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Fetal cardiac dysfunction in preterm premature rupture of membranes

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Background: Preterm premature rupture of membranes (PROM) is associated with one-third of preterm births. In about 50% of preterm PROM cases, the fetuses will elicit a fetal inflammatory response syndrome (FIRS). FIRS is associated with the impending onset of preterm labor, periventricular leukomalacia, neonatal sepsis, and long-term handicap, including the development of bronchopulmonary dysplasia and cerebral palsy. The fetal myocardium is a potential target organ of proinflammatory cytokines released during FIRS. The objective of this study was to determine whether preterm PROM is associated with functional changes in the fetal heart, as determined by fetal echocardiography.

Methods: A retrospective study was conducted to assess the diastolic function of fetuses with preterm PROM with documented microbial invasion of the amniotic cavity ($n = 25$), preterm PROM without microbial invasion of the amniotic cavity ($n = 42$), and fetuses from normal pregnancies (control group = 150). Pregnancies with multiple gestation, fetal distress, fetuses that were small for gestational age, and major congenital anomalies were excluded. Fetal echocardiography studies were performed with two-dimensional ultrasound, color Doppler imaging and pulsed Doppler ultrasound. Non-parametric statistics were used for comparisons. A p value of < 0.05 was considered significant.

Results: The prevalence of positive amniotic fluid cultures for micro-organisms in patients with preterm PROM was 35.8% (24/67). *Ureaplasma urealyticum* was the most frequent isolate, either alone (41.7%; 10/24) or with other micro-organisms (29.2%; 7/24). Fetuses with preterm PROM had a higher delta early diastolic filling/atrial contraction (E/A) peak velocity ratio, a higher delta E/A velocity–time integral (VTI) ratio, a lower delta A peak velocity, a lower delta A VTI, and a lower A VTI/total VTI ratio in the mitral valve compared to those with uncomplicated pregnancies. The delta E/A peak velocity ratio was significantly higher and the delta A VTI significantly lower in fetuses with preterm PROM and microbial invasion of the amniotic cavity than in those with preterm PROM without microbial invasion of the amniotic cavity.

Conclusions: Preterm PROM is associated with changes in fetal cardiac function consistent with increased left ventricular compliance. These observations were also noted in fetuses with microbial invasion of the amniotic cavity. Our findings suggest that fetal cardiac function is altered in preterm PROM and, in particular, in cases with intra-amniotic infection.

Key words: DIASTOLIC DYSFUNCTION; PRETERM PROM; FETAL INFLAMMATORY RESPONSE SYNDROME; FETAL ECHOCARDIOGRAPHY; COLOR DOPPLER; INTRA-AMNIOTIC INFECTION

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INTRODUCTION

Preterm premature rupture of membranes (PROM) remains a leading cause of perinatal morbidity and mortality worldwide[1]. In the USA alone, this condition affects 120 000 pregnancies per year[1]. Microbial invasion of the amniotic cavity (MIAC) is present in one-third of patients with preterm PROM at the time of presentation[2–6] and in 75% of cases at the time of the onset of preterm labor[7]. Patients with MIAC have a shorter amniocentesis-to-delivery interval and a higher rate of perinatal morbidity than those with a sterile amniotic cavity[6].

The fetal inflammatory response syndrome (FIRS), operationally defined as an elevated level of fetal plasma interleukin 6 (IL-6), is present in nearly 50% of fetuses with preterm PROM[8] and is a risk factor for perinatal morbidity after adjusting for gestational age at birth[8]. FIRS is characterized by multi-organ involvement including: endocrine evidence of fetal stress expressed as an abnormal cortisol/dehydroepiandrosterone ratio[9]; neutrophilia[10]; an increased number of nucleated red blood cells[10] without a change in the umbilical vein pH or PO_2[11]; and increased concentrations of fetal plasma matrix metalloproteinases-9, an enzyme implicated in the degradation of extracellular matrix and rupture of membranes[12]. Moreover, fetuses with evidence of a systemic fetal inflammatory response are at increased risk for short-term morbidity, as well as long-term complications such as cerebral palsy[13] and bronchopulmonary dysplasia[14].

A systemic inflammatory response syndrome in adults, often referred to by the acronym 'SIRS', was introduced in 1992 by the American College of Chest Physicians and the Society of Critical Care Medicine to describe a complex set of findings which often involved cardiovascular abnormalities thought to be the result of systemic activation of the innate immune system[15]. The changes, which included fever, tachycardia, hyperventilation and an elevated white blood cell count[15], have been attributed to the effects of cytokines and other proinflammatory mediators[16]. In 2001, this same organization noted that elevation of certain mediators such as IL-6 may be associated with SIRS, and that this observation may bring about a new definition of the syndrome in adult patients, as the clinical and laboratory findings originally proposed to characterize SIRS were non-specific[17].

The heart is a major target organ during SIRS, sepsis with organ dysfunction and septic shock. Indeed, most patients with septic shock die of refractory hypotension and cardiovascular collapse[18], which has been attributed to myocardial depression and failure[18,19]. Despite considerable investigation in adults[18–20], there was a paucity of studies in the fetuses of patients with preterm PROM or those exposed *in utero* to MIAC. In 1999, we communicated to the Society for Maternal Fetal Medicine the observation that a novel form of cardiac changes had been observed in fetuses of mothers with preterm PROM[21]. This article provides the evidence for those conclusions, as well as data demonstrating that such changes are also observed in fetuses with preterm PROM and proven MIAC.

PATIENTS AND METHODS

A retrospective study was conducted to assess the cardiac function of fetuses with preterm PROM with MIAC ($n = 25$), preterm PROM without MIAC ($n = 42$) and those with uncomplicated pregnancies (control group = 150). All patients were admitted to Hutzel Hospital, Detroit, Michigan, and Sotero Del Rio Hospital, Santiago, Chile, between January 1993 and July 1999. The criteria for inclusion were: preterm PROM; fetal echocardiography; and amniocentesis for the assessment of the microbial state of the amniotic cavity and fetal lung maturity. Pregnancies with multiple gestation, fetal distress, fetuses that were small for gestational age (defined as birth weight below the 10th centile for gestational age) and congenital anomalies were excluded. Fetal echocardiography studies were performed using two-dimensional ultrasound, color Doppler imaging and pulsed Doppler ultrasound. Gestational age was confirmed by first- or second-trimester ultrasonography. Preterm PROM was diagnosed by sterile speculum examination with a combination of vaginal pooling, nitrazine and ferning tests, whereas MIAC was defined in the presence of positive amniotic fluid culture and/or Gram stain. The control group consisted of patients with uncomplicated pregnancies who were approached in the prenatal clinic and asked to participate in an observational study involving detailed ultrasonographic examination of the fetal heart. All patients had fetal echocardiography, did not have any obstetric complications and delivered a term neonate appropriate for gestational age. If a congenital anomaly was diagnosed during the ultrasonographic examination, the results were disclosed to the referring physician and the patient was excluded from the analysis. The collection and utilization of the data for research purposes was approved by the Institutional Review Boards of the performing sites, as well as the National Institute of Child Health and Human Development (NIH, DHHS). Informed consent was obtained from all patients.

Ultrasound assessment of the fetal heart and maternal and fetal vessels

Examinations were performed with an ACUSON 128-XP machine (ACUSON Corporation, Mountain View, CA, USA) utilizing a 3.5-MHz or a 5-MHz curvilinear probe. Minimum power was used for color and pulsed Doppler examinations. Fetal biometry was attempted in all cases with the goal of obtaining the following parameters:

biparietal diameter, head circumference, abdominal circumference and femur length. An anatomical survey for structural malformations was carried out. Color imaging and pulsed Doppler interrogation were performed in the mitral inflow tract, tricuspid inflow tract and pulmonary veins. A minimum of three pulsed Doppler waveforms were measured from each site and the mean value was used for analysis. From these waveforms the atrial and tricuspid E and A peak velocity and E/A peak velocity ratio were calculated, as well as the following velocity time integrals (VTI): E VTI, A VTI, E/A VTI ratio and A VTI/total VTI ratio.

Amniotic fluid studies included Gram stain, microbial cultures for aerobic and anaerobic bacteria and genital mycoplasmas, and fetal lung maturity tests. The results were used for subsequent clinical management decisions.

Statistical analysis

Kolmogorov–Smirnov tests were utilized to test for normal distribution of the data. Because the measured Doppler values may change as a function of gestational age, first-, second- and third-order polynomial regression analyses were performed in the control group for all of the pulsed Doppler measurements and computed variables. The regression equation that best described every data set was used to predict the mean value for each gestational age. The delta value for each measurement was subsequently computed as the difference between the expected value from the regression equation and the measured value. The Mann–Whitney U test was used for comparisons. A p value of < 0.05 was considered significant.

RESULTS

Table 1 displays the clinical characteristics of control patients and patients with preterm PROM. Maternal age and parity were not different between the two groups. Patients with preterm PROM delivered considerably earlier (31.2 ± 3.4 weeks vs. 39.4 ± 1.2 weeks; $p < 0.001$) and had neonates with significantly lower birth weight (1697 ± 584 g vs. 3398 ± 402 g, $p < 0.001$) than the control patients.

The prevalence of positive amniotic fluid cultures for micro-organisms in patients with preterm PROM was 35.8% (24/67). *Ureaplasma urealyticum* was the most frequently isolated microorganism, either alone (41.7%; 10/24) or in combination with other micro-organisms (29.2%; 7/24). Other micro-organisms recovered from the amniotic fluid were: *Lactobacillus*, *Candida albicans*, *Streptococcus agalactiae*, *Streptococcus viridans*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. One patient had Gram-positive cocci in the amniotic fluid but the culture was negative. There was no significant difference in gestational age at the time of fetal echocardiography between control and preterm PROM patients. Patients with preterm PROM and MIAC had a significantly shorter amniocentesis-to-delivery interval than those with preterm PROM without MIAC ($p = 0.001$).

Mitral valve

Fetuses with preterm PROM had a higher delta E/A peak velocity ratio, a higher delta E/A VTI ratio, as well as a lower delta A peak velocity, a lower delta A VTI and a lower delta A VTI/total VTI ratio compared to fetuses in the control group (Table 2 and Figure 1). Fetuses with documented MIAC had a higher delta E/A peak systolic velocity ratio and lower delta A VTI than those with preterm PROM without MIAC (Table 3 and Figure 2).

Tricuspid valve

Fetuses with preterm PROM had a higher delta E/A peak velocity and a lower delta A VTI/total VTI ratio compared to those in the control group (Table 4). Fetuses with preterm PROM and MIAC had a lower A VTI compared to fetuses with preterm PROM without MIAC (Table 5).

Table 1 Patient demographics

	Control ($n = 150$)	Preterm PROM ($n = 67$)	p Value
Maternal age (years)†	25.6 ± 6.2	25.9 ± 6.8	0.15
Parity ‡	1 (0 – 7)	1 (0 – 9)	0.06
Gestational age at delivery† (weeks)	39.4 ± 1.2	31.2 ± 3.4	< 0.001*
Birth weight† (g)	3398 ± 402	1697 ± 584	< 0.001*

PROM, premature rupture of membranes

* Statistically significant, $p < 0.05$

†Values expressed as mean $\pm$ standard deviation

‡Values expressed as median (range)

Table 2 Fetal echocardiography of the mitral valve in patients with preterm premature rupture of membranes (PROM) and controls

Doppler parameter (delta values)	Control ($n = 150$)	Preterm PROM ($n = 67$)	p Value
Mitral valve E peak velocity	0.008 (−0.136 to 0.207)	0.027 (−0.126 to 0.217)	0.2
Mitral valve A peak velocity	0.0095 (−0.198 to 0.295)	−0.035 (−0.19 to 0.296)	0.01*
Mitral valve E/A peak velocity ratio	0.009 (−0.228 to 0.262)	0.067 (−0.171 to 0.452)	< 0.001*
Mitral valve E VTI	0 (−0.015 to 0.019)	0.002 (−0.01 to 0.025)	0.3
Mitral valve A VTI	0 (−0.014 to 0.20)	−0.004 (−0.015 to 0.014)	0.01*
Mitral valve E/A VTI ratio	0.0175 (−0.528 to 0.971)	0.17 (−0.549 to 0.656)	0.002*
Mitral valve total VTI	0.0005 (−0.024 to 0.034)	0.015 (−0.022 to 0.037)	0.6
Mitral valve A VTI/total VTI	−0.005 (−0.19 to 0.21)	−0.0552 (−0.16 to 0.07)	< 0.001*

* Statistically significant, $p < 0.05$
Values expressed as delta median (range)
VTI, velocity time integrals

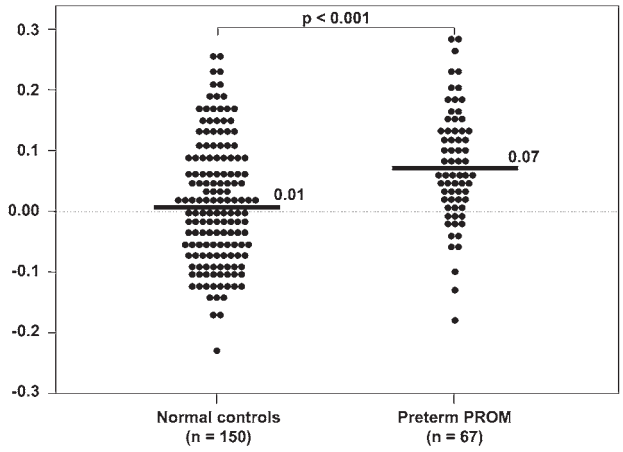

Figure 1 Delta E/A peak velocity ratio of the mitral valve in patients with uncomplicated pregnancies and patients with preterm premature rupture of membranes (PROM). The delta E/A peak velocity ratio in patients with preterm PROM was significantly higher than in those with uncomplicated pregnancies. For values, see Table 1

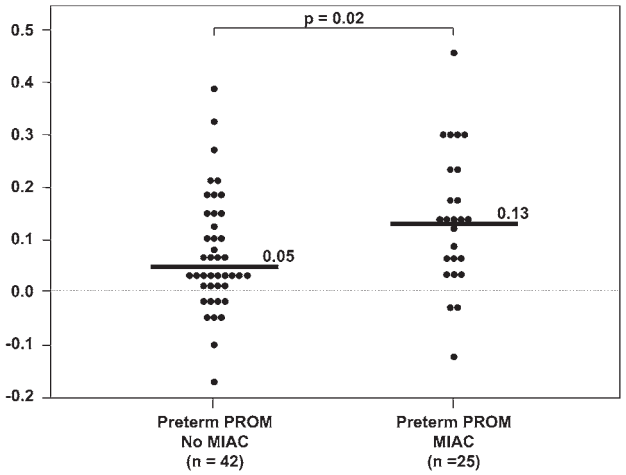

Figure 2 Delta E/A peak velocity ratio of the mitral valve in patients with preterm premature rupture of membranes (PROM) with and without microbial invasion of the amniotic cavity (MIAC). The delta E/A peak velocity ratio in patients with preterm PROM with MIAC was significantly higher than in those without MIAC. For values, see Table 2

Table 3 Fetal echocardiography of the mitral valve in patients with preterm premature rupture of membranes (PROM) with and without microbial invasion of the amniotic cavity (MIAC)

Doppler parameter (delta values)	Preterm PROM without MIAC ($n = 42$)	Preterm PROM with MIAC ($n = 25$)	p Value
Mitral valve E peak velocity	0.028 (−0.11 to 0.158)	0.025 (−0.126 to 0.217)	0.6
Mitral valve A peak velocity	−0.034 (−0.15 to 0.296)	−0.038 (−0.19 to 0.142)	0.4
Mitral valve E/A peak velocity ratio	0.049 (−0.171 to 0.372)	0.131 (−0.129 to 0.452)	0.02*
Mitral valve E VTI	0.002 (−0.01 to 0.024)	0.002 (−0.01 to 0.025)	0.5
Mitral valve A VTI	−0.004 (−0.012 to 0.014)	−0.007 (−0.015 to 0.014)	0.03*
Mitral valve E/A VTI ratio	0.05 (−0.549 to 0.537)	0.202 (−0.19 to 0.656)	0.06
Mitral valve total VTI	0.002 (−0.022 to 0.023)	−0.003 (−0.022 to 0.037)	0.4
Mitral valve A VTI/total VTI ratio	−0.054 (−0.13 to 0.04)	−0.0594 (−0.16 to 0.07)	0.2

* Statistically significant, $p < 0.05$
Delta values expressed as median (range)
VTI, velocity time integrals

Table 4 Fetal echocardiography of the tricuspid valve in patients with preterm premature rupture of membranes (PROM) and controls

Doppler parameter (delta values)	Control ($n = 150$)	Preterm PROM ($n = 67$)	p Value
Tricuspid valve E peak velocity	0.005 (−0.127 to 0.216)	0.016 (−0.208 to 0.252)	0.4
Tricuspid valve A peak velocity	0.0135 (−0.20 to 0.317)	−0.0145 (−0.47 to 0.217)	0.3
Tricuspid valve E/A peak velocity ratio	−0.006 (−0.223 to 0.416)	0.032 (−0.0173 to 0.30)	0.006*
Tricuspid valve E VTI	0.001 (−0.012 to 0.024)	0.003 (−0.017 to 0.042)	0.2
Tricuspid valve A VTI	0 (−0.017 to 0.027)	0.001 (−0.015 to 0.011)	0.7
Tricuspid valve E/A VTI ratio	0.002 (−0.375 to 0.814)	0.98 (−0.377 to 2.331)	0.06
Tricuspid valve total VTI	0.001 (−0.021 to 0.04)	0.004 (−0.02 to 0.033)	0.4
Tricuspid valve A VTI/total VTI ratio	0.0073 (−0.18 to 0.16)	−0.0356 (−0.35 to 0.12)	0.001

* Statistically significant, $p < 0.05$ Delta values expressed as median (range) VTI,velocity time integrals

Table 5 Fetal echocardiography of the tricuspid valve in patients with preterm premature rupture of membranes (PROM) with and without microbial invasion of the amniotic cavity (MIAC)

Doppler parameter (delta values)	Preterm PROM without MIAC ($n = 42$)	Preterm PROM with MIAC ($n = 25$)	p Value
Tricuspid valve E peak velocity	0.017 (−0.21 to 0.252)	0.015 (−0.085 to 0.145)	0.9
Tricuspid valve A peak velocity	−0.011 (−0.47 to 0.217)	−0.033 (−0.192 to 0.208)	0.7
Tricuspid valve E/A peak velocity ratio	0.028 (−0.173 to 0.30)	0.049 (−0.021 to 0.204)	0.3
Tricuspid valve E VTI	0.002 (−0.017 to 0.019)	0.004 (−0.006 to 0.042)	0.7
Tricuspid valve A VTI	0.002 (−0.011 to 0.01)	−0.004 (−0.015 to 0.011)	0.047*
Tricuspid valve E/A VTI ratio	0.049 (−0.377 to 0.790)	0.132 (−0.121 to 2.331)	0.07
Tricuspid valve total VTI	0.0045 (−0.015 to 0.023)	0.002 (−0.02 to 0.033)	0.4
Tricuspid valve A VTI/total VTI ratio	−0.0246 (−0.19 to 0.12)	−0.0612 (−0.35 to 0.08)	0.1

* Statistically significant, $p < 0.05$
Delta values expressed as median (range) VTI,velocity time integrals

Table 6 Fetal Doppler ultrasound examination of the pulmonary veins in patients with preterm premature rupture of membranes (PROM) and controls

Doppler parameter (delta values)	Control ($n = 150$)	Preterm PROM ($n = 67$)	p Value
Pulmonary vein A velocity	−0.1 (−0.08 to 0.154)	0.033 (−0.046 to 0.192)	< 0.001*
Pulmonary vein D velocity	−0.003 (−0.137 to 0.189)	0.038 (−0.123 to 0.22)	0.006*
Pulmonary vein S velocity	−0.001 (−0.149 to 0.199)	0.044 (−0.116 to 0.313)	0.009*

* Statistically significant, $p < 0.05$
Delta values expressed as median (range)

Pulmonary veins

The delta S, D and A velocities were significantly higher in fetuses with preterm PROM compared to those from the control group (Table 6). There was no difference in the parameters of Doppler waveform velocimetry in the pulmonary veins between fetuses with and without MIAC.

DISCUSSION

Our results suggest that fetuses with preterm PROM have changes in the parameters used to evaluate diastolic function, when compared to fetuses of women with uncomplicated pregnancies. Moreover, the changes were also observed in fetuses with documented MIAC. These observations suggest that the fetal heart is a potential organ in preterm PROM, probably due to the effect of

inflammatory mediators produced during the course of intra-amniotic inflammation/infection.

Systolic and diastolic function of the heart

The heart can be considered to have two main functions from a hemodynamic point of view: first, a systolic function consisting of expelling blood during ventricular contraction into the main arteries (pulmonary and aorta); and second, a diastolic function consisting of filling the ventricular cavities after the opening of the atrioventricular (AV) valves. Cardiac performance may be affected by factors independent of 'intrinsic myocardial function', such as the preload and afterload[22]. Therefore, it is possible to have impaired myocardial performance without cardiac failure *per se*. This occurs in patients with severe hypertensive crisis in which the increased afterload prevents adequate emptying of the ventricles[22].

It is now recognized that many adult patients with clinically diagnosed congestive heart failure have normal left ventricular systolic function[23,24]. Indeed, most patients with congestive heart failure have a preserved left ventricular ejection fraction ($\geqslant 50\%$)[23,25,26]. Diastolic dysfunction has been recognized as a major cause of congestive heart failure. In fact, the degree of diastolic dysfunction has been reported to predict outcome regardless of the degree of left ventricular systolic dysfunction in adults[26].

Evaluation of diastolic function of the heart

Diastolic function depends upon the interplay of several factors including ventricular relaxation, suction of blood from the atrium by the ventricles, viscoelastic forces of the myocardium and atrial systole[27,28]. However, the major determinants of ventricular filling are ventricular relaxation and effective chamber compliance[27,28]. Ventricular relaxation, which can be assessed in adults using a high-fidelity manometer-tipped catheter, is reflected by the rate and duration of the fall of ventricular pressure after systolic contraction[29,30]. However, the most common method to assess diastolic function is pulsed Doppler echocardiography, in which the sample volume is placed below the AV valves[27,28]. The parameters derived from this type of interrogation do not measure ventricular diastolic function directly, but rely on the hemodynamic consequences of ventricular diastolic function on the Doppler waveform velocity below the AV valves. The E wave reflects early diastolic filling, while the A wave represents changes in flow velocity due to atrial contraction[28]. There is evidence that parameters of diastolic function derived from Doppler studies correlated well with those obtained from invasive testing in adults[31].

Evaluation of diastolic function in the human fetus

The Doppler velocity waveforms obtained from interrogation of the human fetal atrioventricular valves have been well characterized[32–43] and are different from the patterns observed in newborns[43,44], infants[43] and adults[45]. A widely used parameter to examine diastolic ventricular function is the E/A ratio[46]. The E/A ratio is calculated by dividing the peak velocity of the E wave by the peak velocity of the A wave, which reflects changes in blood velocity during atrial contraction (Figure 3). The fetal E/A ratio increases with gestational age, and there is predominance of the A wave during intrauterine life, while the E wave is predominant in children and adults (Figure 4). The peak velocity and the

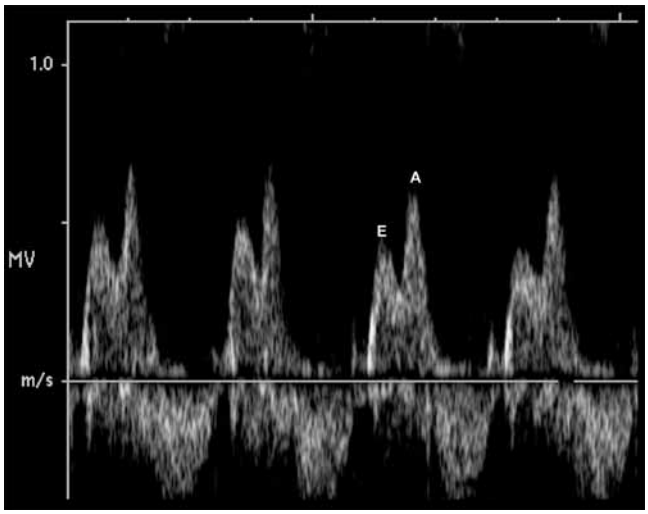

Figure 3 Waveform of the mitral valve in a 34-week fetus from an uncomplicated pregnancy

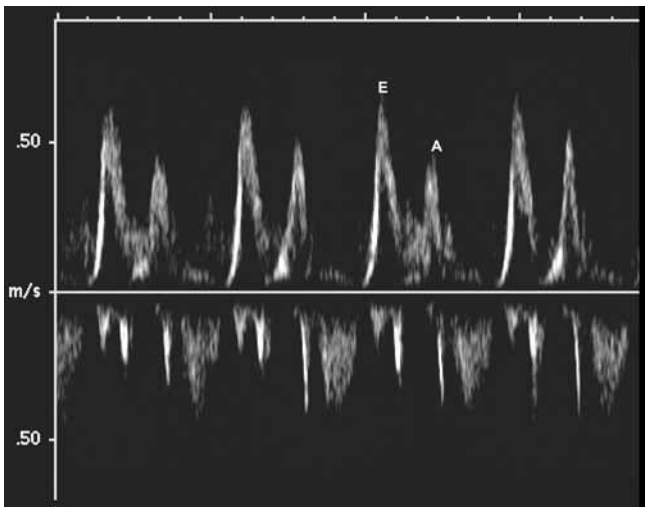

Figure 4 Waveform of the mitral valve in a healthy adult

VTI of the A wave do not change with gestational age, whereas those of the E wave increase with gestational age. Thus, the change in the E/A peak velocity ratio is attributed to the changes in the E wave that reflect early ventricular diastolic filling[36,47]. These changes are more pronounced after 25 weeks[36] and have been attributed to an increase in cardiac compliance. Indeed, Veille and colleagues reported an increase in the left ventricle elastic recoil and a reduction in the ventricular stiffness as gestational age advanced[43], although changes in ventricular relaxation and/or loading conditions[34,37] can also contribute to the increase in the E wave velocity during pregnancy. The functional significance of these observations is that atrial function is important to achieve adequate ventricular filling during fetal life[38]. The E/A peak velocity ratio has been used to assess changes in ventricular diastolic function, and is considered to reflect both ventricular compliance and preloading conditions[48–50]. Another parameter used to examine diastolic function is the VTI, which is the area under the curve of the E and A wave[43]. These parameters (E VTI, A VTI, and combined E and A VTI) are proportional to blood flow during each component of diastole: E VTI for early ventricular filling, A VTI for blood flow during atrial systole, and the sum of E and A for the total blood flow during diastole[45]. The isovolumetric relaxation time[42], the chamber stiffness calculated by the formula $K_{LV} = [(0.08/t_{dec})^2$ mmHg/ml], where t_{dec} represents the time for deceleration of left ventricular early filling[43,51], and the ratio A VTI/total VTI[42,52] have also been used to assess diastolic function. Our study focused on the E/A ratio and the VTI since they are the most widely used Doppler parameters studied in the literature in normal and diseased fetuses.

Diastolic function in fetuses with preterm PROM and intra-amniotic infection

Fetuses with preterm PROM had a higher delta E/A ratio in both ventricles and a higher delta E/A VTI in the left ventricle than normal fetuses. Moreover, fetuses with documented intra-amniotic infection had similar findings in the left but not the right ventricle. To the extent that the E/A ratio is a reflection of diastolic function, our observations suggest that fetuses with preterm PROM have changes in diastolic function when compared to normal fetuses. The changes in the Doppler waveform characteristics suggest an increase in left ventricular compliance in fetuses with preterm PROM, particularly those with proven intra-amniotic infection. Consistent with this interpretation is that a higher degree of left ventricular filling took place during early diastole, as reflected by the higher delta E/A VTI ratio, than that observed in normal fetuses. The change in this ratio can be attributed to the lower ventricular filling during atrial systole, as reflected by the lower A VTI of the left ventricle in fetuses with preterm PROM, as well as in those with documented intra-amniotic infection.

The significance of the diastolic changes observed in fetuses with preterm PROM and intra-amniotic infection

The hemodynamic changes described herein are novel and quite different from those reported in fetuses with intrauterine growth restriction (IUGR) secondary to placental insufficiency. IUGR fetuses typically have lower E, A and E/A peak velocity ratios[35,40,53]. Thus, cardiac dysfunction in the growth-restricted fetus is characterized by increased peripheral resistance and decreased diastolic compliance to blood flow. Therefore, the heart of the IUGR fetus can be described as 'stiff', which is similar to the findings reported in adults with coronary artery disease[45,54–56]. In contrast, fetuses with preterm PROM and those with documented intra-amniotic infection have hemodynamic changes that could be described as a 'floppy heart', likely to be due to higher compliance. The higher S, D and A velocities in the fetal pulmonary veins observed in the PROM group may be due to a lower impedance to blood entering the left atrium during systole and early diastole, and may also be associated with a more compliant left ventricle.

It is unknown why our findings are demonstrable in the left ventricle and not the right in fetuses with preterm PROM as well as those with documented MIAC. In adult patients with septic shock, the right ventricular function does not parallel left ventricular function[18]. Left ventricular afterload is generally low, owing to systemic hypotension, whereas right ventricular afterload is often high as a result of increased pulmonary vascular resistance associated with acute respiratory distress syndrome (ARDS)[18]. ARDS does not exist in the fetus, and systemic fetal hypotension may occur only in severe cases prior to fetal death. Therefore, we propose that three possibilities can be invoked to explain the apparent predominant involvement of the left ventricle in fetuses with preterm intrauterine infection. First, microbial invasion of the fetal lung can create a local inflammatory response, and blood returning from the lung to the left atrium could have higher concentrations of tumor necrosis factor (TNF)-α, IL-1β and other cardiodepressant factors than blood returning to the right ventricle from the systemic fetal circulation. Second, abnormal Doppler findings may be easier to detect below the mitral valve than below the tricuspid valve, because blunting of the E and A waves is more frequent in the right than in the left ventricle in the human fetus. Third, it is possible that a larger sample size is required to detect changes in the right ventricle. In any event, further studies are necessary to investigate the apparent asymmetric cardiac performance during diastole in preterm PROM and intra-amniotic infection.

Why is the fetal heart more compliant in the setting of intrauterine infection/inflammation?

A pattern of myocardial depression characterized by left ventricular dilatation, decreased left ventricular ejection fraction, and a normal or increased cardiac index has been observed within the first several days of septic shock in adults[57]. Acute ventricular dilatation within the first days of septic shock is more frequently observed among survivors. This has been attributed to compensatory ventricular dilatation in order to maintain stroke volume despite a profound loss in myocardial contractility[57]. Therefore, we propose that the changes in cardiac diastolic function observed in human fetuses represent a compensatory mechanism similar to that identified in adults with sepsis. It is possible that fetuses unable to change cardiac compliance in the context of a fetal systemic inflammatory response syndrome may not be able to maintain ventricular stroke volume and cardiac output and, hence, may not perfuse the brain adequately, predisposing to hypotension and brain ischemia *in utero*, which could create conditions for the development of periventricular leukomalacia. Indeed, a linear association has been described in normal fetuses between the mitral E/A peak velocity ratio and cardiac output[33]. The changes in diastolic function reported herein may therefore have protective and even survival value. In cases of overwhelming fetal sepsis – the pathophysiological counterpart to septic shock in adults – myocardial depression may lead to fetal death, which we have observed in cases with preterm PROM. The reason why fetuses without evidence of MIAC also have changes in cardiac function remains unclear. One possible explanation is that a substantial number of fetuses with preterm PROM do not have microbiological evidence of infection, but rather intra-amniotic inflammation[58]. Thus, though culture results may be negative, the fetuses could still have an inflammatory response. There is now evidence that intra-amniotic inflammation is as good a predictor of pregnancy outcome and neonatal outcome as proven MIAC with standard microbiological techniques[59]. Future studies need to examine the relationship between a fetal cytokine response and changes in cardiac function, regardless of the microbial state of the amniotic cavity. Similarly, studies with molecular microbiological techniques allowing the detection of micro-organisms that may not be isolated with cultivation techniques perhaps will yield valuable information to address this question[60,61].

What are the possible mechanisms involved in the changes in cardiac function in fetuses with preterm PROM and intra-amniotic infection/inflammation?

The mechanism by which sepsis induces myocardial depression is not completely understood. The most likely explanation is that the myocardium is depressed by the action of soluble factors such as bacterial products and cytokines, which are elevated in the circulation of patients with septic shock[18–20]. There is now substantial evidence indicating that endotoxin and pro-inflammatory cytokines, such as TNF-α, IL-1β and macrophage inhibitory factor (MIF), play a central role as myocardial depressants in the context of sepsis. Bacterial lipopolysaccharide (LPS) or endotoxin, a component of the cell wall of Gram-negative bacteria, has been implicated in the pathophysiology of preterm labor[62–66], preterm PROM[67] and septic shock[68]. The mechanism by which LPS induces a cellular response has been partially defined. LPS binds to an acute-phase reactant protein, known as LPS-binding protein (LPB), to form an LPS–LBP complex which, in turn, binds to CD14, a receptor present on the surface of neutrophils, monocytes and macrophages[69–72]. The interaction of CD14 with the LPS–LBP complex initiates signal transduction with participation of toll-like receptor-4 (TLR-4)[73]. This eventually leads to activation of the mitogen-activated protein kinase (MAP-kinase) and nuclear factor (NF)-κB pathway[74]. These events result in the production of pro-inflammatory cytokines such as TNF-α and IL-1β[75,76].

Animal models of endotoxin-induced cardiac dysfunction indicate that this proinflammatory cascade may play an important role in the pathogenesis of cardiac dysfunction in septic patients. The evidence in support of this view includes the following: LPS impairs myocardial contractility and relaxation, increasing the proportion of isovolumetric relaxation and contraction times[77]; CD14-deficient mice are protected against LPS-induced left ventricular dysfunction[78]; TLR-4 mRNA and protein are constitutively present in the fetal myocardium[77], and TLR-4-deficient mice do not experience left ventricular diastolic and systolic dysfunction after intraperitoneal injection of LPS[79]; LPS upregulates the production of TNF-α and IL-1β mRNA transcripts and protein in the fetal and adult myocardium[77,78]; and intraperitoneal injection of LPS in mice induces the myocardial expression of mRNA and protein of TNF-α, IL-1β, IL-6 and monocyte chemotactic protein-1[80].

A role for fetal cytokines in the changes in cardiac function

In cases of documented intrauterine infection, the fetus may display a fetal inflammatory response syndrome. FIRS is operationally defined as a fetal plasma IL-6 concentration above 11 pg/ml[8,81]. Its histological hallmark is inflammation of the umbilical cord, or funisitis[82]. We propose that proinflammatory cytokines like TNF-α, IL-1β and MIF may play a role in the pathogenesis of myocardial dysfunction, based upon the following observations: 1) serum and plasma concentrations of TNF-α are elevated in children and adults with sepsis, respectively[83,84]; 2) transgenic mice

engineered to produce myocardial TNF-α develop a dilated cardiomyopathy characterized by ventricular dilatation and decreased ejection fraction[85]; 3) administration of TNF-α to animals produces a dose-dependent myocardial depression[86]; and 4) incubation of TNF-α with human and animal myocardial tissue results in concentration-dependent depression of contractility[87]. Similar observations have been described for IL-1β[88–90]. MIF is constitutively expressed in the myocardium, and the mRNA expression of MIF is also increased after the LPS injection[80]. Furthermore, MIF has potent cardiodepressant effects[91]. We have recently reported that this cytokine is elevated in cases of MIAC[92]. In addition, synergy may also be important in myocardial depression. The combination of TNF-α and IL-1β at concentrations that did not individually lead to myocardial depression resulted in contractile depression of human myocardial tissues suspended in organ baths[93].

Clinical implications

Our observations that fetuses with preterm PROM and intra-amniotic infection undergo changes in cardiac function are consistent with the findings of Yanowitz and colleagues[94], who recently reported that neonates born with histological chorioamnionitis had several hemodynamic abnormalities including a decreased mean and diastolic blood pressure, and that there was a correlation between mean blood pressure and umbilical cord IL-6 concentrations[94]. It is possible that some of these hemodynamic changes are present *in utero* and may contribute to the pathophysiology of periventricular leukomalacia and cerebral palsy[95]. These conditions were originally considered to be due to ischemia/hypoxia and have recently been linked to chorioamnionitis, infection and fetal inflammation. We propose that, in the context of FIRS, the combination of inflammatory changes in the brain and fetal systemic hypotension may increase the likelihood of brain injury.

The current approach of monitoring patients with preterm PROM mainly includes examination of fetal heart rate patterns with the non-stress test and fetal well-being with the biophysical profile. While these tools are useful to detect the fetus at risk for infection and to plan the optimal time of delivery, they are not sufficient to characterize the cardiovascular responses to infection/inflammation. We envision that the future of fetal surveillance will include parameters of cardiovascular function, particularly in very preterm fetuses in which a decision to deliver may be life-threatening. Clearly, longitudinal studies are required to describe the natural history of cardiovascular function in preterm PROM and its relationship to fetal and neonatal outcome.

Monoclonal antibodies against TNF-α have recently been shown to prevent both LPS-induced ventricular dilatation and the reduction in myocardial contractility in an animal model of sepsis[96]. Furthermore, phase III clinical trials with an anti-TNF-α monoclonal antibody therapy in patients with septic shock reported an improvement in the left ventricular function, although this did not improve survival[97,98]. Since some neonates are born with very high concentrations of TNF-α, approaches aimed at modulating the innate immunoresponse with the use of anti-TNF, IL-10 and anti-MIF deserve consideration for the neonate with early sepsis. The recent development of a mouse model of fetal inflammation where fetal cardiac dysfunction was achieved by the intra-amniotic injection of LPS[77] provides a potential tool to explore these therapeutic options.

Finally, the long-term consequences of exposing the fetal myocardium to the effects of systemic inflammation deserve consideration. There is now evidence that TNF-α can induce apoptosis in cardiac monocytes[99]. Further study will be required to determine whether such an effect may cause structural cardiac changes and even re-program some aspects of fetal cardiac development.

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