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Sequelae of congenital cytomegalovirus (cCMV) following maternal primary infection are limited to those acquired in the first trimester of pregnancy

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Summary

In this study, only children infected after a maternal primary infection in first trimester had sequelae at follow-up. For women with seroconversion in second or third trimester, the risk returns to the baseline associated with any pregnancy.

Abstract

Background: Relationship between gestational age at maternal primary infection and outcome in cCMV was based on small retrospective studies between 1980 and 2011. They reported sequelae in 32% and 15% following maternal primary infection in first and second or third trimester respectively. We aimed to revisit this relationship prospectively between 2011 and 2017 using accurate virological tools.

Methods: Women with primary infection and an infected child aged at least 1 year at the time of analysis. Accurate determination of the timing of primary infection was based upon serial measurement of IgM, IgG and IgG-avidity in sera collected at each trimester. Outcome was assessed according to a structured follow-up between birth and 48 months.

Results: 255 women and their 260 fetuses/neonates were included. Dating maternal infection was prospective in 86% of cases and retrospective in 14%. At a median follow-up of 24 months, the proportion of SNHL and/or neurologic sequelae were 32.4% (95%IC 23.72, 42.09) after maternal primary infection in first trimester, and 0 (0, 6.49) and 0 (0, 11.95) after infection in second and third trimester respectively ($p < .0001$).

Conclusion: These results suggest that CMV infection can be severe only when the virus hits the fetus in the embryonic or early fetal period. Recent guidelines recommend auditory follow-up for at least 5 years of all infected children. This raises parental anxiety and generates significant cost. We suggest that auditory and specialized neurologic follow-up may be recommended only to children infected from maternal infection in the first trimester.

Keywords: cytomegalovirus, congenital, first trimester, outcome

Introduction

Human cytomegalovirus (HCMV) is the most prevalent cause of congenital infection and a major cause of neurological disabilities with a worldwide prevalence of 7 cases per 1,000 live births (1). 20% of infected neonates endure long-term sequelae (2). Current knowledge on the relationship between gestational age at maternal primary infection and the risk of long-term sequelae in congenital CMV infection (cCMV) suggests an increased risk in the first trimester but with similar sequelae in 6 to 15% of neonates infected in second or third trimester. Maternal CMV primary infection is usually asymptomatic or with non-specific symptoms and its timing relies on serological investigation. The impact of gestational age at maternal primary CMV infection on outcome rests on cases diagnosed between 1980 and early 2000's (3–8). At that time, accurate serological timing of a silent primary CMV infection was difficult because IgG avidity assays were either not available or in their early stage of development. We therefore assessed the association between gestational age at maternal primary infection and outcome in a large recent cohort including cCMV cases between 2011 and 2017. Timing of maternal infection was accurately determined using recent serological tools and a standardized algorithm.

We show that sequelae at a median of 2 years of age are confined solely to cCMV following maternal primary infection in first trimester of pregnancy. This reminds of the risk associated with congenital rubella (9) although the prevalence of long-term sequelae is lower with cCMV. However, if congenital rubella hold a higher individual risk, the community burden of cCMV is higher because of its higher prevalence (10).

Population and Methods

Population

Pairs of women and fetuses/neonates were included when maternal infection could accurately be dated in first, second or third trimester. All children included were at least 1 year-old and were fully investigated and characterized as symptomatic or asymptomatic.

The cohort was built by pooling cCMV cases from 3 different studies including patients between 2011 and 2017. The Cymeval II (11), Cymepedia (12) and BiocCMV studies (Table S1). Ethics committee approved the 3 studies (2011-001610-34; 2013-A00213-42; 2016-14024) and written informed consent was obtained before inclusion.

These studies are registered in clinicaltrial.gov website under NCT01923636 (Cymepedia); NCT01651585 (Cymeval II) and NCT03090841 (BiocCMV).

Methods

Definitions of a symptomatic neonate and of severe or non-severe cases after termination of pregnancy (TOP) are given in supplementary data.

Determination of the timing of maternal primary CMV infection

Serological assessment was centralized in Necker Reference laboratory. CMV serology testing is not mandatory but often offered to pregnant women at 12 weeks' in France, systematic serology in the second and third trimester are less often done. Most of our cases had routine serology. In cases diagnosed only at birth, 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 easily be tested retrospectively.

CMV IgG and IgM antibodies were determined with LIAISON XL® CMV IgG II and IgM on (Diasorin, Antony, France). IgG avidity was measured by LIAISON® CMV IgG Avidity II and/or by VIDAS® CMV IgG avidity II (BioMerieux, Marcy L'Etoile, France) both assays have high sensitivities to detect primary infection (13,14).

The algorithm for CMV serology interpretation is in Figure 1. For all cases included in this study, maternal serum was tested in the first trimester. Cases with high IgG avidity in first trimester were considered as non-primary infection and were excluded from the study. Cases with seroconversion and/or positive IgG, positive IgM and low or intermediate IgG avidity in first trimester' sera, were considered as primary infection in first trimester. Cases with negative IgG and IgM in first trimester (at 12 to 14 weeks) were classified in either the second or third trimester groups depending on the date of seroconversion. Seroconversion timing was established by testing consecutive monthly sera when available. When not available, the determination of the onset of primary infection was based on IgG and IgM levels (high IgM with low or negative IgG are in favour of very recent primary infection) or avidity value (avidity below 10% is consistent with infection onset within the past 3 weeks) (12).

Follow-up protocol

All infected children were followed with the same protocol including visits at 4, 12, 18, 24, 36 and 48 months (Figure S1). Clinical examination was standardized assessing motor, cognitive, speech and psychological development according to age. Each one of these categories was graded between 1 and 4. Grade 1 was normal for age, grade 2 was slightly below normal, 3 and 4 were considered as neurological sequelae. Audiological assessment and presence of otitis media were recorded at each visit. Auditory Brainstem Responses (ABRs) or audiology was 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 later after otitis healing. Tympanostomy tubes were placed when indicated. Hearing was normal if the child could hear stimulus of between 0 and 20dB. SNHL was mild, moderate, severe and profound for detection of sound within 21 to 30 dB, 31 to 60db, 61 to 90 db and ≥ 91 dB respectively.

Vestibular functions were assessed in children with SNHL and/or delayed walking (15) (see supplementary).

Review of the literature (Table S2) (4–8).

Statistical analysis:

Between groups comparisons were done with Fischer exact tests. Follow-up was compared between groups by the Kruskal Wallis test and Clopper-Pearson methods were used to estimate the 95% exact confidence interval. Only p values <0.05 were considered as significant.

Results

Description of the population (Figure 2)

In 86% (224/260) of cases, maternal primary infection was known prenatally because serology was performed prospectively at the woman request (91%) or because of fetal ultrasound features in few cases (9%). CMV fetal infection was diagnosed by positive PCR in amniotic fluid in 56% (146/260) of the cases. 36/260 cases (14%) were diagnosed only postnatally because of neonatal symptoms mainly small for gestational age (SGA). The diagnosis was made postnatally in 12.6% (16/126), 10% (7/72) and 36% (13/36) in the 234 live born neonates infected following maternal infection in first, second and third trimester respectively. cCMV followed maternal primary infection in first, second and third trimester in 58% (152/260), 28% (72/260) and 14% (36/260) of cases respectively.

The 26 cases with TOP were all severe cases but one and all followed maternal primary infection in first trimester. Median follow-up duration of was 24 months and similar for each group. The proportion of children followed for at least 24 months were 57%, 61% and 56% in each group. 19/234 (8%) children were lost for follow-up.

Clinical neonatal status according to gestational age at maternal primary infection (Table 1)

SGA under the 10th centile was found in 14%, 19% and 36% of neonates infected after maternal infection in the first, second and third trimester respectively. SGA accounted for all symptoms recorded in babies infected in the third trimester. Cases only diagnosed postnatally (mainly because of SGA) were more frequent in the third trimester than in the total population. When the analysis was done after retrieving the 36 cases diagnosed postnatally, the proportion of SGA was 11% (12/110), 12% (8/65) and 17% (4/23) across first, second and third trimester respectively.

SNHL at birth was found only in the first trimester group. It was bilateral in 8 neonates: profound in 6, severe in 1 and moderate in 1. It was unilateral in 15 neonates: profound in 8, severe in 5 and moderate in 2.

Sequelae at follow-up according to gestational age at maternal primary infection (Table 2)

Among the 215 children followed-up, 23 had moderate SNHL at the last visit but in a well-documented context of acute or chronic otitis media. The audiological tests for these children were reported as uninterpretable. SNHL without otitis media (interpretable tests) was reported in 30 children: 16% (30/192) of all children and 27% (30/108) of children infected after maternal infection in first trimester. Late onset SNHL was found in 7/169 (4%) new children (169= 192 minus 23 children already known has having SNHL at birth) and was always unilateral. Late onset was diagnosed at 4-months, 12-months and 18-months visits in 3, 3 and 1 children respectively and all in the first trimester group with an incidence of 7/85 (8.2%) in this group (85= 108 minus 23 children with SNHL at birth). 8 children were death with profound bilateral SNHL and 22 children had unilateral SNHL: 13 profound, 5 severe and 4 moderate. Neurological sequelae were recorded in 15 children (see Table 2).

All SNHL and neurological sequelae occurred in children infected after maternal primary infection in first trimester (see maternal serologies in Table S3). The risk of neurological sequelae and/or SNHL was significantly linked to first trimester.

Among children with no SNHL and no neurological sequelae, a mild motor or speech delay was recorded in 16/180 (8.8%) mostly. The frequency of these borderline delays were not significantly different between groups with 9.5% (8/84), 5% (3/64) and 15% (5/32) following maternal infection in first, second and third trimester respectively ($p = 0.60$; $p=0.75$, $p=0.14$).

Sequelae at follow-up in the subgroup of children that were screened at birth

Between 2014 and 2016, 11,951 consecutive neonates were screened in our maternities (12). 44 babies were infected, among those 21 were infected after a primary infection and included in that study. 7 of them had been infected after a primary infection in first trimester. At 2 years old, 28% (2/7) showed profound unilateral SNHL. This rate of sequelae in the screened group is therefore similar to the one reported for the all population of the study.

Discussion

This cohort of 255 women with primary infection and their 260 infected fetuses/234 infected neonates is the largest reported to date with a thorough evaluation of symptoms at birth and outcome according to precise timing of maternal primary infection. Among the 234 infected neonates, 126, 72 and 36 were infected after maternal infection in first, second and third trimester respectively. This over-representation of cases from the first trimester is due to the fact that elective CMV serology is more often requested in first trimester of pregnancy. The main finding is that SNHL and neurological sequelae were only seen in children who were infected following a primary infection in the first trimester of pregnancy with a risk of neurologic and/or SNHL sequelae of 32.4% in this group.

Previous reports have shown that infection in the first trimester was a risk factor for early and/or late sequelae (3,4,6–8). However, they also reported neurological sequelae and SNHL in between 6 to 15% and 1 to 8% of children infected following maternal infection in second and third trimester respectively (3,4,8). The discrepancy between our study and previous ones is unlikely to be due to its size since our study included more infected children than the most empowered previous study (Table S2). However, other limitations of previous studies could explain this

discrepancy. First, cases included dated back between 1980 and early 2000's (3,4,6,8), when avidity assays, that are key to date CMV primary infection, either did not exist or their pitfalls and limitation were not well known (16,17). It is therefore possible that the timing of maternal primary infection was wrongly assigned in some cases of these older studies. In our study we overcame this difficulty by centralizing serology testing in one reference laboratory using the same assays and the same algorithm for interpretation throughout the study period (2011-2017). The second limitation that could have impacted the results of older studies is the interpretation of audiological tests. Hearing thresholds have been reported as fluctuating from normal to abnormal and back to normal in cCMV infected children of less than 2 years (4). We suspected that the occurrence of otitis media, very frequent at that age, could explain, at least partly, these fluctuations. In our study, the presence of otitis media was scrupulously reported at each hearing assessment in order to not wrongly attribute to cCMV, SNHL that would very likely be due to otitis. Indeed in our study, most children that presented with late onset moderate SNHL in the presence of otitis media had normal hearing when hearing was reassessed after the cure of the otitis. In previous studies reporting the occurrence of SNHL in children infected in second or third trimesters of pregnancy this issue was not raised except in one (4).

Moreover, 8% of children belonging to all 3 trimester groups were recorded as having motor or speech skill slightly below what would be expected for their age group. The prevalence of these mild delays was not significantly different in the 3 groups of children and was not different from an expected prevalence of around 10% in the general population (18).

In this study, no sequelae was seen when primary infection occurred after the first trimester of pregnancy. This suggests that CMV infection can be severe only when the virus hits the fetus in the embryonic or early fetal period. The structure of the inner ear is formed by week 10 and

sensory hair cells are present by week 12, this probably explains why SNHL only develops after maternal infection of first trimester (19). A similar epidemiology is documented for congenital rubella with no sequelae seen when maternal infection occurs after the 16th week; the description of SNHL in rubella maternal infection of the early second trimester might be explained by uncertainty in dating the pregnancy back in these days when ultrasound was not routinely available (9). However, in congenital rubella, infected fetuses following first trimester maternal infection develop sequelae in up to 90% of cases (9). In our study and others, the risk of sequelae after maternal infection in the first trimester was around 30% and the phenotype of these sequelae was highly diverse ranging from severe mental and motor sequelae to isolated unilateral SNHL. The variability of the consequences of primary maternal infections occurring at the same period is not understood. This suggests that the genetic background of the mother or of the fetus might play a role in the defence against fetal infection. Another explanation could be that in some cases even if the primary infection occurs before 14 weeks, fetal infection is delayed up to the second trimester due to placental role of barrier and reservoir. The median time between maternal and fetal infection is around 6 weeks (20) but longer time intervals have also been reported (21) .

In the European population about half of cCMV would follow a non-primary infection mainly by re-infection (12,22,23) with a related outcome as severe as that following primary infection (4,12,24,25). The pathophysiology of non-primary infections is largely unknown but it could be speculated from our results that only those occurring in the first trimester could lead to sequelae. However, since the timing of non-primary infection is practically impossible to achieve, the results from our study could not apply to non-primary infections.

Our results may have major implications for counselling pregnant women with primary infection and for parents dealing with an infected newborn. Women with a primary infection in first

trimester of pregnancy should be aware of the likelihood of sequelae if the fetus is infected. This pleads for proactive counselling of these cases as well as CMV serological screening in first trimester and amniocentesis following early maternal infection. Specific follow-up of infected fetuses in a reference center for prognostic evaluation seems appropriate (26). On the contrary, women with seroconversion in the second or third trimester could be reassured on the outcome of fetal infection, although, the risk of SGA should not be ignored. Infected neonates should be evaluated differently accordingly to the timing of maternal infection. Neonates born to mothers infected in first trimester should benefit from specialized follow-up to assess neurological development and hearing capacity at least up to 5 years of age. Neonates born to mothers infected in second or third trimester should probably not undergo specialized audiological and neurological follow-up. This could lead to change of the recently published recommendations to follow all infected neonates with audiological testing at 6-months interval for the first 3 years of life and then annually (27).

The strength of our study is the size of a well-documented cohort with centralized assessment of the timing of maternal primary infection based upon accurate serological assays and standardized algorithm in the follow-up of infected children. One of the limitations of the study is the length of follow-up with a median of only 24 months. Late-onset SNHL was reported up to the adolescence, with a median age between 33 and 44 months in only one study (28) while in all other studies, most cases were diagnosed by the age of 24 months (25,29). In a case-control study by Lanzieri et al, 13% and 14% of asymptomatic infected children had SNHL by the age of 2 and 5 years respectively. Moreover, the risk of developing SNHL after the age of 5 was not different in infected and uninfected children (29). Finally, investigators from a study with a follow-up longer than 5 years, reported that all significant sequelae were seen before the age of 1 (25).

Another limitation of our study is the absence of a control group of uninfected children to ensure that all sequelae seen in children infected after maternal infection in first trimester are all linked to cCMV.

In conclusion, our results suggest that the potential severity of fetal infection is restricted to maternal primary infection in the first trimester of pregnancy. This should encourage screening before 15 weeks of gestation using accurate and standardized virological methods. Furthermore, prolonged follow-up of audiological and neurological functions is likely to be justified only in infected infants following primary maternal infection before 14 weeks as well as in infants infected following non-primary maternal infection since the timing of these infections is not feasible.

Authors Contribution: Marianne Leruez-Ville (Virologist), Laurence Buissieres (Methodologist), Idir Ghout (Statistician), Jean-Francois Magny (Pediatrician), Marine Parodi (Otologist) and Yves Ville (Obstetrician) designed the study. All co-authors contributed to the data collection. Tiffany Guilleminot (Technician) performed the serology tests. Valentine Faure-Bardon (Obstetrician), Idir Ghout and Marianne Leruez-Ville analysed the data. Yves Ville, Marianne Leruez-Ville and Idir Ghout 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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Conflict of interest :

MLV reports travel fees from live By GI Events, a lecture honorarium from Siemens, and other fees from Abbott and BioMérieux, outside the submitted work. YV reports travels fees from Ferring SAS and Siemens Healthcare SAS, outside the submitted work. Others authors declare no competing interests relevant to this work or outside this work.

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Table 1: Clinical status of infected neonates at birth according to gestational age at maternal CMV primary infection

(1) First trimester was compared to second trimester. (2) First trimester was compared to third

	First trimester (<14 weeks) N=126	Second trimester (≥14 and <28 weeks) N=72	Third trimester (≥28 weeks) N=36	P(1)	P(2)
Symptomatic—n (%)	41 (32.5%)	16 (22.2%)	13 (36.1%)	0.168	0.841
SNHL—n (%)	23 (18.3%)	0	0	< 0.001	0.013
Isolated SGA—n (%)	11 (8.7%)	11 (15.3%)	13 (36.1%)	0.240	< 0.001
Total SGA—n (%)	18 (14.3%)	14 (19.4%)	13 (36.1%)	0.454	0.007
Other*—n (%)	6 (4.8%)	3 (4.2%)	0	> 0.999	0.340

trimester.

* One or more of the following: hepatosplenomegaly, petechia, retinitis, abnormalities of laboratory results but without SNHL

SGA= small for gestational age

Table 2: Sequelae at follow-up according to gestational age at maternal CMV primary infection

	First trimester (<14 weeks) N=119	Second trimester (≥ 14 and <28 weeks) N=64	Third trimester (≥ 28 weeks) N=32	P(1)	P(2)
Neurologic sequelae*	15 (12.6%) 95% CI (7.23,19.64)	0 95% CI (0.5.6)	0 95% CI (0.10.89)	0.007	0.041
SHNL **	30/108 (27.7%) 95% CI (19.59,37.22)	0/55 (0%) 95% CI (0,6.49)	0/29 (0%) 95% CI (0,11.94)	$<.0001$	0.003
Any sequelae **	35/108 (32.4%) 95% CI (23.72,42.09)	0/55 (0%) 95% CI (0,6.49)	0/29 (0%) 95% CI(0,11.95)	$<.0001$	0.001

Data are n (%) or n/N (%) if missing data.

(1) First trimester was compared to second trimester. (2) First trimester was compared to third trimester.

*11 with vestibular disorders, 1 with severe global development retardation, 1 with arm monoplegia, 1 with severe autistic presentation, 1 with spastic diplegia

**11, 9 and 3 children had an uninterpretable audiological test at the last visit because of the presence of otitis media in the first trimester group, second trimester group and the third trimester group respectively

SHNL: SensoriNeural Hearing Loss

Figures' legends

Figure 1: Algorithm for the determination of the timing of maternal infection

Figure 2: Flowchart of the study

*Cymeval II, Cymepedia and BiocCMV studies. Among the 349 women/fetuses/neonates included in the Cymeval II, Cymepedia and BiocCMV studies, 260 fetuses/neonates of 255 women met the inclusion criteria.

**There were 5 twin pregnancies

Cases that were not included were 50 cases of non-primary maternal infections, 27 cases for which pregnancy serum samples were not available to ascertain the date of seroconversion or with an uncompleted assessment at birth or at TOP and 17 children under the age of 1 at the time of analysis.

The outcome of the 260 (152+72+36) cases was 26 TOP and 234 live-born neonates (126+72+36). Among the 234 live born babies, 215 (119 +64+32) were followed up for at least 2 years.

***The median [IQR] follow-up was 24[16-36], 24[12-35] and 24[12-24] months in the first trimester group, second trimester group and third trimester group respectively. The proportions of children lost for follow-up were not different in the 3 groups ($p=0.253$). TI= trimester of infection; TOP= termination of pregnancy.

Figure 1

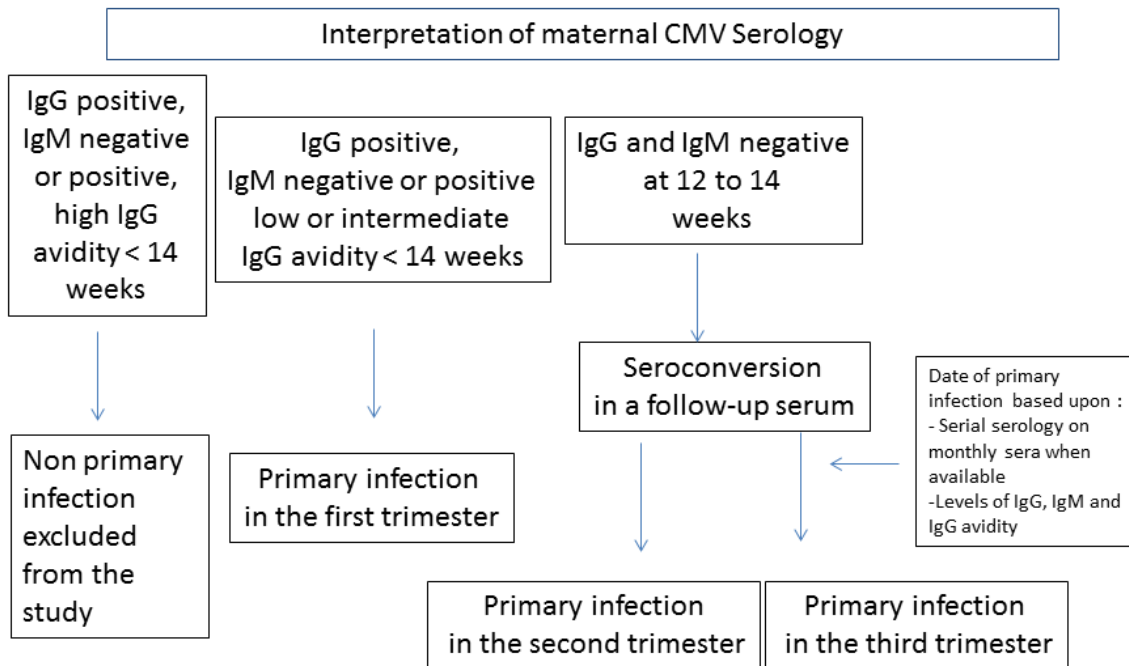


Figure 2

