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Universal newborn screening for congenital cytomegalovirus infection: feasibility and relevance in a French type-III maternity cohort

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Short running title

Universal neonatal cytomegalovirus screening

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ABSTRACT

Objective: Evaluation of relevance and feasibility of universal newborn congenital cytomegalovirus infection (cCMVI) screening in saliva.

Design: Retrospective, population-based cohort study.

Setting: Clamart, France, 2016-2020.

Population: All neonates born consecutively in our level III maternity unit.

Methods: CMV PCR in saliva for all neonates at birth, and, if positive, CMV PCR in urine to confirm or exclude cCMVI. Prospective and retrospective characterization of maternal infections. ROC curve analysis to assess saliva PCR performances. Acceptability of screening among staff members evaluated by a survey.

Main outcome measures: Number of cCMVI neonates; number of expected and unexpected cCMVI.

Results: Among 15,341 tested neonates, 63 had cCMVI (birth prevalence of 0.4% 95CI [0.3 – 0.5]). In 50% of cases, maternal infection was a non-primary infection (NPI) during pregnancy. cCMVI was expected or suspected (maternal primary infection (PI), antenatal or neonatal signs) in 24/63 neonates (38%), and unexpected in 39/63 neonates (62%). The best CMV saliva threshold to predict cCMVI was 356 (2.55 log) copies/mL 95CI [2.52 log – 3.18 log], with an area under the ROC curve of 0.97. Over 90% of the 72 surveyed staff members reported that the screening was easy and quick. No parent refused the screening.

Conclusions: Universal screening for cCMVI with CMV PCR on saliva samples is feasible and highly acceptable to parents and health care providers. Over half (62%) of the cases had no prenatal/neonatal signs of cCMVI, nor a maternal history of CMV infection during pregnancy, and would probably not have been diagnosed without universal screening.

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Keywords congenital cytomegalovirus, neonatal screening, saliva, non-primary infection

Tweetable Abstract

In 62% of congenital cytomegalovirus infection cases, only universal neonatal screening in saliva can detect infection.

INTRODUCTION

Congenital cytomegalovirus infection (cCMVI) is the leading worldwide cause of congenital viral infections, with a birth prevalence ranging from 0.2% to 1%, depending on the country and maternal seroprevalence (1,2). Approximately 15-20% of all cCMVI children will develop long-term disability, mainly late-onset hearing loss and other developmental disorders. Specific follow-up is essential because some neurological defects appear as children grow older (3). Early diagnosis of cCMVI allows early investigation for specific signs, early initiation of follow-up, and also offers the opportunity for appropriate care (4). Antiviral treatment has proven effective only for symptomatic neonates (5). Because more than 85% of cCMVI are asymptomatic at birth, this condition often remains undiagnosed, and many infected infants do not receive appropriate follow-up (1,6–8). Currently, in the prenatal period there are two main opportunities to expect cCMVI: *(1)* maternal primary infection detected by universal serological screening (not recommended in France, but scientific societies have divergent opinions) or infectious symptoms in the mother (9–11), and *(2)* prenatal ultrasound (US) signs that can be related to CMV. Moreover, only maternal primary infection (PI) can be confirmed with serology, while detection of non-primary infection (NPI) is very challenging (12). In countries where maternal CMV seroprevalence is around 50%, approximately half of cCMVI are due to maternal PI during pregnancy, and half to maternal NPI (13–15). However, in countries with high maternal seroprevalence (>90%), almost all cCMVI are due to maternal NPI (16,17). Similarly to what is reported in other western European countries, the birth prevalence of cCMVI in France is estimated to be 0.4% (7). This represents approximately 3,200 affected neonates each year in France, of whom 700 will develop sequelae (2,7). CMV PCR in saliva within the first days of life offers a reliable tool to detect cCMVI and, if positive, should be confirmed by PCR in urine samples collected in the first 2 weeks of life (8,18–20). Although cCMVI meets most criteria for a universal screening program according to the World Health Organization (21), it has never been implemented in any country (7). The French national health authorities have pointed out a lack of feasibility and acceptability data and encourage studies before implementing such screening (10,22).

Here, we report our 4.5 years of experience of systematic screening for neonatal cCMVI in the saliva of all live-born infants delivered in our maternity unit, to evaluate its feasibility, acceptability and relevance.

MATERIALS AND METHODS

Population and management

This retrospective single cohort study was carried out from April 2nd 2016 to October 2nd 2020. It was approved by the local ethics committee (CEROG 2020-OBST-0902) and written informed consent was obtained from all mothers. All live-born neonates were screened. If CMV PCR was positive in saliva at

birth, urine was tested before the 14th day of life. When positive PCR results were obtained after maternity discharge, parents were asked by phone to return for retesting of the neonate.

When cCMVI was confirmed, the neonate was enrolled in our standard cCMVI follow-up program as per our internal clinical protocol. In detail, a complete neonatal clinical exam was performed, including *fundus oculi* examination, auditory brainstem response, total serum bilirubin assay, assay of transaminases, complete blood cell count, and brain US, following international guidelines on point-of-care on neonatal US (23). After this assessment, cCMVI was considered "symptomatic" if one or more of the following signs were present and other causes were excluded: persistent thrombocytopenia, petechiae, hepatomegaly, raised transaminases or bilirubin, splenomegaly, birth weight lower than 5th percentile according to AUDIPOG curves (24), neurological involvement such as microcephaly lower than 5th percentile according to AUDIPOG curves (24), intracranial calcifications, ventriculomegaly, periventricular cysts (>2), chorioretinitis, sensorineural hearing loss (8,19,25). For infants with severe cCMVI disease, according to international consensus recommendations published in 2017 (8,26), valganciclovir was offered as soon as possible before the age of one month. For all infants (irrespective of clinical manifestations), follow-up included serial clinical evaluation and hearing assessments until they were 6 years old. **(Figure S1)**.

Gestational age at delivery and birth weight were collected prospectively for all newborns. For cCMVI newborns, additional information was collected from the mother's medical record: parity, maternal CMV serology, prenatal US and clinical examination at birth.

CMV serology in pregnant women

In our center, pregnant women are offered CMV serology screening as part of current management of pregnancy between 11 and 13^{+6} weeks of gestation (WG), and it is repeated at 20 WG if seronegative at first testing. For each cCMVI case, if maternal status for CMV was not known at birth, maternal infection was retrospectively characterized (PI or NPI) via the stored sera. In France, serum samples are collected at least in the first and third trimesters of pregnancy for serology testing (HIV, HBV…), and women seronegative for toxoplasmosis are tested monthly. All sera are stored for 1 year. CMV-IgG and CMV-IgM were measured in our laboratory with LIAISON XL (LXL, DiaSorin®, Saluggia, Italy), and CMV IgG avidity with LXL and/or VIDAS (bioMérieux®, Craponne, France) following the manufacturer's recommendations (27). CMV IgG avidity allowed primary infection during pregnancy to be excluded or confirmed, as previously described (27).

Sample collection

Saliva samples were collected at delivery (before first feeding) or at least before day 3 of life with a swab (Sigma Virocult®ENT) in the cheek. Samples were immediately stored at 4°C and PCR was performed within 3 days. If PCR was positive on the saliva sample, a urine sample was collected in the first 2 weeks of life in order to confirm or exclude cCMVI.

CMV DNA detection

For nucleic acid extraction, the automated system QIASymphony (Qiagen, Germantown, MD, USA) with the DSP virus/pathogen kit was used following the manufacturer's recommendations. For amplification and detection, the Rotor-Gene Q (Qiagen) with Artus® CMV QS-RGQ kit (Qiagen) was used, following the manufacturer's recommendations (28). The technique allows CMV DNA quantification at a threshold of 200 (2.3 log) copies/mL, but can also detect smaller amounts of DNA. All infants with at least one copy detected in saliva were tested using urine samples.

Evaluation of acceptability and feasibility of the screening

Acceptability of screening was evaluated by: *(1)* the percentage of parental opposition, and *(2)* a survey conducted among physicians and allied healthcare providers of our maternity unit in December 2020 (**Figure S2**). Answers were anonymously collected.

Feasibility was evaluated by the participation rate (percentage of newborns screened out of the total number of live births during the period in the maternity unit).

Statistical analysis

Birth prevalence of cCMVI was defined as the proportion of infected neonates among all live-born infants tested during the study period.

Statistical analysis was performed with RStudio software (version 1.4.110). Proportions were compared with the Chi2 test or Fisher test, as appropriate. Continuous variables were expressed as mean (standard deviation or median [interquartile range]) and then compared with Student, Mann-Whitney or Wilcoxon for paired samples tests, as appropriate. Receiver operating characteristic (ROC) analysis was used to evaluate the ability of CMV PCR in saliva to predict cCMVI. 95% confidence intervals (95% CI) were calculated and $p<0.05$ was considered to be statistically significant.

RESULTS

Performances of screening strategy and cCMVI prevalence

Figure 1 shows the flow chart of our study. During the study period, 15,649 infants were born alive in our maternity ward, and screening was offered to the parents of 15,356 newborns (98.1%). For the 293 other infants, saliva sample testing, although accepted, did not yield results or failed (broken vial, sample not labeled or lost). Exhaustivity of screening increased during the period, from 92.7% in 2016, to 99.5% in 2020 **(Table 1)**.

Among the 15,356 tested saliva samples, 290 (1.9%) were CMV PCR positive. cCMVI was confirmed, by positive CMV PCR on a urine sample collected before the 14th day of life, in 63 cases (true-positive cases, 0.4%). In 212 cases, cCMVI was excluded by a negative CMV PCR on the urine sample, and in 15 cases, the patient never returned to hospital for urine collection (excluded from further analysis). The false-positive rate of screening was therefore 1.4% (212/15,341). cCMVI prevalence in our population was 0.4% (95% CI 0.3-0.5) (63/15,341) **(Table 1)**.

In 75 cases (0.005 %), screening results were not available at the time of maternity discharge, and after they were obtained we had to retest the neonates: in 39 cases because of technical problems with the sample (inverted or unlabeled sample), in 36 cases because PCR was positive in saliva. All but one were finally negative on PCR urine testing. In 20/63 (31.7%) cCMVI neonates, screening was positive in saliva during hospital stay, and therefore urine was tested, but the results were obtained after discharge: parents were contacted by phone to be informed about cCMVI diagnosis and support.

Figure 2A shows the ROC analysis for prediction of cCMVI. The area under the ROC curve (AUC) was 0.97 95CI [0.93 – 1.0]. The best saliva CMV threshold for predicting cCMVI was 2.55 log (356) copies/mL 95CI [2.52 log – 3.18 log] with a sensitivity of 92% [84 – 97%] and a specificity of 98% [95 – 100%]. At this threshold, the positive predictive value was 90.5% 95CI [84.9 – 100%] and the negative predictive value was 97.6% [95.4 – 99.6%] in predicting cCMVI. As **Figure 2B** shows, saliva CMV viral load was significantly lower in non-infected neonates (median viral load: 1.57 log copies/mL, IQR [1.32 log – 1.77 log]) compared to cCMVI neonates (median viral load: 6.51 log copies/mL, IQR [4.32 log – 7.51 log]) (p=0.02). However, in 5/63 (7.9%) true-positive cases, saliva CMV viral load was lower than 2.55 log (356) copies/mL. In contrast, 6/212 (2.8%) false-positive cases had a saliva viral load greater than 2.55 log copies/mL (reaching 3.4 log copies/mL). For cCMVI neonates, median urine viral load was 6.19 log copies/mL IQR [5.07 log – 6.93 log] and median blood viral load was 3.37 log copies/mL IQR [2.73 log – 3.82 log] (p<0.001).

Characterization of maternal infection

The 63 infants with cCMVI were born to 62 women (one woman had twins). In 14 cases (22.5%), maternal seroconversion was known before birth. Retrospective collection of maternal serum samples allowed us

to characterize maternal infection in 40 extra cases (64.5%). Half of the pregnant women had NPI (31/62; 50%) whereas PI occurred during pregnancy for 23 women (23/62, 37.1%). The type of maternal infection remained unknown for 8 women (8/62, 12.9%) (**Table S1**). Focusing on PI during pregnancy, 14/23 (60.9%) were detected by systematic screening, and 9/23 (39.1%) were not diagnosed before delivery. For 8/9 patients, PI occurred late in pregnancy, during the second or third trimester (after systematic serology assay offered at 20th WG in seronegative women), and, for 1/9 patient, CMV PI occurred during the first trimester of pregnancy but was missed by screening. Finally, systematic antenatal serological screening allowed us to detect only 14/62 (22.6%) maternal infections that led to cCMVI. The other 48/62 maternal infections (77.4%) were not identified until neonatal screening.

Antenatal and neonatal features

Gestational age at birth and birth weights of cCMVI newborns compared to non-infected newborns were not significantly different (**Figure S3**).

For 24/63 infants, cCMVI could be suspected before or at birth (**Table 2**). Among them, 14/24 (58.3%) were born to mothers with known PI during pregnancy. Moreover, in 8/24 (33.3%), a prenatal US abnormality was detected: 4 had US features that led to amniocentesis and confirmation of cCMVI by PCR (1 hyperechogenic bowel, 1 multiple paraventricular cysts, 1 splenomegaly, and 1 hepatomegaly). In 4 other cases, intra-uterine growth restriction (IUGR) had been observed, but amniocentesis was not performed; in only 1 of them, cCMVI was suspected because systematic serological screening revealed maternal PI. Remarkably, all the infants with IUGR assessed by prenatal US (4/24) had a birthweight appropriate for gestational age, so 3 of them would probably not have been tested for CMV at birth without universal screening.

In 8/24 (33.3%) infants, clinical signs possibly due to cCMVI were observed at birth: 7 were small for gestational age (birth weight < 5th percentile), of which 5 had preserved head circumference, and none had abnormal antenatal signs. Another one had bilateral profound sensorineural hearing loss (detected by universal newborn hearing screening); his mother was known to have periconceptional seroconversion, and prenatal US revealed hepatomegaly (**Table 2**).

After specific neonatal assessment, 2 infants had multiple paraventricular cysts shown by brain US (detected antenatally in one case, and associated with hearing loss in the other one), and one had transient anomalous laboratory findings. Overall, among the 9/64 symptomatic infants, the 2 with multiple paraventricular cysts were enrolled in a course of antiviral medication.

Acceptability and feasibility of universal cCMVI screening

No parent refused the screening.

Table S2 shows results of a survey conducted in December 2020 among staff members, 72 of whom answered the questionnaire. Most healthcare workers (92.7%) found the salivary test easy or very easy to perform, and 91.4% reported that time devoted to sample collection was acceptable or very acceptable. Time spent obtaining informed consent was acceptable for 78.6% of the respondents, and 95.6% said the screening had no or a very small impact on the mother-to-child relationship.

Costs

The global cost of cCMVI screening for the French health care system is estimated at 41.23 €/neonate. This amount includes swabs, reagents, labor costs, shipping procedures, but also retesting on urine samples for positive saliva tests. A medico-economic analysis currently underway includes the overall costs of neonatal assessment and follow-up in childhood.

DISCUSSION

Main findings

Our study reports implementation of universal neonatal screening for cCMVI in a maternity unit, relying on CMV PCR in saliva. Among 15,341 tested neonates, 63 had cCMVI (birth prevalence of 0.4% 95CI [0.3 – 0.5]). In 50% of cases, maternal infection was an NPI. cCMVI was expected or suspected (because of known maternal PI, antenatal/neonatal signs) in 24/63 neonates (38%), and unexpected in 39/63 neonates (62%). The best CMV saliva threshold to predict cCMVI was 2.55 log (356) copies/mL 95CI [2.52 – 3.18 log] with an area under the ROC curve of 0.97.

Strengths and limitations

From 2016 to 2020, exhaustivity of the screening increased, reaching >99.5% of tested infants in the last two years. During this period, midwives and pediatricians improved screening procedures and awareness especially by checking CMV PCR results before discharge and recalling parents if the saliva test was forgotten during hospital stay. Over the study period, 98.1% of all live-born infants were tested. This high feasibility is an important criterion in ensuring the continuation of screening over time. We also evaluated the acceptability of screening by assessing parental acceptance (22). No parent refused the test, which means that cCMVI screening in saliva is highly acceptable.

One limitation of our study is the high number of false-positive results. Indeed, 212 infants unnecessarily underwent urine tests. We chose a sensitivity of 100% for the saliva test and used urine samples to check all positive saliva tests from 1 copy/mL. Retrospectively comparing viral load of true-positive *versus* false-

positive results, we calculated an optimal cut-off of 2.55 log copies/mL, with the best sensitivity and specificity. By reducing the false-positive rate, this threshold seems reasonable for optimizing screening.

We recognize that a positive CMV PCR on saliva may generate anxiety among parents as they wait for urine PCR results, as may the long auditory follow-up that infants with cCMVI must undergo, when no apparent symptoms are present (29). In our experience, parents rarely expressed anxiety or annoyance. Only qualified pediatricians should announce cCMVI before discharge to deliver detailed and reliable information, and hence limit parental anxiety. Our questionnaire identified the interval between sample collection and PCR results as the main limiting factor of screening. Unfortunately, we sometimes had to announce cCMVI over the phone, after discharge. However, this situation is now rarer and we are currently working with the laboratory to ensure that PCR results are always available within 3 days. Indeed, announcing cCMVI during hospital stay would allow us to provide parents with better support.

False-negatives were not evaluated: only positive saliva led to urine testing.

Interpretation

The birth prevalence of cCMVI in our study (0.4%) is similar to those reported in neighboring countries with the same maternal CMV seroprevalence, ie, from 0.2% to 0.7%. In a review based on 15 studies with 117,986 infants screened, cCMVI birth prevalence was 0.7% (30). In 2017, a French prospective neonatal screening study involving 11,715 consecutive newborns reported a 0.37% cCMVI birth prevalence (7). Puhakka *et al* reported a 0.2% cCMVI birth prevalence in a prospective cohort study that tested 19,868 infants around Helsinki (31). However, cCMVI birth prevalence in some French regions (especially those located in the Indian Ocean or the Americas) may be higher mainly because CMV seroprevalence in the general population is far higher (32). Consequently, in these regions most cCMVI are due to NPI, whereas in our study only half of cCMVI were due to NPI.

We found no significant variations in cCMVI birth prevalence over the years. Recently, early treatment with valaciclovir was reported to be effective in reducing the rate of fetal CMV infection after maternal PI acquired early in pregnancy (33). In our maternity unit, this therapeutic program was implemented in October 2020, after our study period, and did not influence birth prevalence. Moreover, valaciclovir can currently be offered only to mothers with known early PI, and it would have concerned only 7/63 (11.1%) mothers in our study. Anyway, as birth prevalence of cCMVI is quite low, a significant decrease might be observed only in a very large cohort.

Although CMV PCR in urine is the gold standard for cCMVI diagnosis, large prospective studies also show that CMV PCR in saliva performs well for screening purposes (7,8,19,34). False-positive cCMVI diagnosed in saliva could be an issue because CMV is frequently shed in vaginal secretions and breast milk, and

consequently contamination of the newborn's saliva could happen throughout the peripartum period (35,36). We observed 1.4% (212/15,341) of false-positive cases, and a positive predictive value of PCR in saliva of 22.9%. However, in the case of high viral load (>3 log), this rises to 96.8%. Our results therefore confirm previous observations that CMV PCR in saliva may be used for cCMVI screening, but cCMVI diagnosis requires confirmation on urine samples within two weeks following birth (19).

Our study highlights that in the absence of universal screening, most cases of cCMVI remain undiagnosed. Indeed, we report only 24/63 (38%) newborns with antenatal and/or neonatal signs possibly related to cCMVI, and/or born to mothers with PI diagnosed during pregnancy. Moreover, 32/63 (50.6%) cCMVI were due to maternal NPI and cannot be currently and reliably diagnosed (7,12). Hygiene measures, which have proven effective in preventing maternal PI (37), must therefore be applied to all pregnant women, not only seronegative ones, but also to women before pregnancy and to the other parent (2,37,38). Health professionals should be repeatedly trained on this point, because awareness of CMV infection remains low (39). Overall, in the absence of neonatal screening, we would have missed at least 39/63 (62%) cCMVI cases.

Universal neonatal screening leads to early diagnosis of cCMVI, and allows regular follow-up to detect delayed hearing loss, and benefit from early symptomatic intervention. With a 0.4% birth prevalence, cCMVI is estimated to be responsible for moderate to severe injury in 700 newborns each year in France, among which 288 are born asymptomatic. In France, pregnant women are mandatorily screened for four infectious diseases: toxoplasmosis, rubella, syphilis and hepatitis B (40). Additionally, mandatory neonatal screening is performed for five other diseases: phenylketonuria, congenital hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis and bilateral hearing impairment (41). The most frequent is congenital hypothyroidism, with 3.4 cases/10,000 births (about 272 infants each year), which is comparable to the estimated number of asymptomatic newborns who will develop cCMVI sequelae during childhood (42). The most common expected sequelae in asymptomatic infants with cCMVI is sensorineural hearing loss, which occurs in 5 to 15% of cases and is often delayed (43,44). We therefore expect that in our cohort 3 to 9 children will have hearing impairment. If universal neonatal screening were implemented, it would allow early detection and management of these children, whose effectiveness in improving language scores has been demonstrated (45). To investigate the natural history of cCMVI, and to assess whether these identified infants will indeed suffer from specific injury, follow-up of our cohort is ongoing and will be reported in a future publication.

CONCLUSION

Universal neonatal screening for cCMVI is currently not recommended by any public health authority (21). Our experience shows that it is, however, feasible, and highly acceptable to parents and health care providers, and that without this approach, 62% of cCMVI cases would have been missed.

Disclosure of interests

The authors have no competing interest to disclose.

Contribution to authorship

ELR and CVF led the implementation of the screening, co-conceived the study, analyzed and interpreted the data, co-drafted the first draft of the article and critically reviewed final drafts.

CPD analyzed and interpreted the data, did the statistical analysis, co-drafted the first draft of the article and critically reviewed the manuscript for important intellectual content.

LdG collected and analyzed the data of the survey, and critically reviewed the manuscript.

IT, AMRA and AGC critically reviewed the manuscript.

DDL and AB co-conceived the study and critically reviewed the manuscript for important intellectual content.

All authors agree with the manuscript's results and conclusions.

Ethics approval

Data collection and processing was approved by the Ethics and Research Committee in Obstetrics and Gynecology, (CEROG 2020-OBST-0902). An information letter was given to the expectant mothers and written informed consent was obtained from all of them.

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FIGURE LEGENDS

Figure 1: Flow chart of the study

Abbreviations: CMV, cytomegalovirus; PCR, polymerase chain reaction; cCMVI: congenital CMV infection.

Figure 2: Saliva viral load

A: ROC for predicting cCMV infection

Receiver operating curve (ROC) of saliva CMV viral load for prediction of congenital CMV infection. Area under the ROC curve (AUC) is equal to 0.97 95CI [0.93 – 1.0]. The best saliva CMV threshold to predict cCMVI is 356 (2.55 log) copies/mL 95CI [2.52 – 3.18 log] with a sensitivity of 92% [84 – 97%] and a specificity of 98% [95 – 100%]. At this threshold, the positive predictive value is 90.5% 95CI [84.9 – 100%] and the negative predictive value is 97.6% [95.4 – 99.6%].

B: Saliva viral load in true-positive *versus* false-positive cCMVI cases

cCMV cases: n=63. False-positive cases: n=212 (unconfirmed cases were excluded). Saliva viral load is expressed in log copies per milliliter.

*p=0.02. Saliva CMV viral load was significantly lower in non-infected neonates (median viral load=1.57 log copies/mL, IQR [1.32 log – 1.77 log]) compared to cCMV neonates (median viral load= 6.51 log copies/mL, IQR [4.32 log – 7.51 log])

TABLES

Table 1: Exhaustivity of the screening, and birth prevalence of cCMVI over the study period.

Exhaustivity: number of tested infants / number of live births

Year	Live births	Tested infants	Exhaustivity (%)	False positive	True positive	Birth prevalence (%)
2016	2,720	2,522	92.7	26	7	0.3
2017	3,160	3,123	98.8	50	14	0.4
2018	3,484	3,452	99.0	55	10	0.3
2019	3,510	3,499	99.6	53	18	0.5
2020	2,775	2,760	99.5	28	14	0.5
TOTAL	15,649	15,356	98.1	212 (1.4%)	63	0.4

Table 2: Signs related to cCMVI: maternal infection (if known), laboratory signs, ultrasound signs, and clinical signs observed in cCMVI cases.

Abbreviations: T1, T2, T3, respectively first, second and third trimester maternal primary infection detected during pregnancy; PC, periconceptional maternal primary infection detected during pregnancy; IUGR, intra-uterine growth restriction; AGA, appropriate weight for gestational age; SGA, small for gestational age; HC, head circumference; p, percentile

Patient	Known maternal primary infection during pregnancy	Prenatal ultrasound abnormality	Neonatal clinical sign	Abnormality at neonatal work-up
1	T2	0	0	0
2	T1	0	0	0
3	T1	0	0	0
4	T1	0	0	0
5	T2	0	0	0
6	T2	0	0	0
7	T2	0	0	0
8	T2	0	0	0
9	T2	0	0	0
10	T1	4 paraventricular cysts	0	5 paraventricular cysts
11	T1	Splenomegaly	0	0
12	T1	Hyperechogenic bowel	0	0
13	PC	Hepatomegaly	Profound bilateral hearing loss	4 paraventricular cysts
14	T2	IUGR	0 (AGA)	0
15	0	IUGR	0 (AGA)	0
16	0	IUGR	0 (AGA)	0
17	0	IUGR	0 (AGA)	0
18	0	0	SGA <1st p	0
19	0	0	SGA 2nd p	0

20	0	0	SGA<1st p and HC 5th p	Transient thrombocytopenia
21	0	0	SGA 4th p	0
22	0	0	SGA 3rd p and HC 2nd p	0
23	0	0	SGA 2nd p	0
24	0	0	SGA 1st p	0
Total	**14**	**8**	**8**	**3**