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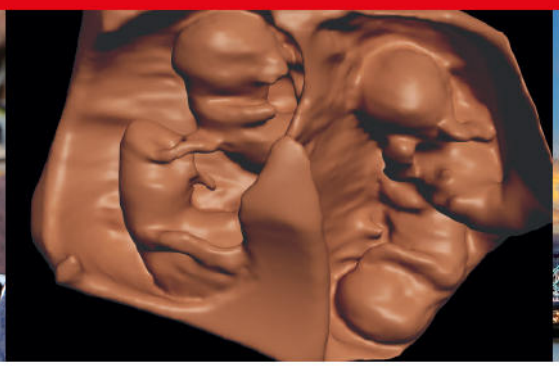


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Prevention of maternal–fetal transmission of cytomegalovirus after primary maternal infection in the first trimester by biweekly hyperimmunoglobulin administration

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KEYWORDS: CMV; cytomegalovirus; first trimester; hyperimmunoglobulin; pregnancy

ABSTRACT

Objective *To examine the efficacy of biweekly hyperimmunoglobulin (HIG) administration to prevent maternal–fetal transmission of cytomegalovirus (CMV) in women with primary first-trimester CMV infection.*

Methods *This was a prospective observational study of women with confirmed primary CMV infection in the first trimester who had the first HIG administration at or before 14 weeks' gestation. All women had biweekly HIG treatment until 20 weeks' gestation at a dose of 200 IU/kg of maternal body weight. Each subject underwent amniocentesis at least 6 weeks after first presentation at about 20 weeks. Primary outcome was maternal–fetal transmission at the time of amniocentesis, and secondary outcome was the frequency of congenital CMV infection at birth. The results were compared with a historic cohort of women with first-trimester CMV infection who did not undergo HIG treatment and who had amniocentesis at about 20 weeks.*

Results *Subjects were 40 pregnant women with a primary CMV infection, with a median gestational age at first presentation of 9.6 (range, 5.1–14.3) weeks. On average, HIG administration started at 11.1 weeks and continued until 16.6 weeks. Within this interval, HIG was administered between two and six times in each patient. While CMV immunoglobulin-G (IgG) monitoring showed periodic fluctuations during biweekly HIG administration cycles, high CMV-IgG avidity indices remained stable over the whole treatment period. Maternal–fetal transmission before amniocentesis occurred in only one of the 40 cases (2.5% (95% CI, 0–13.2%)). At delivery, two additional subjects were found to have had late-gestation transmission. Considering all three cases with maternal–fetal transmission, the transmission rate was 7.5% (95% CI, 1.6–20.4%) in our 40 cases. All infected neonates were asymptomatic at birth. The matched historical control group consisted of 108 pregnancies. Thirty-eight transmissions (35.2% (95% CI, 26.2–45.0%)) occurred in the control group, which was significantly higher ($P < 0.0001$) than the transmission rate in the HIG treatment group.*

Conclusion *After a primary maternal CMV infection in the first trimester, biweekly HIG administration at a dose of 200 IU/kg prevents maternal–fetal transmission up to 20 weeks' gestation.* *Copyright © 2018 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Cytomegalovirus (CMV) primary infection during pregnancy results in 700–1400 children per year being born with developmental disorders in Germany[1]. Primary maternal CMV infection occurs in about 0.5–4% of CMV-seronegative pregnant women, and in about 10–15% of these cases the neonate is symptomatic at birth. These children have a 30–40% risk of long-term sequelae including sensorineural hearing loss,

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developmental delay, psychomotor impairment and cerebral palsy[2,3]. However, the level of this risk depends on the gestational age at the time of maternal infection[3]. In three separate studies that included a total of 600 women with primary maternal CMV infection, the risk for a symptomatic neonate was highest if maternal seroconversion occurred in the first trimester[4–6]. A recent study examined 138 children with congenital CMV infection; amniocentesis was performed in all pregnancies at least 6 weeks after the assumed time of maternal infection, predominantly at about 20–23 weeks' gestation. In the cohort of infants with a negative CMV-DNA amniocentesis result, none of the children had long-term complications after birth. In contrast, in the cohort with a positive CMV-DNA test result, 14% suffered from long-term sequelae[7].

Several studies have examined the efficacy of hyperimmunoglobulin (HIG) to prevent maternal–fetal transmission. In a randomized placebo-controlled study, a reduced rate of congenital infection of the newborns was noted in the treatment group, but this was not significantly lower than that in the placebo-control group[8]. However, limitations of this study included recruitment up to 26 weeks' gestation and a median time interval between the diagnosis of infection and first HIG administration of 5 weeks. Most importantly, the HIG dose administered was only 100 IU per kg of body weight on a 4-weekly basis. The rationale for a 4-weekly algorithm was based on a pharmacokinetic study that found a terminal half-life of HIG of 22.4 days[9]. Accordingly, most studies have used a 4-weekly interval for sequential HIG treatment[8,10,11]. However, in pregnant women receiving monthly HIG transfusions, we have demonstrated that the half-life of HIG (Cytotect, Biotest) was only 11 days[12].

In this study, we examined the efficacy of biweekly HIG administration to prevent maternal–fetal transmission of CMV after a primary maternal CMV infection diagnosed in the first trimester.

METHODS

Study design

This prospective observational study was conducted at the prenatal medicine departments of the Universities of Tübingen, Bonn and Cologne, of the department of virology of the University of Tübingen and of the laboratory Prof. Gisela Enders and Colleagues in Stuttgart, Germany, between 2013 and 2017.

In Germany, there is no antenatal screening program for CMV and such screening in the first trimester is offered only as an individual healthcare service. Pregnant women with a primary maternal CMV infection were referred to one of the three prenatal medicine departments conducting this study. Enrollment criteria were the presence of confirmed primary CMV infection in the first trimester and a gestational age at first HIG administration of ≤ 14 weeks. Before HIG treatment was started, each woman received detailed information about the off-label use of HIG and the potential side-effects. Approval for the study was obtained from the ethics committees of the Universities of Tübingen (541/2017BO2), Cologne (17-415) and Bonn (285/17).

HIG (Cytotect, Biotest, Dreieich, Germany) was administered at 200 IU per kg of maternal body weight intravenously, according to the standard protocol in Germany[10,13]. The treatment was repeated every 2 weeks until 20 weeks' gestation or until maternal mean immunoglobulin-G (IgG) levels were above 100 U/mL 7 days after the last HIG administration. In all cases, amniocentesis was performed at least 6 weeks after the first presentation, generally at about 20 weeks' gestation. Furthermore, the neonates were tested for viral DNA and viral isolates after birth.

The prevalence of maternal–fetal transmission at about 20 weeks in our cohort was compared with that in a historic cohort with first-trimester primary maternal CMV infection who did not undergo HIG treatment. The control cohort was derived from the digital databases of the laboratory Prof. Gisela Enders and Colleagues, Germany, and of the University of Leuven, Belgium. We included only cases in which amniocentesis was performed between 19 and 22 weeks to diagnose maternal–fetal transmission of CMV after a primary infection in the first trimester. Cases with fetal defects, those with seroconversion after 15 weeks, cases in which amniocentesis was not performed between 19 and 22 weeks and cases that had HIG treatment or any other prior intervention were not included. Three experts (K.H., M.E. and K.O.K.) reviewed the cases individually. They focused on the clinical and virologic data, especially on CMV-IgG and immunoglobulin-M (IgM) levels, IgG avidity and, if available, anti gB2-IgG reactivity. Only cases for which all three experts agreed on a first-trimester infection were included in the control group.

The primary outcome was maternal–fetal transmission at the time of amniocentesis, and a secondary outcome was frequency of congenital CMV infection at birth.

Virological studies and inclusion criteria

The following commercial tests were used for the diagnosis of the CMV antibody status: 'ECLIA' Elecsys CMV IgG/IgM/IgG avidity (Roche Diagnostics, Switzerland) using fully automated cobas 6000/cobas e 601 analyzer (Roche, Hitachi); and recomLine CMV IgG/IgM/IgG avidity assay (Mikrogen, Germany). The recomLine immunoblot assay (recIB) detected CMV-specific antibodies to the recombinant CMV proteins IE1, p150, CM2, p65, gB1 and gB2. The assay was used as described previously[14]. In summary, recIB scoring ranges between: 0, when there is no detectable binding compared to test IgG/IgM-control (negative); 1, when binding reactivity is weaker than in the test controls (±); 2, when reactivity is positive (+); 3, when it is strongly positive (++); and 4, when there is very strong reactivity (+++).

The following inclusion criteria indicated a recent primary infection and were necessary for study inclusion: CMV-IgG level (ECLIA) < 60 U/mL, no positive reactivity

against gB2 (Score 0 or 1, recIB), CMV-IgG avidity index (ECLIA) < 45% and IgM index (ECLIA) > 1.0. The latter had to be confirmed by recIB. In three cases with an IgM index < 0.7, an early CMV primary infection was still assumed, as there was anti rec p150 IgM reactivity together with low IgG avidity.

CMV monitoring

Each HIG administration cycle was monitored by determination of CMV-IgG levels and IgG avidity (ECLIA) directly before, and at 2 h and at 7 days after HIG administration.

Amniotic fluid was tested by two polymerase chain reactions (PCRs), nested PCR (nPCR) and quantitative real-time PCR (q-rt-PCR), short-term microculture and long-term culture until generation of a cytopathic effect (CPE) ranging from 18 h to 5 days. Short-term 18-h fibroblast microculture, followed by CMV-IE1 immunoperoxidase staining and virus isolation from amniotic fluid, was performed with a high-speed centrifugation step of 50 000 *g* for 1 h prior to virus inoculation, as described previously for milk whey sample preparation[15]. DNA was purified by spin columns using QIAmp DNA Blood Mini Kit (Qiagen, Germany) and used for qualitative nPCR of the IE1Ex4-target region[15]. The limit of detection (LOD) for nPCR was 200 copies/mL. q-rt-PCR from plasma, serum or whole ethylenediaminetetraacetic acid (EDTA) blood as well as from amniotic fluid was performed using CMV-R-gene™ real-time PCR kits (Argene, France) with a LOD of 600 copies/mL.

After birth, umbilical cord blood, urine and/or saliva were tested for CMV-DNA and for potential viral isolation using short-term microculture with anti-CMV-IE1 immunoperoxidase staining and potential CPE generation via fibroblast microculture[15].

Statistical analysis

Results are given as median (interquartile range (IQR)) or *n* (%). 95% CIs were determined using the Clopper and Pearson method. The proportion of cases with transmission at 20 weeks and at term in the treatment group was compared with the transmission rate in the control group at 20 weeks using Fisher's exact test; the significance level was set at 0.05.

RESULTS

During the study period, 48 women with a first-trimester primary CMV infection were referred to one of the three participating prenatal medicine departments. Eight cases were excluded from further analysis, comprising six that declined HIG administration and two that were terminated before HIG administration started owing to chromosomal abnormality (trisomy 21 and monosomy X, respectively). Thus, our study cohort consisted of 40 women with a first-trimester primary CMV infection.

Characteristics of the study population and their treatment course are summarized in Table 1. Median gestational age at first presentation was 9.6 (range, 5.1–14.3) weeks. HIG administration was started at a median of 11.1 weeks and carried out until 16.6 weeks' gestation. Within this time interval, HIG was administered between two and six times to each patient. Details of the individual characteristics and treatment course of each patient are provided in Table S1.

Table 2 and Figure S1 demonstrate median levels of CMV-IgG, CMV-IgG avidity, CMV-IgM indices and the number of cases with positive CMV-DNA viral load in maternal plasma or serum at diagnosis and CMV-IgG avidity using ECLIA and immunoblotting at amniocentesis.

The results of the recIB-detected CMV-specific IgM and IgG antibodies to the recombinant CMV proteins IE1, CM2, p150, p65, gB1 and gB2 at diagnosis are shown in Figures 1 and 2, respectively. The CMV-specific IgM reactivity showed a distinct pattern: all sera were reactive against the tegument protein p150, and in 82.5% of the cases there was reactivity against the fusion protein CM2. In contrast, no reactivity was observed against the recombinant surface proteins gB1 and gB2 (Figure 1). With respect to the CMV-specific IgG reactivity, there was no reactivity against rec gB2, while all sera contained detectable antibodies against the viral tegument protein p65.

We found that IgG levels fluctuated, while IgG avidity increased after the first HIG administration and remained

Table 1 Characteristics and treatment course of 40 pregnancies with first-trimester primary cytomegalovirus infection that underwent biweekly hyperimmunoglobulin (HIG) treatment

Variable	*Value*
Maternal age (years)	32.9 (30.4–34.6)
Maternal weight (kg)	65.0 (58.0–69.0)
Caucasian ethnicity	40 (100.0)
Parity > 0	38 (95.0)
Had child ≤ 3 years of age at time of primary infection	29 (72.5)
GA at first presentation (weeks)	9.6 (7.5–11.4)
GA at first HIG administration (weeks)	11.1 (8.5–12.4)
Number of HIG courses	
Two	2 (5.0)
Three	16 (40.0)
Four	13 (32.5)
Five	6 (15.0)
Six	3 (7.5)
GA at amniocentesis (weeks)	20.3 (19.4–20.7)
Time interval between first presentation and amniocentesis (weeks)	10.4 (9.0–11.8)
Maternofetal transmission	
At time of amniocentesis	1 (2.5)
At time of delivery	3 (7.5)

Data are presented as median (interquartile range) or *n* (%). GA, gestational age.

Table 2 Virological data of 40 pregnancies with first-trimester primary cytomegalovirus (CMV) infection

Variable	*Value*
CMV viral load in maternal plasma or serum	
CMV-DNA qPCR < 600 copies/mL*	34 (85.0)
CMV-DNA qPCR ≥ 600 copies/mL	6 (15.0)
CMV viral load in cases with CMV-DNA qPCR ≥ 600 copies/mL	1420 (1205–2400)
CMV-IgG level (ECLIA) at first presentation	
Anti-CMV-IgG < 0.5 U/mL†(not reactive)	1 (2.5)
Anti-CMV-IgG ≥ 0.5 U/mL†(borderline and reactive)	39 (97.5)
Anti-CMV-IgG U/mL (ECLIA) in cases with anti-CMV-IgG ≥ 0.5 U/mL	6.6 (3.2–17.1)
CMV-IgG avidity (ECLIA) at first presentation	
Not measurable (CMV-IgG < 1.0 U/mL†)	5 (12.5)
Measurable (CMV-IgG ≥ 1.0 U/mL†)	35 (87.5)
IgG avidity in cases with CMV-IgG ≥ 1.0 U/mL (low avidity, < 45%)	21.7 (10.0–33.17)
CMV-IgM index (ECLIA) at first presentation	
Anti-IgM index < 0.7†‡(not reactive)	3 (7.5)
Anti-IgM index ≥ 0.7†	37 (92.5)
Anti-IgM index in cases with anti-IgM index ≥ 0.7	2.23 (1.2–4.9)
Positive CMV-IgG gB2 reactivity (recIB)	0 (0.0)

Data are presented as *n* (%) or median (interquartile range). *Limit of detection of test system. †Cut-off of test system. ‡In these cases, anti rec p150 immunoglobulin-M (IgM) scores were between + and +++. ECLIA, commercial test for CMV antibody status; IgG, immunoglobulin-G; qPCR, quantitative polymerase chain reaction; recIB, recomLine immunoblot assay.

stable over the whole treatment period. Figure 3 shows the fluctuations in CMV-IgG levels and the CMV-IgG avidity in a typical pregnancy before, during and after HIG treatment.

In all pregnancies, amniocentesis was performed at around 20 weeks, at a median of 10.4 weeks after diagnosis. Maternal–fetal transmission at the time of amniocentesis was noted in only one of the 40 cases (2.5% (95% CI, 0–13.2%)).

The historical control group consisted of two cohorts, one German and one Belgian, comprising a total of 108 pregnancies with first-trimester CMV infection that did not undergo HIG treatment. In the German group, maternal–fetal transmission was observed in 24 (34.3%) of the 70 cases. In the Belgian dataset, transmission occurred in 14 (36.8%) of the 38 pregnancies. Thus, 38 transmissions (35.2% (95% CI, 26.2–45.0%)) occurred in the historical control cohort, which was significantly higher ($P < 0.0001$) than the incidence in our HIG-treatment group.

Delivery occurred at a median of 39.3 (IQR, 38.7–40.1) weeks' gestation. Median birth weight was 3350 (IQR, 3115–3625) g, which corresponds to the 41st centile. One pregnancy had a late termination at 23 weeks owing to structural chromosomal abnormality. In this case, fetal blood tested after delivery lacked CMV. In all cases, the course of the pregnancy was uneventful. None of the women was treated for pre-eclampsia or preterm labor.

At delivery, two additional infants were diagnosed with congenital CMV infection after a negative result

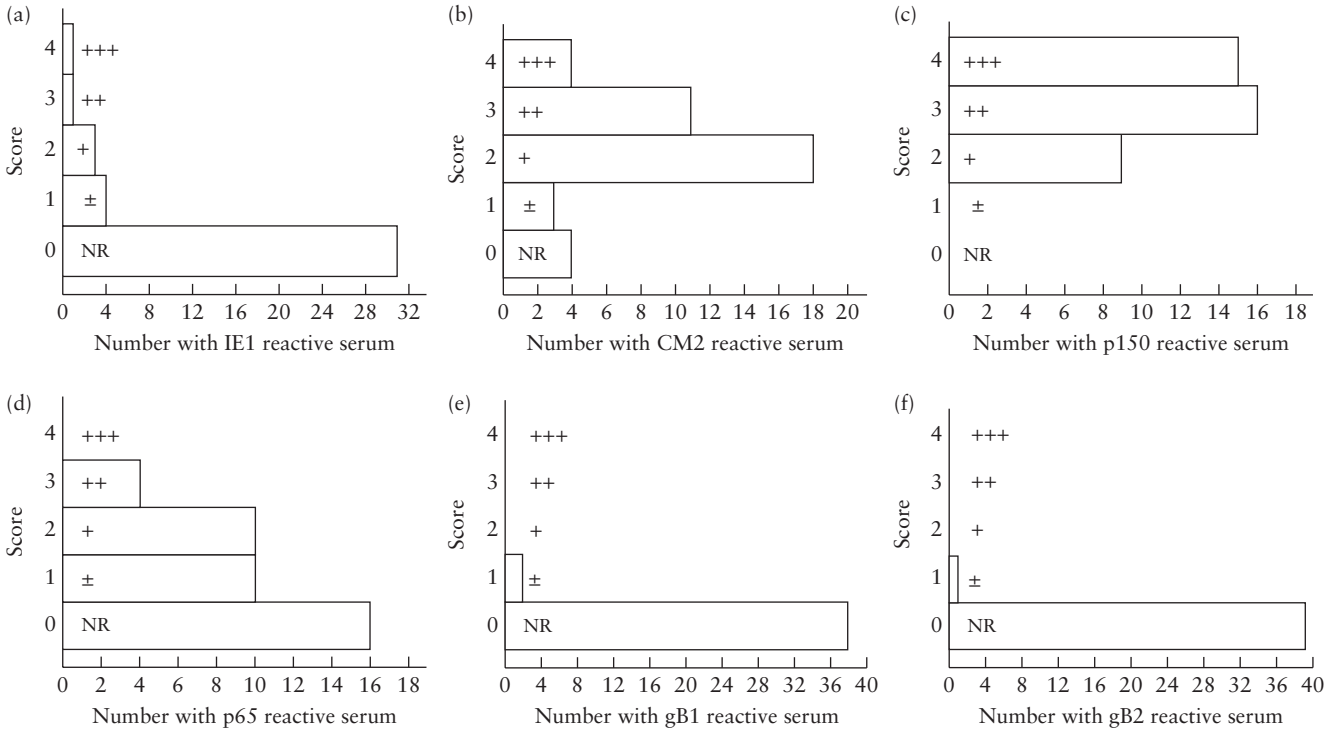

Figure 1 Cytomegalovirus (CMV)-specific immunoglobulin-M (IgM) reactivity against rec IE1 (a), CM2 (b), p150 (c), p65 (d), gB1 (e) and gB2 (f) at first presentation, in 40 women with first-trimester primary CMV infection. NR, non-reactive.

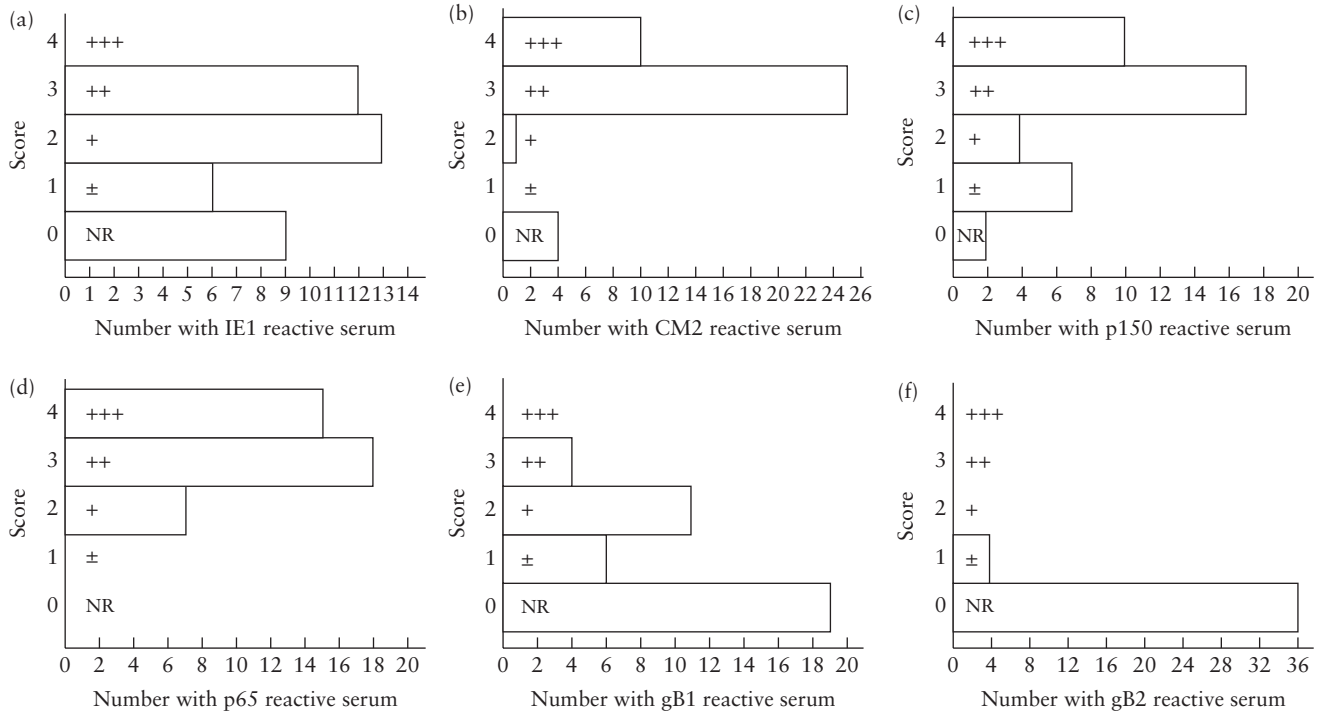

Figure 2 Cytomegalovirus (CMV)-specific immunoglobulin-G (IgG) reactivity against recombinant IE1 (a), CM2 (b), p150 (c), p65 (d), gB1 (e) and gB2 (f) at first presentation, in 40 women with first-trimester primary CMV infection. NR, non-reactive.

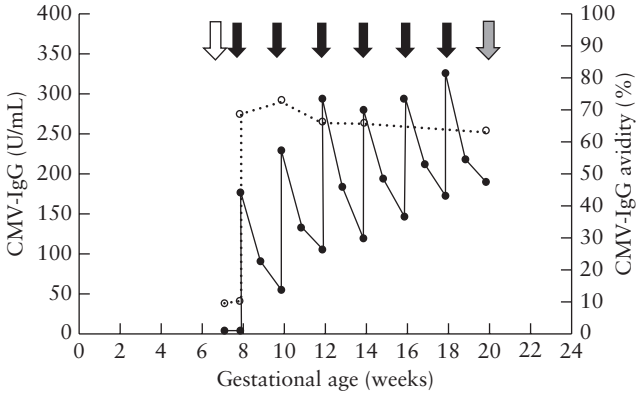

Figure 3 Cytomegalovirus immunoglobulin-G (CMV-IgG) levels (solid line) and respective IgG avidity (dotted line) in woman with first-trimester primary CMV infection, at first presentation (white arrow), during hyperimmunoglobulin treatment (six courses, black arrows) and at amniocentesis (gray arrow).

at amniocentesis. Thus, these were two cases of late-gestation transmission after HIG administration. Overall, maternal–fetal transmission in our cohort occurred in three pregnancies (7.5% of 40 cases (95% CI, 1.6–20.4%)). The CMV transmission rate at term was significantly lower than the transmission rate at 20 weeks in the control group ($P = 0.001$).

None of the three fetuses that experienced CMV transmission showed *in-utero* signs of a CMV infection. They were asymptomatic after birth and did not receive any antiviral medication postpartum. At the time of writing, the children were 26, 20 and 4 months old, respectively; developmental and hearing follow-up evaluations were normal.

DISCUSSION

In this study, we demonstrate that, after a primary maternal infection with CMV in the first trimester, a modified HIG protocol consisting of biweekly administration of 200 IU/kg HIG until 20 weeks' gestation can be followed to prevent successfully maternofetal transmission. Importantly, HIG administration was restricted to a small group of women with very recent primary CMV infection. In our study cohort, CMV-IgG levels were < 60 U/mL, CMV-IgG avidity was low in two independent test systems (ECLIA and immunoblotting), and there was no anti gB2 IgG reactivity. Interestingly, we observed a distinct CMV-specific IgM reactivity pattern: all sera were reactive against the tegument protein p150 and most against CM2, while there was no reactivity against rec gB1 or rec gB2. In terms of the CMV-specific IgG reactivity, we found no IgG antibodies against gB2 while all sera were reactive against p65. These CMV-specific IgM and IgG reactivities together with the concordant low avidity values and the low overall IgG levels emphasize that the maternal primary CMV infection must have occurred within a few weeks prior to diagnosis.

The treatment protocol was developed based on an index case and additional *in-vitro* studies of characteristics of HIG and other standard intravenous immunoglobulin preparations on CMV-specific neutralization capacity[10,12,16]. In the most recent of these studies comparing HIG with standard Ig preparations, we found high CMV-specific IgG antibody-binding capacity as well as very potent antiviral neutralization capacity against human epithelial target cells in both[16]. In the index case, we observed an initial half-life of about 11 days after

administration of 200 IU/kg body weight, indicating that a monthly protocol for the administration of HIG is not sufficient. In addition to strong fluctuations in CMV-IgG levels, we also detected fluctuations in CMV-IgG avidity and anti-CMV-specific neutralization capacity against epithelial ARPE-19 target cells. Therefore, we concluded that monthly administration is insufficient for an effective prevention of maternal–fetal transmission and thus changed our protocol to biweekly HIG administration[8,11].

In our study, HIG administration was safe. Revello *et al.*[8] reported a 15% risk of preterm delivery and a 5% risk of fetal growth restriction in cases treated with HIG. Although our study was not powered to detect side-effects, we did not observe any serious problems in our cohort. This is consistent with a recent study that also used 200 IU/kg body weight HIG, in which HIG treatment was well tolerated and was not associated with prematurity or decreased birth weight or other serious side-effects[17]. Interestingly, there seems to be an increase in birth weight and gestational age at delivery after the mother receives multiple doses of HIG[18].

Our results concerning the effectiveness of HIG are partly consistent with those of previous studies. An editorial commentary on the findings of the published studies highlighted the positive effect of HIG[19]. The first study of HIG observed a reduction in the transmission rate from 40% in the control group to 16% in the HIG prevention group[11]. A second study found a 44% transmission rate in the control group but a 30% rate in the treatment group[8]. With more subjects, these results may have been significant[20]. One review that combined the data of all published studies reported an odds ratio of 0.43 for HIG to prevent maternal–fetal transmission[21]. In three observational studies[10,17,22], the transmission rate was, respectively, 23%, 31% and 41% in the group of women who received HIG treatment. However, the administration schemes varied substantially between these studies; all, except the study of Chiaie *et al.*[17], used a low dose of HIG.

In our cohort, we observed a transmission rate at amniocentesis of only 2.5% and at delivery of 7.5%. We suggest that the considerably lower rate of transmission observed in our study was due to a substantial reduction in the time interval between the diagnosis of primary CMV infection and the first HIG administration, increased frequency of HIG administration and doubling of the dose to 200 IU HIG per kg of body weight. These protocol changes resulted in a stable and high CMV-IgG avidity and a continuously high concentration of neutralizing CMV-specific antibodies, while the CMV antigen-binding capacity was still fluctuating.

A limitation of this study is the lack of randomization to a control group. We conducted a prospective observational study over 4 years and compared our results with a historic cohort that did not receive treatment. However, in Germany and other countries, the use of HIG is widespread and providing HIG after a primary maternal CMV infection reflects common practice. We suggest that our Phase-I results should be confirmed in a larger placebo-controlled randomized trial of HIG *vs* placebo.

Most cases in our cohort underwent amniocentesis at around 10 weeks after the first presentation at about 20 weeks' gestation; however, in four cases, amniocentesis was done at 17 weeks, which was still at least 7 weeks after diagnosis (Table S1). In all four of these cases, maternofetal transmission was excluded at birth. A recent study confirmed that an earlier amniocentesis before 20 weeks can also reliably assess the risk of transmission[23]; therefore, we believe that this limitation did not affect our results.

We observed that two infants were uninfected by CMV at amniocentesis but were infected at birth. These late-gestation infections are unlikely to be related to HIG administration, as a previous study using low-dose HIG observed similar results[7]. Late-gestation fetal infections are common but almost always yield asymptomatic infants who do well in the long term[7].

In conclusion, we have shown that, in pregnant women with primary CMV infection in the first trimester, HIG administration substantially reduces maternal–fetal transmission when administered biweekly at a dose of 200 IU/kg before 20 weeks' gestation. A prospective randomized controlled study should be carried out to confirm our findings.

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Disclosure

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Results of virological studies indicating recent primary cytomegalovirus (CMV) infection necessitating HIG prophylaxis to prevent maternal–fetal transmission in 40 women included in this study: (a) low CMV immunoglobulin-G (IgG) levels (< 60 U/mL) (ECLIA), (b) low CMV-IgG avidity index (< 40%) (ECLIA) and (c) elevated CMV immunoglobulin-M (IgM) indices (ECLIA) at time of first presentation; (d) low CMV plasma viral load, (e) high CMV-IgG avidity index (ECLIA) and (f) RecomLine CMV-IgG avidity index (anti-IE1,-CM2, -p150, -gB2 IgG) ranging between 12 and 14 out of 16 at amniocentesis. Lines inside boxes and upper and lower lines indicate median and interquartile range, respectively, and whiskers are range.

Table S1 Details of individual characteristics and treatment course of each patient

