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Running title: Routine ultrasound ignores most severe fetal CMV infections
Keywords: cytomegalovirus, congenital infection, ultrasound, outcome

Contribution

What are the novel findings of this work

Without CMV serology screening in pregnancy, routine ultrasound examination identified 26% of infected fetuses that developed long-term sequelae, although non-specific infection-related features were reported in 64% without raising suspicion. However targeted ultrasound of known infected fetuses had 91% sensitivity and 96% negative predictive value.

What are the clinical implications of this work

Routine ultrasound screening in pregnancy is not an appropriate screening tool for congenital CMV infection leading to long-term sequelae. Sonologists' awareness and the knowledge of maternal serological status in the first trimester are key to the performance of prenatal ultrasound.

Abstract

Objectives: To compare the ability to identify infected fetuses who will develop long-term sequelae, of routine ultrasound detailed examination performed without the knowledge of maternal serology and the fetal status to that of targeted prenatal imaging performed in prenatal diagnosis units in known cases of fetal infection.

Methods: All prenatal imaging reports were collected for 256 children with congenital CMV in a registered cohort between 2013 and 2017 (NCT01923636). All pregnancies underwent detailed fetal ultrasound examination at 20-24 and 30-34 weeks' as part of routine antenatal care. All cases of known fetal CMV infection underwent targeted prenatal ultrasound. Postnatal structured follow-up up until 48 months included audiology and neurological assessment including Brunet-Lezine scoring. All imaging reports were analyzed retrospectively with the knowledge of congenital CMV infection, searching for features that were or could have been related to fetal infection.

Results: 237 children with complete data were followed-up to a median 24 months, of whom 30% (71/237) and 70% (166/237) were diagnosed prenatally or within 3 weeks of life respectively. 73% (29/40) of children with any sequelae and 74% (14/19) with severe sequelae (bilateral hearing loss and/or neurologic sequelae) were not identified in the prenatal period. In those diagnosed prenatally, sensitivity of prenatal imaging for any and severe sequelae was 91 and 100%. In the group diagnosed postnatally non-specific infection-related features were reported in 48% and 64% with any and severe sequelae without raising suspicion.

Conclusions: Routine ultrasound screening in pregnancy is not an appropriate screening tool for congenital CMV infection leading to long-term sequelae, contrasting with the high performance of targeted prenatal imaging in known cases of fetal infections. Non-specific nature of ultrasound features and their evolution and lack of awareness of caregivers about cCMV are the main explanations. Sonologists' awareness and knowledge of maternal serological status in the first trimester seem key to the performance of routine prenatal ultrasound.

Introduction

Congenital CMV infection (cCMV) affects 0.7% of all newborns worldwide [1] and around 15 to 20% of those suffer long-term sequelae [2]. In high-income countries half of infected newborns are infected following maternal primary infection in pregnancy and the other half following maternal non-primary infection (reactivation or reinfection) [3-4]. All long-term sequelae develop in fetuses that have been infected after maternal infection occurring in the first trimester of pregnancy [5-6]. Around 30 to 35% of newborns infected after maternal primary infection in the first trimester develop significant neurological sequelae [7]. Series of infected fetuses diagnosed prenatally following documented maternal primary infection report high sensitivity and negative predictive value of targeted prenatal ultrasound for the occurrence of symptoms at birth and of sequelae at follow-up [8-12]. Following maternal prenatal serological screening and a positive amniocentesis, ultrasound performed by 23 weeks' identified up to 90% of cases with postnatal sequelae; and even more so by adding MRI examination at 28-32 weeks' [9]. Normal ultrasound follow-up of fetuses known to be infected leaves a residual risk of isolated unilateral neurosensory hearing loss of 14% [8].

No country outside Germany and Italy, have yet implemented prenatal maternal serological screening for CMV [13]. Therefore, most maternal primary infections remain unknown and all maternal non-primary infections remain undiagnosed in the absence of any validated diagnostic tools. Consequently, sonologists are mainly unaware of the maternal status at the time of routine second or third trimester ultrasound examination and only 20% of symptomatic neonates could be identified in one study [14]. However, symptoms at birth are associated with long-term sequelae in

40 to 60% of cases and 10 to 15% of asymptomatic neonates will develop sequelae essentially hearing loss [15]. The value of routine detailed ultrasound examination of the fetal anatomy to identify fetuses who will become infants with long-term sequelae is unknown. The aim of this study was to assess the type and proportion of sequelae that are missed by detailed ultrasound examination in the second and in the third trimester, as part of routine antenatal care offered to all pregnant women in France.

Material and methods

Population

The Cymepedia study included infected neonate/mother pairs in 11 French perinatal centers between 2013 and 2017. Neonates included in the study were diagnosed with congenital CMV either prenatally because of a primary infection in the mother and/or compatible prenatal ultrasound features in the fetus or postnatally. The study was proposed to the parents of all children diagnosed with cCMV and only around 5% of those refused to be included. The cohort was composed of a total of 256 neonates.

The Cymepedia study is registered in clinicaltrial.gov website under NCT01923636. All parents gave written consent for their children to participate to the study. Ethics committee approved the study (2013-A00213-42).

Determination of the timing of maternal CMV infection

CMV serological assessment was centralized in Necker Reference laboratory. Among the 237 cases, maternal serology was screened at 12 weeks' in 78. In the remaining cases, maternal serology results were obtained retrospectively on stored sera. In France, serum samples are systematically collected in first and third trimesters of pregnancy for various serology testing. Moreover, women seronegative for *toxoplasmosis* (70% of the pregnant population) are tested monthly. All sera are stored for 1 year and can therefore easily be tested retrospectively.

Levels of CMV specific IgG and IgM antibodies were determined with LIAISON® CMV IgG II and LIAISON® CMV IgM on Liaison XL platform (Diasorin, Antony, France). IgG avidity was

measured by the LIAISON® CMV IgG Avidity II and/or by VIDAS® CMV IgG avidity II (BioMerieux, Marcy L'Etoile, France) as described elsewhere [16]. The algorithm of CMV serology interpretation is shown in Figure S1.

Follow-up protocol

All infected children were followed with the same protocol including visits at 4, 12, 18, 24, 36 and 48 months of age. Clinical examination was standardized to assess motor, cognitive, speech and psychological development according to age as previously described [6]. Audiological assessment and the presence of otitis media were recorded at each visit. A Brunet-Lezine test which is an early childhood psychomotor development scale covering four areas of neurodevelopment: movement and posture, coordination, language, and sociability was done at M12 and M24 [17]. Auditory Brainstem Responses (ABRs) or audiology tests were done depending on the child's age. Conductive SNHL (Sensorineural Hearing Loss) with presence of otitis media with effusion was considered non-interpretable and testing was repeated 4 to 6 months after otitis healing. Transtympanic drains were placed when indicated. Hearing was considered normal if the child could hear stimuli of between 0 and 20dB. SNHL was mild, moderate, severe and profound for detection of sound within 21 to 30 dB, 31 to 60 dB, 61 to 90 dB and ≥91 dB respectively. Vestibular functions were assessed in children with SNHL and/or delayed walking as previously reported [6].

Sequelae were considered to be mild in cases with isolated unilateral hearing loss and/or vestibular disorders. Sequelae were considered to be severe in cases with bilateral hearing loss and/or neurologic sequelae.

Antenatal ultrasound results

Reports of all routine detailed ultrasound examinations performed in the second trimester (20 to 24 weeks) and in the third trimester (30 to 34 weeks) of pregnancy were identified from the women pregnancy files and collected retrospectively from the sonologists. They are working in different settings including teaching hospitals, regional hospital, private hospital or private clinics all over France. Since the aim of the study was to compare the performance of ultrasound examination in routine setting (maternal serology unknown, fetal infection unknown) to the performance of ultrasound when both maternal and fetal status were known, we deliberately not attempt to review the ultrasound images and only took into account the information obtained at the time of the examination and as written by the operator in the ultrasound report.

Prenatal detailed ultrasound examination, as part of the pregnancy care in France, includes a number of measurements and of features to be made and checked for using standardized ultrasound planes at recommended gestational ages of 20-24 and 30-34 weeks. This is offered to all pregnant women and the examinations are performed by registered sonologists[18]. The items checked for that could be relevant to fetal CMV infection include fetal biometry, size of the cerebral lateral ventricles, aspect of the cerebral midline, aspect and diameter of the cerebellum, size and position of the stomach bubble, aspect of the fetal bowel, placenta and the amount of

amniotic fluid. Therefore, infection-related features that could be suspected include: small for gestational age (<10° percentile), microcephaly (< 2 DS), ventriculomegaly, abnormal cerebral midline, abnormal posterior fossa, abnormal cerebellum, hyperechogenic bowel, hepato-splenomegaly compressing or displacing the stomach bubble, placentomegaly, and oligo or polyhydramnios [18].

When amniocentesis was positive for CMV, the women underwent a follow-up protocol with US scan every 2 to 3 weeks and systematic cerebral MRI between 32 and 34 weeks. Target ultrasound scans were realized by sonographers in fetal medicine Units.

Statistical analysis

The proportions between groups were compared by exact Fischer test. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), negative likelihood ratio (LR-) and positive likelihood ratio (LR+) were calculated. All analyses were conducted using R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Description of the population (Figure 1)

Antenatal ultrasound reports were recovered for 255 mothers of the 256 eligible children. One mother received very limited antenatal care. Among the 255 cases with antenatal ultrasound results available, 16 (6.2%) children were lost for follow-up before the age of one year. In addition, two children were excluded from the study because they were both diagnosed with a genetic disorder associated with neurologic symptoms and neurodevelopmental delay (Silver Russel and Galloway Mowat syndromes respectively).

71/237 (30%) of the cases were diagnosed prenatally with a positive CMV PCR in the amniotic fluid at amniocentesis. Amniocentesis was done because of a serology CMV screening with identification of a primary maternal infection in 89% (63/71) of these cases or because routine ultrasound screening picked up compatible symptom(s) in 11% (8/71). 166/237 (70%) cases were neither identified nor suspected in the antenatal period and were diagnosed only postnatally in the first 3 weeks of life. Among these 166 infected neonates, 38 were recruited through a universal neonatal screening program implemented in 2 centers between 2013 and 2015 [3]; 113 were diagnosed because of compatible symptoms (SGA, hearing loss, thrombocytopenia…) and 15 were diagnosed because of a known primary infection in pregnancy. 45/237 (19%) of the neonates were preterm: 21,19 and 5 were born between 36 and 37 weeks, 31 and 35 weeks and 26 and 31 weeks respectively.

The median follow-up of the 237 children was 24 months (range: 12-48 months), only 22 children were lost for follow-up between 12 and 24 months. 17% (40/237) of the children had

long-term sequelae, mild in 9% (21/237) and severe in 8% (19/237). The detailed prenatal ultrasound findings and the type of sequelae for the 40 children with sequelae are reported in Table 1. 27% (11/40) of cases with any sequelae and 26% (5/19) of cases with severe sequelae were diagnosed prenatally, following maternal serology screening in 9 cases or because of evocative ultrasound features in 2 cases. 73% (29/40) of cases with any sequelae and 74% (14/19) of cases with severe sequelae were not identified in the prenatal period. There was a similar proportion of prematurity in children with sequelae than in those without: 15% (6/40) and 19% (39/197) respectively.

Performance of antenatal imaging in cases diagnosed prenatally (fetuses known as infected at the time of prenatal imaging)

The sensitivity, NPV and LR- of targeted prenatal imaging following a positive prenatal diagnosis were 91%, 96% and 0.21 to diagnose all cases with sequelae and 100%, 100% and 0 for cases with severe sequelae (Table 2). The PPV and the LR+ of imaging features were low when all features were considered. However, the specificity, PPV and LR+ of severe cerebral symptoms to predict a severe outcome reached 96%, 80% and 20 respectively (Table 3). Indeed, among children whom infection was diagnosed prenatally, 5 had severe sequelae and in 4 of the 5, severe cerebral abnormalities were identified by targeted ultrasound and MRI examinations. The one child identified prenatally and who did not show severe cerebral abnormalities, developed bilateral hearing loss with no psychomotor delay.

Performance of antenatal imaging in cases diagnosed at birth (fetuses not known as infected at the time of prenatal imaging)

Based upon the review of ultrasound reports, the sensitivity, NPV and LR- of ultrasound screening could have been 48%, 87% and 0.70 to identify all cases with sequelae and 64%, 95% and 0.49 to identify cases with severe sequelae (Table 2). However, in all of these cases, although ultrasound features of infection were present the sonographer or the attending obstetrician did not raise the suspicion of CMV fetal infection. The sensitivity and NPV of ultrasound screening could have been 37% and 74% respectively to identify cases with severe sequelae in the group of children infected after a maternal primary infection in the first trimester (Table S1).

Comparison of imaging results between cases diagnosed prenatally and those diagnosed only postnatally

Table 4 reports the types and proportion of ultrasound features of infection: SGA, extra cerebral features, non-severe cerebral features and severe cerebral features, in the 2 groups of patients according to the presence of sequelae and to the type and date of maternal infection.

SGA was the most frequent US finding and was detected in similar proportions between the prenatal and the postnatal groups in cases with sequelae (36% Vs 45%) and in cases without sequelae irrespective of the trimester of maternal infection.

However, the presence of at least 1 extra-cerebral feature of infection was significantly more frequent in the prenatal group than in the postnatal one in cases with sequelae (82% Vs 3%,

p<0.001) but also in the subgroup of cases with no sequelae following maternal infection in the first trimester (37% Vs 7%, p=0.002).

In the group of children diagnosed postnatally, no severe cerebral abnormalities were identified in the 29 children who developed sequelae (including the 14 with severe sequelae). None of these cases had access to fetal MRI but postnatal MRI was performed in 13/14 cases and showed severe white matter lesions in 10 of 13. Conversely, in the group diagnosed prenatally, 36% (4/11) of children with sequelae, and 80% (4/5) of those with severe sequelae showed severe white matter abnormalities identified by prenatal MRI (p=0.003 and p=0.001).

Discussion

In this cohort of children with congenital CMV infection, 73% (29/40) of any sequelae and 74% (14/19) of severe sequelae were not anticipated prenatally although prenatal care followed the national French guidelines including 2 standardized detailed ultrasound examinations of the fetal anatomy at between 20-24 and 30-34 weeks. In over half of the cases missed in utero who developed sequelae non-specific ultrasound features were present but was not raised by the sonologist nor by the attending clinician receiving the report. Even assuming that those ultrasound features would have been related to CMV infection, the low sensitivity of ultrasound screening compares poorly with the sensitivity of diagnostic ultrasound performed with the knowledge of fetal infection, that reached 91% in all cases and 100% in severe cases, thus confirming previous reports [9-12]. Therefore, a known infected fetus with a normal ultrasound and MRI was left with a residual risk of 10% of mild sequelae as previously reported [8].

The PPV of extra cerebral and non-severe cerebral features for sequelae were respectively 23% and 20% in our study, while PVV over 60% has been reported in most studies including both termination (TOP) cases and live children [9-12]. One explanation that might reflect a weakness of our study is that our population is a postnatal cohort and therefore TOP cases could not be identified and accounted for in the performance of antenatal assessment. Indeed, the specificity, PPV and LR+ of severe cerebral features, mostly on MRI, to predict severe sequelae were high (96, 80% and 20 respectively).

We reported that with a strategy based on CMV serology screening in the first trimester for maternal primary infection followed by prenatal diagnosis, the risks of neurologic sequelae and

of hearing loss were of 12 and 27% respectively while 17% of affected cases were terminated for severe cerebral lesions [6]. Interestingly, the natural history of cCMV following maternal infection in the first trimester is associated with neurologic sequelae and hearing loss in 32% and 23% respectively [7]. Although no direct comparison can be made, the dramatic difference in neurological sequelae between these populations suggests that cCMV antenatal screening is likely to identify the most severe cases and give parents the alternative choice of terminating the pregnancy or anticipating the birth of a child with potential severe neurologic sequelae. The identification of cases following maternal non-primary infection is more difficult since serology is not helpful [19-20]. However, we show here that around 90% (9/10) of severe cases following maternal non-primary infection presented with at least one feature at ultrasound screening, mainly SGA.

Several hypotheses could be made to explain the poor performance of routine ultrasound examination even when this involves a detailed and structured examination of fetal biometry and anatomy: 1) Ultrasound screening had a poor sensitivity to detect subtle extra cerebral features of infection (hyperechogenic bowel, hepatosplenomegaly) that may be transient: in cases with sequelae they were only reported in 3% of those diagnosed postnatally while they were reported in 82% of those diagnosed prenatally ($p< 0.001$). Conversely, SGA, which was the most frequent warning sign, was reported in the same proportion in cases diagnosed postnatally and prenatally. Ultrasound screening has therefore a good performance to detect SGA related to CMV. However this symptom is frequent and nonspecific and the awareness of the sonologists should therefore be raised regarding its link to the most frequent congenital infection affecting 0.37% of all births

in France [3]. 2) The development of fetal lesions is a continuous and heterogeneous process that may not offer the possibility to be recognized at only two points in time in the pregnancy. Indeed, none of the severe cases in the group that did not benefit from a prenatal diagnosis had severe cerebral lesions picked-up by routine ultrasound examination, while in the group with prenatal diagnosis, 80% of the severe cases had severe cerebral anomalies identified by targeted imaging (ultrasound and/or MRI). Indeed, the knowledge of fetal infection will generate serial and targeted ultrasound follow-up and planned MRI in the third trimester; all more likely to identify even subtle significant lesions.

The prevalence of neonatal infection is 0.37% in France with around 2,960 infected neonates per year. One third of these, therefore 986 neonates, are infected following maternal infection in the first trimester and 315 (32% of 986) are therefore expected to bear long-term neurological sequelae. However, only around 60 cases of TOP in relation with severe cCMV infection are reported annually in France through the mandatory annual report to Health Authorities [21]. This is therefore compatible with our results and also suggests that only around 20% of all severe cases might be identified prenatally also at national level.

Strengths and Limitations

One weakness of this study is that the cases included in this pediatric cohort did not come for a systematic neonatal screening program. It is therefore impossible to estimate the true proportion of severe cases that were missed by routine ultrasound. Another limitation is that the population did not include cases of termination of pregnancy. However, this could also be seen as a strength

since in prenatal cohorts, terminated cases are classified as severe cases based upon the lesions seen at autopsy although it remains difficult to preclude if those interrupted fetuses might have developed long-term sequelae or otherwise. Another limitation of the study is the length of follow-up with a median of only 24 months since late-onset hearing loss has been reported up to5 years of age. However, as extensively documented in the literature late onset hearing loss is rare after 2 years of age [22-23]. Finally, this study is multicentre including 11 different paediatrics centres all over France and the women had their prenatal follow-up in various settings including teaching hospitals, district general hospitals, private hospitals and private clinics therefore reflecting the diversity of medical care.

Conclusion

Our study shows that even a well-organized systematic ultrasound screening in second and third trimester, as it is implemented in France, is not sensitive enough to identify most CMV cases with cerebral lesions that will lead to neurologic long-term sequelae. This calls for raising awareness of all antenatal care providers, and particularly sonologists; this should help identifying severe cases following both primary and non-primary maternal infection. Moreover, we believe that the results of this study should be taken into account in the evaluation of offering maternal serology testing in the first trimester.

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Conflict of interest

MLV reports other from BioMerieux, non-financial support from BioMérieux, non-financial support from Abbott, non-financial support from Ferring SAS, outside the submitted work. YV reports non-financial support from GE Medical, non-financial support from Ferring SAS, non-financial support from Siemens Health care, outside the submitted work. JFM reports personal fees from ABBVIE, outside the submitted work. Other authors report no conflict of interest.

Authors Contribution: Marianne Leruez-Ville (Virologist), Laurence Bussieres (Methodologist), Jean-Francois Magny (Pediatrician), Marine Parodi (Otologist) and Yves Ville (Obstetrician) designed the study. All co-authors contributed to the data collection. Marianne Leruez-Ville and Sally Ren (Midwife) analysed the data. Yves Ville and Marianne Leruez-Ville attest to the data and analysis. Marianne Leruez-Ville and Yves Ville wrote the paper with input from the co-authors. All authors agreed to publish the paper.

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Figure legend

Figure 1: Flow chart of the study

Table 1: Description of cases with sequelae: type and date of maternal infection, type of sequelae and prenatal imaging (N=40)

	Type of maternal infection (date in weeks)	Sequelae	Prenatal US features throughout the pregnancy
Cases diagnosed prenatally (positive CMV PCR in amniotic fluid) N=11			
001-001	PI (8)	Moderate uni HL	SGA, placentamegaly splenomegaly
001-003	PI (7)	Profound uni HL	HEB, hepatomegaly
001-073	PI (2)	Profound uni HL	SGA
001-083	PI (3)	Profound uni HL	SGA, HEB
001-100	PI (10)	Severe uni HL	Placentomegaly, HEB, hepatomegaly, splenomegaly
001-117	PI (2)	Profound Bil HL	SGA, HEB
001-130	NPI	Psychomotor delay	Splenomegaly, mild ventriculomegaly, sub ependymal cysts, LSV
002-020	PI (11)	Moderate uni HL	0
007-008	PI (8)	Moderate uni HL, spastic dysplegia	HEB, sub ependymal cysts, LSV
016-005	PI (4)	Profound bil HL	HEB, hepatomegaly, splenomegaly, Sub ependymal cysts
017-002	NPI	Psychomotor delay	Hepatomegaly, polyhydramnios, microcephaly
Cases not diagnosed prenatally *(*amniocentesis not done) N=29			
001-025	PI (8)	Profound uni HL	0
001-026	Pi (8)	Profound uni HL	0
001-048	NPI	Moderate uni HL vestibular	0

		disorder	
001-053	PI (7)	Profound bil HL, Psychomotor delay	SGA
001-060	UN	Profound uni HL	0
001-076	PI (7)	Profound bil HL	0
001-094	UN	Moderate uni HL Vestibular disorder	SGA
001-110	Pi (10)	Severe uni HL	SGA
002-001	NPI	Profound uni HL, vestibular disorder	0
002-002	PI (9)	Profound Uni HL	0
002-008	NPI	Profound Uni HL	SGA
002-012	NPI	Severe bil HL	0
002-024	NPI	Psychomotor delay	SGA
007-001	NPI	Psychomotor delay	SGA
007-004	PI (9)	Severe uni HL	0
007-005	NPI	Profound bil HL Psychomotor delay	SGA, mild ventriculomegaly
007-009	UN	Psychomotor delay	0
007-010	PI (8)	Moderate uni HL	0
008-001	PI (11)	Profound bil HL Psychomotor delay	Hepatomegaly
008-009	NPI	Psychomotor delay, autism	SGA
013-003	PI (11)	Profound uni HL	0
013-008	PI (9)	Profound uni HL	SGA
013-014	PI (2)	Severe uni HL	0
013-019	PI (1)	Psychomotor delay	0

014-002	PI (3)	Psychomotor delay	SGA
015-001	PI (3)	Severe bil HL Psychomotor delay	0
015-003	NPI	Psychomotor delay	SGA
015-007	PI (1)	Delayed moderate Uni HL	subependymal cysts, LSV
016-003	NPI	Profound bil HL	SGA

PI= primary infection NPI= non primary infection
UN= unknown; ND= not done
HEB= hyperechogenic bowel; SGA= small for gestational age (< 10th percentile); Bil HL= bilateral hearing loss; Uni HL= unilateral hearing loss; LSV= lenticular striated vasculopathy
Cases in bold characters are the ones that were classified in the severe sequelae group
Mild ventriculomegaly = <15 mm

Table 2: Sensitivity, specificity, PPV and NPV of prenatal ultrasound for long-term sequelae

	Se	Sp	PPV	NPV	LR+	LR-
All population N=237/all sequelae n=40	57%	63%	24%	88%	1.54	0.68
All population N=237/ severe sequelae n=19	74%	62%	14%	96%	1.95	0.42
Population diagnosed at birth N=166/ all sequelae n=29	48%	74%	28%	87%	1.85	0.70
Population diagnosed at birth N=166/ severe sequelae n=14	64%	73%	18%	95%	2.4	0.49
Population diagnosed prenatally N=71/ all sequelae n=11	91%	41%	22%	96%	1.54	0.21

Population diagnosed prenatally N=71/ severe sequelae n=5	100%	38%	11%	100%	1.61	0

Se= sensitivity; Sp=specificity; PPV= positive predictive value, NPV= negative predictive value; LR+= positive likelihood ratio; LR-= negative likelihood ratio

Table 3: Sensitivity, specificity, PPV and NPV of specific imaging features for long-term sequelae in the subgroup diagnosed prenatally

N=71 Number of sequelae =11 Number of severe sequelae=5	Se	Sp	PPV	NPV	LR+	LR-
At least one extra cerebral symptom / all sequelae	81%	66%	23%	76%	2.38	0.28
At least one non severe cerebral symptom/ all sequelae	18%	86%	20%	85%	1.28	0.95
At least one severe cerebral symptom /all sequelae	36%	98%	80%	89%	18	0.65
At least one severe cerebral symptom/severe sequelae	80%	96%	80%	98%	20	0.21

Se= sensitivity; Sp=specificity; PPV= positive predictive value, NPV= negative predictive value; LR+= positive likelihood ratio; LR-= negative likelihood ratio

Table 4: Prenatal imaging according to the presence of sequelae and the time and type of maternal infection

N=237	Prenatal diagnosis positive	Prenatal diagnosis not done	P OD CI95%	Prenatal diagnosis positive	Prenatal diagnosis not done	p	Prenatal diagnosis positive	Prenatal diagnosis not done	p	Prenatal diagnosis positive	Prenatal diagnosis not done	p
	PI and NPI with sequelae N=11	PI and NPI with sequelae N=29		PI T1 no sequelae N=30	PI T1 no sequelae N=42		PI T2 /T3 no sequelae N=29	PI T2 /T3 no sequelae N=69		NPI No sequelae N=1	NPI or type of infection unknown no sequelae N=26	
No US features	1/11 (10%)	15/29 (52%)	0.02	13/30 (43%)	35/42 (83%)	<0.001	11/29 (38%)	50/69 (72%)	0.004	1/1	16/26 (61%)	NS
SGA	4/11 (36%)	13/29 (45%)	NS	3/30 (10%)	4/42 (9%)	NS	8/29 (27%)	11/69 (16%)	NS	0	8/26 (31%)	NS
1 extra cerebral feature *	4/11 (36%)	1/29 (3%)	0.01	7/30 (23%)	3/42 (7%)	NS	5/29 (17%)	10/69 (14%)	NS	0	2/26 (7%)	NS
2 extra cerebral features *	4/11 (36%)	0/29 (0%)	0.003	3/30 (10%)	0/42 (0%)	NS	2/29 (7%)	5/69 (7%)	NS	0	0/26	NS
3 extra cerebral features *	2/11 (18%)	0/29 (0%)	NS	1/30 (3%)	0/42 (0%)	NS	3/29 (10%)	0/69	0.02	1/1	1/26 (4%)	NS

At least 1 extra cerebral feature *	9/11 (82%)	1/29 (3%)	<0.001	11/30 (37%)	3/42 (7%)	0.002	10/29 (34%)	15/69 (21%)	NS	1/1		NS
Non-severe cerebral features	3/11 (27%) (LSV n=2, cysts n=3, mild VM n=1)	2 /29 (10%) (LSV + cysts n=1; mild VM n=1)		6/30 (20%) (cysts n=6)	2/42 (5%) (mild VM n=2)	NS	2/29 (7%) (cysts n=1, mild VM n=1)	5/69 (7%) (cysts n=3, mild VM n=2)	NS	0/1	0/26	NS
Severe cerebral features	4/11 (36%) (WMA (n=4), Mc n=1) 4/5 (80%) of cases with severe sequelae	0/29 (0%) 0/14 (0%) of cases with severe sequelae	0.003 0.001	1/30 (3%) (WMA)	0/42 (0%)	NS	0/29	0/69	NS	0/1	0/26	NS

PI= Maternal primary infection, NPI= Maternal non-primary infection, T1= First trimester of pregnancy, T2= Second trimester of pregnancy, T3= Third trimester of pregnancy, LSV= Vasculopathy of the lenticulo-striate vessels, Cysts= sub-ependymal cysts; VM= ventriculomegaly, mild ventriculomegaly (<15 mm), WMA: white matter abnormalities

SGA= small for gestational age (< 10th percentile); Mc= microcephaly; *= excluding SGA