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Title: Clinical value of serial quantitative analysis of cytomegalovirus DNA in blood and saliva over the first 24 months of life congenital infection: the French Cymepedia Cohort

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Conflict of interest

MLV reports non-financial support from BioMérieux, non-financial support from Abbott, non-financial support from Diasorin 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. The other authors declare no conflict of interest.

Running title : Viral load over time in congenital CMV

Previous Presentations: Portions of this study were presented as a <poster/abstract> during the Congress CMV2021, October <dates>, 2021, Rome, Italy.

Abstract (253)

Objective: To evaluate cytomegalovirus (CMV) viral load dynamics in blood and saliva during the first two years of life in symptomatic and asymptomatic infected infants and to identify whether these kinetics could have practical clinical implications.

Study design: The Cymepedia cohort prospectively included 256 congenitally infected neonates followed for two years. Whole blood and saliva were collected at inclusion, months 4 and 12, and saliva at months 18 and 24. Real-time CMV PCR was performed, results expressed as \log_{10} IU/mL in blood and in copies/mL in saliva.

Results: Viral load in saliva progressively decreased from 7.5 \log_{10} at birth to 3.3 \log_{10} at month 24. CMV PCR in saliva was positive in 100% and 96% of infants at 6 and 12 months, respectively. In the first month of life, neonatal saliva viral load $<5 \log_{10}$ was related to a late CMV transplacental passage. Detection in blood was positive in 92% (147/159) of neonates in the first month of life. No viral load threshold values in blood or saliva could be associated with a high risk of sequelae. Neonatal blood viral load $<3 \log_{10}$ IU/ml had a 100% negative predictive value (NPV) for long-term sequelae.

Conclusions: Viral loads in blood and saliva by CMV PCR testing in congenital infection fall over the first 24 months. In this study of infants affected mainly after primary maternal infection during pregnancy, all salivary samples were positive in the first 6 months of life and sequelae were not seen in infants with neonatal blood viral load $<3 \log_{10}$ IU/mL.

Cytomegalovirus infection is the most frequent congenital infection, with a birth prevalence of around 0.7% (1). Congenital CMV is a frequent cause of sensorineural hearing loss and neurological disabilities, with about 20% of infected neonates developing long-term sequelae (1).

CMV PCR performed on a saliva sample is a reliable tool for diagnosing congenital infection at birth (2). However, the evolution of CMV DNA excretion in saliva in the first two years of age and its association with prognosis is poorly known. CMV PCR in blood is not recommended for the neonatal diagnosis of congenital CMV because of its lack of sensitivity. . There are limited and conflicting data on whether CMV viral load in blood at birth or later reflects disease severity among symptomatically and asymptotically infected infants and could be used as an indicator of prognosis.

The French multicenter Cymepedia cohort includes symptomatic and asymptomatic congenitally infected neonates. Within this cohort, samples of whole blood and saliva were prospectively collected at different times over the two first years of life. This constitutes a unique dataset of saliva and blood viral load in symptomatic and asymptomatic infants. The current study explored the kinetics of viral load in blood and saliva from birth to 2 years of age and its correlation with disease severity. The objective was to demonstrate the potential clinical utility of viral load determination in both compartments for the diagnosis and prognosis workup of infected infants.

Materials 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 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 parents declined inclusion. The cohort was composed of a total of 256 neonates.

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

Follow-up:

All infected children were followed with the same protocol, including visits at inclusion, 4, 12, 18, and 24 months of age. Clinical examination was standardized to assess motor, cognitive, speech, and psychological development according to age as previously described (3). Audiological assessment and the presence of otitis media were recorded at each visit. A Brunet-Lezine test was done at months 12 and 24 to evaluate the early childhood psychomotor development scale covering four areas of neurodevelopment: movement and posture, coordination, language, and sociability (4). Auditory Brainstem Responses (ABRs) or audiology tests were performed depending on the child's age. Conductive SNHL (Sensorineural Hearing Loss) with the presence of otitis media with effusion was considered non-interpretable, and testing was repeated 4 to 6 months after resolution of otitis media. Transtympanic drains were placed when indicated. Hearing was considered to be normal if the child could hear stimuli between 0 and 20dB. SNHL was mild, moderate, severe, and profound for detecting 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 (3).

Whole blood and saliva samples were collected at inclusion, second and third visits (month 4 and month 12) for the entire population. In 2015, we changed the protocol to collect saliva samples at the fourth and fifth visits (months 18 and 24). Therefore, only about half the population had saliva samples collected at these two last visits.

Classification of cases:

Children with any of the following sequelae were classified into the “sequelae” group: hearing loss (bilateral or unilateral), motor or cognitive impairment, epilepsy, vestibular disorders, visual impairment linked to retinitis.

Laboratory methods

Type of maternal infection and dating of a maternal primary infection were based on serological results as described elsewhere (3,5).

Saliva samples were collected with flocked swabs and then discharged into transport medium (Copan, ESWABR1, Labelians, Nemours, France). DNA was extracted from 200 μ L of saliva or of whole blood sample on the EasyMag Extractor (BioMérieux, Marcy l’Etoile, France) and amplified using the real time PCR CMV R Gene assay (Argène BioMerieux). The limit of detection of the method is 2.5 \log_{10} copies/mL in saliva and 2.6 IU/mL in whole blood; the limit of quantification is 2.7 \log_{10} copies/mL in saliva and 2.8 IU/mL in whole blood.

Statistical analysis:

Because it was previously demonstrated that in utero valacyclovir treatment decreases viral load in fetal blood (6), CMV PCR results in blood collected at the first visit from neonates treated in utero by valacyclovir were excluded from the analysis. Moreover, blood and saliva samples from children treated by valganciclovir at the time of samples collection were excluded from the analysis.

For comparison of viral loads, all positive viral loads under the limit of quantification of the technique ($<2.7 \log_{10}$ cp/mL or $2.8 \log_{10}$ IU/mL) were assigned half the value of the limit of detection ($1.35 \log_{10}$ copies/mL or $1.4 \log_{10}$ IU/mL).

Medians viral loads were compared between two groups (sequelae/ no sequelae groups and 1st, 2nd, 3rd, 4th and 5th visits groups) using the Mann Whitney test, only two-tailed p values <0.05 were considered significant. Medians viral loads were compared between three or more groups using Kruskal-Wallis test; only p values <0.05 were considered significant.

The proportions between the 2 groups were compared by Fisher exact test; only two-sided p values <0.05 were considered as significant. The proportions between three or more groups were compared by Chi-square test; only p values <0.05 were considered as significant.

Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) were calculated. All analyses were conducted using GraphPad Prism 9 software (GraphPad Software, San Diego, California).

Results

Population

Among the 256 cases, the type of maternal infection was known for 246 cases: 202 were primary infections (99 in the 1st trimester, 63 in the 2nd trimester, 36 in the 3rd trimester, and 4 undetermined); 44 were non-primary infections. For 10 cases, the type of maternal infection was unknown. According to the consensus classification (2), 157 neonates were asymptomatic, 98 were symptomatic at birth (including growth retardation and hearing loss), and for one neonate, the clinical status at birth was unknown.

Mothers of 52 neonates had been treated in utero with valacyclovir; 55 neonates were treated with valganciclovir postnatally for a median of 42 days (range: 10-203). Among the 256 infants,

28 were lost to followup. Among 228 children followed until 24 months, 45 had at least one sequela. Among children with sequelae at month 24, 34 had received valganciclovir treatment in the neonatal period, and among children with no sequelae at month 24, 21 children had been treated in the neonatal period.

Kinetics of CMV DNA in saliva

Results of CMV PCR in infants treated with valganciclovir at the time of sample collection were not included in the analysis. Some infants were not sampled at all visits; however, saliva was collected in 97%, 90%, and 83% of the 256 included infants at the first, second, and third visit, respectively. Only 41% and 49% of the population was tested at fourth and fifth (see the material and methods section)

Kinetics of CMV DNA in saliva are shown in Figure 1. Positive CMV DNA detection in saliva was 100% (240/240) at the second visit (median of 4.5 months). At the third visit, at a median of 12 months, 96% (199/208) of samples were positive. Then the proportion of positive CMV DNA detection decreased to 69% (72/105) at the fourth visit (median 19 months) and to 59% (75/126) at the last visit (median 24 months), respectively (Figure 1A). CMV DNA detection was positive in all the 113 saliva samples collected between 4-5, 58 of samples collected at 5-6, and 15 samples collected at 6-7 months of age; therefore, 100% of CMV PCR tests on saliva were positive up to 7 months. The first negative viral load in saliva was reported at day 338 (> 11 months), but due to the study design, only 13 tests were performed between 7 months and 11 months (Figure 1B). CMV shedding was intermittent in some infants: among the 9 children with a negative CMV PCR on saliva at month 12 visit, 5 were re-tested at month 18, and 3 had a positive saliva PCR. At month 18 visit, 33 infants had a negative PCR on saliva, 27 were re-tested at month 24, and 7 had a positive CMV PCR on saliva.

In positive samples, CMV DNA loads decreased progressively from birth to month 18. Viral load levels significantly decreased from inclusion (median 7.5 log₁₀, IQR 6.7-8.1) to month 4

of age (median 7.1 log₁₀, IQR 6.3-7.6) ($p < 0.0001$), from month 4 to month 12 (median 4.2 log₁₀, IQR 3.6-5.1) ($p < 0.0001$), from month 12 to month 18 (median 3.6 log₁₀, IQR 2.7-4.2) ($p < 0.0001$). Viral load was not significantly different from month 18 to month 24 (median 3.2 log₁₀, IQR 2.8-4.0) ($p = 0.3935$).

Kinetics of CMV DNA in blood

Results of CMV PCR on blood samples collected at the first visit from neonates with in utero valacyclovir were excluded from the analysis. Results of CMV PCR on blood collected from infants being treated with valganciclovir at the time of collection were excluded from the analysis. Some infants were not sampled at all visits; however, a blood sample was collected in 97%, 90%, and 83% of the 256 included at the first, second, and third visit, respectively.

Kinetics of CMV DNA in blood are shown in Figure 2. The proportion of positive CMV PCR tests performed on whole blood decreased significantly from 92% (147/159) to 72% (175/240) and 27% (57/213) at inclusion, months 4 and 12, respectively. In positive samples, median CMV DNA loads decreased significantly from inclusion (median 3.9 log₁₀, IQR 3.5-4.4) to month 4 (median 3.4 log₁₀, IQR 2.9-3.9) ($p < 0.0001$) and from month 4 to month 12 (median 1.4 log₁₀, IQR 1.4-3.1) ($p < 0.0001$).

Comparison of duration and the level of CMV by specimen and patient characteristics

There was no significant difference in positivity rate and viral load level in saliva between children with or without sequelae at any visits (Figure 3; available at www.jpeds.com).

All children with sequelae had a positive PCR in blood with a viral load above 3 log₁₀ IU/mL at inclusion (Figure 4). The median viral load at inclusion (median 13 days) and at the second visit (median 4.5 months) was significantly higher in the group of children with sequelae than in the group of children without sequelae ($p = 0.0013$ and $p < 0.0001$, respectively). At the third

visit (median 12 months), having a positive CMV PCR in blood was significantly more frequent in children with sequelae than in children without sequelae ($p=0.0029$).

The sensitivity, specificity, PPV, NPV for long-term sequelae of a CMV PCR in blood $>3.0 \log_{10}$ IU/mL was 100%, 16%, 18% and 100% respectively. The sensitivity, specificity, PPV, NPV for long-term sequelae of a CMV PCR in blood $>3.5 \log_{10}$ IU/mL was 95%, 33%, 21% and 97% respectively. The sensitivity, specificity, PPV, NPV for long-term sequelae of a CMV PCR in blood $>4 \log_{10}$ IU/ml was 68%, 60%, 24% and 91% respectively.

In the first month of life (inclusion visit), CMV DNA load in saliva was significantly lower in neonates infected after maternal primary infection in the 3rd trimester than in neonates infected after a maternal primary infection in the 1st or 2nd trimesters (Figure 5A). Among children infected after a maternal primary infection in the 3rd trimester, viral loads in saliva significantly increased when the time interval between the date of the maternal infection and birth increased. Median viral load increased from $4.6 \log_{10}$ (IQR 1.4-4.7) to $6.7 \log_{10}$ copies/mL (IQR 5.2-7.7) to reach a plateau at $8.0 \log_{10}$ (IQR 7.5-8.4,) for an interval between the date of the maternal primary infection and birth of 3 to 4 weeks, 5 to 7 weeks and 8 to 12 weeks respectively ($p<0.0001$; $p=0.0059$; $p=0.0045$) (Figure 5B).

From the second visit (at a median of 4.5 months) onward to the last visit (median 24 months), the proportion of positive saliva samples and the median viral load in positive samples were not significantly different between children infected after primary maternal infection in the first, second and third trimester (Figure 6; available at www.jpeds.com).

Only two neonates (2/86, 2%) infected after a maternal primary infection in the first or second trimester had a viral load in saliva below $5.0 \log_{10}$ copies/ml (Figure 5B). Among these 2 cases, 1 had a prenatal diagnosis with a negative CMV PCR in amniotic fluid sampled at 22 weeks suggesting a late passage of the virus that could explain the low viral load at birth (7). Unfortunately, prenatal diagnosis was not done in the other case, and the time of CMV

transplacental passage was not documented. Those 2 cases had a good outcome with no sequelae at 2 years of age.

The proportion of positive CMV PCR in blood and the level of median viral loads in positive samples were not significantly different between children infected after a maternal infection in the first, second and third trimester (Figure 7; available at www.jpeds.com).

At the first visit, newborns treated with valganciclovir had lower saliva viral loads than patients without treatment (Figure 8; available at www.jpeds.com). At the second visit (age 4 months), although viral load levels in positive samples were the same in the 3 groups, we observed fewer positive saliva PCR (41% vs. 100%) in infants who received or had received valganciclovir. From the 3rd visit onwards, there was no difference in saliva PCR positivity rate or viral load level. No difference was observed in viral blood load levels in infants treated with valganciclovir treatment (Figure 9A; online available at www.jpeds.com however, treated infants were more likely to have a positive CMV PCR in blood at month 12 possibly reflecting the rebound in viral load after the end of therapy as reported by Kimberlin et al (8).

In utero valacyclovir treatment had no effect on both positivity rate and viral load level in saliva samples at first visit (Figure 8). However, as expected from previous data (6) in utero valacyclovir treatment was associated with significantly decreased viral load blood level (3.6 vs 4 log IU/mL; $p=0.0464$) in the first 7 days of life (Figure 9B).

Discussion

This study reports new longitudinal data on saliva and blood viral loads within a large prospective cohort of symptomatic and asymptomatic neonates with congenital CMV infection. Studies based on viral culture reported a positive CMV culture in 97% of urine samples and in 87% of oral samples collected in the first year of life (9). Longitudinal data on CMV DNA excretion quantified by PCR in the urine and saliva of children with cCMV are scarce. One

study reported in 33 infants, 100% of CMV DNA excretion in the urine at 3 and 18 months and 100% and 47% at 3 and 18 months respectively in the saliva (10). Another study reported CMV DNA excretion in the urine in 85% and 75% of 33 infants at months 12 and 24 respectively (11). In the current study, we observed a progressive decrease in saliva viral load level from 7.5 \log_{10} copies/mL at birth to 3.3 \log_{10} copies/mL at month 24. No correlation was found between the proportion of positive CMV PCR and the level of viral load in saliva from birth to 2 years of age and the presence of long-term sequelae. CMV DNA was detected in 100% of saliva samples collected up to 6 months. This has value in clinical practice for the retrospective diagnosis of congenital CMV because a repeatedly negative PCR in saliva in the first 6 months of age likely excludes congenital CMV infection as in our cohort, which was predominantly due to primary infection during pregnancy, 100% of salivary samples were positive in the first 6 months.

CMV PCR in blood might be negative although the neonate is infected and shows positive CMV PCR in saliva. A retrospective study in the USA reported 8% of negative PCR in 74 infected children younger than 2 months (12). In our prospective study, using CMV PCR in whole blood, the proportion of negative PCR in blood in congenitally infected children less than 1 month of age was 7% (12/159). The predictive value of neonatal blood viral load for long-term sequelae is still a matter of debate. A link between neonatal blood viral load and the risk of long-term sequelae was reported in two small retrospective studies. Lanari et al (13) were the first to report a 70% risk of sequelae in newborns with a viral load higher than 10,000 copies per 10^5 polymorphonuclear leukocytes. Forner et al (11) reported a risk of sequelae over 50% when CMV DNAemia at birth was $\geq 12,000$ copies/mL. Conversely, Marsico et al (14) failed to find a clinically relevant neonatal viral load threshold predictive of long-term sequelae in a cohort of 120 symptomatic infants. In our large prospective study of 256 infected neonates show that a high viral blood at birth is not a predictor of long-term sequelae in a population

including both symptomatic and asymptomatic neonates. Indeed, although median blood viral loads were significantly higher at inclusion and month 4 in children with sequelae than in children with no sequelae, the viral load levels were largely overlapping between the 2 groups. A clinically relevant threshold could not be found; therefore, high neonatal blood viral load cannot identify, among infected neonates symptomatic or asymptomatic, those with a high risk of long-term sequelae who could potentially benefit from neonatal treatment. Two studies suggested that a low blood viral load ($<3 \log_{10}$ copies/mL and $<3.5 \log_{10}$ genomes/mL respectively) could be associated with a good outcome (14,15). In our large prospective study, a neonatal blood viral load $< 3 \log_{10}$ IU/mL had a 100% negative predictive value for long-term sequelae. Caution is required in generalizing these results to other population and because interassay result comparison remains an issue even with standardized CMV PCR assays expressed in IU/mL (16).

We could not identify any difference in terms of viral load level and the duration of detection in blood and saliva according to the time and type of maternal infection. Except that viral loads in saliva at birth were significantly lower in women infected after a primary infection during the third trimester. This could be explained by the dynamic of CMV fetal replication after maternal primary infection. Our data show that CMV load in saliva is already detectable at a median of 4.6 logs by 3 weeks after primary maternal infection, increased to a median of 6.5 logs by 5 weeks and reaches a plateau 8 weeks after primary maternal infection. Earlier studies reported a similar pattern of CMV DNA kinetics in amniotic fluid after a primary maternal infection, with the first detection of a low level of CMV DNA at 3 to 4 weeks after the primary maternal infection, increasing steadily until it reaches a plateau around 10 to 12 weeks after maternal infection (17,18).

Our study has some limitations. The population of the study was not subjected to a systematic screening at birth and was enriched for primary infection during pregnancy which may affect

generalizability. However, the population was large enough to compare children with and without sequelae. Among the 45 children with sequelae at 24 months, 34 had received neonatal treatment, which could significantly impact the long-term outcome (8). Because valacyclovir treatment decreases blood viral load (6), we chose to exclude baseline neonatal blood viral load data for 51 neonates (including 11 cases with sequelae at 24 months) treated with valacyclovir in utero. Moreover, some children were already treated with valganciclovir at the time of inclusion, and baseline viral load in blood and saliva could not be analyzed for these 20 cases, including 11 children with sequelae at month 24. Finally, because saliva samples were not collected repeatedly in the first 6 months but only at 2 times for each infant, potential intermittency of shedding could not be completely eliminated although the high viral loads detected in saliva in this period suggest it is unlikely.

The strength of our study is its large population of children included prospectively, with a limited number of children lost to followup (10%) at 24 months of age. Moreover, the blood and saliva collection were both collected in 97%, 90%, and 83% of the 256 included children tested at baseline, second and third visits.

Our study demonstrates potential clinical value of performing saliva CMV PCR to 6 months of age in the assessment of infants not screened at birth and presenting with symptoms suggestive of congenital CMV infection. This study suggested that negative saliva collected in the first 6 months of life had a 100% negative predictive value for congenital infection, however further studies are needed to exclude intermittency of viral shedding and to confirm this finding for congenital infections following non-primary infection. Our study also demonstrated that a neonatal blood viral load $<3.0 \log_{10}$ IU/mL had a 100% negative predictive value for the occurrence of long-term sequelae. This threshold expressed in IU, if confirmed in other settings

of congenital CMV infection, potentially could be useful for the management of infected neonates as well as for parents' counseling.

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Figures' legends

Figure 1

A: Proportion of positive CMV PCR in saliva and levels of CMV DNA loads in positive samples over time

**** < 0.0001; ns = not significantly different

B: Kinetics of CMV DNA loads in saliva over time

Dy= day, mth=month

Figure 2: Proportion of positive CMV PCR in blood and levels CMV DNA loads in positive samples over time

Dy= day, mth=month, **** < 0.0001

Figure 3: Comparison of the duration and the level of CMV DNA saliva excretion in children with or without sequelae

ns = not significantly different, dy= day, mth=month

Figure 4: Comparison of the duration and the level of CMV DNA load in blood in children with or without sequelae

Dy= day, mth=month, **** < 0.0001, ** =0.0013 (1st visit), ** = 0.0029 (3rd visit, 22% vs 46%)

Figure 5: Levels of CMV DNA loads in the first month of life according to

A the type and the timing of maternal infection

*= 0.0341 (1st trimester vs 3rd trimester); **= 0.0041 (2nd trimester vs 3rd trimester); *= 0.0414 (3rd trimester vs Non primary infection)

B the interval between the time of maternal infection and birth in cases with primary infection in the third trimester

****<0.0001 (3-4 vs 8-12), ** = 0.0045 (3-4 vs 5-7), **=0,0059 (5-7 vs 8-12)

Figure 6: Proportion of positive CMV PCR in saliva and levels of CMV DNA loads in positive samples according to the type and the timing of maternal infection; A: at the second visit (median age: 4.5 month); B: at the third visit (median age: 12 months); C: at the fourth visit (median age: 19 months); D: at the fifth visit (median age: 24 months)

PI= primary infection, NPI= non primary infection, ns = not significantly different, mth=month, *=0,0176 (1st trimester PI vs NPI), *=0,0157 (2nd trimester PI vs NPI)

Figure 7: Proportion of positive CMV PCR in blood and levels of CMV DNA loads in positive samples according to the type and the timing of maternal infection

A: in the first month of life

B: at the second visit (median age: 4.5 month)

C: at the third visit (median age: 12 months)

PI= primary infection, NPI= non primary infection, mth=month, ns = not significantly different

Figure 8: Proportion of positive CMV PCR in saliva and levels of CMV DNA loads in positive samples according to the type of treatment

med = median, mth=month, dy=day, w/o=without, ns = not significantly different,

****<0.0001, **=0.0050 (1st visit), **=0.0027 (2nd visit)

Figure 9: Proportion of positive CMV PCR in blood and levels of CMV DNA loads in positive samples according to the type of treatment

A : in the first year of life

B : in the first week of life

dy= day, mth=month, ns = not significantly different, **=0.0062, *=0.0464

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References

1. Leruez-Ville M, Foulon I, Pass R, Ville Y. Cytomegalovirus infection during pregnancy: state of the science. *Am J Obstet Gynecol.* sept 2020;223(3):330-49.
2. Rawlinson WD, Boppana SB, Fowler KB, Kimberlin DW, Lazzarotto T, Alain S, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect Dis.* juin 2017;17(6):e177-88.
3. Leruez-Ville M, Ren S, Magny JF, Jacquemard F, Couderc S, Garcia P, et al. Accuracy of prenatal ultrasound screening to identify fetuses infected by cytomegalovirus which will develop severe long-term sequelae. *Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol.* janv 2021;57(1):97-104.
4. Fily A, Pierrat V, Delporte V, Breart G, Truffert P, EPIPAGE Nord-Pas-de-Calais Study Group. Factors associated with neurodevelopmental outcome at 2 years after very preterm birth: the population-based Nord-Pas-de-Calais EPIPAGE cohort. *Pediatrics.* févr 2006;117(2):357-66.
5. Leruez-Ville M, Magny JF, Couderc S, Pichon C, Parodi M, Bussièrès L, et al. Risk Factors for Congenital Cytomegalovirus Infection Following Primary and Nonprimary Maternal Infection: A Prospective Neonatal Screening Study Using Polymerase Chain Reaction in Saliva. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 1 août 2017;65(3):398-404.
6. Jacquemard F, Yamamoto M, Costa JM, Romand S, Jaqz-Aigrain E, Dejean A, et al. Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection. *BJOG Int J Obstet Gynaecol.* sept 2007;114(9):1113-21.
7. Bilavsky E, Pardo J, Attias J, Levy I, Magny JF, Ville Y, et al. Clinical Implications for Children Born With Congenital Cytomegalovirus Infection Following a Negative

- Amniocentesis. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 1 juill 2016;63(1):33-8.
8. Kimberlin DW, Jester PM, Sánchez PJ, Ahmed A, Arav-Boger R, Michaels MG, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. *N Engl J Med.* 5 mars 2015;372(10):933-43.
 9. Pass RF, Stagno S, Britt WJ, Alford CA. Specific cell-mediated immunity and the natural history of congenital infection with cytomegalovirus. *J Infect Dis.* déc 1983;148(6):953-61.
 10. Puhakka L, Pati S, Lappalainen M, Lönnqvist T, Niemensivu R, Lindahl P, et al. Viral shedding, and distribution of cytomegalovirus glycoprotein H (UL75), glycoprotein B (UL55), and glycoprotein N (UL73) genotypes in congenital cytomegalovirus infection. *J Clin Virol Off Publ Pan Am Soc Clin Virol.* avr 2020;125:104287.
 11. Forner G, Abate D, Mengoli C, Palù G, Gussetti N. High Cytomegalovirus (CMV) DNAemia Predicts CMV Sequelae in Asymptomatic Congenitally Infected Newborns Born to Women With Primary Infection During Pregnancy. *J Infect Dis.* 1 juill 2015;212(1):67-71.
 12. Ross SA, Novak Z, Fowler KB, Arora N, Britt WJ, Boppana SB. Cytomegalovirus blood viral load and hearing loss in young children with congenital infection. *Pediatr Infect Dis J.* juill 2009;28(7):588-92.
 13. Lanari M, Lazzarotto T, Venturi V, Papa I, Gabrielli L, Guerra B, et al. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatrics.* janv 2006;117(1):e76-83.
 14. Marsico C, Aban I, Kuo H, James SH, Sanchez PJ, Ahmed A, et al. Blood Viral Load in Symptomatic Congenital Cytomegalovirus Infection. *J Infect Dis.* 16 avr 2019;219(9):1398-406.
 15. Boppana SB, Fowler KB, Pass RF, Rivera LB, Bradford RD, Lakeman FD, et al. Congenital cytomegalovirus infection: association between virus burden in infancy and

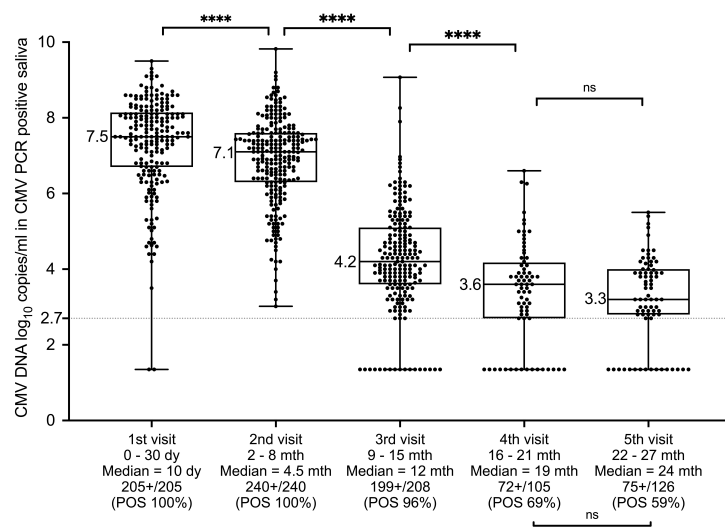
hearing loss. *J Pediatr.* juin 2005;146(6):817-23.

16. Preiksaitis JK, Hayden RT, Tong Y, Pang XL, Fryer JF, Heath AB, et al. Are We There Yet? Impact of the First International Standard for Cytomegalovirus DNA on the Harmonization of Results Reported on Plasma Samples. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 1 sept 2016;63(5):583-9.

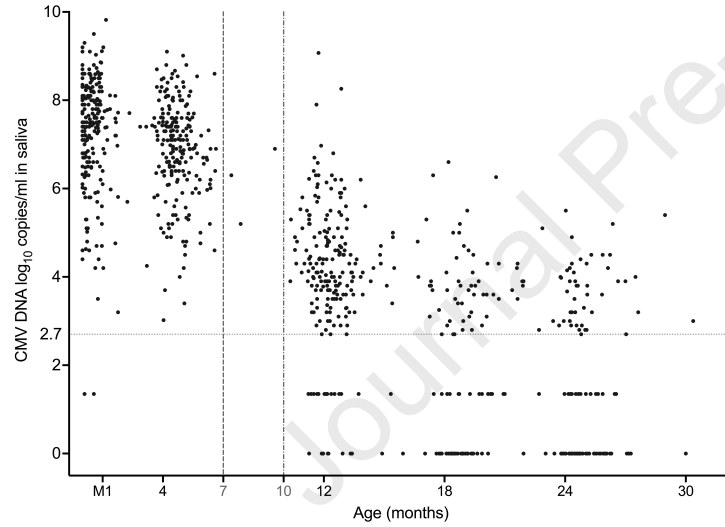
17. Gouarin S, Gault E, Vabret A, Cointe D, Rozenberg F, Grangeot-Keros L, et al. Real-time PCR quantification of human cytomegalovirus DNA in amniotic fluid samples from mothers with primary infection. *J Clin Microbiol.* mai 2002;40(5):1767-72.

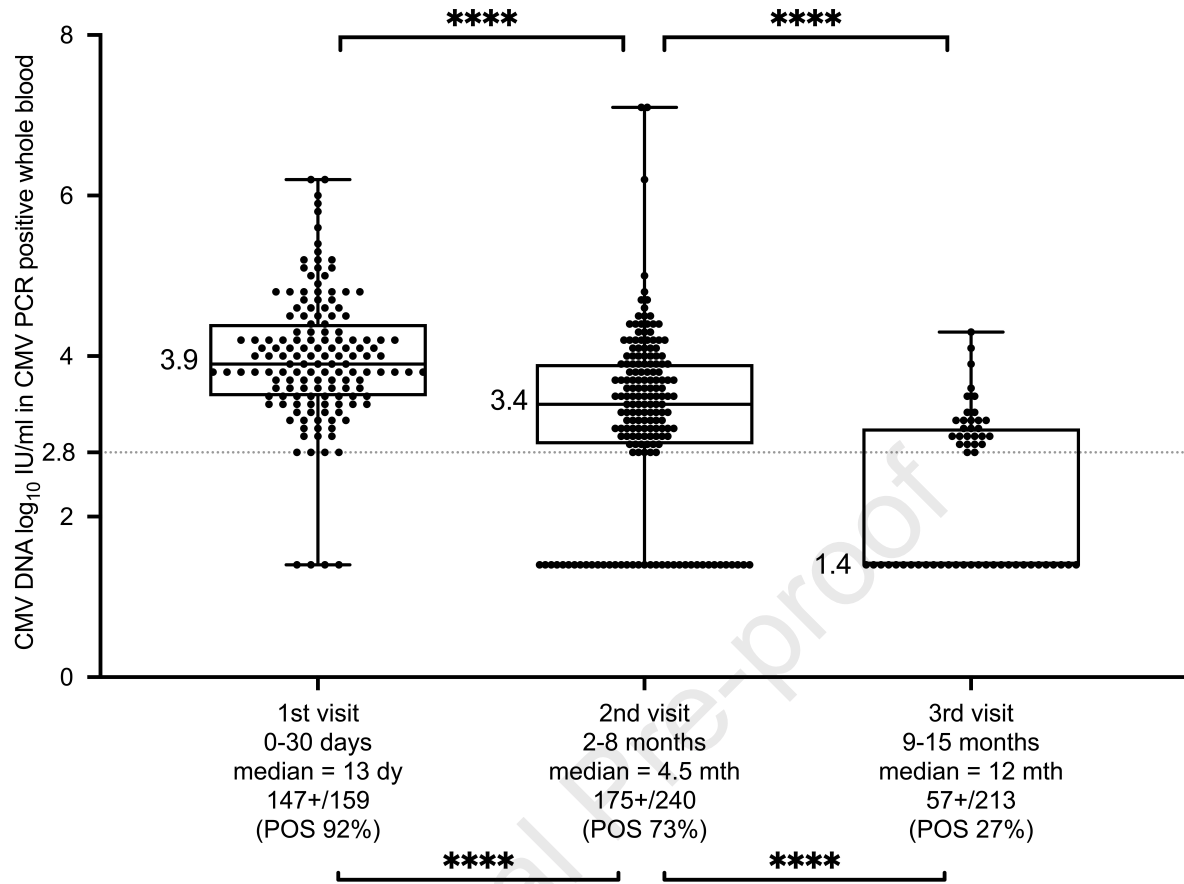
18. Leruez-Ville M, Stirnemann J, Sellier Y, Guilleminot T, Dejean A, Magny JF, et al. Feasibility of predicting the outcome of fetal infection with cytomegalovirus at the time of prenatal diagnosis. *Am J Obstet Gynecol.* sept 2016;215(3):342.e1-9.

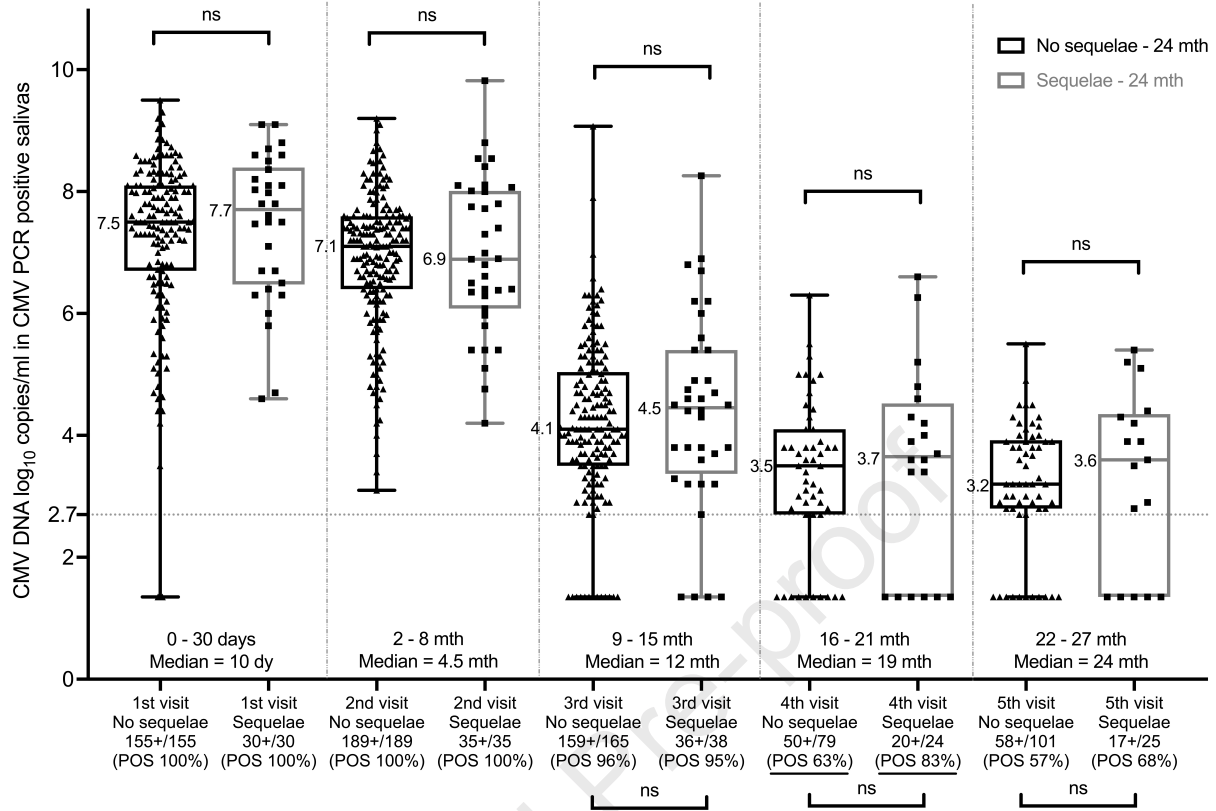
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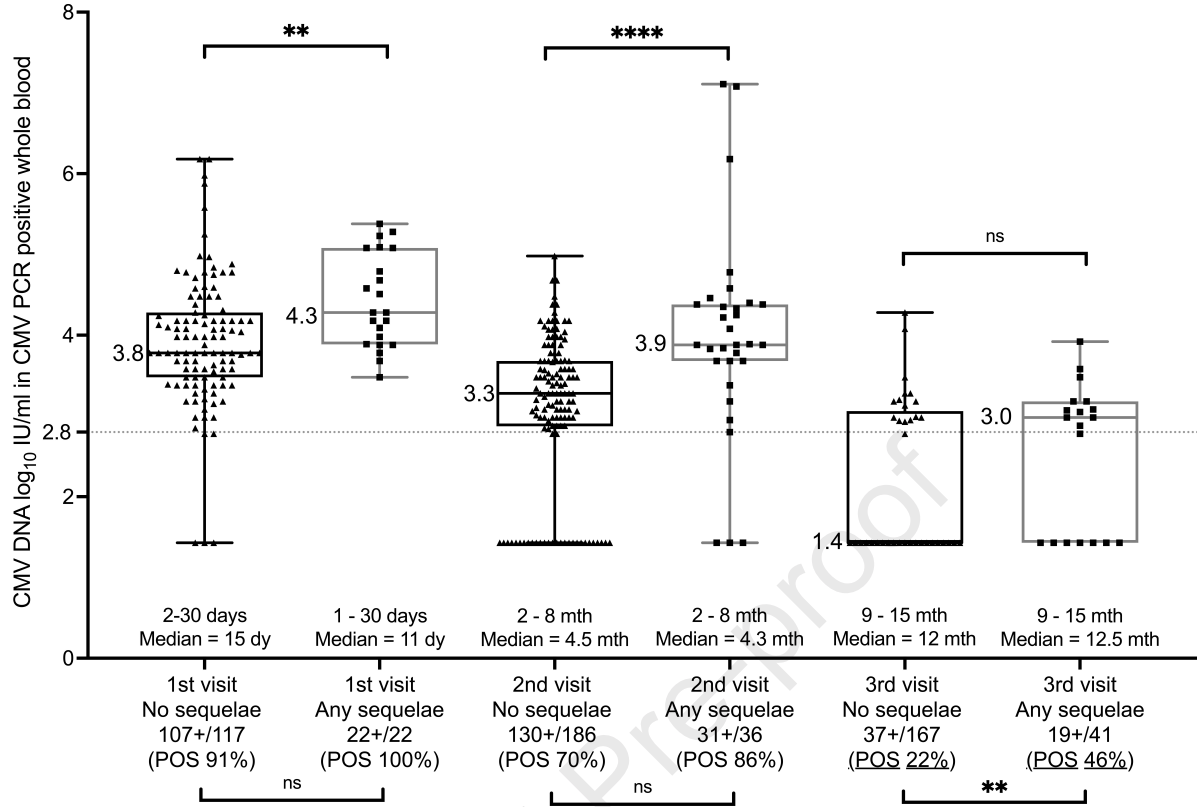


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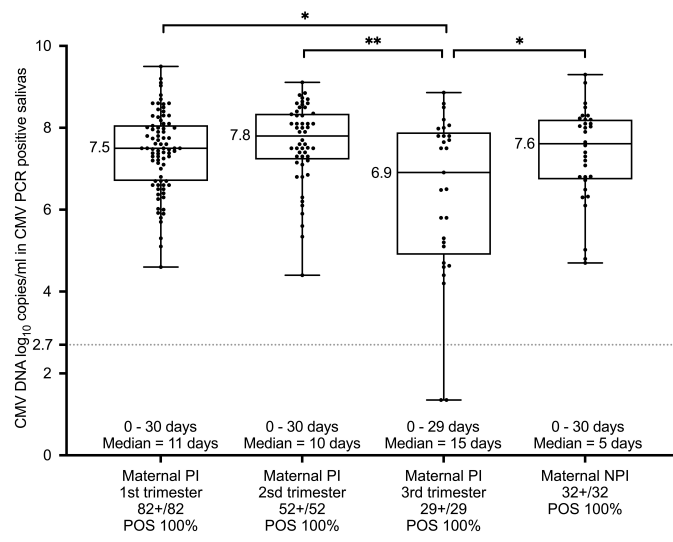




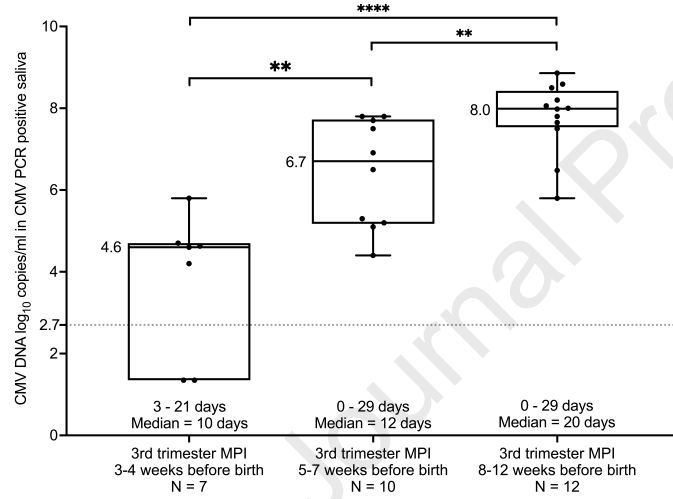


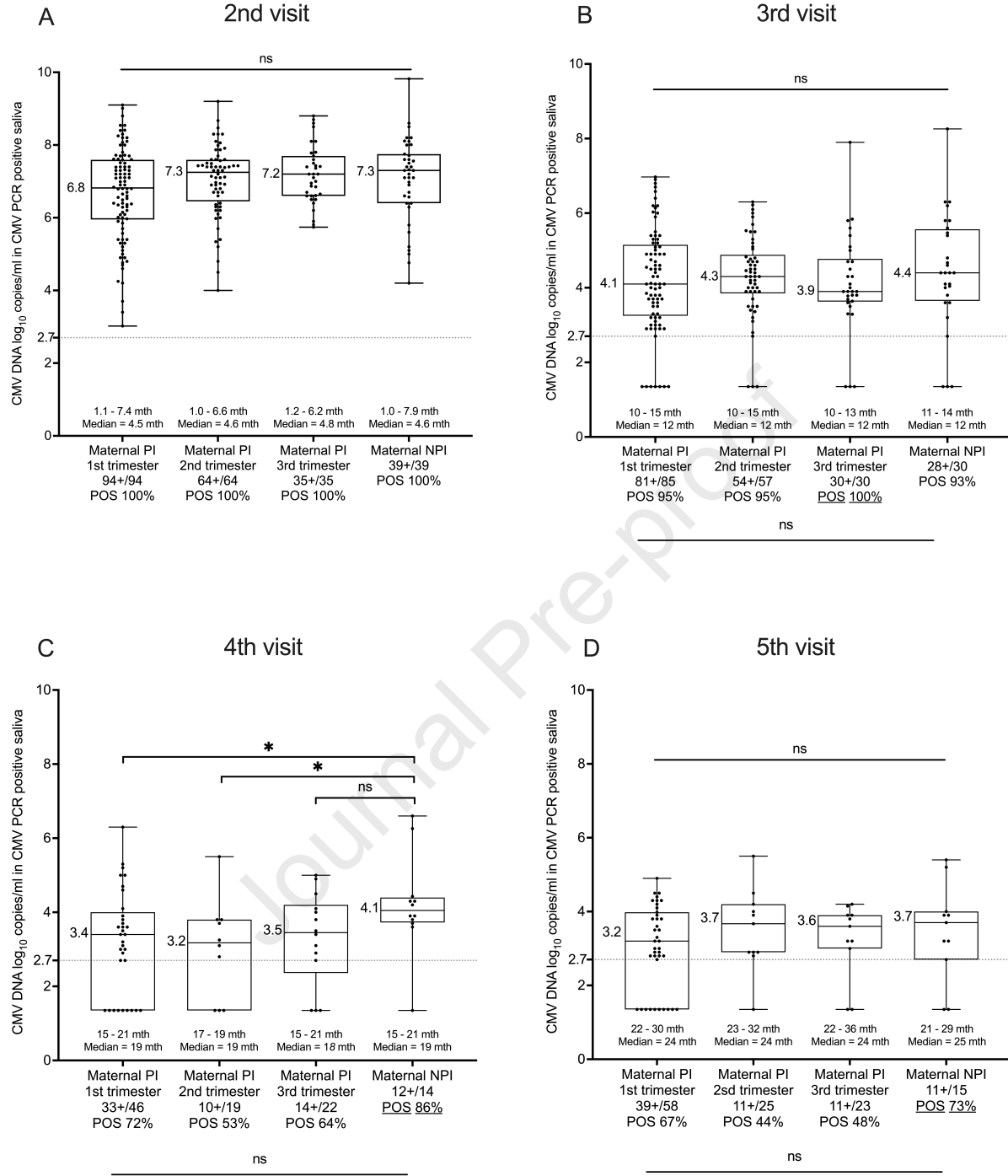


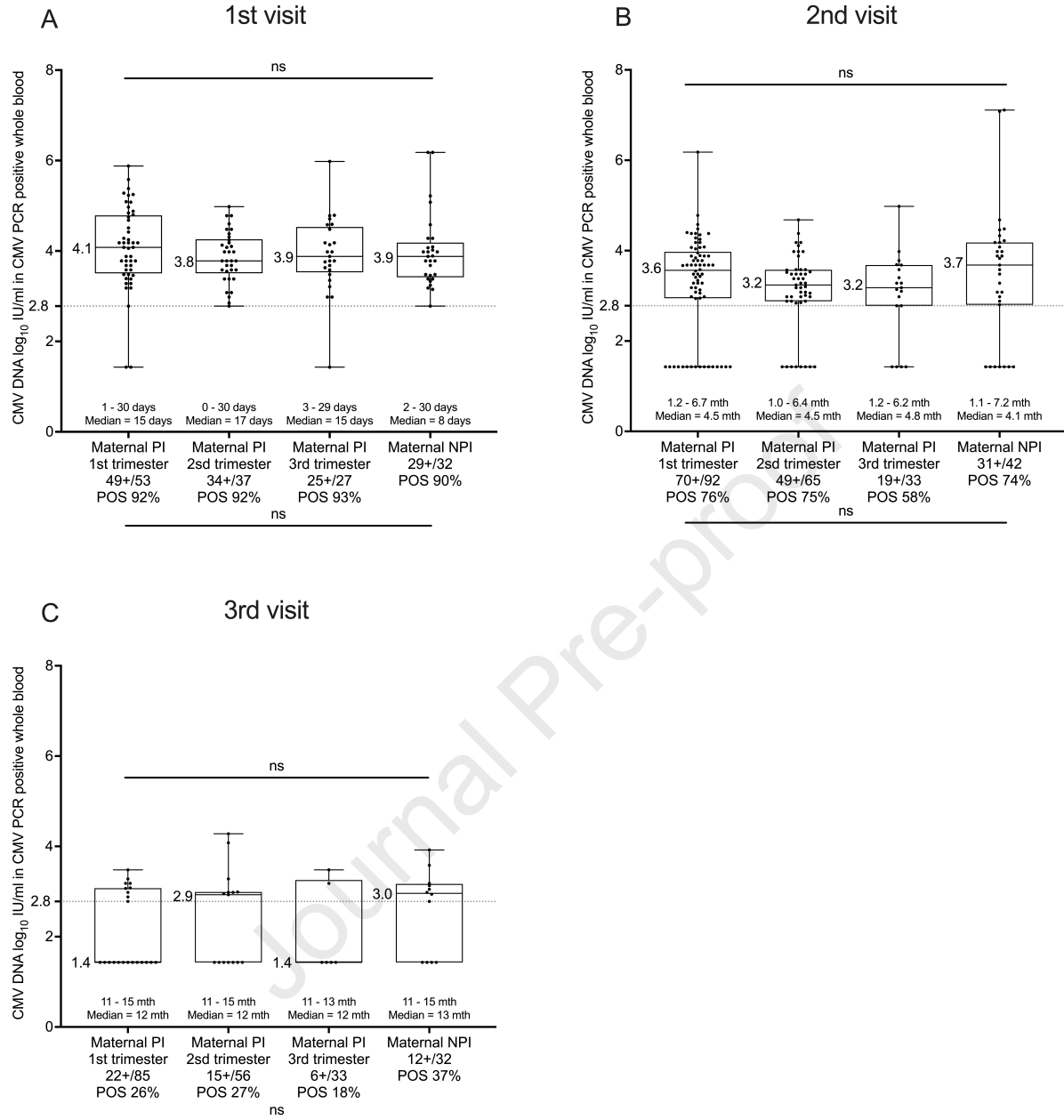
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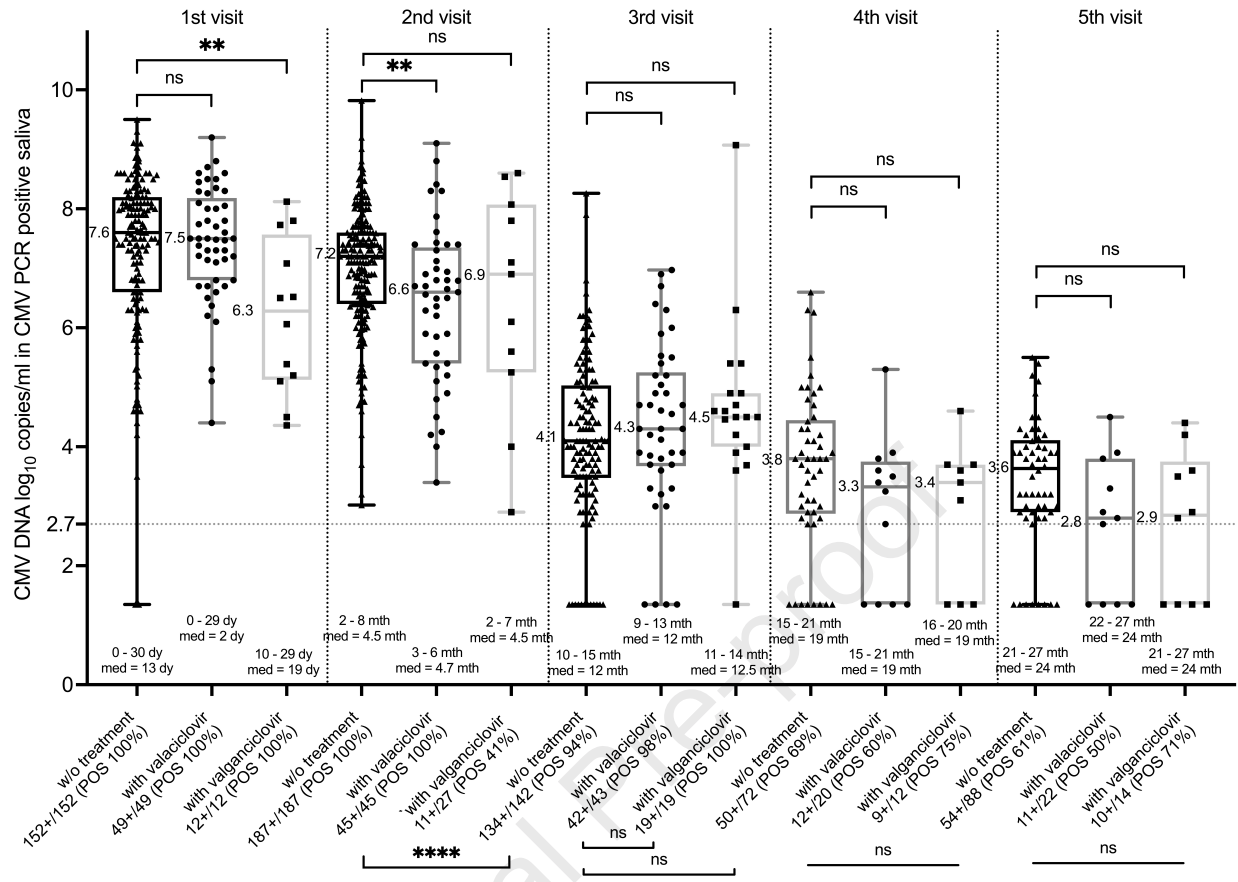


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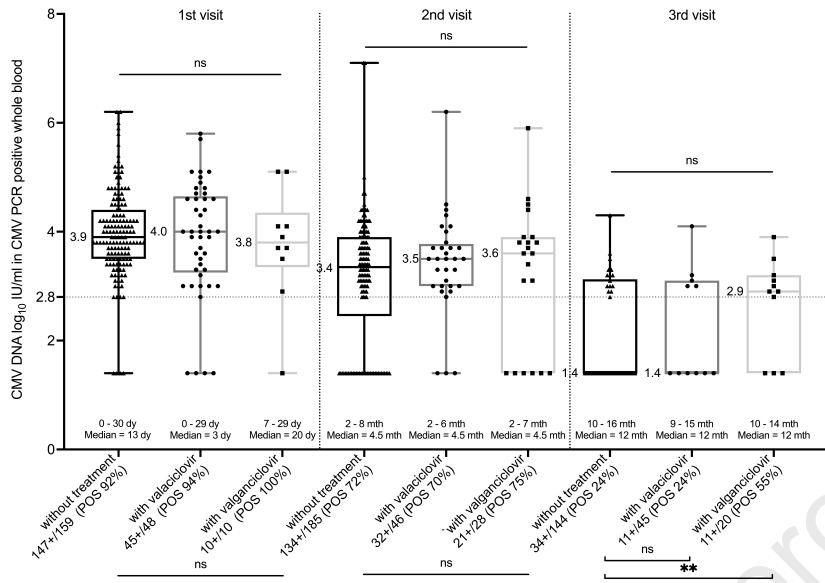








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