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MAJOR ARTICLE

Sequelae of Congenital Cytomegalovirus Following Maternal Primary Infections Are Limited to Those Acquired in the First Trimester of Pregnancy

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(See the Editorial Commentary by Pass on pages 1533–4.)

Background. The known relationship between the gestational age at maternal primary infection an the outcome of congenital CMV is based on small, retrospective studies conducted between 1980 and 2011. They reported that 32% and 15% of cases had sequelae following a maternal primary infection in the first and second or the third trimester, respectively. We aimed to revisit this relationship prospectively between 2011 and 2017, using accurate virological tools.

Methods. We collected data on women with a primary infection and an infected child aged at least 1 year at the time of analysis. An accurate determination of the timing of the primary infection was based upon serial measurements of immunoglobulin (Ig) M and IgG and on IgG avidity in sera collected at each trimester. The case outcome was assessed according to a structured follow-up between birth and 48 months.

Results. We included 255 women and their 260 fetuses/neonates. The dating of the maternal infection was prospective in 86% of cases and retrospective in 14%. At a median follow-up of 24 months, the proportion of sensorineural hearing loss and/or neurologic sequelae were 32.4% (95% confidence interval [CI] 23.72–42.09) after a maternal primary infection in the first trimester, 0 (95% CI 0–6.49) after an infection in the second trimester, and 0 (95% CI 0–11.95) after an infection in the third trimester ($P < .0001$).

Conclusions. These results suggest that a cytomegalovirus infection can be severe only when the virus hits the fetus in the embryonic or early fetal period. Recent guidelines recommend auditory follow-ups for at least 5 years for all infected children. This raises parental anxiety and generates significant costs. We suggest that auditory and specialized neurologic follow-ups may be recommended only in cases of a maternal infection in the first trimester.

Keywords. cytomegalovirus; congenital; first trimester; outcome.

Human cytomegalovirus (CMV) is the most prevalent cause of congenital infections and a major cause of neurological disabilities, with a worldwide prevalence of 7 cases per 1000 live births [1]. Of infected neonates, 20% endure long-term sequelae [2]. The current knowledge of the relationship between the gestational age at a maternal primary infection and the risk of long-term sequelae in congenital CMV (cCMV) infections suggests an increased risk in the first trimester, but with similar sequelae in 6 to 15% of neonates infected in the second or third trimester. A maternal CMV primary infection is usually asymptomatic or has non-specific symptoms, and determining its timing relies on serological investigation. Our knowledge of the impact of the gestational age at a maternal primary CMV infection on the outcome rests on cases diagnosed between 1980 and the early 2000s [3–8]. At that time, the accurate serological determination of the timing of a silent primary CMV infection was difficult, because immunoglobulin G (IgG) avidity assays were either not available or in the early stage of development. We therefore assessed the association between gestational age at a maternal primary infection and outcomes in a large recent cohort that includes cCMV cases between 2011 and 2017. The timing of maternal infections were accurately determined using recent serological tools and a standardized algorithm.

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We show that sequelae at a median of 2 years of age are confined solely to cCMVs following maternal primary infections in the first trimester of pregnancy. This is reminiscent of the risks associated with congenital rubella [9], although the prevalence of long-term sequelae is lower with cCMV. However, if congenital rubella holds 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 the maternal infection could accurately be dated in the first, second, or third trimester. All children included were at least 1 year old; they were fully investigated and were characterized as either symptomatic or asymptomatic.

The cohort was built by pooling 3 different studies of cCMV cases between 2011 and 2017: the Cymeval II [11], Cymepedia [12], and BiocCMV studies (Supplementary Table S1). Ethics committees 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 the clinicaltrial.gov website as NCT01923636 (Cymepedia), NCT01651585 (Cymeval II), and NCT03090841 (BiocCMV).

METHODS

Definitions of a symptomatic neonate and of severe or non-severe cases after the termination of pregnancy are given in the Supplementary Data.

Determination of the Timing of Maternal Primary Cytomegalovirus Infection

The serological assessments were centralized in the Necker Reference laboratory. CMV serology testing is not mandatory, but is often offered to pregnant women at 12 weeks in France; systematic serology in the second and third trimesters 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 the first and third trimesters of pregnancy for various serology tests. 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 infections [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 the first trimester were considered as non-primary infections 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 infections in the first trimester. Cases with negative IgG and IgM levels in the 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

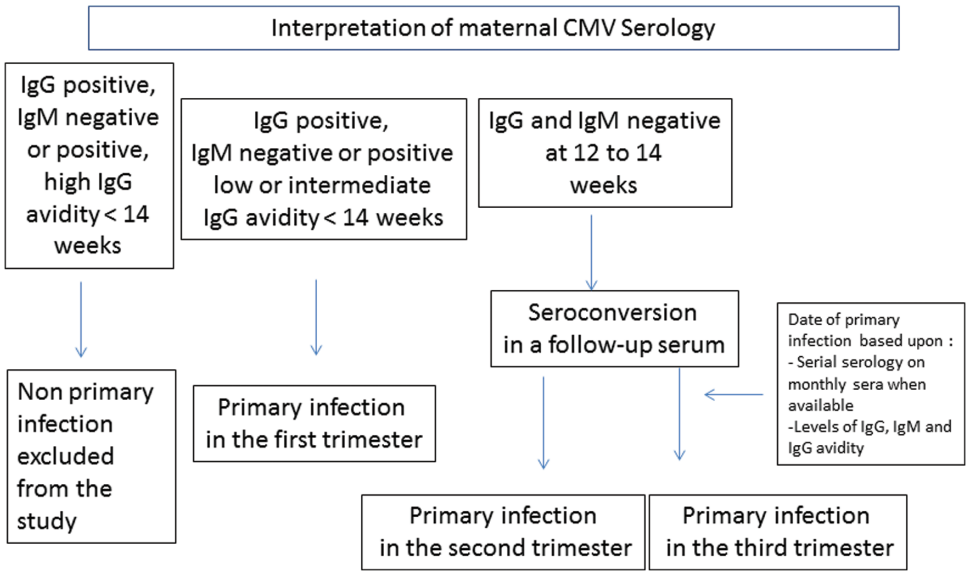

Figure 1. Algorithm for the determination of the timing of maternal infection. Abbreviations: CMV, cytomegalovirus; Ig, immunoglobulin.

the primary infection was based on IgG and IgM levels (high IgM levels with low or negative IgG levels are in favor of very recent primary infections) or avidity values (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 of age (Supplementary Figure S1). Clinical examination was standardized, assessing motor, cognitive, speech, and psychological development according to age. Each of these categories was graded between 1 and 4, where grade 1 was normal for age, grade 2 was slightly below normal, and grades 3 and 4 were considered as neurological sequelae. Audiological assessments and the presence of otitis media were recorded at each visit. Auditory brainstem responses were measured or audiology was done, depending on the child's age. Conductive sensorineural hearing loss (SNHL) with the presence of otitis media with effusion was considered non-interpretable and the testing was repeated 4 to 6 months later, after otitis healing. Transtympanic drains were placed when indicated. Hearing was considered normal if the child could hear a stimulus of between 0 and 20 dB. SNHL was mild, moderate, severe, and profound for the 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 [15] (see Supplementary Material).

A review of the literature is available in Supplementary Table S2 [4–8].

Statistical Analysis

Between-group 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 intervals. Only P values < .05 were considered as significant.

RESULTS

Description of the Population

In 86% (224/260) of cases, the maternal primary infection was known prenatally because serology was performed prospectively at the woman's request (91%) or because of fetal ultrasound features (9%). CMV fetal infections were diagnosed by positive PCRs in amniotic fluid in 56% (146/260) of the cases. In 14% of the cases (36/260), CMV infections were diagnosed only postnatally and because of neonatal symptoms: mainly, the infant being small for their gestational age (SGA). The diagnosis was made postnatally in 12.6% (16/126), 10% (7/72), and 36% (13/36) of the 234 live-born neonates infected following maternal infections in the first, second, and third trimesters, respectively. A cCMV infection followed the maternal primary infection in the first, second, and third trimester in 58% (152/260), 28% (72/260), and 14% (36/260) of cases, respectively. A flowchart of the study is available in Figure 2.

All but 1 of the 26 cases with termination of pregnancy were severe cases, and all followed a maternal primary infection in the first trimester. The median follow-up duration of was 24 months, and was similar for each group. The proportion of children followed for at least 24 months were 57%, 61%, and 56% of those with an infection in the first, second, and third trimester, respectively. Of 234 children, 19 (8%) were lost to follow-up.

Clinical Neonatal Status, According to Gestational Age at Maternal Primary Infection

SGA infants under the 10th percentile were found in 14%, 19%, and 36% of neonates infected after maternal infection in the first, second, and third trimester, respectively (Table 1). Being SGA accounted for all symptoms recorded in babies infected in the third trimester. Cases only diagnosed postnatally (mainly because of being 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 proportions of SGA infants were 11% (12/110), 12% (8/65), and 17% (4/23) across the first, second, and third trimesters, 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

Among the 215 children with follow-ups, 23 had moderate SNHL at the last visit, but in a well-documented context of acute or chronic otitis media (Table 2). The audiological tests for these children were reported as uninterpretable. SNHL without otitis media (interpretable test) was reported in 30 children: 16% (30/192) of all children and 27% (30/108) of children infected after a maternal infection in the first trimester. Late-onset SNHL was found in 7/169 children (4%; 169 = 192 minus 23 children already known to have SNHL at birth), and was always unilateral. Late onset was diagnosed at 4-month, 12-month, and 18-month visits in 3, 3, and 1 children, respectively, all of which were in the first-trimester group, with an incidence of 7/85 (8.2%; 85 = 108 minus 23 children with SNHL at birth) in this group. There were 8 children diagnosed with profound bilateral SNHL and 22 children with 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 a maternal primary infection in the first trimester (see maternal serologies in Supplementary Table S3). The risk of neurological sequelae and/or SNHL was significantly linked to the first trimester.

Among the 180 children with no SNHL and no neurological sequelae, 16 (8.8%) had a mild motor or speech delay recorded. The frequency of these borderline delays were not significantly

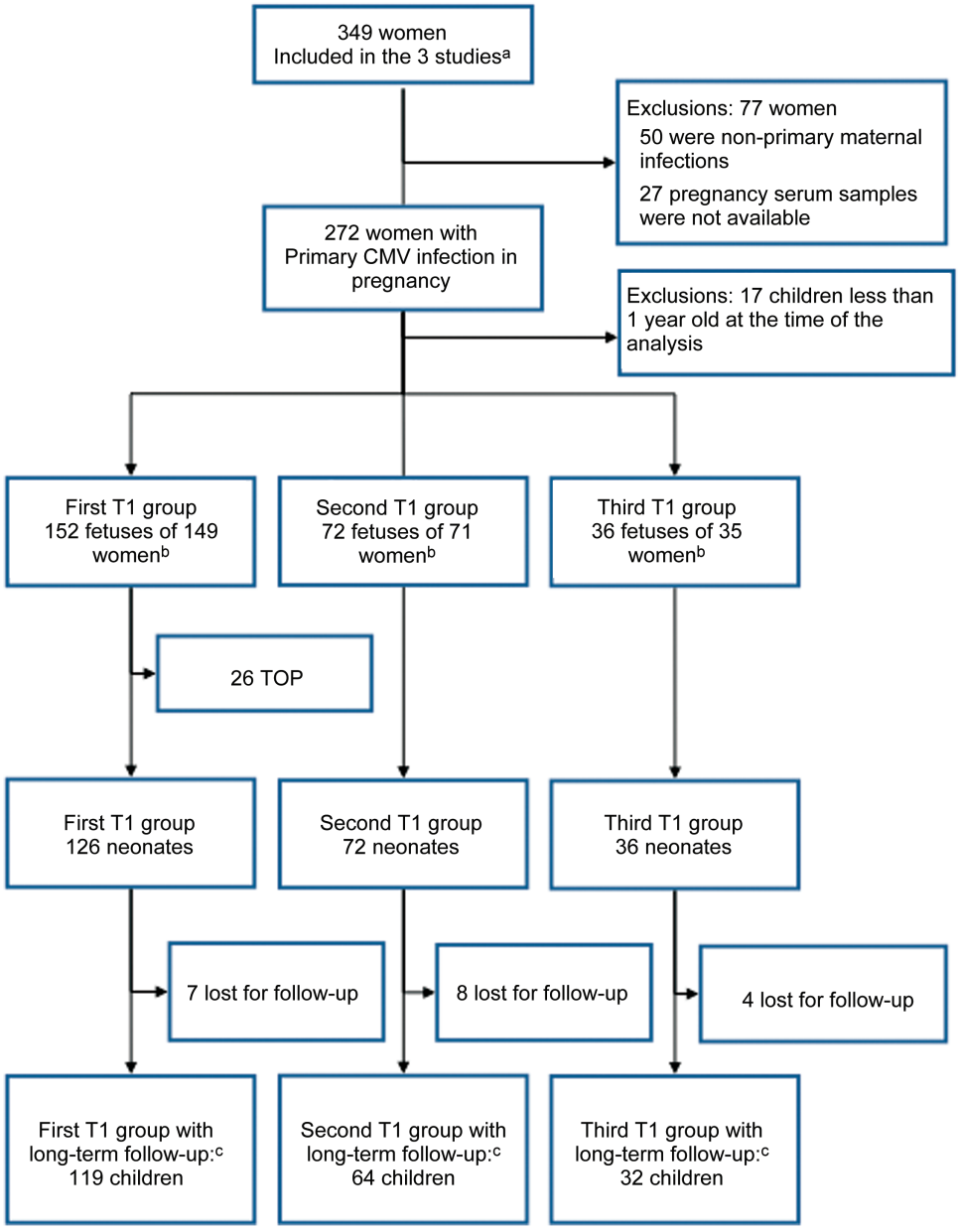

Figure 2. Flowchart of the study. Abbreviations: CMV, cytomegalovirus; TI, trimester of infection; TOP, termination of pregnancy. [a]Cymeval II, Cymepedia, and BiocCMV studies. Among the 349 women/fetuses/neonates included in the Cymeval II, Cymepedia, and BiocCMV studies, 260 fetuses/neonates of 255 women met the inclusion criteria. [b]There were 5 twin pregnancies. The cases that were not included were 50 cases of nonprimary 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 for at least 2 years. [c]The median (interquartile range) follow-ups were 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 ($P = .253$).

different between groups, with 9.5% (8/84; $P = .60$), 5% (3/64; $P = .75$), and 15% (5/32; $P = .14$) following maternal infections in the first, second, and third trimesters, respectively.

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 maternity wards [12]. There were 44 babies infected; among those, 21 were infected after a primary infection, and 7 of them had been infected after a primary infection in the 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 rate reported for the overall population of the study.

DISCUSSION

This cohort of 255 women with primary infections and their 260 infected fetuses/234 infected neonates is the largest

Table 1. Clinical Status of Infected Neonates at Birth, According to Gestational Age at Maternal Cytomegalovirus Primary Infection

	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%)	.168	.841
SNHL—n (%)	23 (18.3%)	0	0	<.001	.013
Isolated SGA—n (%)	11 (8.7%)	11 (15.3%)	13 (36.1%)	.240	<.001
Total SGA—n (%)	18 (14.3%)	14 (19.4%)	13 (36.1%)	.454	.007
Other[a]—n (%)	6 (4.8%)	3 (4.2%)	0	>.999	.340

For *P*(1), the first trimester was compared to the second trimester. For *P*(2), the first trimester was compared to the third trimester.

Abbreviations: SGA, small for gestational age; SNHL, sensorineural hearing loss.

[a]Other indicates 1 or more of the following: hepatosplenomegaly, petechia, retinitis, or abnormalities of laboratory results but without SNHL.

reported to date, with a thorough evaluation of symptoms at birth and of outcomes, according to the precise timing of the maternal primary infection. Among the 234 infected neonates, 126, 72, and 36 were infected after a maternal infection in the first, second, and third trimester, respectively. This overrepresentation of cases from the first trimester is due to the fact that elective CMV serology is more often requested in the 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 sequelea of 32.4% in this group.

Previous reports have shown that infection in the first trimester is a risk factor for early and/or late sequelae [3, 4, 6–8]. However, they also reported neurological sequelae and SNHL in 6–15% and 1–8% of children infected following a maternal infection in the second or third trimester, respectively [3, 4, 8]. The discrepancy between our study and previous studies is unlikely to be due to size, since our study included more infected children than the most empowered previous study (Supplementary Table S2). However, other limitations of previous studies could explain this discrepancy. First, the cases included dated back to between 1980 and the early 2000s [3, 4, 6, 8], when avidity assays, which are key to dating CMV primary infections, either did not exist or their pitfalls and limitations were not well known [16, 17]. It is, therefore, possible that the timing of maternal primary infections were wrongly assigned in some cases of these older studies. In our study, we overcame this difficulty by centralizing serology testing in 1 reference laboratory and 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 aged 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 an otitis-related SNHL to cCMV. Indeed in our study, most children that presented with late-onset, moderate SNHL in the presence of otitis media had normal hearing when their hearing was reassessed after the cure of the otitis. Except in 1 instance [4], previous studies reporting the occurrence of SNHL in children infected in the second or third trimesters of pregnancy did not raise this issue.

Moreover, 8% of children belonging to all 3 trimester groups were recorded as having motor or speech skills slightly below what would be expected for their age group. The prevalences of these mild delays were not significantly different in the 3 groups

Table 2. Sequelae at Follow-up, According to Gestational Age at Maternal Cytomegalovirus 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[a]	15 (12.6%) 95% CI (7.23–19.64)	0 95% CI (0–5.6)	0 95% CI (0–10.89)	.007	.041
SNHL[b]	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	.003
Any sequelae[b]	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	.001

Data are n (%) or n/N (%), if missing data. For *P*(1), the first trimester was compared to the second trimester. For *P*(2), the first trimester was compared to the third trimester.

Abbreviations: CI, confidence interval; SNHL, SensoriNeural Hearing Loss

[a]There were 11 cases with vestibular disorders, 1 with severe global development retardation, 1 with arm monoplegia, 1 with severe autistic presentation, and 1 with spastic diplegia.

[b]There were 11, 9, and 3 children media in the first trimester group, second trimester group, and third trimester group, respectively, who had an uninterpretable audiological test at their last visit because of the presence of otitis.

of children and were not different from an expected prevalence of around 10% in the general population [18].

In this study, no sequelae were seen when the 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 a maternal infection of the first trimester [19]. A similar epidemiology is documented for congenital rubella, with no sequelea seen when maternal infection occurs after the 16th week; the description of SNHL in rubella maternal infections of the early second trimester might be explained by the uncertainty in dating the pregnancy back in the days when ultrasounds were not routinely available [9]. However, in congenital rubella, infected fetuses following first-trimester maternal infections developed sequelae in up to 90% of cases [9]. In our study and others, the risk of sequelae after a 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 defense against a 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 the placental role as a barrier and reservoir. The median time between maternal and fetal infections 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 reinfection [12, 22, 23], with a related outcome as severe as that following a 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 a 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 infections and for parents dealing with infected newborns. Women with a primary infection in the first trimester of pregnancy should be aware of the likelihood of sequelae if the fetus is infected. This pleads for the proactive counselling of these cases, as well as CMV serological screening in the first trimester and amniocentesis following an early maternal infection. Specific follow-ups of infected fetuses in a reference center for prognostic evaluation seems appropriate [26]. In comparison, women with seroconversion in the second or third trimester could be reassured on the likely outcome of a fetal infection, although the risk of an infant being SGA should not be ignored. Infected neonates should be evaluated differently accordingly to the timing of the maternal infection. Neonates born to mothers infected in the first trimester should benefit from specialized follow-ups to assess their neurological development and hearing capacity, at least up to 5 years of age. Neonates born to mothers infected in the second or third trimester should probably not undergo specialized audiological and neurological follow-ups. This could lead to changes in the recently published recommendations to follow all infected neonates, with audiological testing at 6-month intervals for the first 3 years of life and then annually [27].

The strength of our study is the size of the well-documented cohort, with the centralized assessment of the timing of maternal primary infections, based upon accurate serological assays and standardized algorithms in the follow-ups of infected children. A limitation of the study is the length of follow-ups, with a median of only 24 months. Late-onset SNHL has been reported up to adolescence, with a median age between 33 and 44 months in only 1 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 [29], 13% and 14% of asymptomatic, infected children had SNHL by the age of 2 or 5 years, respectively. Moreover, the risks of developing SNHL after the age of 5 were 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 are linked to cCMV.

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

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. L.-V. (virologist), L. B. (methodologist), I. G. (statistician), J.-F. M. (pediatrician), M. P. (otologist), and Y. V. (obstetrician) designed the study. All coauthors contributed to the data collection. T. G. (technician) performed the serology tests. V. F.-B. (obstetrician), I. G., and M. L.-V. analyzed the data. Y. V., M. L.-V., and I. G. attest to the data and

analysis. M. L.-V. and Y. V. wrote the paper, with input from the coauthors. All authors agreed to publish the paper.

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