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MAJOR ARTICLE

High-Dose Cytomegalovirus (CMV) Hyperimmune Globulin and Maternal CMV DNAemia Independently Predict Infant Outcome in Pregnant Women With a Primary CMV Infection

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(See the Editorial Commentary by Schleiss on pages 1499–501.)

Background. After primary maternal cytomegalovirus (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 primary infection. These women 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, 1–8 years). One hundred fifty-seven women were treated with an average of 2 doses (range, 1–6 doses) 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 (adjusted odds ratio [AOR], 5.2; $P < .0001$), a 1.8-fold increase (32% vs 56%) associated with maternal viral DNAemia prior to HIG administration (AOR, 3.0; $P = .002$), abnormal ultrasounds (AOR, 59; $P = .0002$), and diagnosis of maternal infection by seroconversion rather than avidity (AOR, 3.3; $P = .007$). Lack of HIG and abnormal ultrasounds also predicted symptoms ($P = .001$). Long-term sequelae were predicted by not receiving HIG (AOR, 13.2; $P = .001$), maternal infection in early gestation (odds ratio [OR], 0.9; $P = .017$), and abnormal ultrasounds (OR, 7.6; $P < .003$). 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.

Keywords. cytomegalovirus; pregnancy; hyperimmune globulin; DNAemia.

In Europe and the United States, about 80 000 pregnant women annually acquire primary cytomegalovirus (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]. Despite these frequent and severe effects, CMV screening in pregnancy is not routine. In Italy, CMV serologic screening is frequent at the first postconception evaluation, and an increasing number of pregnant women request CMV monthly serologic testing after discovering CMV information on websites or from friends.

Hyperimmune globulin (HIG) administration to pregnant women with primary CMV infection was initiated >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 despite the high cost, HIG is administered off-label worldwide.

At least 2 factors may account for the success of immunotherapy: (1) HIG is obtained from >1000 donors and contains all human high-titer and high-avidity anti-CMV antibodies [16–19]; and (2) HIG may decrease the CMV-induced inflammatory damage in the placenta and fetal organs because it contains immunoglobulin G (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 maternofetal CMV transmission and fetal/neonatal CMV disease appear associated with maternal CMV DNAemia, but there are no reports on the association of HIG, maternal

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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 of pregnant women with a primary CMV infection.

METHODS

Patients

Three hundred four Italian pregnant women with a primary CMV infection were enrolled between 1 October 2010 and 30 June 2017. No patients with human immunodeficiency virus (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 halfway between the last seronegative and the first seropositive serum or the beginning of symptoms (ie, fever, flulike syndrome) or laboratory abnormalities, when these occurred [29]. For women who had both immunoglobulin M (IgM) and IgG antibodies associated with very low avidity in the first 2 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 imaging (MRI). Congenital CMV infection was defined symptomatic if the neonate had 1 or more of the following: ventriculomegaly, periventricular calcifications, microcephaly, leukoencephalopathy, cerebral or cerebellar atrophy, full or partial hearing loss in 1 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 from Disease Diagnostica Senese and DiaSorin, and CMV DNA was detected using real-time polymerase chain reaction 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]; and (3) since the half-life of anti-CMV antibodies in HIG is <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 3 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 2–3 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 brainstem-evoked response studies, and an ophthalmoscopic evaluation. Brain MRI and computed tomography were also performed in symptomatic infants. CMV-infected infants were monitored for 1–8 years (mean, 4 years) by routine clinical evaluation, sensorineural hearing, and eye examinations. The study was approved by the Ethical Committees of University of L'Aquila and the nonprofit association AMICI Onlus (Association of Mother-Infant Cytomegalovirus Infection). 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, version 14.0 (SAS Institute). Fisher exact test and *t* tests were used for

Table 1. Univariate and Multivariate (Logistic Regression) Analysis of Possible Predictors of Cytomegalovirus Infection in Fetuses and Newborns

Predictor Variables	CMV-Infected Fetuses/Newborns	CMV-Uninfected Fetuses/Newborns	Univariate *P* Value	Adjusted OR (95% CI)	Multivariate *P* Value
All women[a], No.	131	173			
Mean maternal age at enrollment, y	32 ± 5 (n = 131)	33 ± 5 (n = 173)	.18	…	ND
Maternal infection, mean weeks' gestation	13.6 ± 7.9 (n = 131)	11.7 ± 7.3 (n = 173)	.02	…	.84
Time of primary infection, No. of women					
≤14 weeks' gestation	70	112	.06	…	.14
>14 weeks' gestation	61	61		…	
No. of primary infections (<14 weeks' gestation), identified by					
Seroconversion[b]	48	53	.03	3.3 (1.75–7.2)	.007
Low avidity	25	55		…	
HIG therapy (No. of mothers)					
Yes	47	110		…	
No	84	63	< .0001	5.2 (2.4–11.5)	< .0001
Mean No. of HIG doses/woman who received HIG	2.2 ± 1.3 (n = 47)	2.0 ± 1.4 (n = 110)	.25	…	ND
Weeks' gestations when HIG given	22.0 ± 7.0 (n = 47)	19.1 ± 6.8 (n = 110)	.02	…	.6
Interval between maternal infection and HIG given, weeks	7.36 ± 2.8 (n = 47)	7.2 ± 2.9 (n = 110)	.8	…	ND
No. of mothers					
With DNAemia	81	67	< .0001	2.9 (1.5–6.6)	.005
Without DNAemia	50	106		…	
No. of mothers with DNAemia first and second tests					
Yes	33	38	.72	…	ND
No	31	30		…	
DNA tested, weeks' gestation after maternal infection	6.9 ± 3.7 (n = 131)	6.8 ± 3.8 (n = 173)	.68	…	ND
Weeks' gestation when first DNA tested	20.1 ± 7.0 (n = 131)	18.5 ± 7.1 (n = 173)	.03	…	.9
Copy number maternal DNA first test	3041 ± 8000 (n = 57)	2415 ± 5000 (n = 51)	.63	…	ND
Weeks' gestation between first DNA-positive and HIG administration	1.81 ± 1.3 (n = 36)	1.62 ± 1.1 (n = 51)	.48	…	ND
Weeks gestation when second DNA tested	25.5 ± 7.7 (n = 67)	22.1 ± 6.4 (n = 64)	.002	…	.12
Weeks gestation between testing first and second DNA	5.4 ± 4.3 (n = 67)	3.8 ± 2.9 (n = 64)	.02	…	.34
Maternal DNAemia second test					
Yes	33	38	.72	…	ND
No	30	31		…	
Copy number of second DNA test	698 ± 974 (n = 24)	307 ± 265 (n = 25)	.07	…	.1
First DNA positive, second DNA positive					
Yes	38	30	.72	…	ND
No	30	33		…	
1–2 weeks' interval from DNA positive to DNA negative					
Yes	6	6	1.0	…	ND
No	11	13		…	
3–4 weeks' interval from DNA positive to DNA negative					
Yes	11	15	.24	…	ND
No	13	8		…	
>4 weeks' interval from DNA positive to DNA negative					
Yes	9	17	.76	…	ND
No	9	12		…	
First avidity	9.9 ± 5.8 (n = 119)	11.1 ± 6.9 (n = 163)	.1	…	.45
Weeks' gestation first avidity	19.3 ± 7.3 (n = 119)	17.5 ± 7.0 (n = 163)	.04	…	.7
Second avidity	38.2 ± 17 (n = 83)	20.5 ± 9.0 (n = 133)	.35	…	ND
Weeks' gestation second avidity	24.4 ± 6.7 (n = 82)	21.7 ± 7.2 (n = 134)	.02	…	.23
Avidity rise	27.15 ± 8 (n = 82)	8.7 ± 17 (n = 134)	.3	…	ND

Table 1. Continued

Predictor Variables	CMV-Infected Fetuses/Newborns	CMV-Uninfected Fetuses/Newborns	Univariate *P* Value	Adjusted OR (95% CI)	Multivariate *P* Value
Interval between first and second avidity	5.6 ± 5.0 (n = 82)	4.5 ± 4.0 (n = 134)	.06	…	.16
First DNA positive, second DNA negative					
Yes	29	31	.49	…	ND
No	41	33		…	
Prenatal disease via ultrasound					
Yes	24	2	< .0001	59 (6.0–488)	< .0001
No	106	171		…	

Data are presented as mean ± standard deviation unless otherwise indicated.

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; HIG, hyperimmune globulin; ND, not determined; OR, odds ratio.

[a]For data analysis, 4 twin pregnancies were treated as single births.
[b]At >14 weeks' gestation, all maternal infections were diagnosed by seroconversion.

univariate analysis. Variables with a univariate *P* value of < .2 were tested in a logistic regression model.

RESULTS

Of the enrolled 304 women with primary CMV infection, 277 delivered 281 infants, 4 of whom were twins. Twenty-seven 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. One hundred forty-eight 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 2–22 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 2 weeks from the first testing, was not statistically significant ($P = .07$).

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. Thirty-one variables were tested including the kinetics of maternal viremia, avidity, receipt of HIG, and quantitative amniocentesis results. For fetal/neonatal infection, 4 factors were independent predictors of fetal infection ($P < .05$) 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 (adjusted odds ratio [AOR], 5.2; $P < .0001$). The second was a 1.8-fold increase (from 32% vs 56%) associated with maternal viral DNAemia prior to HIG administration (AOR, 3.0; $P = .002$). The third was an abnormal US finding (AOR, 59 $P = .0001$). The fourth was the diagnosis of maternal infection by seroconversion rather than avidity ($P = 0.007$, 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 2 independent variables predictive of symptomatic infection. One was a dramatic reduction of symptomatic infections associated with maternal receipt of 1 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 2 other 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, 1–8 years). Long-term sequelae (Table 3) were independently predicted by not receiving HIG (AOR, 13.2; $P < .0018$). 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 1 year of age. A maternal infection in early gestation (mean, 10 [standard deviation {SD}, 6.4] weeks' gestation) was more likely to yield children with long-term abnormalities than was infection later in gestation (mean, 16.2 [SD, 8] weeks' gestation) (odds ratio [OR], 0.9; $P = .014$). Abnormal US findings were a predictor of poor long-term outcome (OR, 7.6; $P < .003$; Table 3).

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 (SD, 454) grams, which was significantly higher ($P < .001$) than the 3091 (SD, 426) grams for infants born of untreated mothers.

Table 2. Univariate and Multivariate (Logistic Regression) Analysis of Possible Predictors of Symptomatic Congenital Cytomegalovirus Infection

Predictor Variables	All Symptomatic Infants	All Asymptomatic Infants	Univariate *P* Value	Adjusted OR (95% CI)	Multivariate *P* Value
All women, No.	25	256		…	
Maternal age at enrollment, y	30 ± 5 (n = 25)	33 ± 5 (n = 256)	.04	…	.76
Maternal infection					
Mean weeks' gestation	11.8 ± 6.8 (n = 25)	13.1 ± 7.8 (n = 256)	.21	…	ND
Time of primary infection, No. of women					
≤14 weeks' gestation	15	110		…	
>14 weeks' gestation	10	146	.4	…	ND
Serologic diagnosis first trimester by					
Seroconversion	10	77		…	
Low avidity	5	68	.4	…	ND
HIG therapy (No. of mothers)					
Yes	1	149	< .001	59 (5–400)	< .001
No	24	107		…	
Mean No. of HIG infusions/woman for women who received HIG	2 ± 0 (n = 1)	2 ± 1.3 (n = 149)	.9	…	ND
Weeks' gestation when HIG first given	13 (n = 1)	20.2 ± 6.9 (n = 149)	.3	…	ND
Interval, weeks, between maternal infection and first HIG given	9 (n = 1)	7.9 ± 8.0 (n = 149)	> .9	…	ND
No. of mothers					
With DNAemia	12	123	1.0	…	ND
Without DNAemia	13	133		…	
First DNA positive, second DNA negative					
Yes	2	55		…	
No	5	61	.45	…	ND
DNA tested (weeks after maternal infection)	7.88 ± 3.9 (n = 25)	6.78 ± 3.9 (n = 256)	.22	…	ND
Weeks' gestation when first DNA tested	19.1 ± 6.5 (n = 25)	19.5 ± 7.3 (n = 256)	.8	…	ND
First DNA-positive mean genome copy number	954 ± 661 (n = 6)	2632 ± 6959 (n = 92)	.45	…	
Weeks' gestation when second DNA tested	26 ± 9.2 (n = 7)	24 ± 7.2 (n = 113)	.45	…	ND
Interval, weeks, between testing first and second DNA	11.2 ± 7.8 (n = 7)	4.4 ± 3.1 (n = 113)	.6	…	ND
Maternal DNAemia second test					
Yes	2	55		…	
No	5	59	.44	…	ND
Second DNA genome copy number	137 (n = 1)	437 ± 512 (n = 44)	1	…	ND
First avidity	12.1 ± 6.9 (n = 22)	10.5 ± 6.5 (n = 240)	.3	…	ND
First avidity, weeks	18.6 ± 7.3 (n = 22)	18.6 ± 7.3 (n = 240)	.5	…	ND
Second avidity	17.8 ± 5.8 (n = 12)	20.1 ± 9.6 (n = 190)	.23	…	ND
Second avidity, weeks	23 ± 5.8 (n = 12)	22.8 ± 7.2 (n = 190)	.89	…	ND
Avidity rise	7.4 ± 5.8 (n = 12)	9.9 ± 8.5 (n = 190)	.01	…	.5
Interval between first and second avidity, weeks	7.0 ± 4.8 (n = 12)	4.8 ± 3.9 (n = 190)	.15	…	.94
Prenatal disease via ultrasound					
No	12	7		…	
Yes	13	248	< .001	26 (6–105)	< .001
Amniotic fluid DNA genome copy number	1 222 941 ± 2 388 146 (n = 16)	789 592 ± 1 267 450 (n = 17)	.52	…	ND

Data are presented as mean ± standard deviation unless otherwise indicated.
Abbreviations: CI, confidence interval; HIG, hyperimmune globulin; ND, not determined; OR, odds ratio.

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 and 2 in late gestation, but all 3 infants were asymptomatic [33]. 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 9.6 weeks, but in our study for women infected in

Table 3. Univariate and Adjusted Multivariate (Logistic Regression) Analysis of Possible Predictors of Cytomegalovirus Disease at Long-term Follow-up

Predictor Variables	Abnormal (n = 21)	Normal (n = 85)	Univariate *P* Value	Adjusted OR (95% CI)	Multivariate *P* Value
Maternal age at enrollment, y	30.3 ± 5.6 (n = 21)	33.0 ± 4.8 (n = 85)	.06	…	.95
Maternal infection, mean weeks' gestation	10.1 ± 6.4 (n = 21)	16.2 ± 7.8 (n = 85)	.009	0.90 (.83–.98)	.017
Serologic diagnosis first trimester by					
Seroconversion	10	24	1.0	…	ND
Low avidity	4	12		…	
No. of HIG-treated mothers					
Yes	1	40		…	
No	20	45	< .0005	13.2 (1.6–110)	< .0018
Mean No. of HIG infusions per HIG -treated woman	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 first HIG	9.8 ± 0 (n = 1)	9.9 ± 13.8 (n = 40)	ND	…	ND
No. of mothers					
With DNAemia	10	55		…	
Without DNAemia	10	31	.25	…	ND
DNA tested					
Weeks' gestation after maternal infection	8.1 ± 4.0 (n = 21)	6.7 ± 3.9 (n = 85)	.05	…	.78
Weeks' gestation when first DNA tested	18.2 ± 6.9 (n = 21)	22 ± 7.0 (n = 85)	.04	…	.38
DNA-positive mean genome copy number first test	835 ± 622 (n = 4)	2828 ± 8802 (n = 41)	.15	…	.83
Weeks' gestation when second DNA tested	25.2 ± 10.8 (n = 5)	27.2 ± 6.89 (n = 49)	.7	…	ND
Interval in weeks between testing first and second DNA	12.2 ± 8.6 (n = 5)	5.3 ± 3.6 (n = 49)	.14	…	.88
DNAemia second test					
Yes	4	24		…	
No	1	25	.35	…	ND
First avidity	13.1 ± 7.2 (n = 18)	10.1 ± 6.4 (n = 78)	.1	…	.1
Weeks' gestation at first avidity	17.0 ± 6.3 (n = 18)	21.3 ± 7.1 (n = 78)	.02	…	.37
Second avidity	20 ± 4.1 (n = 9)	20.0 ± 10.5 (n = 58)	.8	…	ND
Weeks' gestation at second avidity	23.0 ± 5.7 (n = 9)	25.1 ± 6.5 (n = 58)	.34	…	ND
Avidity rise	8.8 ± 6 (n = 9)	10.0 ± 98.9 (n = 58)	.5	…	ND
Interval, weeks, between first and second avidity	7.7 ± 5.3 (n = 9)	5.0 ± 4.0 (n = 58)	.16	…	.77
Prenatal disease via ultrasound					
Yes	8	7	.002	7.6 (1.8–31.6)	< .003
No	13	77		…	
Amniotic fluid DNA genome copy number	553 925 ± 495 708 (n = 12)	1 080 876 ± 2 352 757 (n = 17)	.37	…	ND
Average age of children, y, at final evaluation	4.6 ± 1.8 (n = 21)	4.0 ± 1.5 (n = 85)	.22	…	ND

Data are presented as mean ± standard deviation unless otherwise indicated.
Abbreviations: CI, confidence interval; HIG, hyperimmune globulin; ND, not determined; OR, odds ratio.

the first trimester was 7 weeks; (2) HIG infusions started at 11.1 weeks and were administered at a mean dose per woman of 3.8 in the German study, but at an average gestational age of 15 weeks and given at about twice per woman in our study. However, in our study, the maternofetal 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 ($P = .01$). 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 through the first trimester. After a primary infection in the first 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, 2 randomized trials that unknowingly used low-dose HIG to prevent fetal/neonatal infection failed to observe a statistically significant benefit for HIG [15, 34]. 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 25–48 weeks [29, 35]. 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 maternofetal 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 1 (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 [36]. Two other infants (1.3%) had retinal hemorrhage that resolved within 1 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 is 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; no clinically significant association was observed [37]. 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 reports indicating that the majority of infections with a poor long-term outcome occur in the first trimester [38]. 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/MRI 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

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