Journal of Clinical Virology 119 (2019) 37–43

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Blood parameters in fetuses infected with cytomegalovirus according to the severity of brain damage and trimester of pregnancy at cordocentesis

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ARTICLE INFO

Keywords:
Pregnancy
Fetal cytomegalovirus infection
Fetal blood markers
Fetal thrombocytopenia
Fetal brain damage
Fetal gamma-glutamyl transpeptidase

ABSTRACT

Background and objective: Cytomegalovirus (CMV) remains a major cause of congenital infection and disease. During pregnancy, symptomatic cases can be detected through ultrasound (US) features, nevertheless, prognostic assessment is difficult. The aim of this study was to assess the predictive value of specific blood parameters in CMV infected fetuses.

Study design: Twenty-eight CMV-infected fetuses in which a cordocentesis had been performed were included. Fetuses were considered severely or mildly affected according to prenatal US/MRI brain damage. Fetal blood parameters were assessed for the prediction of severe brain abnormalities, and compared according to the trimester of pregnancy. Logistic regression and receiver operating curve analysis were performed.

Results: Thrombocytopenia ($\leq$100,000/mm^3; *p:0.03*) and high levels of gamma-glutamyl transpeptidase (GGT) ($\geq$151 IU/L; *p:0.02*) signaled severity. For the prediction of brain damage, GGT levels $\geq$ 183 UI/l achieved 71% sensitivity, 83% specificity (*AUC: 0.78*), and OR of 2.05 (95% CI: 1.22–3.43) per 100 IU/l increase, adjusted for gestational age. However, thrombocytopenia (91% vs 50%; *p: 0.04*), β_2 microglobulin > 10.4 mg/l (60% vs 0% *p: 0.03),* CMV-DNA > 50,000 copies/ml (80% vs 25%; *p: 0.02)*, and positive IgM (70% vs 17%; *p: 0.04)* were observed significantly more often in severely damaged fetuses sampled $\leq$28 weeks than thereafter.

Conclusion: In CMV infected fetuses, thrombocytopenia and high levels of GGT are associated with severe US/MRI brain abnormalities. Nevertheless, among severely affected fetuses, blood parameters, with exception of GGT, change according to gestational age. Fetal blood could be less predictive of brain damage in the third trimester.

1. Background

Cytomegalovirus (CMV) is the most common congenital infection, and remains worldwide a major cause of sensorineural hearing loss and neurodevelopmental abnormalities [1]. Since routine maternal screening is currently not recommended, congenital CMV is underdiagnosed. Detection during pregnancy is usually achieved when suggestive sonographic signs are found during routine scans, although their value in the diagnosis of fetal infection is limited [2]. Once fetal infection is confirmed, serial targeted ultrasound (US) examinations, and magnetic resonance imaging (MRI) as a complementary tool, have shown a good sensitivity in the identification of symptomatic newborns. [3–7]. Severe fetal brain lesions have been associated with dismal prognosis, with odds ratio as high as 41 [8]. However, although the brain is a major target of end-organ damage, a precise cellular marker of brain damage remains uncharacterized [9]. The need for a more accurate evaluation has led to investigating postnatal blood parameters in symptomatic neonates, to be applied in infected fetuses, although their predictive value to identify symptomatic newborns is conflicting [8,10–13]. Moreover, the diagnostic performance according to the trimester at cordocentesis has not been yet compared. The aim of this study was to assess the value of haemathological, biochemical, and

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https://doi.org/10.1016/j.jcv.2019.08.008
Received 9 April 2019; Received in revised form 16 August 2019; Accepted 19 August 2019

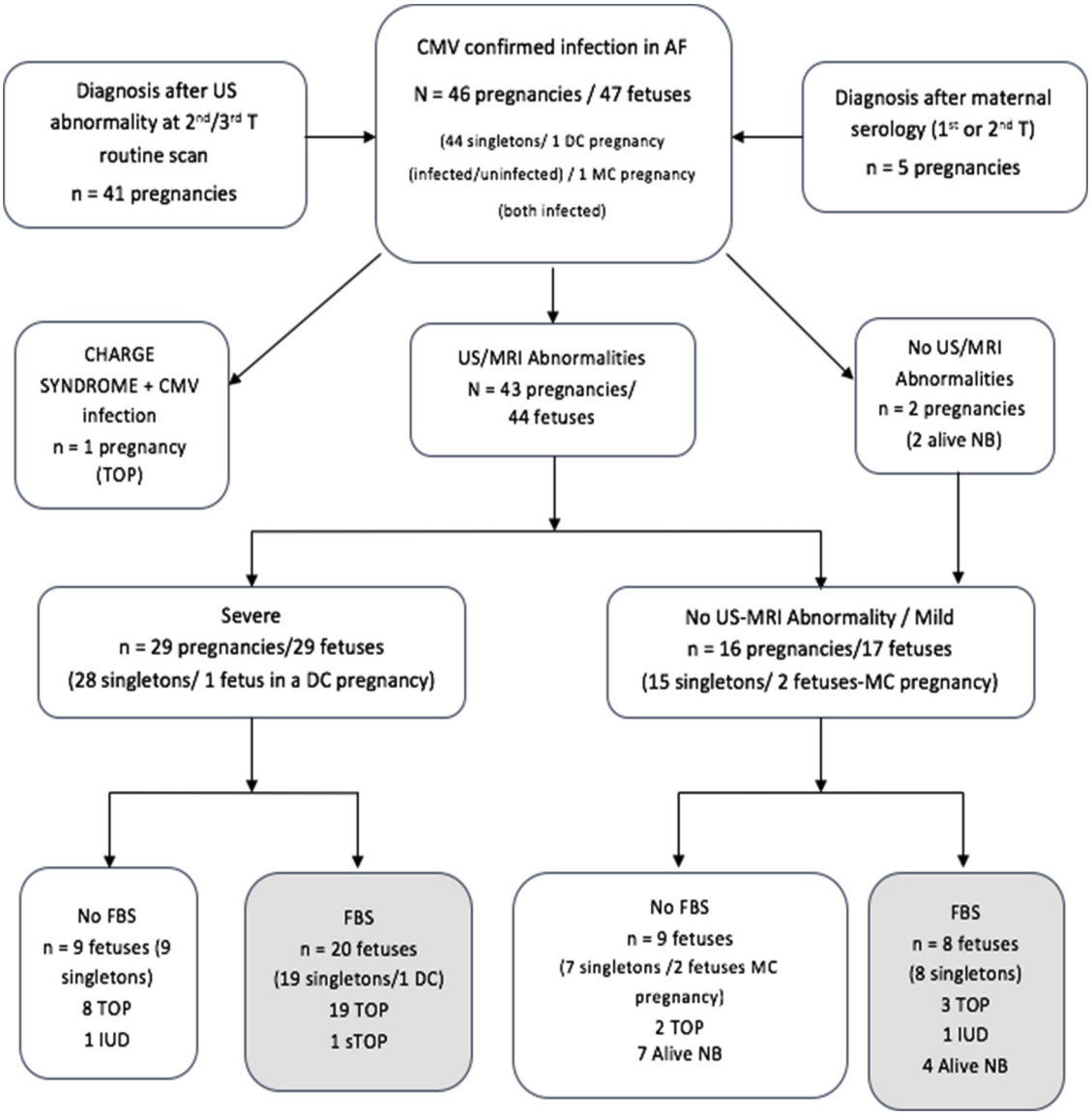

Fig. 1. *Flow chart:* Pregnancies with confirmed fetal CMV infection followed-up at our center between 2006 and 2018. Description of the outcome according to the severity of infection, and cordocentesis performed for fetal blood analysis.
DC: dichorionic, MC: monochorionic, AF: amniotic fluid, US: ultrasound, MRI: magnetic resonance imaging, FBS: fetal blood sampling, TOP: termination of pregnancy, sTOP: selective termination of pregnancy, IUD: intrauterine demise. NB: newborn.

virological blood parameters in CMV infected fetuses to predict severity of fetal brain damage, and to evaluate them according to the gestational age at cordocentesis.

2. Materials and methods

A series of consecutive pregnancies with CMV fetal infection confirmed in amniotic fluid by a positive polymerase chain reaction (PCR), attended at Hospital Clinic, Barcelona, in which fetal blood sampling (FBS) was performed as a complementary investigational tool over a 13-year period (January 2006-December 2018) (Fig. 1). In the absence of routine CMV screening in pregnancy, most of the cases were diagnosed on the evidence of US abnormalities detected during the second or third trimester routine scans. Other causes of fetal defects such as chromosomal abnormalities and toxoplasmosis infection were ruled out at the time of amniotic fluid study.

Fetal examination and follow-up consisted of serial US including a detailed neurosonography. All scans were carried out by experienced examiners using high-resolution US equipment (Voluson 730 Expert and E6 or E8, GE Healthcare, Kretz, Zipf, Austria). In pregnancies reaching the third trimester a fetal MRI was performed at 30–32 weeks (1.5 T GE Sigma Horizon, Echo speed, LX MRI scanner, Milwaukee, WI, USA), or earlier if US examination revealed brain lesions. Fetal infection was classified as severe (fetuses with severe US/MRI brain findings) or mild (fetuses with mild/absent US/MRI brain findings or with extra-cerebral ones) according to Table 1 classification [14].

After fetal imaging, women were counseled about newborn prognosis, and termination of pregnancy (TOP) was discussed according to Spanish laws. Cordocentesis for further studies in fetal blood was offered after evaluating its risks and diagnostic limitations. Written informed consent was obtained, and in most cases, the cordocentesis was undertaken at the time of TOP. Second trimester cordocentesis was defined when performed up to 28.0 gestational weeks, and third trimester when carried out thereafter. Fetal blood was analyzed for platelet count, β_2-microglobulin, gamma-glutamyl transpeptidase (GGT), CMV-DNAemia, and CMV specific-IgM antibodies. Platelet count was determined using ABX Pentra 60 −HORIBA Ltd; Japan. Thrombocytopenia was defined as a platelet-count threshold of

Table 1
Classification of prenatal US and MRI abnormalities in congenital CMV infection.

Severe US/MRI brain abnormalities*	Mild US/MRI brain abnormalities**	Extra-cerebral US abnormalities**
Severe ventriculomegaly (≥ 15 mm)	Intraventricular adhesions	ˆHyperechogenic bowel
Microcephaly (HC ≤ -3 SD)	Mild ventriculomegaly (10-14.9 mm)	ˆˆIntrauterine growth restriction
Periventricular hyperechogenicity	Isolated Calcifications	Hepatomegaly (right lobe ≥ 40 mm)
Enlarged sub-arachnoid space (CCW > 95th centile) (Micrencephaly)	Calcifications of lenticulostriate vessels in basal ganglia	Intra-hepatic calcifications
Porencephaly (porencephalic cysts)	Sub-ependymal cysts	Pleural effusion
	White matter hyperintensity (MRI)	Ascites
Agenesis/dysgenesis of corpus callosum		Pericardial effusion
Cerebellar/vermian hypoplasia (CTD < 5th centile) or cerebellar hemorrhage		Fetal hydrops
Abnormal gyration / Cortical dysplasia (MRI)		† Cardiomegaly
		Oligohydramnios (DVP ≤ 2 cm)
		Polyhydramnios (DVP ≥ 10 cm)
		Placentomegaly ≥ 40 mm

*Lesions of poor prognosis: fetuses with at least one severe brain US or MRI abnormality **Lesions of uncertain prognosis: fetuses with mild brain US or MRI abnormalities or extra-cerebral US abnormalities exclusively.
US: Ultrasound. MRI: Magnetic Resonance Imaging. HC: head circumference; CCW: crania-cortical width; CTD: cerebellar transverse diameter;
DVP: deepest vertical pocket.
ˆ Considered when the echogenicity of the bowel is equal or more intense than that of the fetal bones.
ˆˆ Considered when the estimated fetal weight is below the 10th centile according to specific population tables with or without Doppler ultrasound anomaly.
† Considered if the heart is more than one-third of the thoracic diameter.
Adapted from: 1- Leruez-Ville et al. Prognosis evaluation of fetal CMV infection. Am JObstet Gynecol 2016;215:342.e1-9.
2- Goncé et al. TORCH and B19 Parvovirus infections during Pregnancy. www.fetalmedbarcelona.org.

100,000/mm^3. β_2-microglobulin concentration was measured using Siemens Dimension Vista® N-Latex® β_2-microglobulin reagent cartridge (Siemens Healthineers, Inc., Newark, Del., USA); high-risk levels were considered establishing two different cut-offs: ≥10.4 mg/l and ≥11.5 mg/l as defined by Fabbri et al. [11]. Quantitative determination of GGT was performed using a *in-vitro* diagnostic test [Siemens Dimension® EXL clinical chemistry system Flex® reagent cartridge (Siemens Healthineers, Inc., Newark, Del., USA)]; levels ≥151 UI/L were considered abnormal [15]. Extraction of CMV DNA for all the maternal, fetal, and neonatal samples was performed using QIAsymphony system (Qiagen, Hilden, Germany), and viral load by PCR CMV Real Time (Nanogen Advanced Diagnostics, Italy) with a threshold of 20 copies/ml to define positivity. High viral load in FBS was defined using two thresholds: 30,000 copies/ml (≥ 448 $\log_{10}$ IU/ml) and 50,000 copies/ml (≥ 493 $\log_{10}$ IU/ml), as both have been associated with symptomatic status at birth. [11,14]. CMV-IgM specific antibodies were determined using commercially available enzyme immunoassay [CMV IgM ELISA VIDAS (Biomerieux S.A., Spain)].

In cases of fetal demise and TOP, routine postmortem macroscopic and microscopic examination of the fetus and the placenta was performed, after informed consent obtained from the parents. In cases with an alive newborn, congenital CMV was confirmed by a positive PCR from urine sampled within 2 weeks of birth. Postnataly, the infection was classified as either symptomatic (mild, or moderate to severe), or asymptomatic according to the recent consensus document by Rawlinson *et a*l. [16].

2.1. Ethical approval

This study was approved by the Hospital Clinic ethics committee: **Reg. HCB/2017/0564**.

2.2. Statistical analysis

Results of non-viral and viral assays were compared according to the severity of the congenital CMV infection. Variables were analyzed according to the severity of brain damage in US/MRI imaging. Quantitative variables were assessed using Shapiro-Wilk's test for normality, and normally distributed variables were compared using *t*-test and expressed as mean and standard deviation (SD). Non-normally distributed quantitative variables were compared using U-Mann-Whitney test and expressed as median and interquartile range (IQR: p25-75). Qualitative variables were compared using X^2 and Fisher's exact test. A sub-analysis of fetal cord blood parameters was assessed according to trimester of gestation. Receiver-Operator Curve (ROC) analysis was performed in order to establish the best cut-off point for variables that were significantly different between severely and mildly affected fetuses. Univariate logistic regression was carried out for each independent factor for an outcome of severe brain damage. A robust bias-corrected estimation was used to calculate 95% confidence intervals and p-values. Predictive performance for GGT as a continuous variable was calculated using ROC analysis. Sensitivity, specificity, positive and negative likelihood ratios and as well as area under the ROC curve were calculated. P-value < 0.05 was considered significant. Data were analyzed using STATA, v.15.0 (College Station, Texas)

3. Results

A total of 46 pregnancies and 47 fetuses with CMV infection were included in the study (Fig. 1). With the exception of five pregnancies in which the diagnosis of fetal infection was established after maternal CMV screening in the first or second trimester decided by the patient's practitioner, all other pregnancies were diagnosed on the evidence of US abnormalities, 31 detected during second and 10 during third trimester routine scans. Cordocentesis for further evaluation was accepted by 28 (61%), 20 with severe fetal US/MRI abnormalities, and 8 with non-severe (n = 7) or absent (n = 1) findings. Among the one with absent findings, maternal primary infection was diagnosed after first trimester screening **(Supplementary Tables 1a & 1b).** Baseline characteristics of pregnancies comparing those with and without FBS, and according to the severity of brain-damage are summarized in **Supplementary Table-2 and** Table 2, respectively. Regarding the time of cordocentesis, 18 were performed in the second trimester (12 severely and 6 mildly/non-affected fetuses) at a mean gestational age (MGA) and SD of 23.4 (2.1) weeks, and 10 in the third (8 severely and 2 mildly affected fetuses) at a MGA of 33.2 (2.6) weeks. Twenty-two fetuses (78%) were sampled at the time of TOP. The mean time elapsed between US diagnosis and FBS was 3.4 weeks.

The most frequent fetal brain US abnormality was periventricular hyperechogenicity involving the whole periventricular area (Fig. 2) found in 54% of fetuses, and the most frequent extra-cerebral US abnormality was hyperechogenic bowel (39%). Cerebral MRI was performed in 14 fetuses (50%), between 23.0 and 37.0 weeks, and provided relevant additional information regarding an abnormal cortical

Table 2
Baseline characteristics according to the severity of brain damage in pregnancies with CMV infected fetuses with FBS.

Characteristic	Mild/No US Ab n = 8	Severe US Ab n = 20	*p** value
Maternal Age, years, mean (SD)	30.5 (5.3)	31.4 (5.9)	0.71
Caucasian Ethnicity (%)	100	100	1.00
Low educational level, n (%)**	0 (0)	8/19 (42)	0.08
Multiparity (%)	62	60	0.90
Child at nursery (< 3y child) (%)	50	60	0.27
Gestational age at diagnosis of fetal infection, mean (SD)	22.5 (2.7)	25.5 (4.8)	0.12
≤ 28.0 weeks, n (%)	7 (87)	14 (70)	0.33
Fetal gender (female), n (%)	5 (26)	14 (74)	0.70
AF-CMV million copies/ml, median (IQR)	12.9 (7.2-12.9)	10 (1.14-14)	0.31
Gestational age at cordocentesis, mean (SD)	24.4 (1.8)	27.9 (1.1)	0.11
≤ 28.0 weeks	6 (75)	12 (60)	0.45

Data are presented as mean and standard deviation (SD), frequencies or percentage (%), medians (IQR: interquartile range: p25-75). * *p* value as determined with the *t*-test, Mann-Whitney U, X^2 or Fisher's exact test.
** Low educational level was defined as primary school studies only. FBS: fetal blood sampling. US Ab: ultrasound abnormality. AF: amniotic fluid.

development in 3 (Fig. 3). The US and MRI findings are summarized in **Supplementary-Table 3**.

Concerning FBS results, the only two parameters significantly associated with the degree of brain damage were thrombocytopenia and GGT levels, with a median value significantly higher in the severely affected fetuses **(**Table 3 / Fig. 4**)**. The best performance in the prediction of severe brain damage was achieved with a GGT cutoff-of ≥183 UI/l with 71% sensitivity, 83% specificity, 4.3 positive and 0.34 negative likelihood ratios (LHR) (*AUC: 0.78*), and an OR of 2.05 (95% CI: 1.22–3.43) per 100 IU/l increase. (Fig. 5).

When the distribution of fetal blood parameters was compared among all fetuses according to gestational age at cordocentesis, we observed that in the second trimester, the mean platelet count was lower (69.4/mm^3 vs 117.9/mm^3, *p=0.04),* the median β_2-microglobulin was higher (11.9 mg/l vs 7.5, p < 0.01), and CMV IgM and viral load > 50 000 copies were significantly more frequent (71% vs 13%, p < 0.01 and 79% vs 33%, p = 0.03, respectively) **(Supplementary Table-4)**.

Subsequently, we analyzed the validity of blood parameters according to gestational age among the severely damaged fetuses, and observed that thrombocytopenia, IgM antibodies, and DNAemia > 50 000 copies/ml were obtained significantly more often ≤28 weeks (*p = 0.04, p = 0.04 and p = 0.02, respectively*). Moreover, high levels of β_2 microglobulin appeared exclusively in the second trimester (*p* = 0.03). GGT was the only parameter that did not vary according to the trimester of pregnancy **(**Table 4**).**

4. Discussion

In this series, we observed that among fetuses diagnosed with CMV infection in the second or third trimester of pregnancy, GGT and thrombocytopenia were the only blood parameters significantly associated with severe US/MRI brain lesions. However, while GGT levels showed stable values along the pregnancy, thrombocytopenia, high levels of β_2-microglobuline and DNAemia, and positive IgM antibodies were observed significantly more often in the severely damaged fetuses diagnosed before 28 weeks.

Thrombocytopenia has been reported as an independent factor responsible for a poor perinatal outcome. Fabbri et al and Leurez-Ville et al. found an association between fetal thrombocytopenia in the second trimester and being symptomatic at birth. [11,14]. Leurez-Ville et al. showed that with platelet count ≤114,000/mm3 there was a 62.5% risk of a symptomatic status at birth or at TOP. They concluded that the prognostic assessment for being symptomatic at birth is possible as early as in the second trimester by combining a targeted US-examination, viral-load, and platelet count in fetal blood [14]. In contrast, Enders *et a*l [13] who included second and third trimester FBS, did not observe differences in non-virological markers between fetuses with normal and abnormal US findings. In our series, thrombocytopenia was also observed more often in the severely damaged fetuses though more frequently in the second trimester.

Dreux et al. [17] identified β_2-microglobulin as a reliable marker of fetal CMV infection, and Fabbri et al. reported that β_2-microglobulin was the most reliable non-viral marker for prediction of fetal damage in the second trimester [11]. We did not observe any differences in β_2-microglobulin levels when comparing based on the severity of damage. However, sub-analysis according to the time of cordocentesis in these fetuses, high levels of β_2-microglobulin were found exclusively in those fetuses diagnosed with severe damage in the second trimester.

Regarding virological parameters, higher levels of DNAaemia in symptomatic fetuses have been described [11–14] but with a wide overlap, leading to a poor prognostic value [11,13] as also described in newborns [12]. Different cut-off values have been proposed for fetal prognostic assessment in the second trimester that vary from 4.48 log10 IU/mL to 4.93 log10 IU/mL [11,14], but a cut-off value has not been established in the third trimester. Among our fetuses, there were higher levels in the severely damaged group ≤28 weeks. However, when comparing according to the severity of damage no differences were observed. A few studies have reported that fetuses with US abnormalities have significantly higher values of CMV specific-IgM antibodies

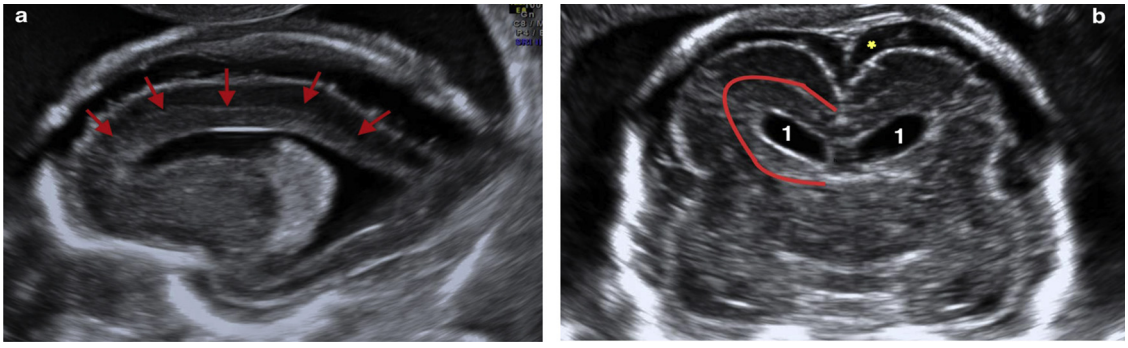

Fig. 2. Fetal neurosonography: transvaginal approach: 24.0 weeks (Case 5 – supplementary table 1a) **(a)** Parasagittal plane of the fetal head in the three horns view showing periventricular hyperechogenicity also known as ∴ periventricular halo ∴ (red arrow heads). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
(b) Coronal transcaudate plane showing dilation of both anterior horns (1) of the lateral ventricles. We delineated the periventricular hyperechogenicity **("halo sign")** in one of the anterior horns. Noted the augmented sinus-cortical space (yellow asterisk) as a sign of micrencephaly.

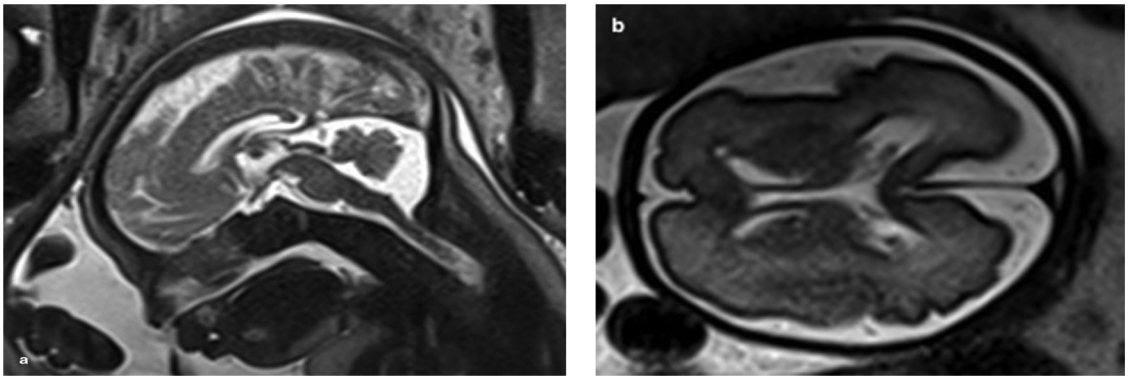

Fig. 3. Fetal MRI image. **(a)** 36.0 weeks. (case 15, supplementary table 1a (3)) - Sagittal plane of the fetal head showing hypoplastic corpus callosum and cortical dysplasia **(b)** 29.0 weeks (case 9, supplementary table 1a (2)) - Axial view showing mild ventriculomegaly, delay in cortical maturation, enlarged subarachnoid space, and white matter hyperintensity.

compared to asymptomatically infected ones, though their usefulness to distinguish symptomatic from asymptomatic newborns is poor [11,13]. We did not observe differences in the presence of CMV-IgM when comparing the severity of damage, but found a significant decrease in IgM positivity in FBS obtained in the third trimester.

According to our results, it could be hypothesized that after an acute phase of the infection, fetal thrombocytopenia recovers, and β_2-microglobulin and DNAemia decrease in the third trimester. Moreover, a longer period elapsed from fetal infection would allow time for IgM to reach its peak earlier in pregnancy, and already be negative in the third trimester.

CMV infection can induce several inflammatory mediators that may induce cytotoxicity [9]. GGT is an enzyme involved in the transfer of amino acids and considered to participate in glutathione-coupled detoxification processes with great activity in biliary epithelial cells, as well as in other tissues, such as the brain [18–20]. GGT does not cross the placenta and cord levels derive entirely from the fetus [15]. There is little information on abnormal fetal values, although high levels of GGT have been reported as a reliable parameter of CMV infection. [11,13,21]. Enders et al. [13] did not observe significant differences in GGT levels according to the presence of US findings in CMV-infected fetuses, although levels above 350 IU/l were found exclusively in those with severe abnormalities. A series of more than 3000 unselected fetal blood samplings showed that the mean GGT value during second and third trimester was 97.5 IU/l with little variation between 20 and 40 weeks [15]. This finding may explain why we observed significant differences according to the severity of damage, but not to the trimester

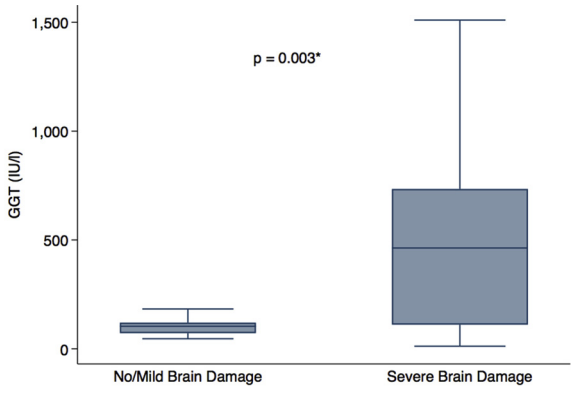

Fig. 4. Gamma-glutamyl transpeptidase levels according to severity of brain damage.
Data are presented as median [IQR]. * *p* value as determined with the *Wilcoxon rank-sum test (Mann-Whitney U), and nonparametric equality-of-medians test.* GGT: Gamma-glutamyl transpeptidase.

at cordocentesis. To the best of our knowledge, an association of the severity of brain lesions with high levels of GGT has never been previously reported. Although it could be interpreted that among the severely damaged fetuses the source of GGT elevated levels is derived from biliary obstruction, our hypothesis is that CMV cerebral-infection

Table 3
Fetal blood sampling results according to the severity of brain damage in CMV infected fetuses.

Characteristic	Mild/No US Ab n = 8		Severe US Ab n = 20		*p** value
Platelet count /mm^3, mean (SD)	125.5 (28.9)		71.3 (15.4)		0.08
Low platelet count (≤ 100,000), n (%)	2/8 (25)		14/19 (74)		0.03*
GGT IU/l, median (p25-75)	103.5 (72-120)		463 (111-734)		0.003*
GGT ≥ 183 IU/l, n (%)	0/6 (0)		10/14 (71.4)		0.011*
GGT ≥ 151 IU/l, n (%)	1/6 (16.7)		10/14 (71.4)		0.024*
GGT ≥ 120 IU/l, n (%)	2/6 (33)		10/14 (71.4)		0.11
β_2 microglobulin (mg/l), median (IQR)	11 (8.7 - 12.7)		9.5 (7.2 - 12.3)		0.41
β_2 microglobulin ≥ 11.5 mg/l, n (%)	2/5 (40)		6/16 (37.5)		0.92
β_2 microglobulin ≥ 10.4 mg/l, n (%)	3/5 (60)		6/16 (37.5)		0.38
CMV-DNA thousands copies/ml, median (IQR)	118 (56-205)		96 (16-428)		0.54
CMV-DNA > 30,000 copies/ml, n (%)	3/5 (60)		11/18 (61)		0.60
CMV-DNA > 50,000 copies/ml, n (%)		3/5 (60)	10/18 (55)		0.47
Positive Fetal IgM n, (%)	3/6 (50)		8/16 (50)	1.00	

Data are presented as mean and standard deviation (SD), percentage (%), medians (IQR: interquartile range: p25-75).
* p value as determined with the *t*-test, Mann-Whitney U, X2 or Fisher's exact test. AF: amniotic fluid GGT: Gamma-glutamyl transpeptidase. US Ab: ultrasound abnormality.

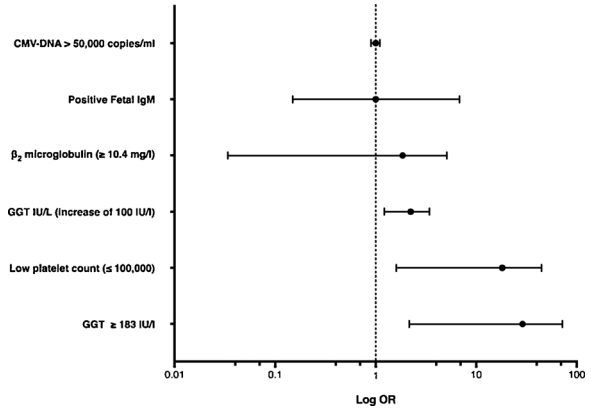

Fig. 5. Individual risk for severe outcome according to fetal blood parameters. All *p* values and confidence intervals were calculated with robust bias-corrected logistic regression

increases GGT expression and activity in the brain, and that it could help discriminate severe cases. Recently, we have obtained similar results when comparing GGT levels at the time of TOP in a group of uninfected fetuses, with (N = 14) and without (N = 20) brain damage [50% sensitivity, 95% specificity, 9.5 positive LHR, and 0.53 negative LHR (*AUC: 0.82*) for the same GGT cutoff-(≥187 UI/l)] [Unpublished data]. These results are still hypothetical, and more research is required.

The strengths of our study are: it is the first one to compare blood parameters in untreated infected fetuses according to the trimester at diagnosis. In addition, it is the first study to establish a cut-off value for GGT levels in the prediction of severe brain lesions. Blood parameters, except for GGT, were nearer normal ranges in the third trimester, also in the severely damaged fetuses. This could be valuable information at the time of considering a cordocentesis for further prognosis assessment as it is an invasive procedure that entails considerable risk.

Our study has several limitations. First, the retrospective nature of the analysis. Second, the small sample size, especially among pregnancies with mildly affected fetuses that are underdiagnosed in the absence of maternal routine screening, and the fact that not all laboratory parameters were available for all fetuses. Third, there could have been an overlap in the grading of severity because it is not known what would have been the evolution of cases classified as mildly damaged in the second trimester if they had survived. This, however, is inherent to a classification of risk in the second trimester. In addition, there was a lack of standardized necropsy protocol for congenital CMV infection.

In conclusion, in CMV-infected fetuses, platelet count and GGT levels were significantly associated with severe US/MRI brain damage. However, thrombocytopenia, high levels of β_2-microglobulin, high CMV-DNAemia, and specific-IgM antibodies were less frequently observed in the third trimester. Our results suggest that with the exception of GGT, blood parameters could be predictive of fetal damage only in the second trimester.

Summary

A series of pregnancies with confirmed fetal CMV infection which underwent cordocentesis. Fetuses were considered severely or mildly affected based on US/MRI findings. Fetal blood parameters were compared according to the severity of brain damage and trimester of pregnancy at cordocentesis.

Ethical approval

This study was approved by the Hospital Clinic ethic committee: **Reg. HCB/2017/0564**.

Credit author statement

All authors fulfill all conditions required for authorship, have seen and approved the manuscript, and all have significantly contributed to the work as follows: Anna Goncé and Ameth Hawkins-Villarreal (conception and design of the study); Ameth Hawkins-Villarreal, Ana L. Moreno-Espinosa, Elisenda Eixarch, Laura Garcia-Otero, Laura Salazar (acquisition of data); Ameth Hawkins-Villarreal, Raigam J. Portilla-Martinez, Anna Goncé, Francesc Figueras (analysis and interpretation of data); M. Angeles Marcos (analysis and interpretation of virological essays). Anna Goncé (supervision). Anna Goncé, Ameth Hawkins-Villarreal, Ana L. Moreno-Espinosa (writing-original draft). Anna Goncé, Ameth Hawkins-Villarreal, Antoni Borrell, Francesc Figueras, Marta Lopez (writing, revision and editing of the submitted article).

Funding

This project has been funded with support of the Erasmus + Programme from the European Union (Framework Agreement number: 2013-0040). This publication [communication] reflects the views only of the author, and the Commission cannot be held responsible for any use, which may be made of the information contained therein. Additionally, the research leading to these results has received funding

Table 4
Fetal blood sampling results according to trimester at cordocentesis in CMV infected fetuses with severe brain damage.

Characteristic	≤ 28.0 weeks n = 12	> 28.0 weeks n = 8	*p** value
Platelet count 10^3 /mm^3, mean (SD)	49.8 (46)	100.9 (83)	0.11
Low platelet count (≤ 100,000), n (%)	**10/11 (91)**	**4/8 (50)**	**0.04***
GGT IU/l, median (IQR)	463 (111-898)	495 (170-698)	0.55
GGT ≥ 183 IU/l, n (%)	7/10 (70)	3/4 (75)	0.85
GGT ≥ 151 IU/l, n (%)	7/10 (70)	3/4 (75)	0.85
GGT ≥ 120 IU/l, n (%)	7/10 (70)	3/4 (75)	0.85
β_2 microglobulin (mg/L), median (IQR)	**11.9 (9.7-13.0)**	**7.5 (6.4-7.7)**	**0.03***
β_2 microglobulin ≥ 11.5 mg/L, n (%)	**6/10 (60)**	**0/6 (0)**	**0.03***
β_2 microglobulin ≥ 10.4 mg/L, n (%)	**6/10 (60)**	**0/6 (0)**	**0.03***
CMV-DNA thousand copies/ml, median (IQR)	147 (89,4-456)	108 (3,2-228)	0.05
CMV-DNA > 30,000 copies/ml, n (%)	8/10 (80)	3/8 (38)	0.07
CMV-DNA > 50,000 copies/ml, n (%)	**8/10 (80)**	**2/8 (25)**	**0.02***
Positive fetal IgM n, (%)	**7/10 (70)**	**1/6 (17)**	**0.04***

Data are presented as mean and standard deviation, frequencies or percentage (%), medians [IQR: interquartile range: p25-75].
* p value as determined with the *t*-test, Mann-Whitney U, X2 or Fisher's exact test.
2nd Trimester: ≤ 28 weeks of gestation, 3rd Trimester: > 28 weeks of gestation. GGT: Gamma-glutamyl transpeptidase.

form “la Caixa” Foundation (LCF/PR/GN14/10270005), Cerebra Foundation for the Brain Injured Child (Carmarthen, Wales, UK) and AGAUR 2017 SGR grant n° 1531. A.H-V. has received grant from Hospital Santo Tomas de Panama and IFARHU.

Declaration of Competing Interest

None.

Acknowledgements

“We are indebted to the IDIBAPS Biobank, integrated in the Spanish National Biobank Network, for the sample and data procurement”.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.08.008.

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