High-dose CMV hyperimmune globulin (HIG) and maternal CMV DNAemia independently predict infant outcome in pregnant women with a primary cytomegalovirus (CMV) infection.

Giovanni Nigro[1,2], Stuart P. Adler[3], for the Congenital Cytomegalic Disease Collaborating Group*

[1]Non-profit Association Mother-Infant Cytomegalovirus Infection (AMICI), Rome, Italy.

[2] Pediatric Unit, University of L'Aquila, Italy

[3] CMV Research Foundation, Richmond, VA, USA.

* Members of the Congenital Cytomegalovirus Collaborating Group are listed under notes*

Correspondence to: Prof. G. Nigro
Tel. +39.3387688054
Email: nigrogio@libero.it

Alternate corresponding author:
Stuart P. Adler
Tel. 804-264-8296
Email: spadler123@gmail.com

Main point:

Among 304 women with a primary CMV infection during pregnancy four factors independently predicted fetal infection: not receiving high-dose immunoglobulin (HIG); maternal viral DNAemia before HIG administration; an abnormal ultrasound; and diagnosis of maternal infection via seroconversion rather than avidity.

Abstract

Background

After a primary maternal CMV infection during pregnancy infants are at risk for disease.

Methods

Factors predictive of infant outcome were analyzed in a database of 304 pregnant women with a primary infection. These were enrolled between 2010 and 2017 and delivered 281 infants, of whom 108 were CMV infected. Long term follow-up occurred for 173 uninfected and 106 infected infants at age 4 years (range 1 to 8 years). 157 women were treated with an average of 2 doses (range 1 to 6) of high dose hyperimmune globulin (HIG: 200 mg/kg/infusion). We used a regression model to define predictors of fetal infection, symptoms at birth, and long-term sequelae. 31 covariates were tested.

Results

Four factors predicted fetal infection: a 1.8 fold increase (30% vs. 56%) in the rate of congenital infection without HIG (P<0.0001, adjusted odd ratio (AOR) =5.2), a 1.8 fold increase (32% vs. 56%) associated with maternal viral DNAemia prior to HIG administration (P=0.002, AOR=3.0), abnormal ultrasounds (P=0.0002, AOR=54.2), and diagnosis of maternal infection via seroconversion rather than avidity (P=0.007, AOR=3.3). Lack of HIG and abnormal ultrasounds also predicted symptoms (P=0.001). Long term sequelae were predicted by not receiving HIG (P=0.001, AOR=13.2), maternal infection in early gestation (P=0.017, OR = 0.9), and abnormal ultrasounds (P<0.003, OR =7.6). Prevalence and copy/number of DNAemia declined after HIG.

Conclusions

Maternal viremia predicts fetal infection and neonatal outcome. This may help patient counseling. High-dose HIG may prevent fetal infection and disease, and is associated with the resolution of DNAemia.

Key words: Cytomegalovirus, Pregnancy, Hyper immunoglobulins, DNAemia

In Europe and the United States about 80.000 pregnant women annually acquire primary CMV infection and transmit the virus to the fetus with transmission increasing from 30% to 73% as gestation progresses. [1-3] Congenital CMV disease, including severe permanent neurologic damage and progressive sensorineural hearing deficit, occurs up to 50% of infected neonates born to mothers who acquired the infection in the first half of pregnancy.[4,5] In spite of these frequent and severe effects CMV screening in pregnancy is not routine. In Italy CMV serologic screening is frequent at the first post-conception evaluation, and an increasing number of pregnant women request CMV monthly serologic testing after CMV information on websites or from friends.

HIG administration to pregnant women with primary CMV infection was initiated over 20 years ago, and several observational studies reported an excellent safety and efficacy profile for the prevention of congenital disease using HIG dosage of 200 units/kg. [6-14] Less clear is the efficacy profile for the prevention of congenital infection using monthly HIG administration of 100 units/kg.[8,13-15] Although not approved by regulatory agencies for use during pregnancy and in spite of the high cost, HIG is administered off-label worldwide.

At least two factors may account for the success of immunotherapy: 1) HIG is obtained from over 1000 donors and contains all human high-titer and high avidity anti-CMV antibodies;[16-19] 2) HIG may decrease the CMV-induced inflammatory damage in the placenta and fetal organs because contains IgG antibodies binding to cellular receptors for cytokines and CD8+ cytotoxic T lymphocytes, then modulating the activity of lymphocytes, cytokines, complement system, and the expression of Fc receptors. [20-27] Both maternal-fetal CMV transmission and fetal/neonatal CMV disease appear associated with maternal CMV DNAemia but there are no reports on the association of HIG, maternal DNAemia, and fetal outcome.[28,29] To determine if HIG, maternal

CMV DNAemia, and other factors are predictive of infant outcome, we analyzed a large registry database comprised of pregnant women with a primary CMV infection.

METHODS

Patients

304 Italian pregnant women with a primary CMV infection were enrolled between October 1, 2010 and June 30, 2017. No patients with HIV or other infections or immunodepressive diseases or therapies were included. The decision to be treated with high-dose HIG to prevent fetal CMV infection or disease was determined by each woman and her physician. For each patient essential data were: maternal age at conception, gestational age at the time of maternal CMV infection, gestational age at the time of the first CMV DNA detection in blood, gestational age at the first HIG infusion, gestational age at the time of the CMV DNA detection after HIG or subsequent DNAemia in controls, viral load in the amniotic fluid, number of subsequent HIG infusions, prenatal manifestations of CMV disease, gestational age at delivery, neonatal birth weight, and clinical and laboratory abnormalities in the infants with congenital infection. The gestational age at maternal infection was estimated after maternal seroconversion and defined as half way between the last seronegative and the first seropositive serum or the beginning of symptoms (i.e. fever, flu-like syndrome) or laboratory abnormalities, when these occurred.[29] For women who had both IgM and IgG antibodies associated with a very low avidity in the first two months of pregnancy, being CMV negative in their previous pregnancy, maternal infection was considered to have occurred just after conception. In fact a natural immune depression, occurring during pregnancy to prevent fetal rejection, may facilitate CMV transmission.[30] Fetal CMV infection was symptomatic if the fetus exhibited by ultrasound (US) ventriculomegaly or echodensities in the brain, bowel or liver or exhibited neuronal migrational disorders by magnetic resonance (MR). Congenital CMV infection was defined symptomatic if the neonate had one or more of

the following: ventriculomegaly, periventricular calcifications, microcephaly, leukoencephalopathy, cerebral or cerebellar atrophy, full or partial hearing loss in one or both ears, and purpura with or without thrombocytopenia. Long-term abnormalities included unilateral or bilateral deafness, psychomotor retardation, cerebral palsies, and visual impairment.

Diagnosis of CMV infection

CMV infection was first determined by diagnostic laboratories then confirmed by reference laboratories. CMV-specific antibodies and avidity were detected using enzyme immunoassays (EIAs) from Disease Diagnostica Senese and DiaSorin, and CMV DNA was detected using real-time PCR from Amplimedical-Bioline and Qiagen (Hoffmann-LaRoche), according to the manufacturers' instructions.

High-dose HIG infusions

Commercial HIG (Cytotect/Megalotect Biotest, Germany) was given for preventive use at 200 units/kg of maternal weight. This dose was used because: 1) the majority of the patients enrolled were treated 6 weeks after their presumed maternal viremia and fetal CMV infection could have already occurred; 2) fetal infection may have occurred even before 6 weeks after maternal viremia, since CMV DNA may be detected in the amniotic fluid almost concomitantly with detection of IgG in serum, and thus HIG infusions might have been therapeutic rather than preventive;[8,10,13] 3) since the half-life of anti-CMV antibodies in HIG is shorter than 22.4 days as reported for nonspecific immunoglobulins, HIG persistence in the maternal blood would have been longer using 200 units/kg instead of 100 units/kg.[31,32] Based on the HIG half-life of about 2 weeks, whenever possible, HIG infusions were repeated every three weeks or until amniocentesis or delivery. If amniocentesis was negative, HIG infusions were stopped. If amniocentesis was positive, another infusion was administered and repeated every two to three weeks if US or MRI abnormalities were also observed.

Study design

For HIG-treated women, CMV IgG, IgM, avidity, and DNA in blood (DNAemia) were measured before and after HIG infusions. In untreated women, CMV DNA was measured twice in the majority of women with DNAemia. Serological testing for each woman was done in the same laboratory. For women with a primary infection in the first trimester amniotic fluid was obtained at 19-21 weeks gestation for detection of CMV DNA. If the amniotic fluid was positive then fetal MRI was performed at 21 and 26-28 weeks'gestation. All neonates were tested for CMV IgG, IgM, and DNA in blood and/or urine. All infected infants received brain and abdominal US studies, auditory brain-stem evoked response (ABR) studies, and an ophthalmoscopy evaluation. Brain MRI and CT scan were also performed in symptomatic infants. CMV-infected infants were monitored for 1 to 8 years (mean 4 years) by routine clinical evaluation, sensorineural hearing and eye exams.

The study was approved by the Ethical Committees of University of L'Aquila and the non-profit association CMV-AMICI onlus. All treated patients gave written informed consent for receipt of high-dose HIG infusions, and provided consent permitting the anonymous use of their medical records.

Statistical analysis

Statistical analyses were performed with JMP software,version14.0 (SAS Institute). Fisher's exact test and t-tests were used for univariate analysis. Variables with a univariate P value of < 0.2 were tested in a logistic regression model.

RESULTS

Of the enrolled 304 women with primary CMV infection, 277 delivered 281 infants, four of whom were twins. 27 women terminated pregnancy after abnormal US findings and/or fetal

infection. As previously reported, there was a reduced rate of elective abortions among HIG-treated women with infected fetuses.[8] Of 157 patients, treated with HIG, 88 patients had DNAemia and 69 lacked DNAemia when first tested. 148 pregnant women with primary CMV infection, who decided not to receive HIG, were followed as comparators. Of these, 61 patients had DNAemia when first tested. 47 patients were retested for DNAemia after 2 to 22 weeks, and 19 (40%) lacked DNAemia. Specifically none of 8 patients who were retested within 2 weeks from the first DNA detection, lacked DNAemia. This finding, although suggestive when compared to 10 of 27 HIG-treated patients who lacked DNAemia after two weeks from the first testing, was not statistically significant (p=0.07). 44/61 (72%) .

Fetal/neonatal infection

We used logistic regression to determine if there were any independent predictors of either fetal or neonatal infection, symptomatic infection, or disabilities after long-term follow up. 31 variables were tested including the kinetics of maternal viremia, avidity, receipt of HIG, and quantitative amniocentesis results.

For fetal/neonatal infection, four factors were independent predictors of fetal infection ($P < 0.05$) in the final logistic regression model (Table 1). The first was a 1.8 fold increase (from 30% to 56%) in the rate of congenital infection without HIG ($P<0.0001$), adjusted odd ratio (AOR = 5.2). The second was a 1.8 fold increase (from 32% vs. 56%) associated with maternal viral DNAemia prior to HIG administration (P=0.002, AOR=3.0). The third was an abnormal US finding (P=0.0001, AOR=54.3). The fourth was the diagnosis of maternal infection via avidity rather then seroconversion (P=0.007, AOR=3.3) .

Symptoms at birth

Table 2 is a similar analysis using neurologic and hearing abnormalities at birth as the outcome

variable. The analysis revealed that there were only two independent variables predictive of symptomatic infection. One was a dramatic reduction of symptomatic infections associated with maternal receipt of one infusion of high-dose HIG. Of HIG-treated infants only 1 of 150 (<1%) had neurological or hearing abnormalities at birth compared to 24 of 131 (18%) infants born of mothers not treated with HIG. Retinal hemorrhage had occurred in other two neonates. As expected the other independent predictor variable of a symptomatic infection was prenatal disease on US.

Long-term follow-up

Long-term follow-up occurred for all 173 uninfected and 106 infected infants evaluated at a mean age of 4 years (range 1 to 8 years). Long term sequelae (Table 3) were independently predicted by not receiving HIG (P<0.0018, AOR=13.2). Only 1 of 41 (2%) children born of mothers who received high-dose HIG had abnormalities at long-term follow up as compared to 21 of 67 (31%) for children of untreated women. Both children with retinal hemorrhage at birth developed normal vision within one year of age. A maternal infection in early gestation (mean of 10 weeks SD = $\pm$6.4 weeks) was more likely to yield children with long-term abnormalities then was infection later in gestation (mean of 16.2, SD =$\pm$8 weeks gestation) (P=0.014, OR = 0.9). Abnormal US findings were a predictor of poor long-term outcome (Table 3. P<0.002, OR =7.6).

HIG adverse events

No immediate or late adverse events occurred during or after HIG infusions. As previously observed, infants born of treated mothers had an average birth weight of 3328 grams (SD = 454 grams) which was significantly higher (P <0.001) than the 3091 grams (SD = 426 grams) for infants born of untreated mothers.

DISCUSSION

HIG is safe and effective for preventing congenital CMV infection after a primary maternal infection or the treatment of fetal disease.[6-14] Using low-dose HIG (100 mg/kg) the rate of maternal-fetal CMV transmission has varied from 16% up to 32%.[8,15] A recent German study observed that high-dose (200 mg/kg) HIG given every 2 weeks in 40 patients after a primary maternal infection at < 14 weeks gestation was 92.5% effective at preventing fetal infection: 1 fetus was infected at amniocentesis, two in late gestation but all 3 infants were asymptomatic.[37] Our study, which also used high-dose HIG, did not achieve this same high rate of HIG efficacy because: 1) the median gestational age of the enrolled women in the German study was of 9.6 weeks, in our study for women infected in the first trimester was 7 weeks; 2) HIG infusions started at 11.1 weeks and administered at mean dose no./women of 3.8 in the German study, at an average gestational age of 15 weeks and given at about twice per women in our study. However, in our study, the maternal-fetal transmission rate for 89 women who received HIG at <14 weeks gestation was 26% compared with a 55% rate for 91 women who did not receive HIG (P=0.01). In particular, there was only a 5% transmission rate among mothers without DNAemia, who were given HIG within 6 weeks from the seroconversion. Hence an enhanced efficacy of HIG can most likely be attributed to prompt HIG administration after a primary infection and/or multiple doses thru the first trimester.

After a primary infection in the 1st half of pregnancy, several studies reported a decrease in neurological and sensorineural sequelae at rates ranging from 3% to 12% in HIG-treated fetuses, as compared to 35% to 50% in the untreated fetuses.[2,3,5,8,10,11,14] In our study there was strong evidence for the efficacy of HIG but not of multiple doses. Without pharmacokinetic data, two randomized trials that unknowingly used low-dose HIG to prevent fetal/neonatal infection failed to observe a statistically significant benefit for HIG.[15,38] These results when contrasted with

those in this study and the German study, indicate the likely benefit of high-dose HIG in a randomized trial.

CMV DNA is present in the blood of pregnant women or immunocompetent adults after a primary infection for as long as 25 to 48 weeks.[27,39] One study reported no association between maternal viremia and viral transmission to the fetus. Another study observed a significant association between CMV DNAemia and mother-to-fetus transmission, independent of maternal viral load.[28] We observed that both HIG treatment and maternal viremia were independent predictors of fetal infection. CMV transmission occurred in 52% of the women with DNAemia and 31% of those without DNAemia. Interestingly in HIG-treated women without DNAemia mother-to-fetus transmission was very low (14%), particularly among women treated within 6 weeks from seroconversion (5%). Thus the indications for HIG infusions may include CMV DNAemia in addition to low maternal IgG avidity and IgM. However, our final logistic regression model (Table 1) shows that even for women with DNAemia, the rates of CMV transmission after HIG treatment were significantly lower than those in untreated women.

This study reinforces that HIG decreases maternal-fetal CMV transmission, especially when administered soon after seroconversion in pregnant women without DNAemia.[8,14] More importantly, HIG administered at 200 units/kg of maternal weight prevented fetal disease among infants infected *in utero*.[8] None of the infants born to 150 HIG treated mothers had neurological abnormalities, and only one (0.7%) had hearing loss. Her mother had a primary infection before 6 weeks gestation, but was treated with HIG at 13 weeks gestation, presumably when developing fetal cochlear cells had irreversible damage by CMV.[40] Two other infants (1.3%) had retinal hemorrhage that resolved within one year.

In our study, of importance were the many factors that were unassociated with fetal infection, symptoms, or disease in the adjusted logistic model. These included: viremia after the first test;

the number of doses of HIG; the interval between maternal infection and DNA testing, HIG administration, and the duration and resolution of viremia; and the copy numbers of DNA in maternal blood and/or amniotic fluid. The validity of these observations are strengthened by our large sample size. In this study birth weight and gestational age at birth were neither appropriate endpoints nor covariates. We previously reported the association of HIG and CMV infections with these variables in a large comprehensive study and no clinically significant association was observed.[33]

This observational study is limited by potential selection bias and lack of an appropriate placebo control. Our study, however has implications for the design of placebo controlled randomized trials. The first is that, for HIG and monoclonal antibodies, use of the optimal dose is based on the half-life of CMV-specific antibodies rather than that of other IgG molecules in immunoglobulin preparations. Second, enrollment using low avidity is not acceptable because it is difficult to estimate accurately the time of maternal infection and thus accurate rates of maternal-fetal transmission. In our study maternal infection diagnosed in the first trimester by low avidity rather than seroconversion was associated with a lower rate of fetal infection, although we enrolled only previously CMV negative women with a very low avidity. Using low avidity as enrollment criteria will result in low infection rates and thus increase the number of subjects needed for a successful trial. Third, immunotherapy, whether active or passive, is optimal when used in early gestation. Our results confirm those of recent report indicating that the majority of infections with a poor long-term outcome occur in the first trimester.[41] Fourth, in clinical trials maternal viremia needs to be monitored so as not to introduce selection bias into a clinical trial. Finally, abnormalities on US/MR exams are an excellent predictor of fetal infection, symptoms, and outcome and thus would be a very appropriate endpoint for clinical trials of active or passive immunization.

In conclusion, although no randomized trial has demonstrated a benefit of CMV HIG on neurodevelopmental outcomes in infants, our findings suggest that these may occur and continued clinical research is required. These data also mean that maternal viremia before HIG administration predicts fetal infection and may be helpful for patient counseling. Finally these data support the efficacy of high-dose HIG to prevent fetal infection, neonatal symptoms, and long-term disabilities.

Notes: Members of the Congenital Cytomegalic Disease Collaborating Group: from the Pediatric Unit, University of L'Aquila, Italy: Stefania Lasorella, Giulia Iapadre, Maria Maresca, Arianna Mareri, Claudia Di Paolantonio, Milena Catenaro, Renato Tambucci, Ivan Mattei; from the Unit of Obstetrics and Gynecology, University of L'Aquila, Italy: Gaspare Carta, Angela D'Alfonso, Felice Patacchiola; from the Department of Pathology, San Salvatore Hospital, L'Aquila, Italy: Maria Aurora Fioroni; from the Department of Radiology, University of L'Aquila, Italy: Lucia Manganaro; from the Unit of Obstetrics and Gynecology, University of Rome, Italy: Antonella Giancotti; from San Giovanni Hospital, Rome, Italy: Daniela Pancallo, Silvia Lauri; from the IRCCS Spallanzani Hospital: Giuseppina Liuzzi; from the University of Perugia and Santa Maria Misericordia Hospital, Perugia, Italy: Gian Carlo Di Renzo, Benedetta Della Torre, Carla Lupi; from the Institutes Microbiology, Obstetrics and Gynecologicy, and Infectious Diseases, University Hospital of Bari: Agata Calvario, Antonella Vimercati, Sergio Carbonara; from the Hospital Ruggi d'Aragona, Salerno: Pasquale Pisano.

Funding: We thank the non-profit onlus association Anticito and the association Pediatria Aquilana for recruitment of patients and partial cost contribution.

Potential conflicts: GN and SPA received a one-time lecture fee from Biortest. SPA reports consultation fees from Merck and Moderna, outside the submitted work.

References

1. Pass RF, Arav-Boger R. Maternal and fetal cytomegalovirus infection: diagnosis, management, and prevention. F1000 Research 2018, 7(F1000 Faculty Rev):255 Last updated: 01 Mar 2018.
2. Bodéus M, Kabamba-Mukadi B, Zech F, Hubinont C, Bernard P, Goubau P. Human cytomegalovirus in utero transmission: follow-up of 524 maternal seroconversions. J ClinVirol 2010;47:201-2.
3. Enders G, Daiminger A, Bäder U, Exler S, Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. J Clin Virol 2011;52:244-6.
4. Nigro G. Maternal-fetal cytomegalovirus infection: from diagnosis to therapy. J Matern Fetal Neonatal Med 2009;22:169-74.
5. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol 2006;35:216-20.
6. Nigro G, La Torre R, Anceschi MM, Mazzocco M, Cosmi EV. Hyperimmunoglobulin therapy for a twin fetus with cytomegalovirus infection and growth restriction. Am J Obstet Gynecol 1999;180:1222-6.
7. La Torre R, Nigro G, Mazzocco M, Best AM, Adler SP. Placental enlargement in women with primary maternal cytomegalovirus infection is associated with fetal and neonatal disease. Clin Infect Dis 2006;43:994-1000.
8. Nigro G, Adler SP, La Torre R, Best AM, Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med 2005;353:1350-62.

9. Nigro G, La Torre R, Pentimalli H, Taverna P, Lituania M, de Tejada BM, Adler SP. Regression of fetal cerebral abnormalities by primary cytomegalovirus infection following hyperimmunoglobulin therapy. Prenat Diagn 2008;28:512-7.
10. Nigro G, Adler SP, Parruti G, Anceschi MM, Coclite E, Pezone I, Di Renzo GC. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy – a case-control study of the outcome in children. J Infect Dis 2012;205:215-27.
11. Visentin S, Manara R, Milanese L, Da Roit A, Forner G, Salviato E, Citton V, Magno FM, Orzan E, Morando C, Cusinato R, Mengoli C, Palù G, Ermani M, Rinaldi R, Cosmi E, Gussetti N. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. Clin Infect Dis 2012;55:497-503.
12. Wagner N, Kagan KO, Haen S, Schmidt S, Yerlikaya G, Maden Z, Jahn G, Hamprecht K. Effective management and intrauterine treatment of congenital cytomegalovirus infection: review article and case series. J Matern Fetal Neonatal Med 2014;27:209-14.
13. Blázquez-Gamero D, Galindo Izquierdo A, Del Rosal T, Baquero-Artigao F, Izquierdo Méndez N, Soriano-Ramos M, RojoConejo P, González-Tomé MI, García-Burguillo A, Pérez Pérez N, Sánchez V, Ramos-Amador JT, De la Calle M. Prevention and treatment of fetal cytomegalovirus infection with cytomegalovirus hyperimmune globulin: a multicenter study in Madrid. J Matern Fetal Neonatal Med 2017 Oct 26:1-9. doi: 10.1080/14767058.2017.1387890.
14. Buxmann H, Stackelberg OM, SchlößerRL, Enders G, Gonser M, Meyer-Wittkopf M, Hamprecht K, Enders M. Use of cytomegalovirus hyperimmunoglobulin for

prevention of congenital cytomegalovirus disease: a retrospective analysis. J Perinat Med 2012;40:439-46.

15. Revello MG, Lazzarotto T, Guerra B, Spinillo A, Ferrazzi E, Kustermann A, Guaschino S, Vergani P, Todros T, Frusca T, Arossa A, Furione M, Rognoni V, Rizzo N, Gabrielli L, Klersy C, Gerna G; CHIP Study Group. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. N Engl J Med 2014;370:1316-26.
16. Fouts AE, Chan P, Stephan JP, Vandlen R, Feierbach B. Antibodies against the gH/gL/ UL128/UL130/UL131 complex comprise the majority of the anticytomegalovirus (anti-CMV) neutralizing antibody response in CMV hyperimmune globulin. J Virol. 2012;86:7444-7.
17. Terrazzini N, Kern F. Cell-mediated immunity to human CMV infection: a brief overview.F1000 Prime Rep 2014;6:28.
18. Nigro G. Hyperimmune globulin in pregnancy for prevention of congenital cytomegalovirus disease. Expert Rev Anti Infect Ther 2017;15:977-86
19. Permar SR, Schleiss MR, Plotkin SA. Advancing our understanding of protective maternal immunity as a guide for development of vaccines to reduce congenital cytomegalovirus infections. J Virol 2018;92. pii: e00030-18. doi: 10.1128/JVI.00030-18. Print 2018 Apr 1.
20. Cekinović D, Golemac M, Pugel EP, Tomac J, Cicin-Sain L, Slavuljica I, Bradford R, Misch S, Winkler TH, Mach M, Britt WJ, Jonjić S. Passive immunization reduces murine cytomegalovirus-induced brain pathology in newborn mice. J Virol 2008;82:12172-80.

21. Cheeran MCJ, Lohensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease, mechanisms and prospects for intervention. Clin Microbiol Rev 2009;22:99-126.
22. Maidji E, Nigro G, Tabata T, McDonagh S, Nozawa N, Shiboski S, Muci S, Anceschi M, Aziz N, Adler SP, Pereira L. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. Am J Pathol 2010;177:1298–1310
23. Marchalonis JJ, Kaymaz H, Dedeoglu F, Schluter S F, Yocum D E, Edmundson A B. Human autoantibodies reactive with synthetic autoantigens from T-cell receptor beta chain. Proc Natl Acad Sci U S A 1992; 89: 332-9.
24. Frank MM, Basta M, Freis LF. The effects of intravenous immunoglobulin on complement-dependent immune damage of cells and tissues. Clin Immunol Immunopathol 1992;62:582-6.
25. Andersson UG, Björk L, Skansén-Saphir U, Andersson JP. Down-regulation of cytokine production and interleukin-2 receptor expression by pooled human IgG. Immunology. 1993;79:211-6.
26. Aukrust P, Frøland SS, Liabakk NB, Müller F, Nordøy I, Haug C, Espevik T. Release of cytokines, soluble cytokine receptors, and interleukin-1 receptor antagonist after intravenous immunoglobulin administration in vivo. Blood 1994;84:2136-43.
27. Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat Rev Immunol 2013;13:176-89.

28. Revello MG, Zavattoni M, Sarasini A, Percivalle E, Simoncini L, Gerna G. Human citomegalovirus in blood of immunocompetent persons during primary infection: Prognostic implications for pregnancy. J Infect Dis 1998;177:1170-5.
29. Delforge ML, Costa E, Brancart F, Goldman D, Montesinos I, Zaytouni S, Marchant A, Donner C. Presence of cytomegalovirus in urine and blood of pregnant women with primary infection might be associated with fetal infection. J Clin Virol 2017;90:14-17.
30. Nigro G, Anceschi MM, Cosmi EV, Congenital Cytomegalic Disease Collaborating Group. Clinical manifestations and abnormal laboratory findings in pregnant women with primary cytomegalovirus infection. Brit J Obstet Gynaecol 2003;110:572-7.
31. Gaunt G, Ramin K. Immunologic tolerance of the human fetus. Am J Perinatol 2001;299-312.
32. Thurmann PA, Sonnenburg-Chatzopoulos C, Lissner R. Pharmacokinetic characteristics and tolerability of a novel intravenous immunoglobulin preparation. Eur J Clin Pharmacol 1995;49:237-242.
33. Hamprecht K, Bissinger AL, Arellano-Galindo J, et al. Intrafamilial transmission of human cytomegalovirus (HCMV): long-term dynamics of epitope-specific antibody response in context of avidity maturation. J Clin Virol 2014;60:119-126.
34. Nigro G, Capretti I, Manganello AM, Best AM, Adler SP. Maternal cytomegalovirus infections during pregnancy: association of CMV hyperimmune globulin with gestational age at birth and birth weight. J Matern Fetal Neonatal Med 2015;28:168-72.
35. Puliyanda DP, Silverman NS, Lehman D, Vo A, Bunnapradist S, Radka RK, Toyoda M, Jordan SC. Successful use of oral ganciclovir for the treatment of

intrauterine cytomegalovirus infection in a renal allograft recipient. Transpl Infect Dis 2005;7:71-4.

36. Leruez-Ville M, Ghout I, Bussières L, Stirnemann J, Magny JF, Couderc S, Salomon LJ, Guilleminot T, Aegerter P, Benoist G, Winer N, Picone O, Jacquemard F, Ville Y. In utero treatment of congenital cytomegalovirus infection with valacyclovir in a multicenter, open-label, phase II study. Am J Obstet Gynecol 2016;215:462.e1–462.e10.
37. Kagan KO, Enders M, Schampera MS, Baeumel E, Hoopmann M, Geipel A, Berg C, Goelz R, De Catte L, Wallwiener D, Brucker S, Adler SP, Jahn G, Hamprecht K. Prevention of maternal-fetal transmission of CMV by hyperimmunoglobulin (HIG) administered after a primary maternal CMV infection in early gestation. Ultrasound Obstet Gynecol 2018 Jun 26. doi: 10.1002/uog.19164.
38. A randomized trial to prevent congenital cytomegalovirus (CMV). Clinical Trials.govNCT01376778, 2019.
39. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF. Asymptomatic primary cytomegalovirus infection: Virologic and immunologic features. J Infect Dis 1999;180:702-7.
40. Powles-Glover N, Maconochie M. Prenatal and postnatal development of the mammalian ear. Birth Defects Res 2018;110:228-45.
41. Faure-Bardon V, Magny JF, Parodi M, Couderc S, Garcia P, Maillotte AM, Benard M, Pinquier D, Astruc D, Patural H, Pladys P, Parat S, Guillois B, Garenne A, Bussières L, Guilleminot T, Stirnemann J, Ghout I, Ville Y, Leruez-Ville M. Sequelae of congenital cytomegalovirus (cCMV) following maternal primary infection are limited to those acquired in the first trimester of pregnancy.

Clin Infect Dis 2018 Dec 31. doi: 10.1093/cid/ciy1128. [Epub ahead of print] PMID: 30596974

Table 1. Univariate and Multivariate (Logistic Regression) analysis of possible predictors of CMV infection in fetuses and newborns

Predictor variables	CMV infected fetuses/ newborns	CMV uninfected fetuses/ newborns	Univariate P value	Multivariate Adjusted P value	Adjusted odds ratio (95% CI)
All women [#]	131	173			
Mean maternal age (years) at enrollment	32±5 (n=131)	33±5 (n=173)	0.18	ND	
Maternal infection – Mean weeks gestation	13.6±7.9 (n=131)	11.7±7.3 (n=173)	P=0.02	0.84	
Time of primary infection. No. women ≤14 weeks gestation >14 weeks gestation	70 61	112 61	P=0.06	P= 0.14	
No. of primary infections (<14 weeks gestation) identified by: Seroconversion[&] Low avidity	48 25	53 55	P=0.03	P=0.007	3.3 (1.75-7.2)
HIG therapy No. mothers: HIG yes HIG no	47 84	110 63	P<0.0001	P<0.0001	5.2 [2.4-11.5]
Mean number of HIG doses/woman who received HIG	2.2±1.3 (N=47)	2.0±1.4 (n=110)	P=0.25	ND	
Weeks gestations when HIG given	22.0±7.0 (n=47)	19.1±6.8 (n=110)	P=0.02	P=0.6	
Interval between in weeks between maternal infection and HIG given	7.36±2.8 (n=47)	7.2±2.9 (n=110)	P=0.8	ND	

[#] For data analysis four twin pregnancies were treated as single births.

[&] At > 14 weeks gestation all maternal infections were diagnosed by seroconversion.

Number of mothers: With DNAemia Without DNAemia	81 50	67 106	P<0.0001	P=0.005	2.9 [1.5-6.6]
Number of mothers with DNAemia 1st and 2nd tests Yes No	33 31	38 30	P=0.72	ND	
DNA tested (weeks gestation after maternal infection)	6.9±3.7 (n=131)	6.8±3.8 (n=173)	P=0.68	ND	
Week gestation 1st DNA tested	20.1±7.0 (n=131)	18.5±7.1 (n=173)	P=0.03	P=0.9	
Copy number maternal DNA 1st test	3041±8000 (n=57)	2415±5000 (N=51)	P=0.63	ND	
Weeks gestation between 1st DNA+ and HIG administration	1.81±1.3 (n=36)	1.62±1.1 (n=51)	P=0.48	ND	
Weeks gestation when 2nd DNA tested	25.5±7.7 (n=67)	22.1±6.4 (n=64)	P=0.002	P=0.12	
Weeks gestation between testing 1st and 2nd DNA	5.4±4.3 (n=67)	3.8±2.9 (n=64)	P=0.02	P=0.34	
Maternal DNAemia 2nd test Yes No	33 30	38 31	P=0.72	ND	
Copy number 2nd DNA test	698±974 (n=24)	307±265 (n=25)	P=0.07	P=0.1	
1st DNA + 2nd + Yes No	38 30	30 33	P=0.72	ND	
1-2 weeks interval DNA+ to DNA negative	6	6			

yes no	11	13	P=1.0	ND	
3-4 weeks yes no	11 13	15 8	P=0.24	ND	
>4 weeks yes no	9 9	17 12	P=0.76	ND	
1st avidity	9.9±5.8 (N=119)	11.1±6.9 (n=163)	P=0.1	P=0.45	
Weeks gestation 1st avidity	19.3±7.3 (n=119)	17.5±7.0 (n=163)	P=0.04	P=0.7	
2nd avidity	38.2±17 (n=83)	20.5±9.0 (n=133)	P=0.35	ND	
Weeks gestation 2 nd avidity	24.4±6.7 (n=82)	21.7±7.2 (n=134)	P=0.02	P=0.23	
Avidity rise	27.15±8 (n=82)	8.7±17 (n=134)	P=0.3	ND	
Interval between 1st and 2nd avidity	5.6±5.0 (n=82)	4.5±4.0 (n=134)	P=0.06	P=0.16	
1st DNA+, 2nd DNA neg Yes No	29 41	31 33	P=0.49	ND	
Prenatal disease via ultrasound Yes No	24 106	2 171	P<0.0001	P<0.0001	59 (6.0,488)

Table 2. Univariate and Multivariate (Logistic Regression) analysis of possible predictors of symptomatic congenital CMV infection

Predictor variables	All symptomatic infants	All asymptomatic infants	Univariate P value	Multivariate P value	Adjusted odds ratio (95% CI)
All women	25	256			
Maternal age at enrollment- Years	30.55 (n=25)	33+5 (n=256)	P=0.04	P=0.76	
Maternal infection - Mean weeks gestation	11.8±6,8 (n=25)	13.1±7.8 (n=256)	P=0.21	ND	
Time of primary infection – No. of women ≤14 weeks gestation >14 weeks gestation	 15 10	 110 146	P=0.4	ND	
Serologic diagnosis 1st trimester by Seroconversion Low avidity	 10 5	 77 68	P=0.4	ND	
HIG therapy – No. mothers: HIG yes HIG NO	 1 24	 149 107	P<0.001	P<0.001	59 (5-400)
Mean number of HIG infusions/woman for women who received HIG	2±0 (n=1)	2±1.3 (n=149)	P=0.9	ND	
Weeks gestation when HIG first given	13 (n=1)	20.2±6.9 (n=149)	P= 0.3	ND	
Interval in weeks between maternal infection and 1st HIG given	9 (N=1)	7.9±8.0 (n=149)	P>0.9	ND	
No. of mothers: With DNAemia Without DNAemia	 12 13	 123 133	P=1.0	ND	
1st DNA+, 2nd DNA neg Yes No	 2 5	 55 61	P=0.45	ND	

DNA tested (weeks after maternal infection)	7.88±3.9 (n=25)	6.78±3.9 (n=256)	P=0.22	ND	
Weeks gestation 1st DNA tested	19.1±6.5 (n=25)	19.5±7.3 (n=256)	P=0.8	ND	
1st DNA+ mean genome copy number	954±661 (n=6)	2632±6959 (n=92)	P=0.45		
Weeks gestation when 2nd DNA tested	26±9.2 (n=7)	24±7.2 (n=113)	P=0.45	ND	
Interval in weeks between testing 1st and 2nd DNA	11.2±7.8 (n=7)	4.4±3.1 (n=113)	P=0.6	ND	
Maternal DNAemia 2nd test Yes No	 2 5	 55 59	P=0.44	ND	
2nd DNA genome copy no.	137 (n=1)	437±512 (n=44)	P=1	ND	
1st avidity	12.1±6.9 (n=22)	10.5±6.5 (n=240)	P=0.3	ND	
1st avidity (weeks)	18.6±7.3 (n=22)	18.6±7.3 (n=240)	P=0.5	ND	
2nd avidity	17.8±5.8 (n=12)	20.1±9.6 (n=190)	P=0.23	ND	
2 nd avidity (weeks)	23±5.8 (n=12)	22.8±7.2 (n=190)	P=0.89	ND	
Avidity rise	7.4±5.8 (n=12)	9.9±8.5 (n=190)	P=0.01	P=0.5	
Interval between 1st and 2nd avidity (weeks)	7.0±4.8 (n=12)	4.8±3.9 (n=190)	P=0.15	P=0.94	
Prenatal disease via ultrasound Yes No	 12 13	 7 248	P<0.001	P<0.001	26 (6-105)
Amniotic fluid DNA genome copy number	1222941 ± 2388146 (n=16)	789592 ± 1267450 (n=17)	P=0.52	ND	

Table 3. Univariate and Adjusted Multivariate (Logistic Regression) analysis of possible predictors of CMV disease at long term follow-up.

Predictor variables	Abnormal N=21	Normal N=85	Univariate P value	Multivariate P value	Adjusted odds ratio (95% CI)
Maternal age at enrollment - Years	30.3±5.6 (n=21)	33.0±4.8 (n=85)	P=0.06	P=0.95	
Maternal infection – Mean weeks gestation	N=21 (10.1±6.4)	N=85 (16.2±7.8)	=0.009	P=0.017	0.90 (0.83, 0.98)
Serologic diagnosis 1st trimester by: Seroconversion Low avidity	 10 4	 24 12	P=1.0	ND	
Number of HIG-treated mothers: HIG yes HIG No	 1 20	 40 45	P<0.0005	P<0.0018	 13.2 [1.6-110]
Mean number of HIG infusions/woman for HIG -treated women	2±0 (n=1)	2.2±1.4 (n=40)	ND	ND	
Weeks gestation when HIG first given	13±0 (n=1)	23.3±6.5 (n=40)	ND	ND	
Interval in weeks between maternal infection and 1st HIG	9.8±0 (n=1)	9.9±13.8 (n=40)	ND	ND	
Number of mothers: With DNAemia Without DNAemia	 10 10	 55 31	P=0.25	ND	
DNA tested: Weeks gestation after maternal infection	 8.1±4.0 (n=21)	 6.7±3.9 (n=85)	P=0.05	P=0.78	
Weeks gestation when 1st DNA tested	18.2±6.9 (n=21)	22±7.0 (n=85)	P=0.04	P=0.38	

DNA+ mean genome copy number 1st test	835±622 (n=4)	2828±8802 (n=41)	P=0.15	P=0.83	
Weeks gestation when 2nd DNA tested	25.2±10.8 (n=5)	27.2±6.89 (n=49)	P=0.7	ND	
Interval in weeks between testing 1st and 2nd DNA	12.2±8.6 (n=5)	5.3±3.6 (n=49)	P=.0.14	P=0.88	
DNAemia 2nd test: Yes No	4 1	24 25	P=0.35	ND	
1st avidity	13.1±7.2 (n=18)	10.1±6.4 (n=78)	P=0.1	P=0.1	
Weeks gestation 1st avidity	17.0±6.3 (n=18)	21.3±7.1 (N=78)	P=0.02	P= 0.37	
2nd avidity	20±4.1 (n=9)	20.0±10.5 (n=58)	P=0.8	ND	
Weeks gestation 2nd avidity	23.0±5.7 (n=9)	25.1±6.5 (n=58)	P=0.34	ND	
Avidity rise	8.8±6 (n=9)	10.0±98.9 (n=58)	P=0.5	ND	
Interval in weeks between 1st and 2nd avidity	7.7±5.3 (n=9)	5.0±4.0 (n=58)	P=0.16	P=0.77	
Prenatal disease via ultrasound Yes No	8 13	7 77	P=0.002	P<0.003	7.6 (1.8-31.6)
Amniotic fluid DNA genome copy number	553925 ± 495708 (n=12)	1080876 ± 2352757 (n=17)	P=0.37	ND	
Average age of children in years at final evaluation	4.6+1.8 (n=21)	4.0+1.5 (n=85)	P=0.22	ND	