UOG26173

Ultrasound Obstet Gynecol 2023; **0**: 000–000
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.26173

Halo sign in fetal cytomegalovirus infection: cerebral imaging abnormalities and postmortem histopathology in 35 infected fetuses

•A. HAWKINS-VILLARREAL[1,2,3,4#], K. CASTILLO[1,2#], A. NADAL[2,5,6], S. PLANAS[7], A. L. MORENO-ESPINOSA[1,3,4], A. ALARCÓN[1,8], M. REBOLLO-POLO[9,10], F. FIGUERAS[1,2], E. GRATACÓS[1,2], E. EIXARCH[1,2] and A. GONCÉ[1,2]

[1]*BCNatal: Fetal Medicine Research Center, Hospital Clínic and Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain;* [2]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain;* [3]*Fetal Medicine Service, Obstetrics Department, Hospital Santo Tomás, University of Panama, Panama City, Panamá;* [4]*Iberoamerican Research Network in Obstetrics, Gynecology and Translational • Medicine;* [5]*Department of Basic Clinical Practice, University of Barcelona, Barcelona, Spain;* [6]*Department of Pathology, Hospital Clínic, Barcelona, Spain;* [7]*Department of Anatomical Pathology, Hospital Sant Joan de Déu, Barcelona, Spain;* [8]*Institut de Recerca Sant Joan de Déu, Barcelona, Spain;* [9]*Diagnostic Imaging and Image Guided Therapy, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain;* [10]*Diagnostic Imaging Department, Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain* •

KEYWORDS: fetal cytomegalovirus infection; fetal histopathology; fetal MRI; fetal ultrasound; periventricular halo; pregnancy; prognosis

CONTRIBUTION

What are the novel findings of this work?
This study reports on the largest cohort of cytomegalovirus (CMV)-infected fetuses with periventricular echogenic halo in which targeted histopathological examination of the fetal brain was performed. The halo sign, when observed as an isolated finding, was detected exclusively during the second trimester and was correlated with mild ventriculitis and mild histopathological staging of brain damage.

What are the clinical implications of this work?
In CMV-infected fetuses with isolated periventricular echogenic halo in the second trimester, a detailed neurosonographic follow-up every 3–4 weeks and complementary magnetic resonance imaging are indicated. The prognostic significance of the halo sign as an isolated finding is still to be determined.

ABSTRACT

Objective *To evaluate the correlation of periventricular echogenic halo (halo sign) with histopathological findings and its association with other brain imaging abnormalities in fetuses with cytomegalovirus (CMV) infection.*

Methods *This was a retrospective study of fetuses diagnosed with severe CMV infection based on central nervous system (CNS) abnormalities seen on ultrasound, which had termination of pregnancy (TOP) or fetal demise at a single center from 2006 to 2021. All included cases had been evaluated by conventional complete fetal autopsy. A maternal–fetal medicine expert reanalyzed the images from the transabdominal and transvaginal neurosonography scans, blinded to the histological findings. The halo sign was defined as the presence of homogeneous periventricular echogenicity observed in all three fetal brain orthogonal planes (axial, parasagittal and coronal). Cases were classified according as to whether the halo sign was the only CNS finding (isolated halo sign) or concomitant CNS anomalies were present (non-isolated halo sign). An expert fetal radiologist reanalyzed magnetic resonance imaging (MRI) studies when available, blinded to the ultrasound and histological results. Hematoxylin–eosin-stained histologic slides were reviewed independently by two experienced pathologists blinded to the neuroimaging results. Ventriculitis was classified into four grades (grades 0–3) according to the presence and extent of inflammation. Brain damage was categorized into two stages (Stage I, mild; Stage II, severe) according to the histopathological severity and progression of brain lesions.*

Correspondence to: Dr A. Goncé, BCNatal, Hospital Maternitat del Clínic, Carrer Sabino de Arana 1, 08028 Barcelona, Spain (e-mail: agonce@clinic.cat)

#A.H.-V. and K.C. contributed equally to this study.

Accepted: 19 January 2023

Results *Thirty-five CMV-infected fetuses were included in the study, of which 25 were diagnosed in the second and 10 in the third trimester. One fetus had intrauterine demise and 34 TOP. The halo sign was detected on ultrasound in 32 (91%) fetuses (23 in the second trimester and nine in the third), and it was an isolated sonographic finding in six of these cases, all in the second trimester. The median gestational age at ultrasound diagnosis of the halo sign was similar between fetuses in which this was an isolated and those in which it was a non-isolated CNS finding (22.6* vs *24.4 weeks;* P = *0.10). In fetuses with a non-isolated halo sign, the severity of additional ultrasound findings was not associated with the trimester at diagnosis, except for microencephaly, which was more frequent in the second compared with the third trimester (10/18 (56%)* vs *1/8 (13%);* P = *0.04). With respect to histopathological findings, ventriculitis was observed in all fetuses with an isolated halo sign, but this was mild (Grade 1) in the majority of cases (4/6 (67%)). Extensive ventriculitis (Grade 2 or 3) was more frequent in fetuses with a non-isolated halo sign (21/26 (81%)) and those without a periventricular echogenic halo (2/3 (67%);* P = *0.032). All fetuses with an isolated halo sign were classified as histopathological Stage I with no signs of brain calcifications, white-matter necrosis or cortical injury. On the other hand, 25/26 fetuses with a non-isolated halo sign and all three fetuses without a periventricular echogenic halo showed severe brain lesions and were categorized as histopathological Stage II. Among fetuses with a non-isolated halo, histological brain lesions did not progress with gestational age, although white-matter necrosis was more frequent, albeit non-significantly, in fetuses diagnosed in the second* vs *third • trimester (10/15 (67%)* vs *3/11 (27%);* P = *0.06).*

Conclusions *In CMV-infected fetuses, an isolated periventricular echogenic halo was observed only in the second trimester and was associated with mild ventriculitis without signs of white-matter calcifications or necrosis. When considering pregnancy continuation, detailed neurosonographic follow-up complemented by an MRI examination in the early third trimester is indicated. The prognostic significance of the halo sign as an isolated finding is still to be determined. © 2023 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Cytomegalovirus (CMV) is the most common congenital infection worldwide, represents the leading cause of non-genetic sensorineural hearing loss and is a major cause of neurodevelopmental disability in children. Prenatal counseling on the prognosis of congenital CMV is challenging and is largely based on fetal imaging. Ultrasound (US) abnormalities are seen in only a small proportion of CMV-infected fetuses, and subtle or non-specific US features are likely to remain undetected[1,2]. While severe abnormal fetal cerebral US and magnetic resonance imaging (MRI) findings have been well defined and are the most significant predictive markers for adverse outcomes, less severe abnormalities carry a more uncertain prognosis[3–5].

Periventricular hyperechogenicity (halo sign), defined as homogeneous echogenicity surrounding the lateral ventricles observed in all three orthogonal planes[6,7], is one of the most common abnormalities in fetuses infected with CMV. The sign was first described by Malinger *et al.*[6] in 2003, although it was first named periventricular echogenic 'halo' by Simonazzi *et al.*[2] in 2010. The halo, which is better depicted by the transvaginal route in the parasagittal plane and is not visible on MRI, has been described as a sign of ventriculitis[2,7], germinal matrix injury[8] and white matter lesion[2,9,10]. Thus, the halo sign is generally considered to be a marker of poor prognosis, but the specific underlying brain histopathology is unknown.

Histological postmortem examination of central nervous system (CNS) tissue of fetuses with periventricular halo would provide a better understanding of the pattern and severity of brain damage in such cases. Nevertheless, very few studies, mainly case reports or those including a broader evaluation of CMV infection and not solely focusing on the halo sign, have performed this examination[2,8–10]. The objective of this study was to analyze the correlation of the halo sign in CMV-infected fetuses with histopathological findings in order to define its prognostic value.

METHODS

We retrospectively evaluated fetuses with severe CMV infection (based on CNS abnormalities on ultrasound) diagnosed at a single center (Hospital Clínic, Barcelona, Spain) between 2006 and 2021, which ended in intrauterine demise (IUD) or termination of pregnancy (TOP), which was performed in full compliance with Spanish legislation. We included only cases that underwent postmortem examination. This study was approved by the Ethics Committee of Hospital Clínic in Barcelona (HCB/2020/0322).

Initial fetal examination and follow-up consisted of serial US scans, including detailed neurosonography (NSG). Fetal infection had been confirmed in all cases by extraction of CMV-DNA from amniotic fluid samples using QIAsymphony (Qiagen, Hilden, Germany) and determination of viral load by real-time-polymerase chain reaction, as previously described[11]. All US scans were performed by experienced examiners using high-resolution US equipment (Voluson 730 Expert and E6 or E8; GE Healthcare, Kretz, Zipf, Austria). All NSG examinations were performed by the transabdominal approach, and in cases with vertex presentation, this was followed by the transvaginal approach. In pregnancies reaching the third trimester, fetal MRI was performed at 30–32 weeks using a 1.5T GE Sigma Horizon, Echo speed, LX MRI scanner (Milwaukee •, WI, USA). In cases with inconclusive NSG in the late second trimester, MRI was performed earlier, at 26–28 weeks.

In the present study, a maternal–fetal medicine expert (E.E.) reanalyzed the transabdominal and transvaginal

neurosonographs obtained in the included cases, blinded to the histological results. The halo sign was defined as the presence of homogeneous bilateral periventricular echogenicity, identified in the axial, parasagittal and coronal planes, with the parasagittal plane depicting the halo in its entirety (Figure 1). When periventricular echogenic halo was the only CNS finding, it was defined as an isolated halo sign; when concomitant CNS anomalies were observed, it was defined as a non-isolated halo sign. The corpus callosum, cerebellum, cerebellar vermis and gyral pattern were also evaluated[11]. Extra-CNS findings were evaluated but not considered for the analysis. The severity of fetal CMV infection was re-evaluated •and was confirmed as severe (severe brain US/MRI findings) in all cases according to the categorization of Leruez-Ville *et al.*[4], considering the presence of isolated periventricular echogenic halo as a sign of a poor prognosis. In cases in which MRI had been performed, an expert fetal radiologist (M. R.-P.), blinded to the NSG and histological results, reanalyzed the images to evaluate the presence of CNS abnormalities. The cut-off for the US diagnosis of microcephaly was defined as a fetal head circumference of three SD below the mean for gestational age (GA)[12]. On NSG, microencephaly was defined as an enlarged subarachnoid space with a cranial–cortical width $> 95^{th}$ percentile[12], and on MRI, as a brain biparietal diameter $< 1^{st}$ percentile[13].

All included cases had been evaluated by conventional complete fetal autopsy. Stored hematoxylin–eosin-stained histologic slides were reviewed independently by two pathologists (A.N., S.P.) blinded to the US/MRI results, and a final diagnosis was reached by consensus. Histological cerebral lesions (microglial nodules, perivascular infiltrates, necrosis or calcification in the cortex or white matter, polymicrogyria and CMV-infected diagnostic cells) were classified according to their presence and extent as: absent, mild (present in only one slide) or severe (extensive in a single slide or present in multiple slides) (Figure 2). Ventriculitis was classified into four grades: Grade 0, absent; Grade 1, identified only by immunohistochemistry; Grade 2, focal; and Grade 3, extensive or seen in multiple preparations. Cases with histologically normal periventricular tissue were further investigated for the presence of cytotoxic T-cells by immunohistochemistry with specific antibodies against CD-8 (CD-8 rabbit monoclonal antibody, clone sp57; Roche, Tucson, AZ, USA). Microencephaly was defined as an encephalic weight less than two SDs below that expected for GA, according to Bartosch *et al.*[14].

In order to classify the severity or histological progression of the histological brain lesions, pathologic findings were tabulated and cases were sorted from those with the least number of different microscopic lesions to those with macroscopic anomalies. The combination of lesions observed among the cases with the smallest number of lesions was classified as mild or less severe (Stage I), while all other combinations of lesions were classified as severe (Stage II). As such, fetuses with histopathological Stage I had normal macroscopic appearance and showed only ventriculitis, microglial nodules and/or viral inclusions on microscopy. Fetuses classified as histopathological Stage II exhibited macroscopic lesions, such as microencephaly, porencephaly and ventriculomegaly, and/or microscopic lesions, such as perivascular infiltrates, calcifications, white-matter or cortical necrosis and polymicrogyria.

Statistical analysis

Histological and NSG imaging findings in CMV-infected fetuses were compared according to the presence or absence of the halo sign and whether the halo sign was isolated or non-isolated. Qualitative variables were compared using the χ^2 test or Fisher's exact test and are expressed as *n* (%). Data were analyzed using STATA version 15.0 (StataCorp., College Station, TX, USA), and $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Population characteristics

Thirty-five CMV-infected fetuses that had TOP in the second ($n = 23$) or third ($n = 11$) trimester or IUD in the third trimester ($n = 1$) were included. Diagnosis of fetal infection was made based on the evidence of CNS abnormalities on routine second- and third-trimester US scans in 22 and seven pregnancies, respectively.

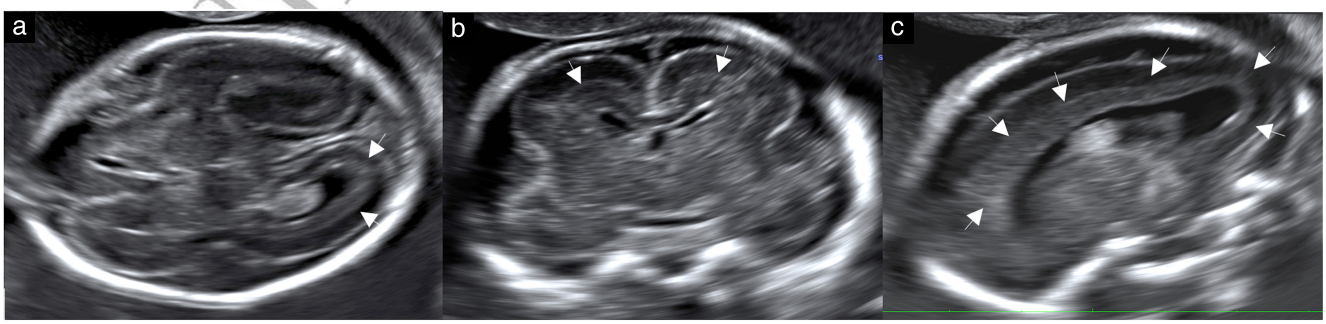

Figure 1 Transvaginal ultrasound images in axial (a), coronal (b) and parasagittal (c) planes, showing periventricular hyperechogenicity at 21 weeks' gestation in a cytomegalovirus-infected fetus with isolated halo sign. Note the clear delimitation between the periventricular zone with the halo sign and the white matter (arrows). The halo sign is visualized in all three orthogonal planes.

Color Figure - Print and Online

In the remaining six cases, all of which had isolated periventricular echogenic halo, congenital CMV was diagnosed based on extra-CNS abnormalities detected during the routine second-trimester US scan ($n = 2$), maternal symptomatology in the first trimester ($n = 2$), close contact with a CMV-infected individual in the first trimester ($n = 1$) and first-trimester CMV screening decided by the patient's practitioner ($n = 1$). With respect to the type of maternal infection, 18 (51%) patients had a confirmed first-trimester primary CMV infection, one (3%) patient had a first-trimester non-primary infection, and in 16 (46%) cases, the type of maternal infection was unknown.

The median GA at diagnosis of congenital CMV infection in the whole cohort was 22.1 weeks (interquartile range (IQR), 21.0–26.5 weeks). The halo sign had been diagnosed in 32 (91%) fetuses and was confirmed retrospectively in this study in all cases. The median GA

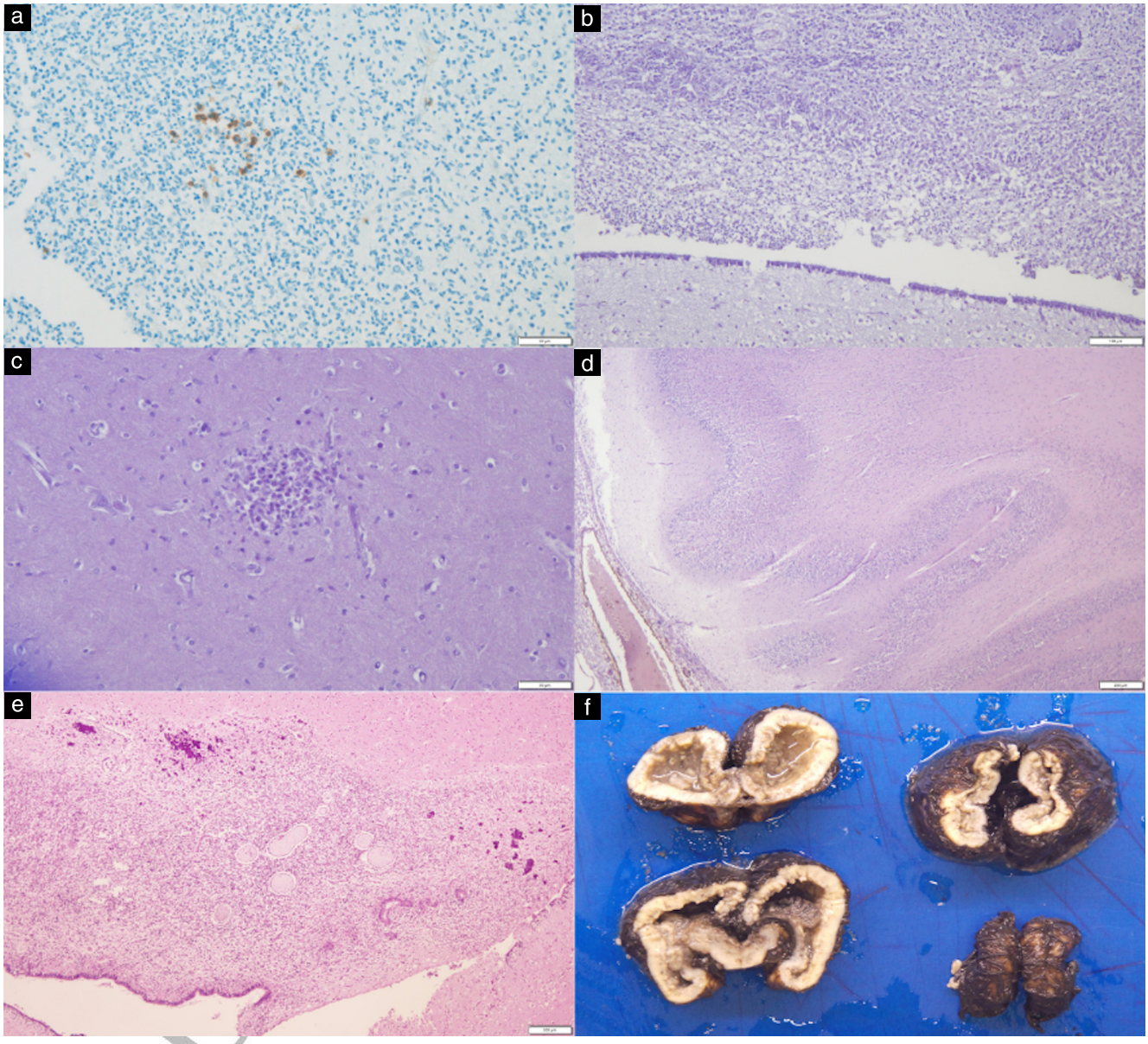

Figure 2 Histological (a–e) and macroscopic (f) images of brain sections from fetuses with cytomegalovirus infection. (a) Anti-CD-8 immunohistochemistry with hematoxylin counterstain in fetus with Grade 1 ventriculitis, showing clusters of cytotoxic T-lymphocytes in the germinal matrix identified by the brown staining in the cytoplasm (original magnification ×20). (b) Hematoxylin and eosin (H&E) staining in fetus with severe ventriculitis, showing discontinuity of the ependymal layer with cell overgrowth through the gaps in the ependymal (top half of the picture); continuous ependymal layer lines the cerebral tissue in the lower part of the picture (original magnification ×10). (c) H&E staining in fetus with microglial nodules, showing round-shaped cluster of microglial cells within the white matter (original magnification ×20). (d) H&E staining in fetus with polymicrogyria, showing cortical cell layers following a microgyria (festooned) pattern with an excessive number of abnormally small gyri; note the meningeal coverings in the lower left corner (original magnification ×4). (e) H&E staining showing Grade 2 ventriculitis (multiple ependymal pseudoducts, vascular dilatation and cellular scarcity) and presence of microcalcifications (irregularly shaped dark and deeply hematoxylin stained) in the border between periventricular germinal matrix and white matter (original magnification ×4). (f) Sections obtained from a fixed brain show the macroscopic appearance of severe bilateral ventriculomegaly.

at diagnosis of the halo sign was 24.0 weeks (IQR, 22.6–28.9 weeks). Of the 32 fetuses with the halo sign present, this was a non-isolated finding in 26 cases and was diagnosed at a median GA of 24.4 weeks (IQR, 22.8–30.1 weeks), while six fetuses had an isolated halo sign with a median GA at diagnosis of 22.6 weeks (IQR, 22–23.4 weeks) ($P = 0.10$). The three fetuses without a diagnosis of periventricular echogenic halo underwent NSG at 21, 26 and 35 weeks, respectively. In all three cases NSG was performed using the transvaginal approach. The median time from NSG to TOP or IUD in the whole cohort was 5 days (IQR, 2–7 days). The median GA at postmortem examination was 24.4 weeks (IQR, 22.8–30.4 weeks).

Ultrasonographic findings

The main NSG findings associated with CMV infection in fetuses with non-isolated and those with absent halo sign are shown in Table 1. A non-isolated halo sign was associated with severe CNS abnormalities (cerebellar and/or vermian hypoplasia (73%), corpus callosum agenesis/dysgenesis (58%), abnormal sulcus gyration (58%), microencephaly (42%), microcephaly (35%)) in all cases except one fetus that had only mild ventriculomegaly. All three fetuses without the halo sign also showed severe brain abnormalities (cerebellar and/or vermian hypoplasia (100%), microcephaly (100%), corpus callosum agenesis/dysgenesis (67%), abnormal sulcus gyration (67%), microencephaly (33%)). When comparing the two groups, microcephaly was significantly more frequent among fetuses without a periventricular echogenic halo ($P = 0.029$).

When evaluating the severity of associated US findings in relation to trimester at diagnosis in fetuses with a non-isolated halo, we observed that severity was not related to trimester at diagnosis, with the exception of microencephaly, which was more frequent in the second compared with the third trimester (56% *vs* 13%; $P = 0.04$) (Table S1). There were no significant differences in the presence of concomitant extra-CNS abnormalities between the three study groups (Table S2).

Table 1 Associated neurosonographic findings in 29 cytomegalovirus-infected fetuses, according to presence of non-isolated periventricular echogenic halo (halo sign)

	Halo sign		
Ultrasound features	*Non-isolated (n = 26)*	*Absent (n = 3)*	P†
Severe			
Severe VMG	4 (15.4)	0 (0)	0.464
Microcephaly	9 (34.6)	3 (100.0)	0.029
Microencephaly (enlarged SAS)	11 (42.3)	1 (33.3)	0.672
Porencephaly	3 (11.5)	0 (0)	0.534
Corpus callosum abnormality*	15 (57.7)	2 (66.7)	0.765
Cerebellar and/or vermian hypoplasia	19 (73.1)	3 (100.0)	0.557
Abnormal sulcus gyration	15 (57.7)	2 (66.7)	0.765
Mild			
Mild VMG	7 (26.9)	1 (33.3)	0.814
Intraventricular adhesions	3 (11.5)	1 (33.3)	0.300
Isolated calcifications	7 (26.9)	0 (0)	0.302
Lenticulostriate vasculopathy	14 (53.8)	2 (66.7)	0.672
Subependymal cysts	2 (7.7)	1 (33.3)	0.167
Anterior horns hyperechogenicity	2 (7.7)	0 (0)	0.948

Data are presented as *n* (%). *Dysgenesis or agenesis (< 5th percentile). †*P* determined using χ^2 or Fisher's exact test. Fetuses could have a severe central nervous system (CNS) finding together with mild CNS abnormalities. SAS, subarachnoid space; VMG, ventriculomegaly.

MRI findings

Fetal MRI was performed in 13 (37%) cases at a median GA of 29.3 weeks (IQR, 27.7–31.0 weeks). These comprised 12 fetuses with the halo sign (11 non-isolated and one isolated based on US) and one fetus without a periventricular echogenic halo. The main MRI abnormalities in fetuses with the halo sign were abnormal cortical development in 75% of cases (comprising mostly polymicrogyria (89%)), microcephaly (67%), microencephaly (58%), and cerebellar hypoplasia (50%) (Table S3). Fetal MRI confirmed the severity of US brain abnormalities in 11 fetuses (10 with a non-isolated halo and one without a halo). Moreover, in one fetus with the halo sign and mild unilateral ventriculomegaly diagnosed on US at 29 weeks, MRI at 30 weeks detected diffuse abnormal cortical development compatible with polymicrogyria. In the remaining fetus, which had an isolated halo sign, MRI at 26 weeks did not show additional brain abnormalities.

Histopathological findings

Histological lesions were diagnosed in all 35 cases, with variable extent and morphology. The most prevalent histopathological lesions were ventriculitis in 31 (89%) cases and microglial nodules in 29 (83%), followed by CMV inclusions in 24 (69%), polymicrogyria in 21 (60%), cortical foci of necrosis in 16 (46%), white-matter foci of necrosis in 14 (40%), perivascular inflammatory infiltrates in six (17%), and calcifications (periventricular in three (9%), white matter in three (9%) and cortical in one (3%)). Macroscopic lesions were observed in 19 (54%) cases.

All fetuses with an isolated halo sign showed evidence of ventriculitis, which was mild (Grade 1) in most (4/6 (67%)) cases (Table 2). The majority of fetuses with a non-isolated halo sign (23/26 (88%)) or without the halo sign (2/3 (67%)) presented ventriculitis, which was of higher grade (Grade 2 or 3) compared with the isolated-halo-sign group ($P = 0.032$). Diffuse microglial nodules and CMV inclusions were identified in 80 •% and 50% of fetuses with an isolated halo sign, respectively. These fetuses did not show other brain parenchymal lesions, calcifications or perivascular infiltrates.

In fetuses with a non-isolated halo sign, microglial nodules were observed in 81% cases and were focal

in half of them, while CMV-inclusion cells were seen in 73% cases. White-matter necrosis, cortical necrosis and polymicrogyria were found in •50%, 54% and 73% cases, respectively. When evaluating histological findings according to trimester at diagnosis of CMV infection, white-matter necrosis was more frequent, albeit non-significantly, in fetuses diagnosed in the second *vs* the third • trimester (10/15 (67%) *vs* 3/11 (27%); $P = 0.06$) (Table S4). Likewise, there were no significant differences in the histopathological severity stage of brain lesions between fetuses that had TOP or IUD in the second *vs* third trimester (Table S5).

All fetuses without the halo sign presented focal microglial nodules, and CMV inclusions were present in two-thirds. White-matter necrosis was found in one-third, whereas cortical necrosis and polymicrogyria were each detected in two-thirds of cases with absent halo sign.

In the whole cohort, seven cases presented ventriculitis and/or microglial nodules as the only histopathological finding and were categorized as histopathological Stage I (Table 3). Of these, six had an isolated halo sign and the remaining fetus showed the halo sign in addition to other brain abnormalities on US/MRI (intraventricular adhesions, temporal lobe cysts and white-matter hyperintensity in the frontal, temporal and occipital lobes), together with microcephaly and microencephaly on both US and MRI studies, which were not confirmed by postmortem examination (Figure S1). In the whole series, histopathological Stage-I brain lesions were associated with the presence of an isolated halo sign ($P < 0.001$). All the remaining fetuses (25 with non-isolated halo sign and all three without the halo sign) showed additional microscopic or macroscopic lesions and were classified as histopathological Stage II.

DISCUSSION

This is the largest study to date reporting on detailed histological examination of fetal brains obtained from cases of CMV infection identified during second- or third-trimester ultrasound scans. Although previous research considered the presence of a periventricular echogenic halo to be a sign of severe CNS injury, this

Table 3 Histopathological stage of brain damage in 35 cytomegalovirus-infected fetuses, according to presence of non-isolated or isolated periventricular echogenic halo (halo sign) or absence of halo sign on ultrasound

	Halo sign		
Stage	*Isolated* (n = 6)	*Non-isolated* (n = 26)	*Absent* (n = 3)
Stage I*	6 (100.0)	1 (3.8)	0 (0)
Stage II	0 (0)	25 (96.2)	3 (100.0)

Data presented as *n* (%). *$P < 0.001$ for •Stage-I brain damage in isolated *vs* non-isolated and absent halo sign groups.

Table 2 Main brain histological findings in 35 cytomegalovirus (CMV)-infected fetuses after termination of pregnancy or intrauterine demise, according to presence of non-isolated or isolated periventricular echogenic halo (halo sign) or absence of halo sign on ultrasound

	Halo sign			
Characteristic	*Isolated* (n = 6)	*Non-isolated* (n = 26)	*Absent* (n = 3)	P*
Ventriculitis grade				0.032
0	0 (0)	3 (11.5)	1 (33.3)	
1	4 (66.7)	2 (7.7)	0 (0)	
2	1 (16.7)	7 (26.9)	1 (33.3)	
3	1 (16.7)	14 (53.8)	1 (33.3)	
Microglial nodules	5 (83.3)	21 (80.8)	3 (100.0)	0.288
Focal	1/5 (20.0)	10/21 (47.6)	3/3 (100.0)	
Diffuse	4/5 (80.0)	11/21 (52.4)	0 (0)	
CMV inclusions	3 (50.0)	19 (73.1)	2 (66.7)	0.436
Focal	2/3 (66.7)	5/19 (26.3)	0 (0)	
Diffuse	1/3 (33.3)	14/19 (73.7)	2/2 (100.0)	
Periventricular calcifications	0 (0)	3 (11.5)	0 (0)	0.987
Perivascular infiltration	0 (0)	6 (23.1)	0 (0)	0.797
White-matter calcifications	0 (0)	3 (11.5)	0 (0)	0.967
Cortical calcifications	0 (0)	1 (3.8)	0 (0)	0.859
White-matter necrosis	0 (0)	13 (50.0)	1 (33.3)	0.065
Focal	0 (0)	6/13 (46.2)	1/1 (100.0)	
Diffuse	0 (0)	7/13 (53.8)	0 (0)	
Cortical necrosis	0 (0)	14 (53.8)	2 (66.7)	0.043
Focal	0 (0)	5/14 (35.7)	1/2 (50.0)	
Diffuse	0 (0)	9/14 (64.3)	1/2 (50.0)	
Polymicrogyria	0 (0)	19 (73.1)	2 (66.7)	0.017
Focal	0 (0)	3/19 (15.8)	0 (0)	
Diffuse	0 (0)	16/19 (84.2)	2/2 (100.0)	
Macroscopic lesions	0 (0)	16 (61.5)	3 (100.0)	0.004

Data are presented as *n* (%) or *n*/*N* (%). *P for comparison between the three groups was determined using χ^2 or Fisher's exact test.

was not confirmed by our study. In the present series, the halo sign as an isolated NSG finding was mostly associated with mild ventriculitis, microglial nodules and CMV-infected cells and, contrary to previous reports, we did not observe white-matter necrosis in these cases.

Cerebral damage was assessed extensively in each brain region, and histopathological severity staging was established. While all fetuses with an isolated halo sign showed a mild histopathological severity stage of brain damage, microglial nodules suggesting immune-mediated damage were more often diffuse in these cases compared to in fetuses without the halo sign or in those with additional severe imaging abnormalities. Although severe parenchymal lesions were not observed, diffuse microglial nodules in the brain of CMV-infected fetuses have been correlated with direct injury[9,10]. Gabrielli *et al.*[10] described a classification system of CNS lesions including an inflammation score in midterm CMV-infected fetuses with or without sonographic findings. They observed that the inflammatory response was associated with the severity of brain damage; however, we did not confirm this finding, possibly owing to methodological differences.

In our study •, fetal CMV infection was diagnosed mainly by the presence of CNS abnormalities during routine second- or third-trimester transabdominal US scans. Since the halo sign is better identified by the transvaginal approach, it was mostly diagnosed in association with other severe brain abnormalities, while cases of milder fetal infection with an isolated halo sign were probably missed. Probably because of this, the histopathological staging of brain damage was severe in almost all cases with a non-isolated halo sign, with findings including polymicrogyria in nearly three-quarters of cases and white-matter and cortical necrosis each seen in half of the cases. This finding is in agreement with the those of Simonazzi *et al.*[2], in which the halo sign, as a mid-gestation finding, was associated with other severe CNS abnormalities and white-matter injury.

The halo sign was absent in less than 10% of cases in our cohort, confirming that this sign is quite prevalent in fetuses with CMV infection, mainly at midterm, as described previously[2]. Among fetuses without a periventricular echogenic halo, histopathological findings did not show a resolution of the inflammatory process, and two out of three of these fetuses • were in the second trimester, therefore it is unlikely that the sign had already disappeared. Indeed, 90% of fetuses in the third trimester still showed the halo sign and displayed analogous severe brain imaging abnormalities to those without the halo sign. Moreover, the absence of the halo sign was not associated with less severe CNS histological findings, since all the fetuses without a periventricular echogenic halo had severe macroscopic lesions. Possible explanations for non-visualization of the halo sign in our third-trimester fetus • are: (1) development into periventricular cysts; (2) an inflammatory reaction during the second trimester impairing neuronal migration and resulting in malformation of cortical development; (3) increased complexity of the white matter[7,15]. However, our sample did not allow us to demonstrate a distinctive histological pattern between fetuses without a periventricular echogenic halo and those with a non-isolated halo sign.

Since all cases with an isolated halo sign were diagnosed in the second trimester, it could be hypothesized that it is an early sign of immune-mediated damage that would have evolved to more severe brain damage and histological lesions if the fetuses had survived to the third trimester. Nevertheless, this hypothesis is uncertain because in almost all fetuses with a halo sign in the second trimester, severe brain abnormalities were present on NSG and severe histological brain damage had already occurred. Moreover, in fetuses with a non-isolated halo, microencephaly detected by NSG was significantly more frequent in the second than in the third trimester. Our study did not provide data to support either progression of the halo sign to severe brain damage or remission.

In CMV-infected fetuses, MRI performed early in the third trimester has been shown to increase the rate of detection of CNS abnormalities, especially those involving the temporal lobes and cortical development. Fetal MRI as a complementary tool to US improves the prediction of symptoms at birth and long-term sequelae[16–19], although this has not been confirmed in all studies[20]. In our series, MRI in the early third trimester helped confirm severe brain abnormalities in fetuses showing the halo sign together with other US abnormalities, although in one case microencephaly was diagnosed by both US and MRI but was not confirmed at autopsy. We hypothesized that it could be a false-positive result or the result of different diagnostic approaches between imaging and postmortem examination.

The main strength of this study is that it reports on the largest cohort of CMV-infected fetuses with the halo sign detected by US during the second or third trimester, in which targeted histopathological examination of the brain was performed. The large number of cases is unlikely to be reproducible in subsequent studies. Moreover, the neuroimaging and pathology specialists were blinded to each other's findings.

Among the limitations of this study, we first acknowledge the retrospective nature of the analysis. Second, the small sample size of the groups with an isolated halo sign and absent halo sign, and the limited number of available MRI examinations in these cases •, prevent significant conclusions being drawn •. Third, the halo sign is a subtle sonographic marker that may be observer dependent. However, all US images in this study were evaluated by the same observer. A standardized quantitative analysis would have helped to correlate the intensity of the halo sign with the degree of inflammation. Further studies are warranted to establish the correlation between the degree of inflammation in the periventricular area and the prognosis. Additionally, we only included fetuses with TOP or IUD, which might have biased our findings to the more severe end of the spectrum of the disease. However, this could have been a conservative bias, since the correlation between the halo sign and ventriculitis in less affected fetuses is likely to be even weaker than in severely affected

ones. Finally, under the hypothesis of the periventricular echogenic halo being a transient sonographic sign, and since prenatal CMV screening is not recommended, we do not know how many infected fetuses may have had a transient halo during the study period.

Follow-up of fetuses and newborns with an isolated halo sign would be key to understanding the real significance of this sign, as has been demonstrated in children born with other sonographic CMV findings, such as lenticulostriate vasculopathy or periventricular pseudocysts[21,22]. However, this would only be feasible under a universal first-trimester screening program, justified after the demonstration of over a 60% reduction of vertical transmission in mothers with primary infection treated with valacyclovir[23,24]. The follow-up of infected fetuses should include NSG, preferably carried out by the transvaginal approach, which would enable the detection of the halo sign. Since some centers rarely perform the transvaginal approach, we encourage the addition of both the parasagittal and coronal planes to the standard axial plane when using the transabdominal approach to confirm the halo sign.

In conclusion, our data show that CMV-infected fetuses with an isolated halo sign tend to present mild histopathological brain lesions, though its prognostic value within a clinical context is yet to be determined. When considering continuation of a pregnancy with fetal CMV infection, detailed NSG follow-up and MRI early in the third trimester are required to counsel parents.

ACKNOWLEDGMENTS

AQ16 We are indebted to the Pathology service • of the Hospital Clínic, Barcelona. This project was funded with the support of the Erasmus + Programme of the European Union (Framework Agreement number: 2013-0040). This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained herein. Additionally, the research leading to these results has received funding from the '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.L.M.-E. and A.H.-V. received financial support from the Secretaría Nacional de Ciencia y Tecnología de Panamá (SENACYT) grant No. 270-2017-294, from the Hospital Santo Tomas de Panamá and Instituto Nacional para la formación y aprovechamiento de Recursos Humanos de Panamá (IFARHU), respectively. E.E. received funding from the Departament de Salut under grant SLT008/18/00156.

REFERENCES

1. Guerra B, Simonazzi G, Puccetti C, Lanari M, Farina A, Lazzarotto T, Rizzo N. Ultrasound prediction of symptomatic congenital cytomegalovirus infection. *Am J Obstet Gynecol* 2008; **198**: 380.e1–7.
2. Simonazzi G, Guerra B, Bonasoni P, Pilu G, Lazzarotto T, Santini D, Rizzo N. Fetal cerebral periventricular halo at midgestation: an ultrasound finding suggestive of fetal cytomegalovirus infection. *Am J Obstet Gynecol* 2010; **202**: 599.e1–5.
3. Leruez-Ville M, Ville Y. Fetal cytomegalovirus infection. *Best Pract Res Clin Obstet Gynaecol* 2017; **38**: 97–107.
4. Leruez-Ville M, Stirnemann J, Sellier Y, Guilleminot T, Dejean A, Magny J-F, Couderc S, Jacquemard F, Ville Y. Feasibility of predicting the outcome of fetal infection with cytomegalovirus at the time of prenatal diagnosis. *Am J Obstet Gynecol* 2016; **215**: 342.e1–9.
5. Esteban H, Blondiaux E, Audureau E, Sileo C, Moutard ML, Gelot A, Jouannic JM, Ducou Le Pointe H, Garel C. Prenatal features of isolated subependymal pseudocysts associated with adverse pregnancy outcome. *Ultrasound Obstet Gynecol* 2015; **46**: 678–687.
6. Malinger G, Lev D, Zahalka N, ben Aroia Z, Watemberg N, Kidron D, ben Sira L, Lerman-Sagie T. Fetal cytomegalovirus infection of the brain: the spectrum of sonographic findings. *AJNR Am J Neuroradiol* 2003; **24**: 28–32.
7. Malinger G, Lev D, Lerman-Sagie T. Imaging of fetal cytomegalovirus infection. *Fetal Diagn Ther* 2011; **29**: 117–126.
8. Guibaud L, Atiia-Sobol J, Buenerd A, Foray P, Jacquet C, Champion F, Arnould P, Pracros JP, Golfier F. Focal sonographic periventricular pattern associated with mild ventriculomegaly in foetal cytomegalic infection revealing cytomegalic encephalitis in the third trimester of pregnancy. *Prenat Diagn* 2004; **24**: 727–732.
9. Gabrielli L, Bonasoni MP, Lazzarotto T, Lega S, Santini D, Foschini MP, Guerra B, Baccolini F, Piccirilli G, Chiereghin A, Petrisli E, Gardini G, Lanari M, Landini MP. Histological findings in foetuses congenitally infected by cytomegalovirus. *J Clin Virol* 2009; **46**: S16–21.
10. Gabrielli L, Bonasoni MP, Santini D, Piccirilli G, Chiereghin A, Petrisli E, Dolcetti R, Guerra B, Piccioli M, Lanari M, Landini MP, Lazzarotto T. Congenital cytomegalovirus infection: patterns of fetal brain damage. *Clin Microbiol Infect* 2012; **18**: E419–427.
11. Hawkins-Villarreal A, Moreno-Espinosa AL, Eixarch E, Marcos MA, Martinez-Portilla RJ, Salazar L, Garcia-Otero L, Lopez M, Borrell A, Figueras F, Goncé A. Blood parameters in fetuses infected with cytomegalovirus according to the severity of brain damage and trimester of pregnancy at cordocentesis. *J Clin Virol* 2019; **119**: 37–43.
12. Guibaud L, Lacalm A. Diagnostic imaging tools to elucidate decreased cephalic biometry and fetal microcephaly: a systematic analysis of the central nervous system. *Ultrasound Obstet Gynecol* 2016; **48**: 16–25.
13. Kyriakopoulou V, Vatansever D, Davidson A, Patkee P, Elkommos S, Chew A, Martinez-Biarge M, Hagberg B, Damodaram M, Allsop J, Fox M, Hajnal JV, Rutherford MA. Normative biometry of the fetal brain using magnetic resonance imaging. *Brain Struct Funct* 2017; **222**: 2295–2307.
14. Bartosch C, Vilar I, Rodrigues M, Costa L, Botelho N, Brandão O. Fetal autopsy parameters standards: biometry, organ weights, and long bone lengths. *Virchows Arch* 2019; **475**: 499–511.
15. Cheeran MC-J, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 2009; **22**: 99–126.
16. Lipitz S, Elkan Miller T, Yinon Y, Weissbach T, De-Castro H, Hoffman C, Katorza E, Weisz B. Revisiting short- and long-term outcome after fetal first-trimester primary cytomegalovirus infection in relation to prenatal imaging findings. *Ultrasound Obstet Gynecol* 2020; **56**: 572–578.
17. Lipitz S, Hoffmann C, Feldman B, Tepperberg-Dikawa M, Schiff E, Weisz B. Value of prenatal ultrasound and magnetic resonance imaging in assessment of congenital primary cytomegalovirus infection. *Ultrasound Obstet Gynecol* 2010; **36**: 709–717.
18. Lipitz S, Yinon Y, Malinger G, Yagel S, Levit L, Hoffman C, Rantzer R, Weisz B. Risk of cytomegalovirus-associated sequelae in relation to time of infection and findings on prenatal imaging. *Ultrasound Obstet Gynecol* 2013; **41**: 508–514.
19. Faure-Bardon V, Millischer AE, Deloison B, Sonigo P, Grévent D, Salomon L, Stirnemann J, Nicloux M, Magny JF, Leruez-Ville M, Ville Y. Refining the prognosis of fetuses infected with cytomegalovirus in the first trimester of pregnancy by serial prenatal assessment: a single-centre retrospective study. *BJOG* 2020; **127**: 355–362.
20. Birnbaum R, Winsteen A, Brusilov M, Wolman I, Krajden Haratz K, Ben-Sira L, Malinger G. Subtle findings on fetal brain imaging in CMV infected pregnancies: what is the clinical significance? A retrospective analysis with outcome correlation. *Prenat Diagn* 2020; **40**: 447–453.
21. Giannattasio A, di Costanzo P, Milite P, de Martino D, Capone E, Romano A, Bravaccio C, Capasso L, Raimondi F. Is lenticulostriated vasculopathy an unfavorable prognostic finding in infants with congenital cytomegalovirus infection? *J Clin Virol* 2017; **91**: 31–35.
22. Amir J, Schwarz M, Levy I, Haimi-Cohen Y, Pardo J. Is lenticulostriated vasculopathy a sign of central nervous system insult in infants with congenital CMV infection? *Arch Dis Child* 2011; **96**: 846–850.
23. Shahar-Nissan K, Pardo J, Peled O, Krause I, Bilavsky E, Wiznitzer A, Hadar E, Amir J. Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial. *Lancet* 2020; **396**: 779–785.
24. Faure-Bardon V, Fourgeaud J, Stirnemann J, Leruez-Ville M, Ville Y. Secondary prevention of congenital cytomegalovirus infection with valacyclovir following maternal primary infection in early pregnancy. *Ultrasound Obstet Gynecol* 2021; **58**: 576–581.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Ultrasound (US; a–c) and magnetic resonance (MRI; d–f) images in axial (a,d), coronal (b,e) and parasagittal (c,f) planes, obtained at 28 weeks of gestation in fetus with non-isolated halo sign. Enlarged subarachnoid space on US (b) and skull biparietal diameter (BPD) and brain BPD < 1st centile for gestational age on MRI indicated microencephaly; however, this was not confirmed at postmortem examination. Note intraventricular adhesions (arrows) and bilateral temporal lobe cyst (arrowheads). On MRI, white-matter hyperintensity in frontal (d,f), temporal (d,e,f) and occipital (d) lobes is also seen.

Table S1 Frequency of severe neurosonographic findings in 26 cytomegalovirus-infected fetuses with a non-isolated halo sign, according to trimester of pregnancy

Table S2 Extra-central nervous system findings in 35 cytomegalovirus-infected fetuses

Table S3 Main magnetic resonance imaging findings in cytomegalovirus-infected fetuses with the halo sign

Table S4 Main histological brain findings in 26 cytomegalovirus-infected fetuses with a non-isolated halo sign, according to diagnosis of infection in the second *vs* third trimester

Table S5 Histopathological stage of brain lesions in 26 cytomegalovirus-infected fetuses with non-isolated halo sign, according to trimester at termination of pregnancy or fetal demise

WILEY

Author Query Form

Journal: UOG

Article: 26173

Dear Author,

During the copyediting of your manuscript the following queries arose.

Please refer to the query reference callout numbers in the page proofs and respond.

Please provide your responses to the queries in an email message or Word file. PLEASE DO NOT ANNOTATE THE PDF FILE.

Many thanks for your assistance.

AUTHOR: Please note that missing content in references have been updated where we have been able to match the missing elements without ambiguity against a standard citation database, to meet the reference style requirements of the journal. It is your responsibility to check and ensure that all listed references are complete and accurate.

Query References	Query	Remarks
AQ1	Please check if link to ORCID is correct.	
AQ2	Would it be possible to provide an address for this institution?	
AQ3	Please check that all affiliations are correct and complete	
AQ4	'third' added here; OK?	
AQ5	Could you provide the name of the company that makes LX scanners?	
AQ6	Based on your response to my previous query, I added 'was re-evaluated and was confirmed as...' here and moved this sentence to this paragraph that reports on the reassessment of the NSGs for the purposes of this study. Do you agree? (RK)	
AQ7	According to Table 2 diffuse microglial nodules were seen in 80% of those with microglial nodules (n = 5), but considered as the whole group (isolated halo sign) it's 4/6, so should 80% here be 67%?	
AQ8	According to Table 2, of 26 fetuses with a non-isolated halo there were 13 with white-matter necrosis, so 48% here changed to 50% (as T-2); OK?	
AQ9	'third' added here; OK?	
AQ10	'for Stage-I brain damage in isolated vs non-isolated and absent halo sign groups' was added to clarify the P-value given. Is this correct? (RK)	
AQ11	I amended this and the next sentence based on your response to my editing query. Please check if your meaning has been retained.(original text: 'Since the halo sign is better identified by the transvaginal approach and fetal CMV infection was diagnosed mainly by the presence of CNS abnormalities during routine second- or third-trimester US scans, in our study, the halo sign was associated with more severe US findings in over three-quarters of the cases') (RK)	
AQ12	'of these' added here before fetuses; is that the correct sense?	

AQ13	Is singular ‘fetus’ correct here?	
AQ14	‘in these cases’ was added here. Correct? (RK)	
AQ15	Based on your response to my previous query, would it be correct to add here:‘...prevent significant conclusions with regards to the added value of MRI from being drawn’? (RK)	
AQ16	‘of the Hospital Clínic, Barcelona’ added here; is that correct?	

Funding Info Query Form

Please confirm that the funding sponsor list below was correctly extracted from your article: that it includes all funders and that the text has been matched to the correct FundRef Registry organization names. If no FundRef Registry organization name has been identified, it may be that the funder was not found in the FundRef registry, or there are multiple funders matched in the FundRef registry. If a name was not found in the FundRef registry, it may not be the canonical name form, it may be a program name rather than an organization name, or it may be an organization not yet included in FundRef Registry. If you know of another name form or a parent organization name for a ‘‘not found’’ item on this list below, please share that information.

Funding Agency	**FundRef Organization Name**
Cerebra Foundation for the Brain Injured Child (Carmarthen, Wales, UK) and AGAUR 2017 SGR	‘‘Not Found’’
Erasmus + Programme of the European Union	‘‘Not Found’’
La Caixa Foundation	‘‘Not Found’’
Secretaría Nacional de Ciencia y Tecnología de Panamá (SENACYT)	‘‘Not Found’’

JOHN WILEY & SONS, LTD., THE ATRIUM, SOUTHERN GATE, CHICHESTER P019 8SQ, UK

*****PROOF OF YOUR ARTICLE ATTACHED, PLEASE READ CAREFULLY*****

After receipt of your corrections your article will be published initially within the online version of the journal.

PLEASE AIM TO RETURN YOUR CORRECTIONS WITHIN 48 HOURS OF RECEIPT OF YOUR PROOF, THIS WILL ENSURE THAT THERE ARE NO UNNECESSARY DELAYS IN THE PUBLICATION OF YOUR ARTICLE

❑ **READ PROOFS CAREFULLY**

ONCE PUBLISHED ONLINE OR IN PRINT IT IS NOT POSSIBLE TO MAKE ANY FURTHER CORRECTIONS TO YOUR ARTICLE

- This will be your only chance to correct your proof
- Please note that the volume and page numbers shown on the proofs are for position only

❑ **ANSWER ALL QUERIES ON PROOFS** (Queries are attached as the last page of your proof.)

- List all corrections and send back via e-mail to the production contact as detailed in the covering e-mail, or mark all corrections directly on the proofs and send the scanned copy via e-mail. Please do not send corrections by fax or post

❑ **CHECK FIGURES AND TABLES CAREFULLY**

- Check size, numbering, and orientation of figures
- All images in the PDF are downsampled (reduced to lower resolution and file size) to facilitate Internet delivery. These images will appear at higher resolution and sharpness in the printed article
- Review figure legends to ensure that they are complete
- Check all tables. Review layout, title, and footnotes

❑ **COPYRIGHT**

- Your article cannot be published unless we have received your completed license agreement. If you have not already done so, please login into Author Services (http://authorservices.wiley.com) and click on 'My Publications' to use the Wiley Author Licensing Service (WALS). If you have any queries, please contact the Production Editor.

❑ **OFFPRINTS**

- Free access to the final PDF offprint or your article will be available via Author Services only. Please therefore sign up for Author Services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers.

Additional reprint and journal issue purchases

- Should you wish to purchase additional copies of your article, please click on the link and follow the instructions provided: http://www.sheridan.com/wiley/eoc
- Corresponding authors are invited to inform their co-authors of the reprint options available.
- Please note that regardless of the form in which they are acquired, reprints should not be resold, nor further disseminated in electronic form, nor deployed in part or in whole in any marketing, promotional or educational contexts without authorization from Wiley. Permissions requests should be directed to mailto: permissionsuk@wiley.com
- For information about 'Pay-Per-View and Article Select' click on the following link: http://www3.interscience.wiley.com/aboutus/ppv-articleselect.html

WILEY AUTHOR DISCOUNT CLUB

We would like to show our appreciation to you, a highly valued contributor to Wiley's publications, by offering a **unique 25% discount** off the published price of any of our books*.

All you need to do is apply for the **Wiley Author Discount Card** by completing the attached form and returning it to us at the following address:

The Database Group (Author Club)
John Wiley & Sons Ltd
The Atrium
Southern Gate
Chichester
PO19 8SQ
UK

Alternatively, you can **register online** at www.wileyeurope.com/go/authordiscount
Please pass on details of this offer to any co-authors or fellow contributors.

After registering you will receive your Wiley Author Discount Card with a special promotion code, which you will need to quote whenever you order books direct from us.

The quickest way to order your books from us is via our European website at:

http://www.wileyeurope.com

Key benefits to using the site and ordering online include:
- Real-time SECURE on-line ordering
- Easy catalogue browsing
- Dedicated Author resource centre
- Opportunity to sign up for subject-orientated e-mail alerts

Alternatively, you can order direct through Customer Services at:
cs-books@wiley.co.uk, or call +44 (0)1243 843294, fax +44 (0)1243 843303

So take advantage of this great offer and return your completed form today.

Yours sincerely,

Verity Leaver
Group Marketing Manager
author@wiley.co.uk

*TERMS AND CONDITIONS

This offer is exclusive to Wiley Authors, Editors, Contributors and Editorial Board Members in acquiring books for their personal use. There must be no resale through any channel. The offer is subject to stock availability and cannot be applied retrospectively. This entitlement cannot be used in conjunction with any other special offer. Wiley reserves the right to amend the terms of the offer at any time.

WILEY

REGISTRATION FORM
For Wiley Author Club Discount Card

To enjoy your 25% discount, tell us your areas of interest and you will receive relevant catalogues or leaflets from which to select your books. Please indicate your specific subject areas below.

Accounting [ ]
- Public [ ]
- Corporate [ ]

Chemistry [ ]
- Analytical [ ]
- Industrial/Safety [ ]
- Organic [ ]
- Inorganic [ ]
- Polymer [ ]
- Spectroscopy [ ]

Encyclopedia/Reference [ ]
- Business/Finance [ ]
- Life Sciences [ ]
- Medical Sciences [ ]
- Physical Sciences [ ]
- Technology [ ]

Earth & Environmental Science [ ]

Hospitality [ ]

Genetics [ ]
- Bioinformatics/ Computational Biology [ ]
- Proteomics [ ]
- Genomics [ ]
- Gene Mapping [ ]
- Clinical Genetics [ ]

Medical Science [ ]
- Cardiovascular [ ]
- Diabetes [ ]
- Endocrinology [ ]
- Imaging [ ]
- Obstetrics/Gynaecology [ ]
- Oncology [ ]
- Pharmacology [ ]
- Psychiatry [ ]

Non-Profit [ ]

Architecture [ ]

Business/Management [ ]

Computer Science [ ]
- Database/Data Warehouse [ ]
- Internet Business [ ]
- Networking [ ]
- Programming/Software Development [ ]
- Object Technology [ ]

Engineering [ ]
- Civil [ ]
- Communications Technology [ ]
- Electronic [ ]
- Environmental [ ]
- Industrial [ ]
- Mechanical [ ]

Finance/Investing [ ]
- Economics [ ]
- Institutional [ ]
- Personal Finance [ ]

Life Science [ ]

Landscape Architecture [ ]

Mathematics [ ]
Statistics [ ]

Manufacturing [ ]

Materials Science [ ]

Psychology [ ]
- Clinical [ ]
- Forensic [ ]
- Social & Personality [ ]
- Health & Sport [ ]
- Cognitive [ ]
- Organizational [ ]
- Developmental & Special Ed [ ]
- Child Welfare [ ]
- Self-Help [ ]

Physics/Physical Science [ ]

Please complete the next page /

I confirm that I am (*delete where not applicable):

a **Wiley** Book Author/Editor/Contributor* of the following book(s):
ISBN:
ISBN:

a **Wiley** Journal Editor/Contributor/Editorial Board Member* of the following journal(s):

SIGNATURE: …… Date: ……

PLEASE COMPLETE THE FOLLOWING DETAILS IN BLOCK CAPITALS:

TITLE: (e.g. Mr, Mrs, Dr) …… FULL NAME: ……

JOB TITLE (or Occupation): ……

DEPARTMENT: ……

COMPANY/INSTITUTION: ……

ADDRESS: ……

……

TOWN/CITY: ……

COUNTY/STATE: ……

COUNTRY: ……

POSTCODE/ZIP CODE: ……

DAYTIME TEL: ……

FAX: ……

E-MAIL: ……

YOUR PERSONAL DATA
We, John Wiley & Sons Ltd, will use the information you have provided to fulfil your request. In addition, we would like to:

1. Use your information to keep you informed by post of titles and offers of interest to you and available from us or other Wiley Group companies worldwide, and may supply your details to members of the Wiley Group for this purpose.
   [ ] Please tick the box if you do **NOT** wish to receive this information

2. Share your information with other carefully selected companies so that they may contact you by post with details of titles and offers that may be of interest to you.
   [ ] Please tick the box if you do **NOT** wish to receive this information.

E-MAIL ALERTING SERVICE
We also offer an alerting service to our author base via e-mail, with regular special offers and competitions. If you **DO** wish to receive these, please opt in by ticking the box [ ].

If, at any time, you wish to stop receiving information, please contact the Database Group (databasegroup@wiley.co.uk) at John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, PO19 8SQ, UK.

TERMS & CONDITIONS
This offer is exclusive to Wiley Authors, Editors, Contributors and Editorial Board Members in acquiring books for their personal use. There should be no resale through any channel. The offer is subject to stock availability and may not be applied retrospectively. This entitlement cannot be used in conjunction with any other special offer. Wiley reserves the right to vary the terms of the offer at any time.

PLEASE RETURN THIS FORM TO:
Database Group (Author Club), John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, PO19 8SQ, UK author@wiley.co.uk
Fax: +44 (0)1243 770154