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Preliminary Evaluation of the Safety and Efficacy of Standard Intravenous Immunoglobulins in Pregnant Women with Primary Cytomegalovirus Infection

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Hyperimmune globulins were reported to prevent and treat fetal cytomegalovirus (CMV) infection during pregnancy. Here, we report that infusions of standard human intravenous immunoglobulin significantly increase CMV IgG titers and avidity indexes in pregnant women, paving the way to their use for passive transfer of maternal CMV humoral immunity to fetuses. Preliminary data on perinatal outcomes of the first 67 newborns are encouraging.

Since an efficacious vaccine for cytomegalovirus (CMV) disease is still lacking, immunoglobulins are yearly used to treat thousands of transplant patients to prevent CMV reactivation and reinfection (16). These immunoglobulins are generally referred to as hyperimmune globulins (HIG), are collected only from selected donors with high-titer IgG antibodies after CMV infection, and appeared to be safe, being the most purified among blood derivatives (2, 7, 13, 14, 16). In recent years, animal and human pivotal studies on women with primary CMV infection during pregnancy suggested that fetal CMV infection might be both prevented and treated using HIG for passive transfer of humoral immunity (13–15, 18). More recently, it has been proposed that standard intravenous immunoglobulins (IVIG), obtained from unselected donor pools, including a varying proportion of donors previously exposed to CMV, may be a valuable and less expensive alternative to HIG for the prevention of CMV disease in transplant patients (12, 16). As the potential benefit of IVIG has not yet been explored in pregnant women, we performed a longitudinal prospective study on IVIG for the prevention and/or therapy of fetal CMV infection (4, 11).

The study protocol was authorized by the local ethical committee on 18 October 2010; published on ClinicalTrials.gov with the serial number NCT01659684 as of 7 August 2012; and carried out at the Infectious Disease Unit of Pescara General Hospital, Pescara, Italy, since December, 2010. Human IVIG were offered monthly to consecutive enrolled pregnant women with confirmed primary CMV infection at any stage, for the prevention and treatment of fetal CMV infection. Primary infection was defined by positive CMV IgM antibodies with absent or low titers of CMV IgG antibodies and low (<40%) CMV IgG avidity indexes. In addition, women with an indefinite (>40, <50) avidity index and positive CMV DNA detection in urine and/or blood samples were also considered for treatment (5). Standard human IVIG (Kiovig, Baxter AG, Vienna, Austria) were chosen as an alternative to CMV HIG for their safety and efficacy, well documented in other settings, in addition to a lower cost than HIG (16, 17). IVIG from a single batch (no. LE12L058AF) were used to perform all the infusions in the study, undiluted after reconstitution in accordance with instructions of the manufacturer. The mean titer of CMV IgG antibodies in the batch was 187 ± 15 U/ml, with a mean IgG avidity index of 85.3% ± 4.2%. In comparison, the mean titer of CMV IgG antibodies in 3 batches of HIG (Cytotect, Biotest Inc., Dreiech, Germany), assayed in parallel under identical experimental conditions, was 380 ± 36.1 U/ml, with a mean IgG avidity index of 72.3% ± 9.4%. As a consequence, we chose to perform IVIG infusions using 0.5 g/kg of body weight, to make sure that a dose of specific CMV IgG at least comparable with that carried by HIG was infused at each time point. Infusions lasted 4 to 5 h, using a double lumen line to infuse approximately 800 to 1,000 ml of either 5% glucose or saline solution in parallel with the undiluted IVIG preparation, to reduce the risk of infusion reactions.

CMV IgG and IgM antibodies and IgG avidity indexes were assayed by Enzygnost kits (Siemens, Marburg, Germany), both before and after each IVIG infusion, within 15 min, the average time it took to collect a control sample after the end of each infusion. The Enzygnost kit was used in accordance with procedures recommended by the manufacturer, providing a 98% positive predictive value (PPV) for recent infection for avidity indexes of ≤40% (3, 5, 8, 9). Quantitative CMV DNA was amplified from whole-blood and urine samples, from both pregnant women and neonates, using the real-time PCR Alert kit (Nanogen, Turin, Italy), and on samples of amniotic fluid from women who required amniocentesis (6).

We prospectively enrolled and followed-up with 113 pregnant women; of these, 104 (92.0%) had a primary CMV infection (avidity index of <50 and amplifiable CMV DNA either on plasma and urine); 2 (1.8%) had documented fetal infection at amniocentesis; the remaining 7 (6.2%) patients had an avidity index at diagnosis of ≥50%, so that recurrent infection could not be excluded. Among enrolled women, 45 (39.8%) were diagnosed

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TABLE 1 Characteristics of the 113 consecutive enrolled pregnant women

Variable	Value
Age in yrs of pregnant women, mean (SD)	32.3 (5.5)
Gestational week at diagnosis, mean (SD)	15.7 (7.2)
No. of weeks after diagnosis at first infusion, mean (SD)	5.6 (4.3)
Pregnancy order, %	
First	37.2
Second	47.1
Third	15.7
Gestational age at diagnosis, %	
First trimester	39.8
Second trimester	45.1
Third trimester	15.1
No. of infusions, %[a]	
One	30.1
Two	27.4
Three or more	42.5
Outcome at birth, no. (%) (data available for 67 newborns)	
Infected	27 (40.3)
Asymptomatic	22 (81.5)
Impaired otoemissions	2 (7.4)
Mild growth retardation	3 (11.1)

[a] Reinfusions were delivered approximately 4 weeks after the previous one.

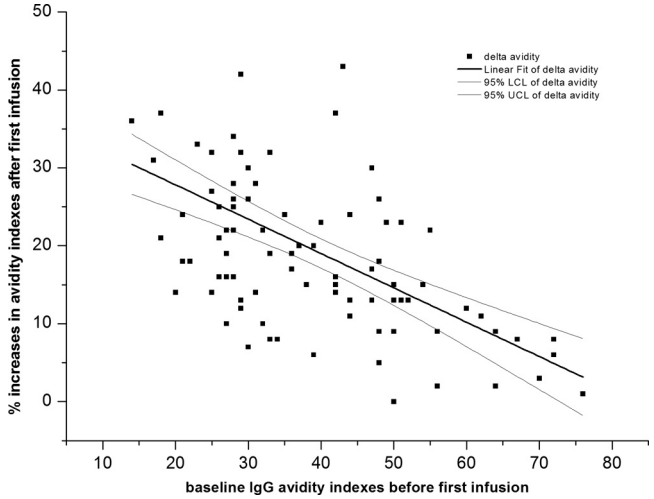

FIG 1 Scatter plot of increases of CMV IgG avidity indexes after the first infusion of IVIG (*y* axis) relative to the preinfusion avidity index of each patient (*x* axis). Overlapped is the curve of Spearman index with 95% confidence intervals.

in their first trimester of gestation (Table 1). Twenty-eight women underwent amniocentesis, 18 (64.3%) with confirmed fetal CMV infection. All women received at least 1 infusion of IVIG, 5.6 ± 4.3 (mean ± standard deviation [SD]) weeks after diagnosis of CMV infection (range, 0 to 21 weeks); 31 (27.4%) received 2 infusions; 48 (42.5%) received ≥3 infusions (Table 1). Infusions were significantly more numerous in patients enrolled in their first trimester of gestation than in patients diagnosed later (*P* value for trend = 0.03). No serious adverse events were recorded during infusions; in 5 cases, mild nausea occurred; in 2 of such cases, infusions were temporarily interrupted due to vomiting and completed on the following day. Mean IgG antibody titers and mean avidity indexes at diagnosis were 41.0 ± 57.2 U/ml and 26.3% ± 16.4%, respectively. Paired blood samples (before and after the first infusion) available for 85 women revealed that mean CMV IgG avidity indexes increased from 38.9% ± 14.4% to 57.2% ± 12.1% (paired *t* test; $P < 0.001$; Table 2). A significant inverse correlation between preinfusion IgG avidity indexes and postinfusion increases in avidity (Δav) was observed (Spearman Rho = −0.54; $P < 0.001$; Fig. 1). In the 59 women evaluated after the second or third infusion, performed with the same modalities after an approximate 4-week interval, the mean CMV IgG avidity index increased from 45.8% ± 13.1% to 58.4% ± 13.4% ($P < 0.001$; Table 2). Indeed, infusions of IVIG significantly increased CMV IgG titers and avidity indexes at all time points considered (Table 2). Essentially identical results were obtained when the 7 women with possible recurrent infection were excluded (data not shown). At present, data are available for 67 neonates only. Of these, 27 (40.3%) were infected at birth: 22 (81.5%) asymptomatic, 2 (7.4%) with abnormal otoemissions, and 3 (11.1%) with a mild growth retardation (Table 1).

In general, the congenital infection rate in CMV-seropositive pregnant women is <2% (2). Infections under such circumstances are frequently asymptomatic or cause mild to moderate neurosensory deficits in fetuses (2). Interestingly, infants born from preexposed mothers do not receive *in utero* immune cells but only maternal antibodies (10). Such epidemiological evidence, together with data from reports on the protective role of transfusions of CMV antibodies in neonates, led to the hypothesis that passive immunization of pregnant women with HIG could prevent or attenuate CMV congenital infection (1, 2, 19). This hypothesis has been supported so far by 5 reports in the literature (7, 13–15, 18). In particular, Nigro et al. recently suggested that the only risk factor for an affected child was the mother not receiving immunoglobulins (15). Our observation demonstrates for the

TABLE 2 CMV IgG titers and avidity indexes at investigated time points

	Mean CMV IgG titer, U/ml (SD) by no. of paired observations			Mean % avidity index (SD) by no. of paired observations		
Time point	87	55	57	85	55	59
Pre-1st infusion	81.8 (67.3)			38.9 (14.4)		
Post-1st infusion	134.3 (72.4)	146.5 (81.3)		57.2 (12.1)	58.4 (10.8)	
Pre-2nd/3rd infusion[a]		90.4 (46.7)	89.4 (46.5)		45.3 (12.9)	45.8 (13.1)
Post-2nd/3rd infusion[a]			127.1 (53.2)			58.4 (13.4)
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Reinfusions were delivered approximately 4 weeks after the previous one.

first time that infusions of standard human IVIG in women with primary CMV infection significantly increase CMV IgG titers and avidity indexes on blood samples collected 10 to 15 min after the end of each infusion. The tested IVIG preparation, having an avidity index of 85.3% ± 4.2%, was effective in influencing the final avidity indexes after distribution in the approximately 6-liter volume of human extracellular fluids. The effect of passive CMV immunization, likely to improve placental function and supplies of fetal oxygen, substrates, and nutritional elements, may well reduce the probability of vertical infection and the severity of CMV-related lesions in the infected fetuses (2, 13). To our knowledge, no randomized trials for immunoglobulin therapy of CMV-infected fetuses are ongoing; our prospective large series of women with primary CMV infection (confirmed or possible in 93.8% of women in our series, based on the presence of CMV IgM, amplifiable CMV DNA, and a CMV IgG avidity index either low or inconclusive) is planned to include at least 300 consecutive enrollees, and results on the efficacy of IVIG on fetal outcomes are due at the end of 2014. By the present time, this report provides preliminary evidence that IVIG may serve the purpose of passive CMV immunization of pregnant women at remarkably lower costs than HIG. Indeed, the use of HIG would have caused an increase in costs of 83%; that is, each infusion would have cost 2,428 Euros instead 1,323 Euros on average.

In conclusion, our data provide initial evidence that infusions with IVIG may significantly enhance IgG titers and CMV avidity indexes in women with primary CMV infection during pregnancy.

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Written informed consent was obtained from each patient for publication of data.

We declare that we have no conflicting interests to disclose.

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