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Pulsation of the fetal splenic vein – a potential ultrasound marker of histological chorioamnionitis and funisitis in women with preterm prelabor rupture of membranes

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Abstract

The fetal spleen is involved in the response to intrauterine infection and inflammation. The flow pattern of its vein is not pulsatile in normal conditions. The aim of the study was to determine whether the presence of histological chorioamnionitis and funisitis is associated with a continuous or pulsatile flow pattern in the fetal splenic vein. We performed a prospective study including 79 women with preterm prelabor rupture of membranes. We found a relation between pulsation in the splenic vein and histological chorioamnionitis (likelihood ratio 13.2), as well as funisitis (likelihood ratio 5.7). Ultrasound evaluation of the splenic vein could be a non-invasive tool for the prediction of these inflammatory complications.

Abbreviations: CI, confidence interval; PPROM, preterm prelabor rupture of membranes.

Introduction

Preterm prelabor rupture of membranes (PPROM) complicates 2–4% of all singleton pregnancies and accounts for one-third of preterm deliveries. The presence of bacteria in amniotic fluid, which is found in approximately 30% of PPROM pregnancies, triggers an intra-amniotic inflammatory response with the subsequent development of histological chorioamnionitis (1), characterized by an intensive neutrophil infiltration in the fetal membranes and placenta. It is normally subdivided to two subgroups, according to the type of host response. The maternal response is limited to infiltration of the chorion/decidua, chorionic plate and amniotic epithelium. In contrast, the finding of neutrophils

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in the umbilical cord (funisitis) is regarded as a histological hallmark of a fetal response (2). Funisitis is the most serious form of histological chorioamnionitis.

Histological chorioamnionitis leads to an activation of the fetal hypothalamic–pituitary–adrenal axis, resulting in typical morphological multi-organ features, such as thymic involution, adrenal gland enlargement and splenic depletion. Moreover, fetal cardiac function can be affected, which is assumed to be manifested in altered fetal hemodynamics (3). Both morphological and functional alterations are reflected by fetal blood flow changes.

Chorioamnionitis is a risk factor for perinatal mortality, short-term morbidity and also for long-term sequelae, such as brain injury and bronchopulmonary dysplasia. Unfortunately, many cases of histological chorioamnionitis remain clinically silent. By ultrasound examination, fetal organ and hemodynamic changes associated with the presence of histological chorioamnionitis and funisitis can be visualized (4,5). The fetal spleen, as an organ of the immune and hematopoietic systems, is involved in the response to intrauterine infection. Little is known about the fetal splenic vein flow pattern, but it is expected to be continuous (non-pulsatile) in normal conditions. To date, there have been no reports of fetal splenic blood flow changes in pregnancies complicated by chorioamnionitis. However, morphological splenic depletion as a response to chorioamnionitis has previously been described (6). The objective of this study was to investigate whether the presence of histological chorioamnionitis and funisitis is associated with changes in the pattern of fetal splenic vein flow.

Material and methods

A prospective cohort study was performed. Pregnant women with PPROM and gestational ages between 24^{+0} and 36^{+6} weeks admitted to the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic, from May 2010 to July 2011, were eligible for the study if they had a singleton pregnancy and the estimated fetal weight was between the 10th and the 90th percentiles for gestational age. Exclusion criteria were vaginal bleeding, signs of fetal hypoxia, and fetal structural malformations or chromosomal abnormalities. Preterm prelabor rupture of membranes was diagnosed by sterile speculum examination that confirmed the presence of amniotic fluid in the vagina, along with a positive test for the presence of the insulin-like growth factor-binding protein (ACTIM PROM test; Medix Biochemica, Kauniainen, Finland) in the vaginal fluid. Samples from the placenta, the fetal membranes and the umbilical cord were obtained at delivery. This study was approved by the Institutional Review Board committee (19 March 2008; no. 200804 SO1P), and written informed consent was obtained from all participants.

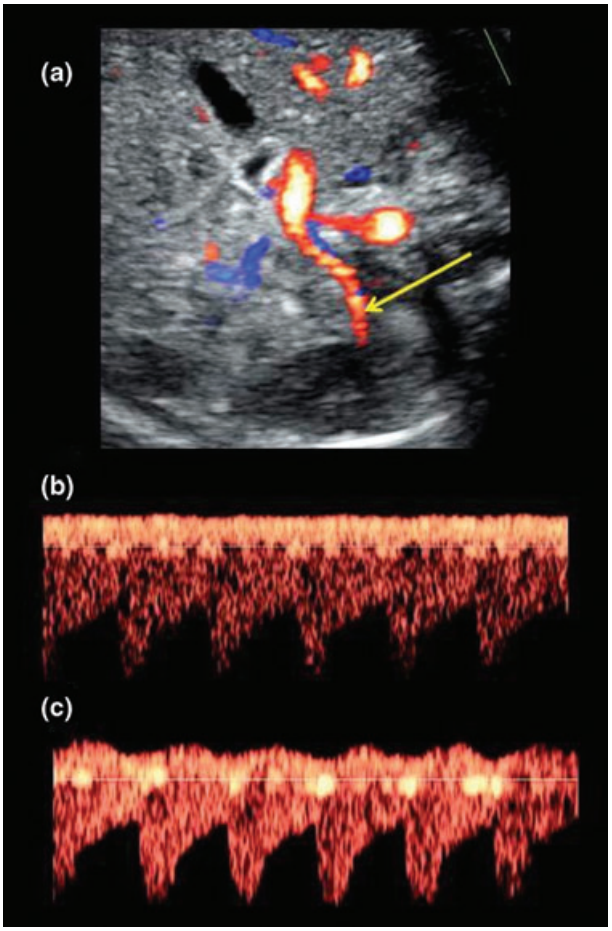

Figure 1. Colour Doppler image of the fetal splenic vein showing the site of measurement (arrow) of flow patterns (a), with continuous (b) and pulsatile waveforms (c). A colour version of this figure is available online.

All ultrasound evaluations were performed by two experienced sonographers. All women, after diagnosis of PPROM and upon admission, were examined in the delivery room using either an Aplio SSA-77A (Toshiba, Tokyo, Japan) with a convex transabdominal probe 3.5–7 MHz or a Voluson E8 Expert (GE Healthcare, Milwaukee, WI, USA) and a 2.1–6.1 MHz transabdominal matrix array volume probe. Pulsed-wave Doppler was employed; sample volume ranged from 3 to 4 mm. All flow-velocity waveforms were obtained in the absence of fetal breathing and movements.

The splenic vein was identified in an oblique plane of the fetal abdomen. The Doppler sample was placed at its origin close to the spleen (Figure 1a). The flow-velocity waveform pattern was defined as continuous when no change in velocity was found within a single cardiac cycle (Figure 1b). The pulsatile pattern was defined as a monophasic pattern with one negative deflection synchronized with fetal heart rate (Figure 1c). Assessment of whether or not pulsation was present was performed visually.

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Histological examination of the placenta, the fetal membranes and the umbilical cord was performed in all cases. Sections of tissue blocks were stained with Hematoxylin and Eosin. The degree of polymorphonuclear leukocyte infiltration was assessed separately in the free membranes, the chorionic plate and the umbilical cord according to the criteria given by Salafia et al. (7). A diagnosis of histological chorioamnionitis was determined based on the presence of histological grades of chorion-decidua 3–4 and/or chorionic plate 3–4 and/or umbilical cord 1–4 and/or amnion 1–4. Funisitis was diagnosed as umbilical cord grade 1–4. Histopathological examination was performed by a perinatal pathologist blinded to the clinical status of the women.

Continuous variables were compared using Student's unpaired *t*-tests (values are given as means ± SD) or the non-parametric Mann-Whitney *U*-test [values are given as medians (range)]. Categorical variables were compared using Fisher's exact test and given as percentages (%). Binary logistic regression was used for the adjustment for potential confounders (variables that were significant in the univariate analysis). The normality of the data was tested using the D'Agostino and Pearson omnibus normality test and the Shapiro–Wilk test. Differences were considered significant at $p < 0.05$. All *p*-values were from two-sided tests, and statistical analyses were performed using GraphPad Prism 5.03 for Windows (GraphPad Software Inc., San Diego, CA, USA) and SPSS 19.0 for Mac OS X (SPSS Inc., Chicago, IL, USA).

Results

Demographic data and clinical characteristics of the women and newborns according to the presence and the absence of histological chorioamnionitis and funisitis are presented in Table 1. All women were self-reported Caucasians. Among 79 women, 64% (51 of 79) displayed histological chorioamnionitis and 24% (19 of 79) displayed funisitis. Infants born from pregnancies with histological chorioamnionitis had a lower birthweight than those who were not. There was no difference in the interval between ultrasound measurement and delivery between women with and without histological chorioamnionitis (women with histological chorioamnionitis, median 23 h vs. women without, median 62 h; $p = 0.22$). The group with funisitis had a higher proportion of microbial invasion of the amniotic cavity, were treated more frequently with antenatal corticosteroids and tocolytics and exhibited lower gestational age and birthweight. Women with funisitis did not have a different interval between the ultrasound measurement and delivery compared with those without funisitis (women with funisitis, median 54 h vs. women without, median 53 h; $p = 0.85$).

Table 1. Maternal and newborn characteristics according to the presence and absence of histological chorioamnionitis and funisitis.

Characteristic	Presence of histological chorioamnionitis (*n* = 51)	Absence of histological chorioamnionitis (*n* = 28)	*p*-Value	Presence of funisitis (*n* = 19)	Absence of funisitis (*n* = 60)	*p*-Value
Maternal age (years)	31.5 ± 5.7	30.7 ± 5.4	0.56	29.4 ± 4.6	31.7 ± 5.7	0.17
Primiparous (%)	27 (53%)	16 (57%)	0.39	9 (47%)	34 (57%)	0.60
Pre-pregnancy body mass index (kg/m^2; range)	22.3 (16.8–40.6)	20.9 (16.3–38.6)	0.38	20.1 (16.8–36.8)	21.6 (16.3–40.6)	0.08
Gestational age on admission ($weeks^{+days}$)	31^{+5} (24^{+5}–36^{+4})	33^{+0} (24^{+0}–36^{+4})	0.15	30^{+5} (24^{+5}–35^{+5})	33^{+0} (24^{+0}–36^{+4})	0.04*
Gestational age at delivery ($weeks^{+days}$)	32^{+0} (25^{+0}–36^{+6})	33^{+1} (24^{+0}–36^{+4})	0.16	31^{+0} (25^{+0}–35^{+0})	33^{+2} (24^{+0}–36^{+6})	0.04*
Maternal serum C-reactive protein on admission (mg/L; range)	5.0 (0–82.0)	5.0 (1.0–12.0)	0.40	6.1 (1.0–21.0)	4.7 (0–82.0)	0.37
Smoking during pregnancy	10 (20%)	4 (14%)	0.63	5 (26%)	9 (15%)	0.31
Antepartum corticosteroids	33 (65%)	13 (46%)	0.19	16 (84%)	30 (50%)	0.02*
Antepartum antibiotics	49 (96%)	25 (89%)	0.78	18 (95%)	56 (93%)	1.00
Tocolytics	33 (65%)	13 (46%)	0.19	16 (84%)	30 (50%)	0.02*
Spontaneous delivery	38 (75%)	20 (71%)	0.89	12 (63%)	45 (75%)	0.37
Cesarean delivery	13 (25%)	8 (29%)	0.97	7 (37%)	15 (25%)	0.37
Amniotic cavity microbial invasion	23 (45%)	7 (25%)	0.17	14 (74%)	17 (28%)	0.001*
Puerperal endomyometritis	3 (6%)	0 (0%)	0.20	1 (5%)	2 (3%)	0.57
Birthweight (g; ± 1 SD)	1721 ± 580	2161 ± 637	0.004*	1473 ± 478	2003 ± 625	0.001*
Apgar score at five minutes	9 (3–10)	9 (6–10)	0.65	9 (5–10)	9 (3–10)	0.22
Apgar score at 10 minutes	10 (7–10)	10 (7–10)	0.16	10 (7–10)	10 (7–10)	0.13

Continuous variables were compared using Student's unpaired *t*-tests (values are presented as means ± SD) or the non-parametric Mann–Whitney *U*-test [values are presented as medians (range)]. Categorical variables were compared using Fisher's exact test and are presented as numbers (%).
* Significant results ($p < 0.05$).

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A pulsatile flow pattern in the fetal splenic vein was found in 47% (24 of 51) of women with histological chorioamnionitis. In contrast, 4% of women without histological chorioamnionitis displayed pulsatile flow waveforms (one of 28). The association between the presence of histological chorioamnionitis and pulsatile pattern was significant ($p < 0.0001$). The presence of a pulsatile flow pattern had a sensitivity of 47% [95% confidence interval (CI) 33–62%], specificity 96% (95% CI 82–100%), positive predictive value 96% (95% CI 80–100%), negative predictive value 50% (95% CI 36–64%) and likelihood ratio 13.2 (95% CI 1.9–92.3) for the prediction of histological chorioamnionitis.

Among women, 84% of those with funisitis had a pulsatile splenic vein pattern (16 of 19). In contrast, a pulsatile pattern was found in 15% (nine of 60) of women without funisitis. There was a significant association between pulsatile splenic vein pattern and the presence of funisitis ($p < 0.0001$). The pulsatile flow pattern had a sensitivity of 84% (95% CI: 61–97%) and specificity of 85% (95% CI: 73–93%), positive predictive value 64% (95% CI: 42–82%), negative predictive value 94% (95% CI: 85–99%) and likelihood ratio 5.7 (95% CI: 3.0–10.6) for the prediction of funisitis. The results were significant in crude analysis (odds ratio 30.2; 95% CI 7.7–125.3), as well as after adjustment for gestational age, antenatal corticosteroids, tocolytics and the presence of microbial invasion of the amniotic cavity (odds ratio 28.3; 95% CI 5.5–125.3; $p < 0.0001$).

Discussion

The fetal splenic vein drains the blood from the spleen and the superior part of the large gastric curvature. It represents one of three affluent branches of the main portal vein, which also receives blood from superior and inferior mesenteric veins.

The flow pattern of the fetal splenic vein was observed as continuous at its origin in 96% of healthy women (8). However, the flow pattern is more frequently pulsatile with a monophasic pattern close to the entrance of the splenic vein into the main portal vein. Pulsation has been found in this part of the splenic vein in more than 38% of healthy fetuses (8). The mechanism of the pulsatile flow pattern in the splenic vein has not been entirely explained. The most plausible explanation seems to be backward transmission of pulsations from the main portal vein (9). The nature of pulsations in the main portal vein is not completely clear, but two potential mechanisms have been considered: first, mirroring of the atrial contraction (a-wave) through the ductus venosus to the portal system and, second, transmission of pulsation from the hepatic artery adjacent to the main portal vein (10,11).

With regard to these important findings, the sample volume was strictly placed only at the origin of the fetal splenic vein (Figure 1a), where the continuous flow pattern is predominant. Nevertheless, a pulsatile flow pattern was frequently found in fetuses of women with PPROM complicated by histological chorioamnionitis and funisitis. The explanation for this finding remains unclear because of the lack of data regarding fetal splenic vein flow changes in inflammatory conditions. We assume that pulsations could reflect fetal venous system changes transmitted through the main portal vein rather than the splenic circulation itself. Unfortunately, we can only speculate about this mechanism, owing to the lack of data regarding flow characteristics in the main portal vein, its left portal branch and the ductus venosus. We are also aware that blood flow pattern is influenced by vessel compliance, which is determined by the cross-sectional area of the vessel and the stiffness of its wall, as well as the intravascular pressure. Thus, the changes in splenic vein compliance associated with the presence of histological chorioamnionitis and funisitis also have to be considered as a potential contributor to the pulsatile flow pattern.

A strength of this study is that it provides unique preliminary information concerning flow patterns in the fetal splenic vein based on the presence of histological chorioamnionitis and funisitis in PPROM pregnancies. Another strength is the short interval between ultrasound measurement and delivery that results from the active management of PPROM pregnancies. Therefore, the ultrasound measurements could be related to the histopathological findings. Another advantage is that the results were adjusted for corticosteroid and tocolytic treatment, as well as for microbial invasion of the amniotic cavity. However, there are also some limitations. First, the flow pattern was determined only by visual assessment, noting whether one negative deflection in each cardiac cycle was present (or not), without any flow quantification. Second, we did not evaluate the changes in venous system adjacent to the splenic vein, which could be helpful in explaining our findings.

We believe these preliminary results may be clinically important, but they must be validated in a replication cohort. The evaluation of splenic vein flow pattern seems to be a rapid, non-invasive approach for detection of the presence of chorioamnionitis and of funisitis. This study has shown a possible association between a pulsatile flow pattern in the splenic vein and the presence of chorioamnionitis and funisitis in women with PPROM. Ultrasound evaluation of the splenic vein flow pattern could represent a tool for prenatal detection of chorioamnionitis and funisitis in PPROM pregnancies.

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