Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther
DOI: 10.1159/000489881

Received: November 30, 2017
Accepted after revision: May 8, 2018
Published online: July 20, 2018

Fetal Brain Injury Associated with Parvovirus B19 Congenital Infection Requiring Intrauterine Transfusion

Emeline Maisonneuve[a, b] Catherine Garel[c] Stéphanie Friszer[a, b] Cécile Pénager[a] Bruno Carbonne[d] Françoise Pernot[a, b] Flore Rozenberg[e] Aurélie Schnuriger[f] Anne Cortey[a, b] Marie-Laure Moutard[g] Jean-Marie Jouannic[a, b]

[a]Department of Fetal Medicine, Hôpital Armand-Trousseau, Paris, France; [b]Centre National de Référence en Hémobiologie Périnatale (CNRHP), Hôpital Armand-Trousseau, Paris, France; [c]Department of Radiology, Hôpital Trousseau, Paris, France; [d]Department of Obstetrics and Gynecology, Princess Grace Hospital, Monaco, Monaco; [e]Department of Virology, Hôpital Cochin, Paris, France; [f]Department of Virology, Hôpital Armand-Trousseau, Paris, France; [g]Department of Neuropediatrics, Hôpital Armand-Trousseau, Paris, France

Keywords

Parvovirus B19 · Fetal anemia · Hydrops fetalis · Intrauterine transfusion · Cerebral lesions · Cerebellar hemorrhage · Brain

Abstract

Background: Infection with parvovirus B19 (B19V) during pregnancy may cause severe fetal anemia, hydrops, and fetal death. Furthermore, neurodevelopmental impairment among survivors may occur despite appropriate prenatal management, including intrauterine transfusion (IUT). ***Objectives:*** Our primary objective was to describe cerebral lesions on MRI in fetuses with severe anemia requiring IUT for B19V infection. Our secondary objective was to search for clinical and biological characteristics associated with the occurrence of such lesions. ***Study Design:*** We performed a retrospective review of data on fetuses infected with B19V and requiring at least one IUT between 2005 and 2016. Fetuses with abnormal cerebral MRI results in the 3rd trimester were compared to those with normal MRI results. ***Results:*** Of 34 transfused fetuses, 26 children were born at full term. Five intrauterine fetal deaths, 1 neonatal death, and 2 terminations of pregnancy occurred. Cerebral anomalies were observed in 7/27 fetuses on MRI, including cerebellar hemorrhage or a small cerebellum. Only viral load in fetal blood appeared to be associated with brain lesions (11.5 $\log_{10}$ copies/mL [10.5–12.5] in case of abnormal MRI results vs. 9.5 $\log_{10}$ copies/mL [7.8–10.0]; $p = 0.05$). ***Conclusions:*** Among the fetuses transfused for B19V infection, 26% presented with prenatal abnormal cerebral imaging results. In our study, viral load in fetal blood appeared to be the only factor associated with fetal brain lesions.

KARGER

E-Mail karger@karger.com
www.karger.com/fdt

Emeline Maisonneuve
Department of Fetal Medicine, Hôpital Armand-Trousseau
26 avenue du Dr Arnold Netter
FR–75012 Paris (France)
E-Mail emelinem@yahoo.com

Introduction

Human parvovirus B19 (B19V) is a small single-stranded DNA virus, which is responsible, mainly in children, for erythema infectiosum or fifth disease. During pregnancy, infection with B19V can be transmitted vertically from mother to fetus in up to 30–50% of cases and cause severe fetal complications, such as severe anemia secondary to fetal erythroid progenitor cell infection and apoptosis [1–5]. Severe anemia may cause high-output cardiac failure and nonimmune hydrops. B19V may also directly infect myocardial cells and lead to myocarditis that further worsens the cardiac failure. Severe anemia and/or myocarditis are high-risk factors for intrauterine fetal death (IUFD). Intrauterine transfusion (IUT) is commonly used for the treatment of severe fetal anemia, with an overall reported fetal survival rate of 75–90% [1, 6, 7]. Nevertheless, among the survivors of 3 cohorts of B19V-infected fetuses, 16% of the infants presented with neurodevelopmental impairment at a median age of 5 years (range: 13 months to 13 years) [7–10]. However, the pathophysiological mechanisms of brain injury induced by fetal B19V infection remain unclear in the literature [9, 11]. There are only few reports of isolated cases of prenatal neuroimaging findings related to congenital B19V infection [11–14].

We aimed to describe the imaging patterns of fetal brain damage on ultrasound and MRI in a consecutive series of fetuses infected with B19V. Our secondary objective was to search for clinical and biological characteristics associated with the occurrence of such lesions: the severity and earliness of anemia, viral replication, and hemodynamic changes generated by IUT.

Subjects and Methods

Patients

The perinatal data on patients with hydrops fetalis and/or severe fetal anemia following B19V infection and managed at our National Reference Center (CNRHP, Centre National de Référence d'Hémobiologie Périnatale) between January 2005 and December 2016 were retrospectively reviewed. The diagnostic criterion for congenital B19V infection was positive B19V polymerase chain reaction (PCR) results in fetal blood (FB) samples or amniotic fluid (AF). We included 34 infected fetuses alive at the time of diagnosis of severe anemia and who underwent at least one IUT.

Management of Fetal B19V Infection at Our Center

A serological diagnosis of primary B19V infection during pregnancy was performed because of either maternal symptoms (rash, fever, arthralgia, etc.) or fetal signs (IUFD, fetal hydrops, and/or anemia). According to the published guidelines [5], when maternal infection had occurred, weekly ultrasound investigation of the fetus up to 12 weeks after infection was performed to identify signs of moderate-to-severe fetal anemia defined as a peak systolic flow velocity of the middle cerebral artery >1.5 MoM [15] and/or fetal hydrops (meeting at least 2 criteria of the following: ascites, pericardial or pleural effusion >3 mm, and prefrontal subcutaneous edema >5 mm). In case of suspected severe fetal anemia, the treatment was emergency IUT. Severely anemic fetuses were transfused with adult donor packed red blood cells, crossmatched with the mother, with a hematocrit >70–80%. Following IUT, fetal follow-up included weekly monitoring of the peak systolic flow velocity of the middle cerebral artery during the first month, and ultrasound focused on the fetal brain every month. A second-line neuroimaging examination including ultrasound and MRI was systematically performed in cases of fetal anemia requiring IUT. After birth, the neonates underwent clinical assessment by a neuropediatrician, brain MRI at 2 months of age, and neurological follow-up in case of cerebral anomalies.

Data Collection

Of each patient, the following characteristics were recorded: gestational age at the time of diagnosis of severe fetal anemia; the number of IUTs and gestational age at the IUTs; fetal hemoglobin at FB sampling before and after transfusion; reticulocyte, leukocyte, and platelet counts; and the viral load in FB and/or in AF. In cases of IUFD occurring before IUT, only amniocentesis was performed. We considered the following criteria to define the severity of fetal anemia: gestational age at diagnosis (<24 weeks of gestation); the presence of hydrops fetalis; the hemoglobin level before the first IUT (<2 g/dL); a low reticulocyte count (<10% of the red blood cell count); and the need for a second or third IUT. The criteria associated with viral activity were a viral load in FB and/or AF and severe thrombocytopenia (platelet count $<50 \times 10^3/mm^3$) before the first IUT; the criterion reflecting hemodynamic changes was an increase in hemoglobin level at the first IUT.

For each fetus, we collected ultrasound and MRI findings in the 3rd trimester. The fetal brain was examined according to the guidelines of the International Society of Ultrasound in Obstetrics and Gynecology [16]. Ultrasonography was performed by an expert in fetal neuroimaging using a Toshiba (Aplio; Toshiba, Tokyo, Japan) ultrasound system with transabdominal probes (3.5–14 MHz) or transvaginal probes (6 MHz) when required in cephalic presentations. MRI scans were performed using a 1.5-T unit (Philips Medical System or General Electric Optima). The prenatal MRI protocol included at least T1-weighted fast spin echo sequences, T2-weighted ultrafast spin echo single-shot sequences, and gradient echo T2 sequences. The postnatal MRI protocol included at least T1-weighted, T2-weighted, and susceptibility-weighted imaging sequences. The ultrasound and MRI data on the fetuses with abnormal findings were reviewed by an expert in fetal neuroimaging (C.G.).

We collected data on the neurological examination at birth and on follow-up by a neuropediatrician for all children, as well as data on postnatal cerebral MRI (performed between 1 and 6 months of age) for those with cerebral anomalies diagnosed in the prenatal period. For the children with normal fetal MRI and normal clinical examination results at birth, the follow-up data were provided by the corresponding physicians. In IUFD cases, postmortem examination results were collected, unless the parents declined.

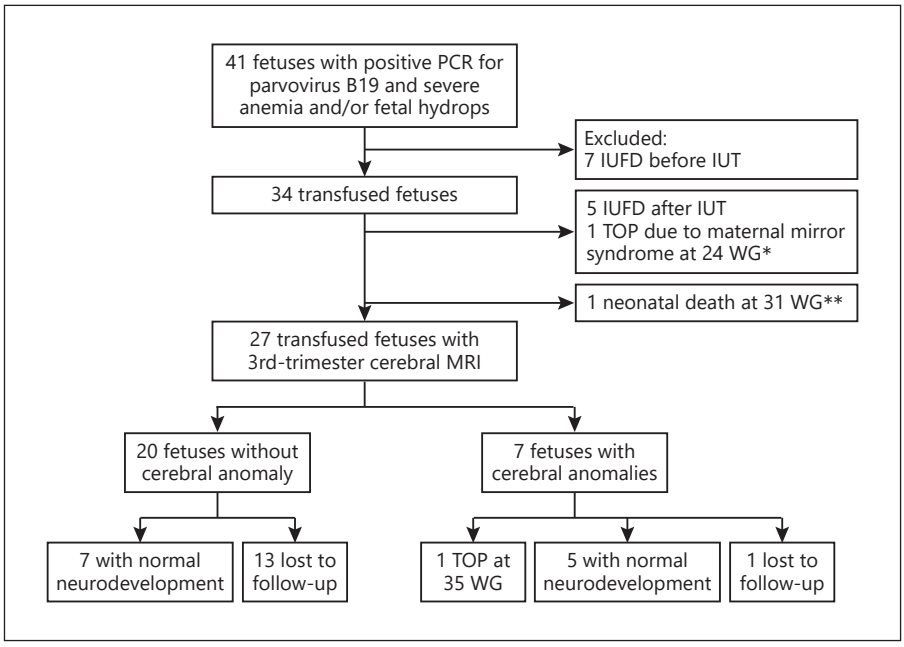

Fig. 1. Flowchart of the study. PCR, polymerase chain reaction; IUFD, intrauterine fetal death; IUT, intrauterine transfusion; TOP, termination of pregnancy; WG, weeks of gestation. * TOP was performed due to maternal mirror syndrome (preeclampsia and HELLP syndrome) associated with fetal hydrops at 24 weeks. ** This fetus presented with hydrops at 30 weeks and a normal brain at ultrasound. He was transfused at 30 WG, which allowed increasing hemoglobin from 2.5 to 10 g/dL. An emergency caesarean section was performed in another center at 31 WG because of an abnormal fetal heart rate, with the newborn presenting a hemoglobin level of 4 g/dL at birth, and which resulted in a neonatal death. Cerebral MRI was not performed in this case, owing to time constraints.

Table 1. Clinical, virological, and biological characteristics of the 34 fetuses transfused in utero for anemia related to parvovirus B19 infection

Gestational age at seroconversion, WG	16 [12.5–18]
Gestational age at diagnosis of severe anemia, WG	22 [22–24]
Gestational age at first IUT, WG	23 [22–24]
At least one serosal fluid accumulation	33/34 (97%)
Hydrops fetalis	28/34 (82%)
Viral load in amniotic fluid at first IUT, $\log_{10}$ copies/mL	7.8 [7.0–8.5]
Viral load in fetal blood at first IUT, $\log_{10}$ copies/mL	10.0 [8.4–11.0]
Hemoglobin level before first IUT, g/dL	2.9 [1.9–3.3]
Hemoglobin level before second IUT, g/dL	5.3 [4.7–6.2]
Hemoglobin level before third IUT, g/dL	6.5 [5.0–8.0]
Platelet count at first IUT, /mm^3	55,000 [40,000–86,000]
Leucocyte count at first IUT, /mm^3	2,165 [1,812–2,675]
Gestational age at delivery for live births, WG	39 [38–40]
Birth weight for live births, g	3,180 [2,985–3,358]

Values are presented as the median [IQR] unless specified otherwise. WG, weeks of gestation; IUT, intrauterine transfusion; IQR, interquartile range.

Statistical Analysis

After having collected the descriptive statistics on the cohort of 34 fetuses with hydrops fetalis and/or severe anemia, we conducted a case-control study on the fetuses transfused for severe anemia caused by B19V infection and who underwent cerebral MRI in the 3rd trimester. The cases corresponded to fetuses with cerebral damage on MRI and the controls were those with normal MRI results. We excluded IUFDs before or after IUT because they occurred before the 3rd trimester MRI examination could be performed.

To assess a possible relationship between cerebral lesions and both clinical and viral criteria, we performed univariate analyses. Significant effects were based on the χ^2 test to compare categorical variables, and Fisher's exact test was used when the assumptions of the χ^2 test were violated. Continuous variables were compared with the Wilcoxon rank-sum test because their distributions were not parametric. The statistical analyses were performed with R software version 3.2.3. A p value <0.05 was considered statistically significant.

Fetal Diagn Ther
DOI: 10.1159/000489881

Table 2. Description of the 7 cases with cerebral lesions on cerebral MRI

Case No.	GA at first IUT, WG	Hb and platelets at first IUT	IUT, *n*	Viral load, log_{10} copies/mL	2nd-trimester fetal ultrasound before IUT	3rd-trimester fetal ultrasound	3rd-trimester fetal cerebral MRI	Cerebral MRI between 1 and 6 months of age	Neuro-development
1	25	Hb = 4.4 Plt = 25	2	NA	NA	32 WG[a]: HC 5th perc	32 WG: encephalic biometry <3rd perc, cerebellar vermis and TDC <3rd perc	Lost to follow-up	Lost to follow-up
2[b]	23	Hb = 3.3 Plt = 49	2	11.5 in FB	23 WG: normal	32 WG: normal	32 WG: T2-weighted hypointensity signal of the hypothalamus and the caudate nucleus	Not done	Normal neurologic examination at 3 months
3[b]	23	Hb = 2.0 Plt = 74	2	8.2 in AF	23 WG: TCD <5th perc	33 WG[a]: HC 43rd perc, asymmetrical cerebellar hemispheres	30 and 33 WG: right cerebellar hemisphere <3rd perc, cerebellar hemorrhagic damage, normal vermis	3.5 months: asymmetrical cerebellar hemispheres, unilateral cerebellar hemorrhagic damage	Normal neurologic examination at 2 years; walking at 20 months
4[b]	27	Hb = 1.7 Plt <10	3	12.5 in FB	27 WG: HC <5th perc, TCD not measured	32 WG[a]: BPD and HC 24th perc TCD <3rd perc	32 WG: TCD <3rd perc 36 WG: TCD between 3rd and 5th perc	2 months: normal	Normal at 9 months
5	22	Hb = 2.1 Plt = 40	1	7.5 in AF	22 WG: normal	33 WG: normal	32 and 34 WG: vermis and TCD <3rd perc, atrophy of the anterior lobe of the vermis	TOP (no postmortem neuropathologic examination)	TOP
6	17	Hb = 2.9 Plt = 124	1	5.5 in AF 10.5 in FB	16 WG: TCD 55th perc 21 WG: TCD 20th perc, bilateral cerebellar hemisphere hemorrhage	27 and 32 WG[a]: bilateral cerebellar hemisphere hemorrhage, normal vermis	32 WG: bilateral cerebellar hemisphere hemorrhage, normal vermis, TCD 15th perc	6 months: bilateral cerebellar hemorrhagic damage	Normal neurologic examination at 2 years; normal development at 3 years
7	24	Hb = 3.3 Plt = 52	1	7.3 in AF	22 WG: HC <5th perc, TCD <5th perc	32 WG[a]: HC 47th perc, cerebellar biometry <3rd, unilateral cerebellar hemorrhage of the outer part of the right hemisphere, bilateral occipital pseudocysts	28 and 32 WG: encephalic biometrics 5th perc, TCD <3rd perc, normal vermis, bilateral occipital pseudocysts, unilateral cerebellar hemorrhage of the right hemisphere	1 month: cerebellar hemorrhagic damage, pseudocysts disappeared	Normal neurologic examination at 2 months; normal development at 13 months

Hemoglobin levels are expressed in grams per dL. Platelet counts are expressed as $n \times 10^3/mm^3$. GA, gestational age; IUT, intrauterine transfusion; WG, weeks of gestation; Hb, hemoglobin; FB, fetal blood; AF, amniotic fluid; NA, not available; BPD, biparietal diameter; HC, head circumference; TCD, transverse cerebellar diameter; TOP, termination of pregnancy; perc, percentile.
[a] Ultrasound examination performed by expert pediatric radiologist (C.G.). [b] Cases 2, 3, and 4 of the present cohort were already cited in a previous series, which was not focused on cerebral findings [35].

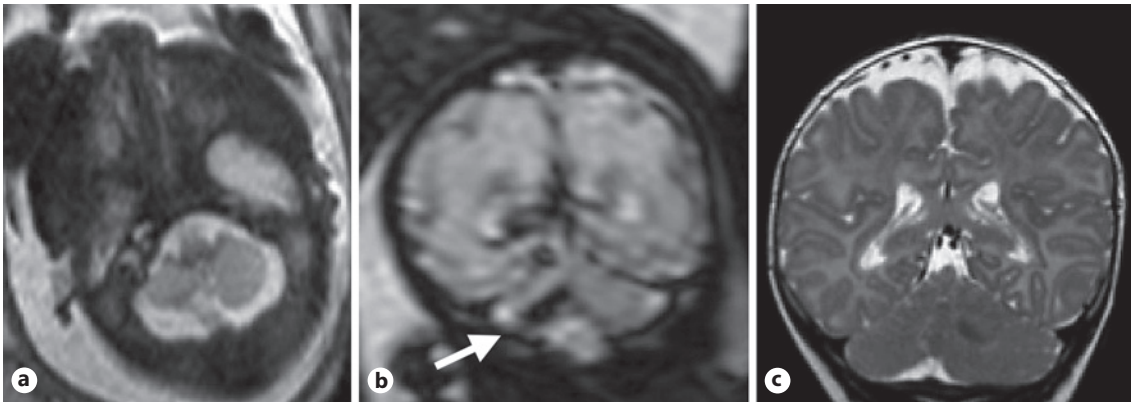

Fig. 2. Case 3. Asymmetrical cerebellar hemispheres resulting from right hemispheric hemorrhage; normal vermis. Ultrasound at 30 weeks of gestation demonstrated a small right cerebellar hemisphere (not shown). **a**, **b** MRI at 30 weeks of gestation. **a** T2-weighted axial slice showing a small right cerebellar hemisphere with an irregular border. **b** Gradient echo T2 coronal slice showing a marked hypointensity signal at the inferior border of the right cerebellar hemisphere, in keeping with hemorrhage (arrow). **c** Postnatal MR image at 3 months of life. The T2-weighted coronal slice shows asymmetrical cerebellar hemispheres. The right hemisphere is smaller and displays abnormal foliation. The vermis (not shown) is normal.

Results

Among the 41 fetuses with severe B19V infection, 7 IUFDs (17%) were diagnosed before IUT; PCR was positive for B19V in the AF in these cases (Fig. 1). The clinical, hematological, and virological characteristics of the cohort of 34 transfused fetuses are presented in Table 1. We observed 5/34 IUFDs (15%) following an IUT (at 22, 23, and 26 weeks following the first IUT, and at 23 and 24 weeks following the second IUT). One neonatal death occurred at 30 weeks of gestation (WG). Two terminations of pregnancy (TOP) were conducted, at 24 and 35 WG, respectively (Fig. 1; Table 2; case 5). The overall survival rate following IUT was 76%.

MRI examinations were performed at 30–34 WG in 27 cases. Cerebral anomalies were observed in 7/27 cases (26%) on MRI in the 3rd trimester (Table 2). Anomalies at the supratentorial level alone were found in only 1 case with a T2-weighted hypointensity signal of the hypothalamus and the caudate nucleus, suggesting hemorrhagic damage (case 2). Anomalies of the cerebellum were found in 6/7 cases (5 living children and 1 TOP). The transverse cerebellar diameter was below the 3rd percentile in 3 cases. In case 3, the right hemisphere was reduced in size and presenting hemorrhage (Fig. 2). Bilateral hemorrhage with a normal cerebellar biometry was found in case 6 (Fig. 3). In 5/6 cases, the cerebellar vermis was normal. In cases 1, 3, 4, 6, and 7 (Fig. 4), the cerebellar anomalies were first diagnosed by 3rd-trimester ultrasound and later confirmed by MRI. Conversely, in case 5, the ultrasound examination result was normal at 33 WG, whereas fetal MRI at 32 WG showed atrophy of the anterior lobe of the vermis, confirmed at 34 WG. TOP was performed at 35 WG. The parents declined an autopsy.

Fetal karyotyping was normal and cytomegalovirus PCR was negative in all cases with cerebral anomalies. Follow-up by a pediatric neurologist ranged between 3 months and 3 years, and postnatal cerebral MRI was performed between 1 and 6 months of age on 4 children.

Among the 5 cases with cerebellar hemispheric anomalies, 4 had a normal neurodevelopmental status and 1 was lost to follow-up (case 1). The child with an anomaly of the caudate nucleus presented with normal development at 3 months of age (case 2).

Considering clinical and biological characteristics potentially associated with the occurrence of cerebral lesions, univariate analysis showed that only viral load in FB appeared to be correlated with the occurrence of abnormal MRI results in the 3rd trimester (11.5 $\log_{10}$ copies/mL [interquartile range: 10.5–12.5] in case of abnormal MRI results vs. 9.5 $\log_{10}$ [7.8–10.0] in case of normal MRI results; $p = 0.05$) (Fig. 5). We observed no association with criteria related to the severity of anemia, gestational age at the first IUT, severity of thrombocytopenia, viral load in AF, and a great increase in hemoglobin during IUT (Table 3).

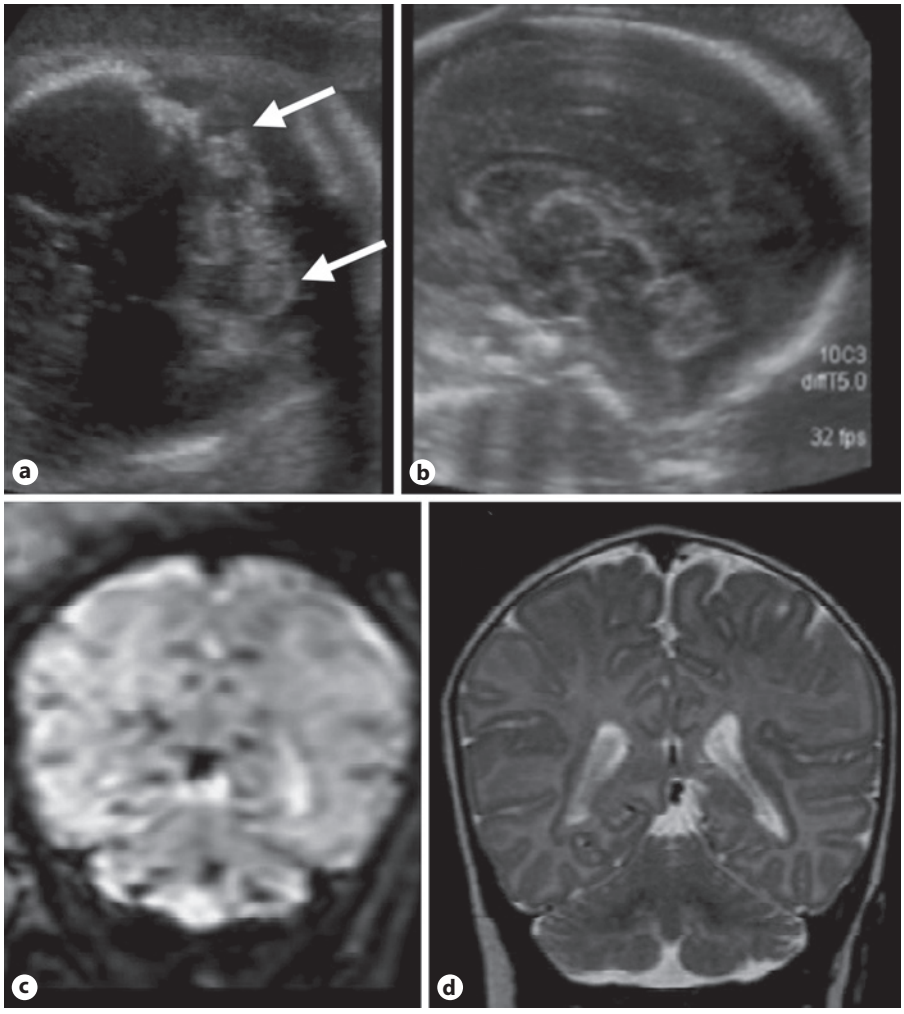

Fig. 3. Case 6. Bilateral cerebellar hemorrhage with a normal vermis and biometry. **a**, **b** Ultrasound at 23 weeks of gestation. **a** Axial view at the level of the cerebellum showing bilaterally increased echogenicity of the lateral parts of both cerebellar hemispheres (arrows). **b** Midline sagittal slice showing a normal vermis. **c** MRI at 32 weeks of gestation. Gradient echo T2-weighted coronal slice showing a marked hypointensity signal in the inferolateral part of both cerebellar hemispheres. **d** Postnatal MRI at 3 months of age. T2-weighted coronal slice showing a slight irregularity of the inferior border of the cerebellar hemispheres.

Discussion

In our series of fetuses transfused for B19V infection, cerebral anomalies were observed in 7/27 fetuses (26%) on cerebral MRI between 30 and 34 WG; the only factor which was associated with fetal brain lesions was a high viral load in FB.

Among the 7 cases with cerebral anomalies observed on 3rd-trimester MRI, 5 cases had been previously diagnosed at the ultrasound examination, while the ultrasound result had been considered normal in 2 cases. MRI specifically revealed an atrophy of the anterior lobe of the vermis, which had not been seen on ultrasound. This supports the added value of MRI for fetuses with severe B19V infection.

Among the 7 cases of cerebral anomalies, 6 were located in the cerebellum, suggesting that the cerebellum may be more susceptible to parvovirus-related injuries than the rest of the brain. In the literature, only isolated cases of fetal cerebellar hypoplasia or uni- or bilateral cerebellar hemorrhage have been reported in association with B19V infection. Sanapo et al. [12] described 1 case with MR images at 34 WG showing bilateral hypoplasia of the cerebellar hemispheres (–2 SD), which was more pronounced inferiorly and associated with a vermis smaller than expected (height of the vermis –4 SD). Postnatal MRI confirmed atrophy of the cerebellar hemispheres and an inferior vermis. The result of a clinical neurological evaluation at 4 months of age was appropriate for the infant's age. Glenn et al. [11] reported 2 cases of cerebral anomalies associated with B19V infection: one case with unilateral cerebellar hemorrhage and bilateral ventriculomegaly, and the other one with bilateral cerebellar hemorrhage and hypoplasia. In both cases, the

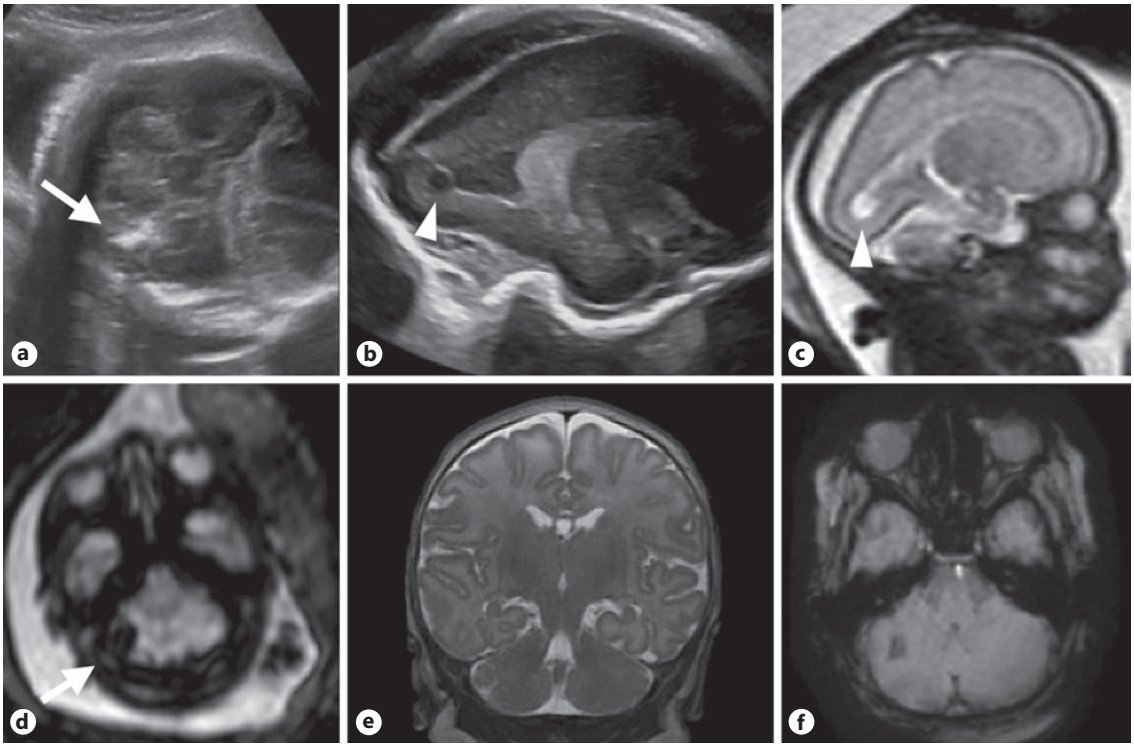

Fig. 4. Case 7. Cerebellar hemorrhage, occipital pseudocyst, and normal vermis. The encephalic and cerebellar biometry values were below the 3rd percentile. **a**, **b** Ultrasound scans at 28 weeks of gestation. **a** Axial slice at the level of the cerebellum showing increased echogenicity (arrow) in the lateral part of the right cerebellar hemisphere, suggesting a hemorrhagic insult. **b** Parasagittal ultrasound view at the level of a lateral ventricle demonstrating a pseudocyst (arrowhead) facing the occipital horn. **c**, **d** MRI at 28 weeks of gestation. **c** T2-weighted parasagittal slice at the same level as in **b**; the occipital pseudocyst is well depicted (arrowhead). **d** Gradient echo T2-weighted axial slice at the level of the cerebellum. The hemorrhage in the right cerebellar hemisphere is markedly hypointense (arrow). **e**, **f** MRI at 1 month of age. **e** T2-weighted coronal slice showing abnormal foliation of the lateral part of the right cerebellar hemisphere. **f** Susceptibility-weighted axial slice at the level of the cerebellum showing a hypointense signal within the right cerebellar hemisphere in keeping with the hemorrhagic damage detected prenatally. The occipital pseudocysts were no longer visible on this MRI scan.

pregnancy was terminated and no autopsy was available. In our series, we found 4 cases presenting with one or both cerebellar hemispheres reduced in size, but we did not use the term "hypoplasia." Indeed, a small transverse cerebellar diameter was associated with abnormal cerebellar echogenicity and an irregular border, thus suggesting ischemohemorrhagic insults to the inferior parts of the hemispheres. Conversely, hypoplasia refers to primitive underdevelopment or incomplete development of an organ [17].

In addition, other kinds of cerebral damage associated with congenital B19V infection have been reported. Courtier et al. [13] described 1 case with a prenatal diagnosis of bilateral polymicrogyria, which was observed at 23 weeks on MRI only and confirmed by autopsy after TOP. Polymicrogyria was also diagnosed in 2 other cases

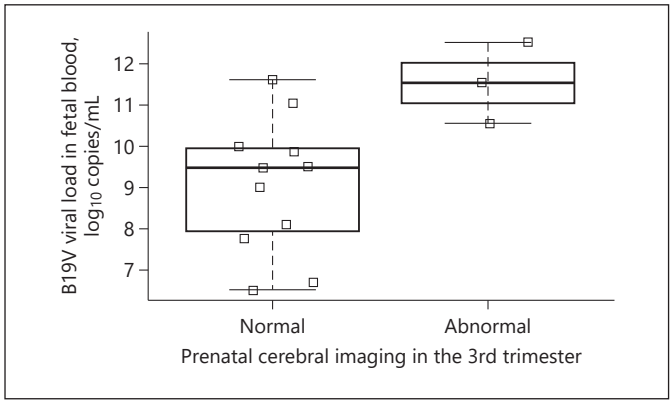

Fig. 5. Distribution of parvovirus B19 viral loads in fetal blood according to the findings of prenatal cerebral imaging at third trimester. B19V: Parvovirus B19. Ultrasound and Magnetic Resonance Imaging were performed between 30 and 34 weeks of gestation.

Table 3. Association between clinical and biological parameters related to fetal parvovirus B19 infection and the cerebral lesions diagnosed on fetal MRI in the 3rd trimester: univariate analysis

	Normal fetal brain MRI result (n = 20)	Abnormal fetal brain MRI result (n = 7)	p
Severity and earliness of fetal anemia			
Gestational age at first IUT, WG	22 (21–23)	23 (22–24)	0.38
Gestational age <24 WG at first IUT	13/20 (65%)	4/7 (57%)	1
Hb before first IUT, g/dL	3.0 (2.2–3.8)	2.9 (2.3–5.2)	0.90
Hb before first IUT <2.0 g/dL	6/20 (30%)	1/6 (14%)	0.63
Fetal hydrops	15/20 (75%)	6/7 (86%)	1
Reticulocytes, $\times 10^3/mm^3$	39.0 (9.3–59.1)	23.0 (12.7–33.4)	0.25
Reticulocyte count <10% of red blood cell count	5/12 (42%)	4/7 (57%)	0.65
Number of IUTs ≥2	5/20 (25%)	4/7 (57%)	0.18
Viral activity			
Platelet count, $\times 10^3/mm^3$	67 (44–90)	49 (34–64)	0.19
Platelet count $<50 \times 10^3/mm^3$	5/18 (28%)	4/7 (57%)	0.21
Viral load in fetal blood, $\log_{10}$ copies/mL	9.5 (7.8–10.0)	11.5 (10.5–12.5)	0.05
Viral load in amniotic fluid, $\log_{10}$ copies/mL	7.8 (7.3–8.3)	7.4 (7.0–7.8)	0.18
Hemodynamic changes related to IUT			
Hemoglobin level increase at first IUT, g/dL	7.8 (5.8–8.7)	7.2 (5.4–9.1)	0.61

Clinical and biological parameters are expressed as the median with 1st and 3rd interquartile range in parentheses. Significant effects were based on the χ^2 test to compare categorical variables, and Fisher's exact test was used when the assumptions of the χ^2 test were violated. Continuous variables were compared with the Wilcoxon rank-sum test because their distributions were not parametric. IUT, intrauterine transfusion; Hb, hemoglobin; WG, weeks of gestation.

of full-term live births. The first child was treated with IUT at 18 weeks and was reported to have a normal early development at 1 year of age [18]. The second child had normal prenatal ultrasound results until 39 weeks, when enlarged cerebral ventricles and enlarged heart chambers were noted. He was born with severe anemia and presented with seizures and neurodevelopmental impairment [14]. In an additional case report, a woman underwent a caesarean section at 27 weeks for mirror syndrome caused by fetal hydrops secondary to infection with B19V [19]. The child died at 9 h of life from a severe respiratory distress syndrome. On autopsy, B19V genomic DNA was found in the multinucleated giant cells and solitary endothelial cells scattered in the frontal lobes. Furthermore, Katz et al. [20] reported 2 cases of hydrocephalus associated with B19V infection, with neonatal deaths following emergency cesarean sections without IUT at 28 and 34 weeks. There are also numerous reports of isolated cases of cerebral anomalies associated with B19V infection in children and adults, such as encephalitis, encephalopathy, meningitis, meningoencephalitis, ataxia, seizures [21, 22], and cerebral ischemic stroke involving endothelial lesions of the cerebral arteries [23, 24]. However, the underlying pathogenic effect of B19V on cerebral tissues has not been demonstrated yet. In children and adults, several possible mechanisms of pathogenesis including direct infection and indirect/epigenetic, reactivation, and autoimmune/inflammatory cytotoxicity, as well as effects due to infection of other organs, have been suggested. In large studies, B19V DNA has been detected in two different regions of the brain including the dorsolateral prefrontal cortex and the cerebellum [25]. Replication of B19V leads to expression of its NS1 protein. B19V NS1 has been shown to induce apoptosis in permissive and nonpermissive cell types. Thus, NS1-induced cytotoxicity is likely a major mechanism by which B19V could cause cerebral lesions [25, 26]. This hypothesis of direct cytotoxicity is supported by the case reports of neonates presenting with cerebral lesions associated with congenital B19V infection and who did not receive any IUT [13, 14, 19, 20]. In our series, the head circumference and transverse cerebellar diameter were already below the 5th per-

centile before IUT in 3 and 2 cases, respectively. However, there was no contributive neuropathological examination in this series to support our hypothesis of B19V neurotropism – especially for the cerebellum, because the fetal brains were too macerated to perform neuropathological examinations in case of IUFD and because the parents did not request an autopsy in case of TOP for cerebellar anomalies.

In terms of prenatal counseling, the following information was provided to the parents according to the type of cerebral lesion: in case of hemispheric unilateral and stable lesions of the cerebellum, the prognosis was considered favorable in the first years of life; in case of vermis impairment, the increased risk of poor neurological prognosis was explained [27]. Indeed, according to a few reported cases, cerebral damage secondary to congenital B19V infection seems to carry a prognosis similar to that of other etiologies of cerebellar injury. In their series of 28 children treated with IUT in the context of B19V infection, de Jong et al. [9] found severe neurodevelopmental impairment in 3 children. One postnatal MRI performed on one of these children revealed atrophy of the cerebellar vermis. Similarly, in another cohort of 16 children under long-term follow-up, 2 children presented with severe developmental delay, including one with a postnatal MRI showing atrophy of the cerebellar vermis [8].

Since the pathophysiological mechanisms of brain injury induced by fetal B19V infection remain unclear in the literature [9, 11], our aim was to explore the hypotheses that have been previously suggested: (1) fetal cerebral injuries could be caused by ischemic stroke associated with severe anemia, similar to cases of severe fetal anemia associated with red blood cell alloimmunization [28–30]; (2) cerebral injuries could be linked to a direct effect of B19V [19]; and (3) cerebral injuries could be related to hemodynamic changes in cerebral blood flow when IUTs are performed, with sudden high increases in hemoglobin level [11, 12].

In our study, the viral load in FB was the only factor appearing to be correlated with the occurrence of brain lesions. This supports the hypothesis of direct cerebral injury caused by the virus. Besides, an association between viral activity and cerebral lesions was also found for congenital cytomegalovirus infection: Fabbri et al. [31] demonstrated that a viral load >30,000 copies/mL in FB was associated with symptomatic fetuses (prenatal cerebral ultrasound or MRI alterations, extracerebral ultrasound alterations, hearing impairment, jaundice, and low birth weight). We did not find any correlation between cerebral anomalies and levels of viral load in AF, which was also not found in cytomegalovirus congenital infections [32]. Viral load in AF reflects an accumulation of the virus and depends on fetal diuresis and the time elapsed between infection and amniocentesis.

Our results concerning the absence of an association between severe anemia or thrombocytopenia and brain anomalies are in line with the study by Nagel et al. [8]. In their study on 24 transfused fetuses, the median fetal hemoglobin concentration was 4.5 g/dL and the platelet count 79×10^9/L before IUT. Among the 16 survivors included in the follow-up, 5 children demonstrated delayed psychomotor development (2 mild and 3 severe delays). Neurodevelopmental status was not correlated with pre-IUT hemoglobin, platelet, or blood pH values.

Finally, although the authors of two studies [11, 12] speculated that hemodynamic change related to the severe anemia and subsequent IUT could have led to hemorrhagic reperfusion injury to the cerebellar germinal zones, our results did not find that a surge in hemoglobin level at the first IUT was associated with brain injury. However, the cerebellum is a rapidly growing, vulnerable brain structure with fragile developmental layers and vulnerable capillaries that can easily rupture, resulting in cerebellar hemorrhage. In preterm infants, hemodynamic factors/changes seem to play a role in the development of these hemorrhages [33]. There are likely many possible mechanisms, both direct and indirect, by which viruses such as B19V may contribute to neuropathology.

The strengths of our study include the fact that it comprised a consecutive series of cases evaluated at a single reference center involving experienced operators [34] and the detailed collection of pregnancy data, hematologic data, and ultrasound and cranial MRI findings in the 3rd trimester. To our knowledge, this is the first study that correlates hematologic and virological data, fetal ultrasound and MRI in the 3rd trimester, and neurodevelopmental status [8, 9].

The limits of our study are the small sample size of the cohort and the absence of contributive neuropathological examinations in case of cerebellar anomaly. Among the fetuses with cerebral abnormalities, viral load assessments were performed on FB in 3 cases and on AF in 4 cases. Moreover, the follow-up for neurodevelopmental assessment comprised only 5 infants of the 6 who had presented with fetal cerebral anomalies and were born alive. A longer follow-up is needed to rule out any neurodevelopmental impairment at an older age.

Conclusions

In our series of 27 fetuses transfused for B19V infection and evaluated by cerebral MRI in the 3rd trimester, 26% presented with cerebral abnormalities such as a cerebellar biometry below the 3rd percentile and uni- or bilateral cerebellar hemorrhage. In this study, the only factor that appeared to be associated with fetal brain lesions was a high viral load in FB.

Our findings emphasize the importance of prenatal identification of brain abnormalities after fetal B19V infection. The cerebellum should be carefully examined by fetal MRI in infected fetuses, particularly following IUT. Long-term follow-up of children with and without cerebral anomalies is mandatory to explore their neurodevelopment and the correlation with cerebellar anomalies.

Statement of Ethics

The subjects' parents have given their informed consent and the study protocol has been approved by our institutional committee.

Disclosure Statement

The authors report no conflict of interest and no financial support for this research.

References

1 Enders M, Weidner A, Zoellner I, Searle K, Enders G: Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1,018 cases. Prenat Diagn 2004;24:513–518.
2 Morel O, Chagnaud S, Laperrelle J, Clément D, Malartic C, Akerman G, et al: Parvovirus B19 in pregnancy: literature review (in French). Gynecol Obstet Fertil 2007;35:1095–1104.
3 Gratacós E, Torres PJ, Vidal J, Antolín E, Costa J, Jiménez de Anta MT, et al: The incidence of human parvovirus B19 infection during pregnancy and its impact on perinatal outcome. J Infect Dis 1995;171:1360–1363.
4 de Jong EP, de Haan TR, Kroes AC, Beersma MF, Oepkes D, Walther FJ: Parvovirus B19 infection in pregnancy. J Clin Virol 2006;36:1–7.
5 Crane J, Mundle W, Boucoiran I; Maternal Fetal Medicine Committee: Parvovirus B19 infection in pregnancy. J Obstet Gynaecol Can 2014;36:1107–1116.
6 de Haan TR, van den Akker ES, Porcelijn L, Oepkes D, Kroes AC, Walther FJ: Thrombocytopenia in hydropic fetuses with parvovirus B19 infection: incidence, treatment and correlation with fetal B19 viral load. BJOG 2008;115:76–81.
7 Ornoy A, Ergaz Z: Parvovirus B19 infection during pregnancy and risks to the fetus. Birth Defects Res 2017;109:311–323.
8 Nagel HT, de Haan TR, Vandenbussche FP, Oepkes D, Walther FJ: Long-term outcome after fetal transfusion for hydrops associated with parvovirus B19 infection. Obstet Gynecol 2007;109:42–47.
9 de Jong EP, Lindenburg IT, van Klink JM, Oepkes D, van Kamp IL, Walther FJ, et al: Intrauterine transfusion for parvovirus B19 infection: long-term neurodevelopmental outcome. Am J Obstet Gynecol 2012;206:204.e1–e5.
10 Dembinski J, Haverkamp F, Maara H, Hansmann M, Eis-Hübinger AM, Bartmann P: Neurodevelopmental outcome after intrauterine red cell transfusion for parvovirus B19-induced fetal hydrops. BJOG 2002;109:1232–1234.
11 Glenn OA, Bianco K, Barkovich AJ, Callen PW, Parer JT: Fetal cerebellar hemorrhage in parvovirus-associated non-immune hydrops fetalis. J Matern Fetal Neonatal Med 2007;20:769–772.
12 Sanapo L, Wien M, Whitehead MT, Blask A, Gallagher M, DeBiasi RL, et al: Fetal anemia, cerebellar hemorrhage and hypoplasia associated with congenital Parvovirus infection. J Matern Fetal Neonatal Med 2017;30:1887–1890.
13 Courtier J, Schauer GM, Parer JT, Regenstein AC, Callen PW, Glenn OA: Polymicrogyria in a fetus with human parvovirus B19 infection: a case with radiologic-pathologic correlation. Ultrasound Obstet Gynecol 2012;40:604–606.
14 Schulert GS, Walsh WF, Weitkamp JH: Polymicrogyria and congenital parvovirus B19 infection. AJP Rep 2011;1:105–110.
15 Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al: Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. N Engl J Med 2000;342:9–14.
16 International Society of Ultrasound in Obstetrics and Gynecology Education Committee: Sonographic examination of the fetal central nervous system: guidelines for performing the "basic examination" and the "fetal neurosonogram." Ultrasound Obstet Gynecol 2007;29:109–116.
17 Garel C: Posterior fossa malformations: main features and limits in prenatal diagnosis. Pediatr Radiol 2010;40:1038–1045.
18 Pistorius LR, Smal J, de Haan TR, Page-Christiaens GC, Verboon-Maciolek M, Oepkes D, et al: Disturbance of cerebral neuronal migration following congenital parvovirus B19 infection. Fetal Diagn Ther 2008;24:491–494.
19 Isumi H, Nunoue T, Nishida A, Takashima S: Fetal brain infection with human parvovirus B19. Pediatr Neurol 1999;21:661–663.
20 Katz VL, McCoy MC, Kuller JA, Hansen WF: An association between fetal parvovirus B19 infection and fetal anomalies: a report of two cases. Am J Perinatol 1996;13:43–45.
21 Yoto Y, Kudoh T, Haseyama K, Tsutsumi H: Human parvovirus B19 and meningoencephalitis. Lancet 2001;358:2168.
22 Barah F, Whiteside S, Batista S, Morris J: Neurological aspects of human parvovirus B19 infection: a systematic review. Rev Med Virol 2014;24:154–168.
23 Guidi B, Bergonzini P, Crisi G, Frigieri G, Portolani M: Case of stroke in a 7-year-old male after parvovirus B19 infection. Pediatr Neurol 2003;28:69–71.
24 Fullerton HJ, Luna JM, Wintermark M, Hills NK, Tokarz R, Li Y, et al: Parvovirus B19 infection in children with arterial ischemic stroke. Stroke 2017;48:2875–2877.
25 Grant JK, Yin NC, Zaytoun AM, Waseem H, Hobbs JA: Persistent adeno-associated virus 2 and parvovirus B19 sequences in post-mortem human cerebellum. Cerebellum 2009;8:490–498.

26 Hobbs JA, Waseem H: How do viruses really cause neuropathology? Is there more to the story. J Neuroinfect Dis 2016;7:1000228.
27 Massoud M, Cagneaux M, Garel C, Varene N, Moutard ML, Billette T, et al: Prenatal unilateral cerebellar hypoplasia in a series of 26 cases: significance and implications for prenatal diagnosis. Ultrasound Obstet Gynecol 2014; 44:447–454.
28 Carbonne B, Nguyen A, Cynober E, Castaigne V, Cortey A, Brossard Y: Prenatal diagnosis of anoxic cerebral lesions caused by profound fetal anemia secondary to maternal red blood cell alloimmunization. Obstet Gynecol 2008; 112(pt 2):442–444.
29 Simonazzi G, Bernabini D, Curti A, Bisulli M, Pilu G, Brill CB, et al: Fetal cerebellar damage in fetuses with severe anemia undergoing intrauterine transfusions. J Matern Fetal Neonatal Med 2016;29:389–392.
30 Ghi T, Simonazzi G, Perolo A, Savelli L, Sandri F, Bernardi B, et al: Outcome of antenatally diagnosed intracranial hemorrhage: case series and review of the literature. Ultrasound Obstet Gynecol 2003;22:121–130.
31 Fabbri E, Revello MG, Furione M, Zavattoni M, Lilleri D, Tassis B, et al: Prognostic markers of symptomatic congenital human cytomegalovirus infection in fetal blood. BJOG 2011;118:448–456.
32 Revello MG, Gerna G: Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 2002;15:680–715.
33 Steggerda SJ, De Bruïne FT, van den Berg-Huysmans AA, Rijken M, Leijser LM, Walther FJ, et al: Small cerebellar hemorrhage in preterm infants: perinatal and postnatal factors and outcome. Cerebellum 2013;12:794–801.
34 Girault A, Friszer S, Maisonneuve E, Guilbaud L, Cortey A, Jouannic JM: Intrauterine blood transfusion: status report of 4 years of practice in France (2011–2014) (in French). J Gynecol Obstet Hum Reprod 2017;46:119–124.
35 Macé G, Sauvan M, Castaigne V, Moutard ML, Cortey A, Maisonneuve E, et al: Clinical presentation and outcome of 20 fetuses with parvovirus B19 infection complicated by severe anemia and/or fetal hydrops. Prenat Diagn 2014;34:1023–1030.