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Research Article

One Timely High-Dose Hyperimmune Globulin May Prevent Congenital Neurological Sequelae and Decrease Fetal Transmission and Hearing Deficit After Primary Cytomegalovirus Infection in The First Trimester of Pregnancy: Long-Term Follow-Up

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Abstract

Background: The rate and severity of disabilities caused by congenital cytomegalovirus (CMV) infection when primary maternal infection in the first trimester of pregnancy are up to 40%. Two randomized trials using monthly infusions of low-dose (100 Units or mg/kg) hyperimmune globulin (HIG) showed nonsignificant results for preventing mother-to-infant CMV transmission. Conversely, observational studies reporting significantly decreased rates of fetal CMV infection following bi-weekly infusion of high-dose (HD: 200 Units/kg) HIG. However, both HIG therapies reported neurological disabilities and were costly. **Objective:** Since HIG displays both antiviral and immunomodulatory activities, and the efficacy appeared to be higher if given soon after maternal infection, we performed a prospective cohort study administering only one timely HD-HIG infusion to evaluate: **1)** the incidence of symptomatic congenital CMV disease; **2)** maternal-fetal transmission rates; **3)** safety profile; and **4)** cost-benefit ratio. **Study Design:** 156 pregnant women were offered one HD-HIG infusion: 76 women (2 twin pregnancies) accepted, while 80 (1 twin pregnancy) did not. Their infants were followed up for a mean of 4.6 years. **Results:** Clinical and/or image abnormalities occurred in 3 fetuses/neonates (3.8%) from HIG treated mothers, but at long-term follow-up all children had normal psychomotor development and 2 developed left deafness. On the contrary, the number of fetuses/neonates with CMV disease from non-HIG-treated mothers was statistically higher (16 vs. 3; $p<0.003$) as well as the children at follow-up (10/74 vs. 2/72; $p<0.019$). CMV was transmitted to 15/78 fetuses/neonates

(19.2%) from HD-HIG treated mothers, but to none of 20 infants from mothers treated within 5 weeks from maternal infection. Among nonHIG-group CMV transmission occurred in 27/81 fetuses/neonates (33.3%), being slightly statistically higher (p=0.044) when compared to HD-HIG-treated patients. In addition, no patient experienced significant adverse events related to HD-HIG administration. **Conclusions:** In pregnant women with primary CMV infection in the first trimester, one timely HD-HIG infusion prevented the development of neurological sequelae, reduced rate and severity of deafness, decreased significantly the rate of fetal infections, especially in women treated within 5 weeks from their infection, was safe, and inexpensive.

Keywords: Primary cytomegalovirus infection in the first trimester of pregnancy; Congenital cytomegalovirus infection; Hyperimmune globulin in pregnancy; Neurological disabilities; Hearing loss.

Introduction

Human Cytomegalovirus (CMV) is the most common cause of congenital infections. There are two primary reasons for this: **1)** CMV is the only pathogen capable of infecting the fetus during both primary and non-primary (recurrent) maternal infections; **2)** the virus can be shed for years from children with infection acquired prenatally or in the first years of life. Congenital CMV infection occurs in approximately 0.5%-2% of all liveborn neonates, but in about 40% of infants born to mothers with primary infection [1]. In these women, the viral transmission rates are increasingly associated with the progression of pregnancy and placental aging, while the symptomatology of the infected infants is inversely correlated with the weeks of pregnancy [2]. When transmitted in the first three months of pregnancy, because of the high susceptibility of the embryonal cells to CMV and the lack of maternal and fetal immune defenses against the virus, CMV can cause severe neurological and sensorineural sequelae up to 40% in infected newborns [3,4].

Primary CMV infection is asymptomatic in most of pregnant women. A flu-like syndrome with lymphocytosis and elevated amninotransferases occurs in some patients [5]. Due to the lack of a universal screening for CMV in pregnancy, these manifestations are underestimated and the diagnosis may be missed. In many countries, however, CMV screening has gained increased importance (notably, universal screening was introduced in Italy in December 2023) and therapeutic approaches have been reported for years [6-14]. Hyperimmune globulin (HIG) administration to pregnant women with primary CMV infection was initiated over 25 years ago. Several controlled or observational studies reported an excellent safety and efficacy profile for the prevention of congenital CMV disease using high dosage (HD: 200 units/kg) of HIG [6-8,11,12]. Less clear is the efficacy profile to prevent mother-to-fetus CMV transmission using monthly HIG administration of 100 units/kg [7,9,14].

Since the antiviral and immunomodulatory efficacy of HIG appeared to be higher if given soon after maternal infection, and there was no significant difference between the number and the frequency of HIG infusions, we performed a long-term cohort study by giving only one timely HD-HIG infusion to 76 women with primary CMV infection in the first trimester. 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, thus HD-HIG infusion might have been therapeutic rather than preventive**; 2)** A single HD-HIG infusion has been previously reported to be therapeutic in pregnant women with fetal CMV disease [7]. First aim was to prevent the development of congenital CMV disease, mostly including neurological and hearing disabilities. Other objectives were prevention of mother-to-fetus CMV transmission, safety, and cost-benefit ratio. As controls 80 women, who did not accept to be treated with HIG, were followed. All the infants, from mothers HD-HIG treated or not, were followed up for a mean of 4.6 years.

Materials and Methods

Study Design

From July 1, 2017 to June 30, 2022, 1246 pregnant women with suspected CMV infection asked for consultation the first author by mails, messages and calls, including 168 (13.5%) with confirmed primary CMV infection in the first trimester. CMV infection was first determined by detection of CMV IgG and IgM in diagnostic laboratories then confirmed by detection of antibodies and IgG-avidity, before HD-HIG infusion or alternative therapy. Detection of CMVDNA in blood (DNAemia) was also suggested but not performed by all women. Twelve (7.1%) women decided to interrupt their pregnancy before 12 weeks of gestation. All 156 (92.9%) women, who continued their pregnancy, were suggested to receive one infusion of HD-HIG for prevention of fetal CMV infection or disease in Villa Mafalda Clinic, Rome, Italy, 4 to 9 weeks after the presumed maternal infection (MI), for the prevention of fetal CMV infection or disease. None patient had HIV infection or immunosuppressive disease or any other current disease. The treatment group was self-selected; the decision to receive HD-HIG was made by each patient following counselling with 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 HD-HIG infusion or date of consultancy or start of alternative therapy, gestational age at the time of CMV DNA detection in blood, viral load in the amniotic fluid, prenatal manifestations of CMV disease, gestational age at delivery, neonatal birth weight, clinical and laboratory abnormalities in the infants with congenital infection, and follow-up for at least two years.

The gestational age at maternal infection was estimated after seroconversion 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 (i,e. elevated transaminases, lymphocytosis), when these were reported [5]. For women who had both IgM and IgG antibodies associated with a very low avidity in their first serological testing, being CMV negative in a previous pregnancy and having a child under 3 years attending a nursery, the date of maternal infection was estimated to be 4 weeks prior to testing to account for the window of viremia and subsequent IgM/IgG production from B-lymphocytes. If only CMV antibodies, followed by IgG detection, the maternal infection was anticipated of 2 weeks. Maternal infection was considered to have occurred just after conception. In mothers with positive IgG and IgM within 6 weeks of pregnancy, maternal infection was considered to occur soon after conception, because of the natural immune depression aimed to prevent fetal rejection since the fetus is like a 50% allograft [3].

Amniocentesis was suggested at 19-20 weeks gestation for detection of CMV DNA in the amniotic fluid. If amniotic fluid was positive, regardless of fetal abnormalities by ultrasound (US), fetal Magnetic Resonance Image (MRI) was suggested to at 20-21 weeks to exclude cerebral malformations and offer the option of legal termination of pregnancy (TOP), which is within 22 weeks in Italy. All neonates were tested for CMV IgG, IgM, and DNA in blood and/or urine. Fetal CMV infection was considered symptomatic if the fetal US scan showed ventriculomegaly or echodensities in the brain, bowel or liver, or if the brain MRI revealed abnormalities like leukodystrophy, cerebral and cerebellar dystrophy or neuronal migrational disorders [15]. Intrauterine growth restriction was defined as head and abdominal circumferences that were below the 10th percentile. All infected infants received brain US and MRI scan, abdominal US, auditory brain-stem evoked response (ABR) studies, and an ophthalmoscopy evaluation. CMV-infected infants were monitored for 2 to 8 years by routine clinical evaluation, sensorineural hearing and eye examinations.

All HD-HIG treated patients gave written informed consent for receipt of hyperimmune globulin infusions. All patients provided consent permitting the anonymous use of their medical records. The study was approved by the Internal Review Board of the University of L'Aquila, Italy (protocol number: 37/109, March 14, 2009), which covered the years of patient enrolment and follow-up, and by the Ethical Committee of the non-profit Association Mother-Infant Cytomegalovirus Infection (AMICI; Registration No. 220615).

Laboratory Diagnosis of CMV Infection

CMV-specific antibodies were detected by enzyme immuno-assays from Diesse Diagnostica Senese (Siena, Italy) and DiaSorin (Saluggia, Italy). CMV IgG avidity was examined using the diagnostic kit (low <0.15%, high >0.25%) from Bouty (Milan, Italy). CMV DNA genome copies/ml were detected by Real-Time PCR from Amplimedical - Bioline (Turin, Italy) and Qiagen (Hoffmann LaRoche). Both serological and virological testing was performed according to manufacturer's instructions.

Hyperimmune Globulin

Commercial hyperimmune globulin (Cytomegatect Biotest, Germany) was given at the dosage of 200 Paul Ehrlich units/kg of maternal weight. To avoid immediate or late side effects related to the immunotherapy, the infusion speed was gradually increased from 20 ml/h in the first 15 minutes, to 30 ml/hour in the minutes 16-30, to 40 ml/h in the minutes 31-45, and to 50 ml/h until the end of the infusion. The duration of the infusion was related to the HD-HID dosage, then to the weight of the pregnant women, who were dismissed after 30 minutes.

CMV Disease

Symptomatic congenital CMV disease was defined as neurological involvement, including microcephaly (head circumference below the 5th percentile for gestational age), periventricular calcifications, cerebral or cerebellar atrophy, cerebral abnormalities (polymicrogyria, lissencephaly, pachygyria), leukodystrophy, ventricular and subependymal abnormalities, seizures in an infant with CMV DNA in cerebrospinal fluid, full or partial hearing loss in one or both ears, chorioretinitis, and purpura with or without thrombocytopenia. The infants were considered asymptomatic when mild hepatosplenomegaly or US findings resolved over the first weeks of life.

CMV disease at ≥ two year of age was defined by mental retardation (speech delay, repetitive behaviors, social withdrawal, intense outburst, IQ below 70 in children aged ≥6 years) or moderate to severe motor delay, and auditory or visual impairment. The threshold by ABR for normal hearing was defined as 0 to 20 dB; abnormal responses were defined as mild (threshold, 21 to 45 dB), moderate (threshold, 46 to 70 dB), or severe (threshold, at least 71 dB).

Statistical Analysis

Qualitative variables were expressed as the number of cases or percentages. Differences between groups for qualitative variables were assessed using Pearson's chi-squared test or Fisher's exact test for small frequencies. For the multivariable logistic regression, the model included maternal age, gestational age at infection, and timing of HD-HIG administration as pre-specified covariates to control for potential confounding. Continuous variables were represented as mean and standard deviation (SD). Parameters were tested for normality with Shapiro-Wilk test and the rank-sum test or t-test were used to compare data between groups as appropriate. Multivariate logistic regression analysis was conducted to identify independent variables correlated with CMV infection or disease in fetuses and newborns. For each analysis, an alpha level of 0.05 was considered to be statistically significant. The statistical analysis was performed using the STATA/BE 18 software for Windows.

Results

Enrolment and Data: As reported in Table 1, 76 pregnant women (two had a twin pregnancy) accepted to be treated with only one HD-HIG infusion at a mean gestational week of 14.4 (range: 8-21), after 6.8 mean gestation week from the presumed maternal infection. Seroconversion occurred in 43 patients (56.6%). All but five pregnant women were Italian and were assisted during the infusions by G.N. None had any HD-HIG-associated side effects during the infusion or later: all were in contact with the author by cell phone. As controls 81 women (one had a twin pregnancy), who did not accept to be treated with HD-HIG, were followed-up from a mean gestational week of 15.1 (range: 7-22), after 7.5 mean gestation weeks from the maternal infection. Of these women, 51 (63.75%) decided to have no therapy, 20 (25%) received non-specific immunoglobulin, 9 (11.25%) were treated with valacyclovir.

Predictor variables	HIG-treated pregnant women	Control pregnant women	Univariate P value	Multivariate Adjusted P value	Adjusted odds ratio (95% CI)
No. subjects	76	80			
No. fetuses	78	81			
Mean maternal age (years) at enrollment	33.9 ± 4.5	32.0 ± 4.5	**0.010**	0.144	0.93 (0.84 - 1.01)
Maternal CMV infection (weeks of gestation)	7.4 ± 3.2	7.3 ± 3.8	0.712	0.894	1.01 (0.88 - 1.16)
Mean interval (weeks of HD-HIG infusion or consultancy/IVIG/VAC	6.8 (range: 4-10)	7.7 (range: 3-12)	**0.004**		
No. of primary infections identified by: Seroconversion (%) Low avidity + high IgM and low IgG values (%)	 43 (56.6) 33 (43.4)	 43 (53.75) 37 (46.25)	 0.722 0.723		
No. subjects with CMV DNA+ in amniotic fluid/ No. tested subjects (%)	13/56 (23.6)	11/57 (19.3)	0.611		
No. of fetuses with abnormalities by US and/or MRI (%)	2 (2.8)	11 (13.6)	**0.012**		
No. of intrauterine death	0	2 (2.5)	0.163		
No. of termination of pregnancy	6 (7.9)	4 (5.1)	0.461		
No. of subjects with vaginal delivery (%)	54 (77.1)	52 (69.3)			

No. of subjects who delivered by Caesarean Section (%)	16 (22.9)	23 (30.7)			
Neonates alive Stillborn (%) CMV DNA+ CMV DNA+ in urine (%) Mean genome CMV DNA+ copies/ml in neonates Mean birth weight: All infants CMV-positive infants CMV-negative infants	72 None 9 (12.5) 11.379.000 3.230 2.889 3.280	74 1 (1.4) 21 (28) 20.312.221 3.191 3.045 3.242	0.321 **0.018** **0.041** 0.473 0.572 0.546		
No. of CMV+ alive neonates with clinical and/or US/MRI abnormalities (%)	2 (22.2)	11 (55)	0.489		
Total number of maternal/fetal CMV transmission during pregnancy (%)	15 (19.5)	27 (33.3)	**0.044**		
Total number of CMV+ fetuses/neonates with clinical and/or US/MRI abnormalities (%)	3 (20)	16 (59.2)	**0.003**		
No. of infants with abnormal outcome >2 years of follow-up (%): Psychomotor retardation Deafness: unilateral bilateral total no.	2 (2.8) 0 2 (2.8) 0 2(2.8)	10 (13.5) 7 (9.5) 6 (8.1) 1 (1.35) 7(9.5)	**0.018** **0.008** 0.157 0.322 0.093		
Valganciclovir therapy	2/9 (22.2)	10/20 (50)	0.160		
Mean year follow-up	5	4.3	0.090		
MRI: Magnetic Resonance Image US: ultrasound; VAC: valacyclovir					

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

Mother-to-fetus CMV transmission and disease: As shown in Table 1, 13 of 56 HD-HIG-treated mothers (23.2%) had positive CMV DNA in the amniotic fluid. Six women (46.1%), one of whom had ultrasound fetal abnormalities, requested TOP. Of 72 neonates, 9 (12.5%) were CMV positive. Totally, CMV was transmitted to 15/78 fetuses/neonates (19.2%). Notably, in the subgroup of 20 patients treated within 5 weeks of the presumed maternal infection, the vertical transmission rate was 0%. This reduction was statistically significant when compared to patients treated after 5 weeks (0% vs. 25.9%; $p<0.013$). Clinical/image abnormalities occurred in 2 neonates (17%), both with left deafness which persisted after valganciclovir therapy. The longterm outcome showed that all children had a normal psychomotor development (Tables 1 and 4).

Among the control women, 11 of 57 (19.3%) had positive CMV DNA in the amniotic fluid: 4 women (30.8%) opted for TOP, and 2 of them showed fetal ultrasound/MRI abnormalities. Two intrauterine fetal deaths, and one stillborn also occurred (Table1). Of 74 alive neonates, 20 (27%) were CMV-positive and 11 of them (55%) were symptomatic. Totally, CMV transmission occurred in 27/81 fetuses/neonates (33.3%), being significantly higher ($p=0.044$) than those from HD-HIG-treated women. In particular,

maternal-fetal CMV transmission was significantly higher (40% vs 0%; p=0.002) in the control women than in HD-HIG-treated women within 5 weeks from the maternal infection (Table 2). Moreover, it is statistically significant the high number of fetuses/neonates with CMV diseases from control women compared to HD-HIG treated mothers (59.2% vs 20%; p=0.003). Of 11 CMV-infected symptomatic neonates, 10 (90.9%) were treated with valganciclovir: 9 (81.8%) had neurological abnormalities and/or abnormal hearing (Table 5). In particular, the difference in the occurrence of abnormal neurological outcome is highly significant (7 vs. 0; p=0.008).

No. of weeks between maternal infection and HIG infusion	76 HIG-treated pregnant women (78 fetuses)	80 Control pregnant women (81 fetuses)	Univariate P value	Multivariate P value	Adjusted odds ratio (95% CI)
≤ 5 WG Seroconversions Fetal infections TOP/Disease	20 12 (60.0%) 0 0	15 8 (53.3%) 6 (40%) 2 (1 left deafness, 1 stillborn)	0.279 0.313 **0.002** 0.093	0.721	0.79 (0.21 -2.94)
≤ 6 WG (%) Seroconversions Fetal infections TOP/Disease	14 12 (85.7%) 3 (21.4%) 1 TOP	15 8 (53.3%) 7 (46.7%) 5 (1 IUD, 1 psychomotor retardation, 1 right deafness, 1 right hypoacusia and psychomotor retardation, 1 visual impairment and psychomotor retardation)	0.926 0.060 0.153 0.082	0.276	2.00 (0.57 -6.96)
≤ 7 WG Seroconversions Fetal infections TOP/Disease	15 (16 fetuses) 8 (53.3%) 4 (26.7%) 2 TOP, 1 left deafness	16 12 (75%) 3 (18.75%) 3 (1 IUD, 1 right hypoacusia, 1bilateral deafness and psycho-motor retardation)	0.905 0.280 0.669 0.082	0.925	1.06 (0.29 -3.88)
≤ 8 WG Seroconversions Fetal infections TOP/Disease	18 (19 fetuses) 7 (38.9%) 6 (33.3%) 2 TOP, 1 left deafness	20 7 (35%) 7 (35%) 3 (1 psychomotor retardation,1 psychomotor retardation and left deafness, 1 psychomotor retardation and left deafness)	0.961 0.839 0.584 0.476	0.211	2.13 (0.65 -6.94)
≤ 9 WG (%) Seroconversions Fetal infections TOP/Disease	12 2 (19.4%) 2 (19.4%) 1 TOP	14 (15 fetuses) 7 (50%) 3 (20%) 2 TOP (14.3%)	0.219 **0.039** 0.897 0.573	-	-

TOP: Termination of pregnancy; IUD: Intrauterine death

Table 2: Interval in weeks gestation (WG) between Maternal Infection and infusion of hyperimmune globulin (HIG) or controls (date of consultancy or beginning of therapy) as a possible predictor of congenital CMV infection or disease at birth and follow-up.

CMV DNAemia: As reported in Table 3, this testing was performed in 67 HD-HIG-treated women (88.1%), 41 of whom (61.2%) were positive and 13 (31.7%) transmitted CMV to their fetuses. Mean genome/ml values were 2.454 (range 24-14.047). Mother-to-fetus CMV transmission was significantly lower (p=0.006) in the women who had negative CMV DNAemia at their first testing, since it occurred in only 1 of 26 (3.8%) women having negative CMV DNAemia but in 12 with positive DNAemia (32.5%).

Among controls, CMV DNAemia was detected in 57 women (71.25%), 35 of whom (61.4%) were positive. Mean genome/ml values were 848.8 (range 45-13.110). There was no statistically significant difference between non-HIG-treated patients having positive (36.4%) or negative (27.3%) DNAemia in mother-to-fetus CMV transmission (p=0.486). Conversely, there was a statistically significant difference (p=0.006) between HD-HIG-treated mothers having negative DNAemia (3.8%) and non-HIG-treated women having negative CMV DNAemia (27.3%).

IgG-avidity: As reported in Table 3, this test was performed before and after HD-HIG infusions in 53 subjects, and twice in 52 controls. There was an increase in the mean avidity values in both groups. Nevertheless, the increased avidity after HD-HIG infusion (from 0.10% to 0.24) was statistically significant (p=0.021), contrary to the raised avidity (from 0.11% to 0.17%) in the controls.

Predictor variables	HIG-treated pregnant women	Control pregnant women	Univariate P value	Multivariate P value	Adjusted odds ratio (95% CI)
No. subjects	75	80			
DNAemia performed DNAemia positive DNAemia negative Mean DNAemia (g/ml) Not tested	66 (88%) 40 (60.6%) 26 (39.4%) 2.511 9 (12%)	57 (71.25%) 34 (59.6%) 23 (40.3%) 849 23 (28.76%)	0.009 0.990 0.990 0.274 0.003	0.207 0.467	3.3 (0.52 - 21.11) 1.00 (1.00 – 1.00)
Subjects with M/F CMV transmission related to DNAemia: DNAemia positive DNAemia <500 g/ml DNAemia >500 g/ml DNAemia negative Not tested	 13/40 (32.5%) 3/15 (20%) 10/25 (40%) 1/26 (3.8%)* 1/9 (11.1%)	 13/34 (38.2%) 9/23 (39.1%) 4/11 (36.4%) 7/23 (30.4%)** 8/23 (34.8%)	 0.609 0.221 0.839 **0.013** 0.188		
1st avidity	0.10± 1.25 (53 subjects)	0.11± 0.05 (54 subjects)	0.093	0.568	0.01 (0 - 81356.13)
2nd avidity	0.24± 0.09 (53 subjects)	0.17±0.07 (54 subjects)	**0.021**	0.586	0.08 (0 - 825.07)
Mean interval (weeks) between 1st and 2nd avidity	4± 2.3	3.1± 2.4	0.304	0.519	1.09 (0.84 - 1.41)

M/F: Maternal/Fetal; g/ml: genome copies/ml; *Statistical comparison between HIG-treated patients having positive or negative DNAemia (32.5% vs. 3.8%): p=0.006; ** Statistical comparison between non-HIG-treated patients having positive or negative DNAemia (36.4% vs. 27.3%): p=0.486

Table 3: Univariate and Multivariate (Logistic Regression) analysis of DNAemia and IgG avidity as possible predictors of congenital CMV infection/disease in fetuses and neonates.

Safety and Cost/Efficacy Ratio

HD-HIG infusions were safe in all patients, in spite of the fact that many of them came back to their cities from Rome in the same day of the infusion. The cost/efficacy ratio was fully achieved, since the mean cost of one HD-HIG infusion is 4.250 euros, which is lower than the cost of at least two infusions of non-specific immunoglobulin, valacyclovir therapy, and mostly of disabilities caused by congenital CMV infection.

Discussion

This prospective cohort study, featuring a robust long-term follow-up, showed that a single, timely HD-HIG infusion significantly prevents congenital CMV disease following first-trimester primary infection. During the study free non-specific immunoglobulin or valaciclovir were available in Italy for use in pregnancy, so a randomized trial with placebo was impossible. In spite of this limitation, we observed significantly lower numbers of fetuses/infants with CMV disease at birth from HD-HIG treated mothers than from control mothers (3.9% vs. 19.6%; p= 0.003). Moreover, although the non-randomized nature of the study may introduce selection bias, the stark contrast in transmission rates (0% in the early treatment group) and the long-term neurological outcomes provide compelling evidence for the efficacy of timely HD-HIG intervention.

Further, after long-term evaluation none of 72 children born to HD-HIG treated mothers developed neurological abnormalities, and only two had unilateral deafness. Also, the CMV transmission rate was significantly (p=0.044) decreased among all fetuses or infants born of HD-HIG-treated mothers compared to infants from controls. The transmission rate of CMV in non-HD-HIG-treated women was 33.3%, [95% CI: 26.2-45.0%]), which was significantly higher than in the control group (19.5%).

Mother years	**wg Maternal Infection**	**wg CMV DNA g/ml in blood**	**wg Interval maternal infection / HD-HIG infusion**	**wg CMV DNA copies/ml in amniotic fluid or US/MRI abnormalities**	**wg Delivery**	**Neonates CMV DNA g/ml in urine**	**Neonatal sex, birth weight, symptoms**	**Outcome (years of follow-up)**
1-35	8	14: **1708**	16 (8)	20: 1.650.000	**TOP**	NA	NA	NA
2-33	14 SC	19: **3576**	20 (6)	22**: 20.460**	**TOP**	NA	NA	NA
3-30	3	8: **935**	9 (6)	19: **3.300.000**	VD 40	**69.300.000**	M 3.055 Normal	Normal (3)
4-34	5	9: **528**	13 (8)	20: **2.370**	**TOP**	NA	NA	NA
5-33	6 SC	12: **24**	13 (7)	20: **1.343.070**	**TOP**	NA	NA	NA
6-31	6	NP	14 (8)	22: **1.796.000**	VD 39	**9.760.350**	M 3140 Normal	Normal (8)
7-36	6 SC	11: **515**	14 (8)	NP	VD 38	**4.000.000**	M 3.180 Normal	Normal (3)
8-29	7 SC	14: **12.870**	15 (8)	20: **663.000**	CS 38	**3.731.000**	M 3.400 **Left deafness**	VGC **Left deafness** (4)
9-36	12 SC	16: **3410**	18 (6)	20: **54.800**	VD 40	**13.500.000**	F 3.270 Normal	Normal (6)
10-35	6	13 negative	15 (9)	18: **Ascites, Ventriculomegaly** 19: **1.753.781**	**TOP**	NA	NA	NA
11-36	4	9: **1.260**	14 (10)	20: **857.000**	CS 38	**2.530.000**	F 3.000 Normal	Normal (4)
12-38	7 SC	11: **13.000**	14 (7)	21: **1.132.000**	**TOP**	NA	NA	NA
13-32	4	11: **613**	12 (8)	20 negative	VD 37	**14.110.000**	F 2.790 Normal	Normal (3)

14-35	11 SC	16: **476**	18 (7)	21: **240**	VD 39	**2.370.000**	F 3.050 Normal	Normal (8)
15-37	8 SC	13: **178**	15 (7)	17:**Intestinal echodensities 21:12.358.000**	CS 31	**75.670.000**	M 1.120 **Leukodystrophy Left deafness**	VGC **Left deafness** (3)
CMV: cytomegalovirus; F: female; g/ml: genome copies/ml; HIG: hyperimmune globulin; M: male; MRI: Magnetic Resonance Imaging; NP: not performed; SC: seroconversion; TOP: termination of pregnancy; US: ultrasound; VGC: valganciclovir; WG: weeks of gestation.								

Table 4: The outcome of pregnant women with primary CMV infection before 14 weeks' gestation who were treated with hyperimmune globulin before amniocentesis to prevent maternal-fetal transmission.

Mother years	wg Maternal Infection	wg CMV DNA g/ml in blood	wg Interval Maternal Infection / consultancy or IVIG or VAC therapy	wg CMV DNA g/ml in fluid amniotic	wg Fetal abnormalities by ultrasound and/or MRI	wg Delivery	Neonates CMV DNA g/ml in urine	Neonatal sex, birth weight, symptoms a	Outcome (years of follow-up)
1-27	5	12: **378**	13 (8) VAC	20: **482.550**	no	CS 38	**47.350.000**	M 3.350	Normal (2)
2-27	13 SC	20 negative	20 (6) VAC	21: **153.720**	no	CS 38	**909.000**	M 2950	Normal (2)
3-36	6 SC	NP	12 (6)	NP	14: **IU death**	NA	NA	NA	NA
4-39	9 SC	23: **224**	18 (9)	NP	24: **Intestinal echodensities**	VD 40	**24.647.000**	F 3.160 **Leukodystrophy Right hypoacusia**	**Right hypoacusia Psychomotor retardation** (2)
5-28	6 SC	20: **768**	14 (8)	NP	22: **Intestinal echodensities Ventriculomegaly Polymicrogyria**	**TOP**	NA	NA	NA
6-34	12 S	20 negative	20 (8)	NP	no	VD 38	**17.361.000**	M 3.270	Normal (5)
7-29	5 SC	13 negative	14 (9)	21: **4.608.272**	no	**TOP**	NA	NA	NA
8-33	3	NP	11 (8)	20: **445.000**	32: **Temporal pseudocysts Ventriculomegaly**	VD 41	**65.800.000**	M 2.810 **Ventriculomegaly Temporal dysplasia Leukodystrophy**	VGC **Psychomotor retardation** (3)
9-23	5	NP	11 (6)	19: **40.275**	no	VD 41	**480.000**	M 3.780 **Ventriculomegaly Cerebral calcifications**	VGC **Psychomotor retardation** (7)
10-35	6	10: **211**	10 (4) VAC	20 negative	no	VD 37	**4.233.488**	F 2.890	Normal (3)
11-31	7 SC	16 negative	14 (7)	NP	23: **Hepatic and intestinal echodensities** 32: **Ventriculomegaly**	VD 39	**75.320.418**	M 2.830 **Polymicrogyria Ventriculomegaly Bilateral deafness**	VGC **Bilateral deafness Psychomotor retardation** (4)

12-28	7 SC	10: **400**	16 (9)	NP	25: **Choroidal echodensity Ventriculomegaly**	CS 36	**7.210.854**	M 2.570 **Ventriculomegaly Choroidal cysts**	VGC Normal (8)
13-35	11 SC	16 negative	16 (5)	21 negative	no	CS 38	**1.550.000**	F 4.000	Normal (7)
14-34	3	9: **270**	11 (8)	20: **1.744.000**	**31: Leukodystrophy**	CS 39	**50.000.000**	F 3.270 **Leukodystrophy Ventriculomegaly Left deafness**	VGC **Psychomotor retardation Left deafness** (2)
15-30	14 SC	20: **2.116**	20 (6) IVIG	NP	no	CS 38	**1.832.000**	M 3.100	Normal (3)
16-33	8 S	15: **200**	14 (6) VAC	21: **1.727.381**	no	VD 38	**10.952.000**	M 2.875 **Ventriculomegaly Right deafness**	VGC **Right deafness** (3)
17-35	12 SC	NP	17 (5)	18 negative	no	CS 30	**210.000**	M 1.580	Normal (3)
18-28	5 SC	9: **224**	12 (7)	20: **8.110.250**	23: **Intestinal echodensities** 29: **Ventriculomegaly**	CS 36	**55.431.000**	M 3.175 **Ventriculomegaly**	**Right hypoacusia** (5)
19-25	12 SC	17: **128**	17 (5) VAC	20 negative	20 negative	VD 38	**1.531.529**	F 3.180	Normal (2)
20-31	7	NP	14 (7)	NP	16: **Hydrocephalus**	**TOP**	NA	NA	NA
21-42	12 SC	NP	19 (7)	NP	21: **IU death**	NA	NA	NA	NA
22-33	7 SC	NP	11 (4)	NP	no	VD 40	**49.911.950**	F 3.280 **Left deafness**	VGC **Left deafness** (4)
23-35	9 SC	NP	17 (8) IVIG	21: **785.000**	no	VD 39	**2.321.000**	M 3.300	Normal (4)
24-33	4	15 negative	10 (6)	NP	no	VD 37	**18.565.402**	M 3.460 **Thrombocytopenia Ventriculomegaly**	VGC **Right hypoacusia Psychomotor retardation** (7)
25-40	11 SC	20: **241**	20 (9)	21: **1.197.200**	no	**TOP**	NA	NA	NA
26-31	13	16: **630**	16 (3)	**21: 130.000**	25: **Intestinal echodensities** 26: **Ventriculomegaly**	VD 38	**17.645.385**	M 2.600 **Stillborn**	NA
27-31	9	13: **13.110**	15 (6)	NP	no	CS 36	**5.940.000**	F 1.349 **SGA Ventriculomegaly Retinopathy Liver disease Anemia Thrombocytopenia**	VGC **Visual impairment Psychomotor retardation** (4)

CMV: cytomegalovirus; F: female; g/ml: genome copies/ml; IVIG: intravenous immune globulin; IU: intrauterine; M: male; MI: maternal infection; MRI: Magnetic Resonance Imaging; NP: not performed; SC: seroconversion; SGA: small for gestational age; TOP: termination of pregnancy; US: ultrasound; VAC: valacyclovir; VGC: valganciclovir; WG: weeks of gestation.

Table 5: The outcome of pregnant women with primary CMV infection before 14 weeks' pregnancy who were not treated with hyperimmune globulin (HIG) to prevent maternal- fetal transmission.

Moreover, one HD-HIG infusion was associated with a higher reduction in mother-to-fetus transmission in the first trimester when HD-HIG was administered within 5 weeks from maternal infection. Likely, in mothers with primary CMV infection in the first trimester, fetal infection may occur after 5 weeks from the maternal infection. Hence if HD-HIG is given before 5 weeks of maternal infection, it may prevent viral transmission to the fetus by displaying both antiviral and immunomodulatory effects in the maternal blood and placenta. Antiviral activity is due to the high anti-CMV antibody titers and neutralizing antibodies, including those anti-gB and anti-pentamer (anti-gH, gL, UL128, UL130, and UL131). Immunomodulatory activities are explained by IgG antibodies binding to cellular receptors for complement, cytokines and CD8+ cytotoxic T lymphocytes, which decrease the CMV-induced inflammatory damage in the placenta and fetal organs [16-19].

The efficacy of one timely HD-HIG administration was further evidenced by the significantly lower (p=0.017) number of CMV transmission from HD-HIG-treated mothers having negative CMV DNAemia at enrolment and by the significant increase (p=0.021) of the IgG avidity, compared to non-HIG-treated mothers. HIG efficacy against CMV is supported by studies in vitro and ex vivo, and by randomized experiments in guinea pigs, rhesus monkeys and newborn mice, showing reduced rates of maternal viremia, fetal deaths and infections, and prevention of CMV-associated brain abnormalities [20-23].

Two randomized trials that used monthly low dose (100 units or mg/kg) HIG to prevent fetal/neonatal infection after primary CMV infection until 26 or 24 weeks of gestation, respectively, failed to observe a statistically significant benefit for HIG [9,14]. However, one randomized trial reported a decreased rate of congenital infections in the HIG treated group (from 44% to 30%) [9]. These infection rates are similar to the rates previous observed by us [7]. Had this randomized trial enrolled only a few more patients, statistical significance may have been achieved [9]. Another randomized trial enrolled 394 patients, 95% of whom based on low initial avidity. This caused a very long interval between maternal infection and HIG treatment (mean of 25±9 days). This means that many enrolled fetuses were infected before HIG was administered. This reliance on avidity for enrolment rate also accounts for why the placebo transmission rate was only 19%, less than half the expected rate of 40% using seroconversion [9,14].

In conclusion, our study showed that only one HD-HIG infusion within 9 weeks from the maternal infection after primary CMV infection in the first trimester of pregnancy obtained the following results: **1)** Normal psycho-motor development in all children; **2)** Decreased number and severity of deafness; **3)** Low maternal-fetal CMV transmission, particularly if HD-HIG infusion occurs within 5 weeks from the maternal infection; **4)** Safety; **5)** High cost/efficacy ratio.

Notes

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