of expectant management was contemplated. At delivery, gestational age, birthweight, Apgar scores, and umbilical pH were recorded. *Perinatal death* was defined as either intrauterine death or neonatal death within the first 28 days of life.[14] *Bronchopulmonary dysplasia* was defined by an oxygen dependency at 36 weeks of corrected gestational age plus compatible clinical and radiographic changes.[25] *Neonatal intraventricular hemorrhage* was defined according to Papile's criteria.[26,27] Necrotizing enterocolitis was classified according to modified Bell's stages.[28] *Adverse perinatal outcome* was defined by the presence of perinatal death, bronchopulmonary dysplasia, neonatal intraventricular hemorrhage grade 3 or 4, or necrotizing enterocolitis.

Ultrasonographic evaluation

The ultrasound studies were performed with a Siemens Sonoline Antares system (Siemens Medical Systems, Malvern, PA, USA) or a Voluson 730 Expert system (GE Medical Systems, Milwaukee, WI) with 6-4 MHz linear curved array probes. All estimations were done in the absence of fetal corporal and respiratory movements and with the mother in voluntary suspended respiration. The angle of insonation was kept <30 degrees in all measurements. The mechanical and thermal indices were maintained at <1, and the wall filter was set to 70 Hz. Routine ultrasound examination included fetal weight calculation, amniotic fluid index, and Doppler examination of the umbilical artery in a free loop of the umbilical cord, middle cerebral artery in a transverse view of the fetal skull at the level of the circle of Willis, and ductus venosus in a mid sagittal view of the fetal abdomen before its entrance to the inferior vena cava.

Echocardiographic measurements

Cardiac function was assessed by ultrasound with 2-dimensional and Doppler ultrasound modes in 80 term AGA and 81 IUGR fetuses. For term AGA fetuses, only 1 ultrasound scan was performed. For IUGR fetuses, there was a median of 3 examinations in the 2-week interval before delivery. If the IUGR fetus remained in the same severity stage as defined earlier during several examinations, the median of each cardiac parameter that was obtained at that stage was considered as the representative value.

Echocardiographic measurements included left MPI, E/A ratios, and cardiac output. Mod-MPI was obtained in a cross-sectional image of the fetal thorax, with the Doppler sample volume placed on the medial wall of the ascending aorta to include the leaflets of the aortic and mitral valves.[29] The clicks of the valves that registered in the Doppler trim were used as landmarks to calculate the isovolumetric contraction and relaxation times and the ejection time. Mod-MPI was calculated in the following manner: (isovolumetric contraction + isovolumetric relaxation time)/ejection time. Right and left E/A ratios were estimated in an apical 4-chamber view with the Doppler sample volume placed just below the atrioventricular valves.[30] Diameters of the aortic and pulmonary valves were measured 3 times in frozen real-time images during systole by the leading-edge method and with the mean as representative.[8] Aortic systolic time-velocity integral was obtained in a long axis of the left ventricle, and pulmonary artery systolic time-velocity integral was obtained in a short-axis view of the fetal heart. Then, left and right cardiac outputs were calculated in the following manner: $\pi \times$ (aortic or pulmonary valve diameter)$^2 \times$ (aortic or pulmonary artery systolic time-velocity integral) $\times$ heart rate. The combined cardiac output was calculated as the sum of both.[11,31]