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MATERNAL-FETAL MEDICINE

Splenic artery blood flow as a potential marker for materno-fetal transmission of a primary CMV infection

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Abstract

Objective To examine the blood flow in the splenic artery as marker for materno-fetal transmission at about 20 weeks following a maternal first-trimester primary CMV infection.

Methods This is a retrospective study at the prenatal medicine unit at University of Tuebingen, Germany. Women were included who underwent an amniocentesis to examine the fetal infection status following a maternal primary CMV infection in the first trimester. In all cases, amniocentesis was done at about 20 weeks and at least 6 weeks after the maternal infection. As part of the detailed ultrasound examination prior to each amniocentesis, we examined the peak systolic velocity flow (PSV) and the pulsatility index (PI) of the splenic artery. Measurements were transformed into MoMs according to the normal curves of Ebbing et al.

Results 81 Women fulfilled the inclusion criteria. Maternal and gestational age was 31.9 years and 20.6 weeks' gestation. Maternal–fetal transmission occurred in 13 of the cases. In fetuses without and with a CMV infection, mean PI was 0.98 MoM and 0.89 ($p=0.081$). Mean PSV was significantly higher in the group of infected fetuses than in those without (1.24 vs. 0.94 MoM, $p=0.026$).

Conclusion The PSV may be a marker for maternal–fetal CMV transmission following a first-trimester maternal infection.

Keywords CMV · Pregnancy · Antenatal · Splenic artery · Peak systolic velocity · Pulsatility index

Introduction

Primary cytomegalovirus (CMV) infection is the most common viral infection in pregnancy, which has adverse effect on the developing fetus. In Germany alone, about 700–1400 children are born each year with developmental disorders that are directly attributable to this infection [1]. The cases with most severe postnatal sequelae are usually due to maternal infections in the first trimester and where maternal–fetal transmission occurs prior to 20–23 weeks of gestation [2, 3].

To look for evidence of maternal–fetal transmission, an amniocentesis is currently recommended to be done at least 6 weeks after a suspected first-trimester maternal CMV infection [4]. There are only a few studies, which have looked at ultrasound markers of fetal infection at this point in gestation. In the largest study, Guerra et al. retrospectively reviewed the results of the fetal sonograms from 600 mothers with a primary CMV infection. In all cases, an amniocentesis was performed at 20- to 21-week gestation. The ultrasound examination was found to be normal in 549 pregnancies. Still, the virus was found in the amniotic fluid in about 24% of these cases. In 51 pregnancies, ultrasound abnormalities compatible with a fetal CMV infection were detected prior to the amniocentesis. However, an infection could be documented by amniocentesis in only 45% of the cases.

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Many more studies have evaluated ultrasound markers and their utility in predicting the outcome after a proven maternal–fetal transmission. Leruez-Ville et al. [5] grouped the ultrasound findings based on the severity of brain anomalies as well as the presence of extra-cerebral ultrasound findings. Within the extra-cerebral group, fetal splenomegaly is one of the most common findings that is associated with a fetal CMV infection. Therefore, splenomegaly could potentially be used as non-invasive marker of a fetal CMV infection in the second trimester.

The pathophysiology of fetal spleen enlargement in these cases is not fully understood. However, irrespective of the exact etiology, it is likely that the enlargement is associated with an altered blood flow in the splenic artery.

In this study, we set out to examine whether Doppler evaluation of the blood flow in the splenic artery is helpful in detecting fetal CMV infections. For this purpose, we examined the pulsatility index (PI) and the peak systolic velocity (PSV) of the splenic artery prior to a mid-second-trimester amniocentesis in patients with a history of a documented first-trimester CMV infection. We compared the results in infected fetuses to those where an infection was not documented by amniocentesis. We also looked for differences in infected fetuses with and without anomalies identified on ultrasound.

Methods

This retrospective study was performed at the prenatal medicine unit at University of Tuebingen, Germany. In this study, we included women who underwent an amniocentesis to examine the fetal infection status following a proven maternal primary CMV infection in the first trimester. In all cases, the amniocentesis was done at least 6 weeks after the maternal infection.

In our prenatal medicine unit, which is a tertiary referral center, we perform a detailed ultrasound examination prior to each amniocentesis. If the amniocentesis is performed due to a suspected primary CMV infection, ultrasound findings are grouped according to a classification proposed in a study by Leruez-Ville et al. [5]. Briefly, ultrasound findings are classified as severe brain anomalies (such as ventriculomegaly > 15 mm or microcephaly), mild brain anomalies (ventriculomegaly 10–15 mm) and/or extra-cerebral ultrasound anomalies (such as hepatomegaly and splenomegaly).

The spleen may be difficult to visualize using gray-scale ultrasound only. Therefore, we use color Doppler to search for the splenic artery, which begins as a branch of the celiac artery. We also examined the blood flow in the splenic artery using pulsed Doppler. The evaluation is performed in the first third of the splenic artery after it is originated from the celiac artery. The Doppler gate was placed so the angle of insonation is < 30 degrees with respect to the longitudinal axis of the splenic artery (Fig. 1). The pulsatility index (PI)

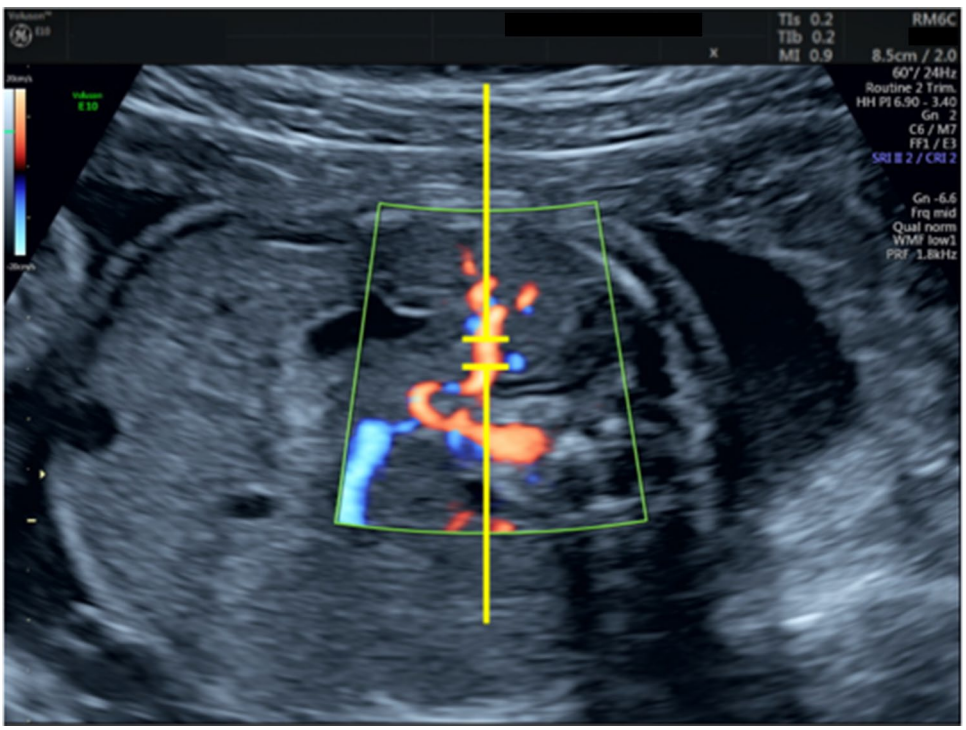

Fig. 1 Ultrasound image of the splenic artery including the Doppler gate for the spectral Doppler analysis

was calculated and peak systolic velocity (PSV) was measured after adjustment for the angle of insonation.

Splenomegaly is diagnosed if the longest distance of the spleen is 40 mm or more [5].

In all cases, amniotic fluid was tested using qualitative nested PCR (nPCR) and quantitative real-time PCR (q-rt-PCR). Short-term microculture and long-term culture were also performed until the generation of a cytopathic effect (CPE) ranging from 18 h to 5 days.

Short-term 18-h fibroblast microculture, followed by CMV IE1 immunoperoxidase staining and virus isolation from amnion fluid, is performed with a high-speed centrifugation step of 50,000×*g* for 1 h prior to virus inoculation as described previously for milk whey sample preparation [6]. DNA was extracted automatically using QIAamp® DNA Blood Mini Kit and QIAcube (QIAGEN) extractor for amplification by nPCR of the viral IE1Ex4-target region [6] and quantitative CMV real-time PCR (qPCR). The limit of detection (LOD) for nPCR is 200 copies/ml. Quantitative real-time PCR from plasma, serum, or whole EDTA blood as well as from amnion fluid was performed using CMV-R-gene™ real-time PCR kits (Argene, France) with an LOD of 600 copies/ml.

Approval for the study was obtained from the ethical committee of the University of Tuebingen (541/2017BO2).

Statistical analysis

The pregnancies were grouped according to the presence or absence of a fetal infection. The PI and the PSV were transformed into MoM values based on the normal curves of Ebbing et al. [7]. Due to the absence of normal Gaussian distribution, the MoM values were compared with a Mann–Whitney *U* test. We used a ROC curve analysis to assess the effect of the splenic artery blood flow in identifying infected fetuses prior to the amniocentesis. Results are shown as mean with standard deviation. The significance level was set at 0.05.

Results

The search of the database identified 81 pregnant women who underwent an amniocentesis between 2016 and 2018 due to a suspected CMV infection. Median maternal and gestational age was 31.9 years and 20.6 weeks' gestation, respectively. Table 1 gives further description of the study population.

Amniocentesis results confirmed maternal–fetal transmission in 13 (16%) of the cases. The ultrasound findings at the time of the amniocentesis in these cases are summarized in Table 2. In seven pregnancies, at least one ultrasound sign of a CMV infection was detected. In the remaining six cases, the ultrasound examination was within normal limits

Table 3 demonstrates the blood flow in the splenic artery in fetuses without and with a CMV infection. In the first group, mean PI was 1.38 or 0.98 MoM. Mean PI measurements in the group of infected fetuses were lower (1.29 or 0.89 MoM). However, the difference did not reach statistical significance ($p=0.081$). In the subgroups of infected fetuses with and without ultrasound signs, PI measurements were 0.94 and 0.83 MoM.

Mean PSV of the splenic artery was significantly higher in the group of infected fetuses than in those without infection (1.24 vs. 0.94 MoM, $p=0.026$). In the subgroups of infected fetuses with and without ultrasound signs, mean PSV was 1.13 and 1.36 MoM, respectively.

In 60 cases, the PSV of the middle cerebral artery was available. In these cases, the PSV of the splenic artery did not correlate with the PSV of the middle cerebral artery ($p=0.324$). This is also the case, in the subgroup of pregnancies with and without transmission.

Figure 2 shows the ROC curve of the PSV and the PI MoM for the detection of a fetal CMV infection. The area under the curve for PSV was 0.696 (95% CI 0.505–0.888, $p=0.026$) and for PI MoM was 0.650 (95% CI 0.459–0.841, $p=0.088$). This indicates that PSV may be useful identifying CMV-infected fetuses. For a false-positive rate of 10%, the detection rate is 53.8%.

Table 1 Maternal and pregnancy characteristics of the study population

	Uninfected fetuses $n=68$	Infected fetuses $n=13$
Maternal age in years median (IQR)	31.7 (29.5–34.6)	34.3 (31.2–38.1)
Maternal weight in kg median (IQR)	65.2 (59.0–72.1)	65.0 (60.3–76.4)
Gestational age in weeks median (IQR)	20.6 (20.3–21.6)	21.7 (20.3–23.3)
Parity median (IQR)	2 (1–2)	2 (1–2)
Children < 3 years *n* (%)	46 (67.6)	6 (46.2)

Table 2 Ultrasound findings in the 13 cases with materno-fetal CMV transmission

Case no.	Maternal age in years	Gestational age in weeks	Splenomegaly	Ultrasound findings
1	20.4	27.1	Yes	Microcephaly, microcephaly, CNS calcifications, abnormal brain topography (neuronal migration defect), hypoplastic cerebellum
2	34.3	20.1	Yes	Cardiomegaly, tricuspid and mitral insufficiency, echogenic bowel
3	35.6	22.3	Yes	Echogenic bowel
4	39.8	27.3	No	Echogenic bowel, oligohydramnios
5	41.9	20.1	No	Echogenic bowel, oligohydramnios
6	33.0	21.7	No	Echogenic bowel, periventricular hyperechogenicity
7	16.6	16.6	No	Hepatomegaly, placentomegaly, echogenic bowel, oligohydramnios
8	34.4	20.3	No	No abnormalities
9	38.1	21.1	No	No abnormalities
10	27.6	23.3	No	No abnormalities
11	31.2	27.3	No	No abnormalities
12	38.4	21.1	No	No abnormalities
13	32.2	23.1	No	No abnormalities

Table 3 Splenic artery blood flow in CMV-infected fetuses and uninfected pregnancies

	Splenic artery blood flow			
	Pulsatility index		Peak systolic velocity	
	Absolute	MoM	Absolute	MoM
Uninfected fetuses mean (SD)	1.38 (0.33)	0.98 (0.21)	18.6 (6.5)	0.94 (0.30)
Infected fetuses mean (SD)	1.29 (0.15)	0.89 (0.25)	27.0 (12.4)	1.24 (0.46)
Infected fetuses with ultrasound symptoms mean (SD)	1.30 (0.20)	0.94 (0.32)	24.3 (11.9)	1.13 (0.39)
Infected fetuses without ultrasound symptoms mean (SD)	1.28 (0.10)	0.83 (0.12)	30.1 (13.2)	1.36 (0.54)

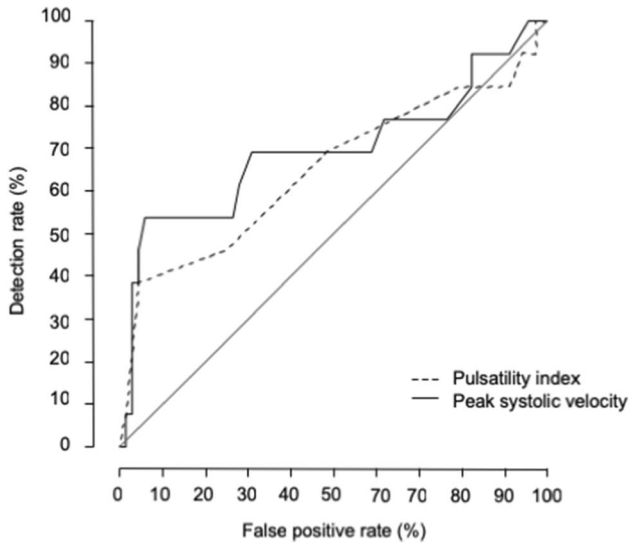

Fig. 2 ROC curve analysis showing the detection rate of materno-fetal transmission by the pulsatility index (dashed line) and by the peak systolic velocity (bold line)

Discussion

The focus of our study was to evaluate the splenic circulation using Doppler ultrasound at the time of the amniocentesis as a possible marker for fetal CMV infection. Our data show that the PSV is significantly higher in the presence of a fetal CMV infection. For a false-positive rate of 10%, the detection rate was 53.8%. However, amniocentesis, which is performed at least 6 weeks after the maternal infection, continues to be the gold standard for diagnosing a fetal CMV infection.

The pathophysiology of the splenomegaly in case of a CMV infection is not fully understood. Based on what we know about CMV infections in immunocompetent adults, it may be that the spleen enlargement is due to the absolute lymphocytosis and splenic lymphoid hyperplasia which are known to be associated with this infection. The increase in lymphatic activity may be associated with increased blood perfusion and diminished vascular resistance in the spleen,

which would explain the findings in our study. Splenic artery blood flow measurements, specifically PSV, have been used by others to predict the presence of fetal anemia in Rhesus disease and to evaluate fetuses that are suspected to be small for gestational age (SGA). In both, SGA and anemic fetuses, the PSV is increased [8–10].

Our study confirms the findings of previous studies, which demonstrate that the utility of ultrasound in diagnosing CMV infections is limited. Guerra et al. reviewed the ultrasound findings prior to mid-trimester amniocentesis in 600 women with CMV seroconversion. The detection rate was only about 15% for a false-positive rate of 6% [4]. Enders et al. [11] reported on the course of 115 pregnancies with a CMV infection. In their cohort, 25 fetuses were infected. The infection rate was 64% in cases with ultrasound abnormalities and 34% in those with normal ultrasound. In a series with 61 CMV-infected fetuses, Leyder et al. [12] detected 44% of those with clinical impairment or histological CNS lesions by ultrasound. Lipitz et al. used ultrasound and fetal MRI in 145 fetuses with a primary CMV infection. In fetuses where both ultrasound and MRI findings were normal, the rate of sequelae was 15.6% in those cases where the infection occurred in the first-trimester and only 2.0% for second-trimester infections. The rate of sequelae increased to 25% and 16% in the presence of abnormal ultrasound and/or MRI findings, respectively [13]. Leruez-Ville et al. reviewed the utility of ultrasound findings as well as the amniotic fluid and fetal blood analysis for the prediction of adverse outcome after a fetal CMV infection. The authors classified the ultrasound findings based on the severity of fetal brain anomalies and on the presence or absence of extracerebral abnormalities. Other useful predictors for either symptomatic status at birth or significant findings at termination of pregnancy were fetal platelet count of 114,000/mm^3 or less and fetal blood DNA load of 4.93 log 10 IU/ml or higher. The negative predictive value of ultrasound plus fetal blood parameters was 100%. In fetuses presenting with non-severe ultrasound features, the positive predictive values of ultrasound alone and in combination with fetal blood parameters were 60%, and 79%, respectively [5].

One of the strongest predictors for transmission and for a symptomatic infection after birth is the gestational age at the time of maternal infection. In the first trimester, the transmission rate is about 30% and the rate increases to 70% in third trimester. Fortunately, the impact of maternal–fetal transmission on the developing fetus decreases with advancing gestation being the most severe in the first trimester when the transmission is the lowest [13–15].

We acknowledge that our study has some weaknesses. Firstly, it is a retrospective study at a single center and secondly, the number of fetuses with documented maternal–fetal transmission is relatively small. We did include a reasonably large number of cases with a primary maternal CMV infection in the first trimester.

However, we now routinely administer immunoglobulins to women with serologically documented, very recent first-trimester CMV infections, which reduce the maternal–fetal transmission rate to less than 5% [16, 17]. We use a protocol for administration of immunoglobulins that differs from the one in the RCT study by Revello et al. First, we administer immunoglobulins only to patients with the first-trimester CMV infections (up to 14 weeks' gestation) and the treatment starts as soon as possible after seroconversion is documented. Second, we use 200 units per kg bodyweight, and repeat the treatment every 2 weeks up to 20 weeks' gestation [18].

In conclusion, we have shown that the PSV of the splenic artery may be a marker for maternal–fetal transmission of CMV following a first-trimester maternal infection. Larger studies are necessary to confirm our findings and to establish whether this measurement can be used as a categorical variable only or as a continuous categorical and if the latter correlates with severity of the disease.

Authors contributions NP: Data collection, manuscript writing and editing. JS: manuscript writing and editing. PW: Data collection, manuscript writing and editing. MH: manuscript writing and editing. HA: manuscript writing and editing. KH: manuscript writing and editing. KOK: Conceptualization, project development, formal analysis, manuscript writing and editing.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This is a retrospective study that was approved by the local ethical committee (541/2017BO2).

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