

# Revised Protocol for Secondary Prevention of Congenital Cytomegalovirus Infection With Valaciclovir Following Infection in Early Pregnancy

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**Background.** A previous randomized placebo-controlled study found valaciclovir to be effective in reducing the rate of vertical cytomegalovirus transmission from mother to fetus. The better results in women infected in the first trimester compared to the periconception period were attributed to the timing of treatment. The aim of the present study was to evaluate valaciclovir efficacy in this setting using a revised protocol.

**Methods.** All pregnant women treated with valaciclovir in 2020–2022 who met the same criteria as in the original study were identified retrospectively from the database of the same medical center. Treatment, however, was initiated earlier: up to 9 weeks or 8 weeks from the presumed time of infection in women infected in the periconception period or the first trimester, respectively. The primary endpoint was rate of vertical cytomegalovirus transmission. Results were compared with the placebo arm in the previous study.

**Results.** Among 178 women who completed valaciclovir treatment, amniocentesis was positive for cytomegalovirus in 14 women (7.9%), significantly ( $P < .001$ ) lower compared with 14 of 47 (30%) in the placebo arm in the previous study. The proportion of positive amniocentesis in the valaciclovir was significantly lower than the placebo arm both among women infected in the first trimester (14/119 vs 11/23; odds ratio [OR] = 0.15; 95% confidence interval [CI]: .05–.45,  $P < .001$ ), as well as among those infected in the periconception period (0/59 vs 3/24, OR = 0; 95% CI 0–.97,  $P = .02$ ).

**Conclusions.** This study provides further evidence of the efficacy of valaciclovir in preventing vertical transmission of cytomegalovirus after primary maternal infection. Efficacy is improved with earlier treatment.

**Keywords.** valaciclovir; cytomegalovirus prevention; congenital cytomegalovirus; congenital infection; pregnancy.

Cytomegalovirus (CMV) is the most frequent cause of congenital viral infection. The risk of serious sequelae is greatest when primary maternal infection occurs early, in the periconception period or during the first trimester [1–4]. The high potential morbidity of congenital CMV infection warrants antenatal prevention of maternal infection or of vertical transmission of the virus to fetus is warranted.

There is no available approved vaccine for primary prevention of maternal CMV infection. Use of CMV hyper-immune globulin was investigated for secondary prevention of viral transmission to the fetus, with controversial results [5–8]. A recent randomized placebo-controlled trial (RCT) from our medical center found valaciclovir to be effective in reducing the risk of congenital CMV infection in women infected early in pregnancy. The rate in the

study group was significantly lower than in the placebo group, with an odds ratio (OR) of 0.29 for vertical viral transmission to the fetus (95% confidence interval [CI] .09–.90,  $P = .027$ ) [9]. These results were supported by another case-controlled study using a similar treatment protocol compared to no treatment (OR 0.318 [95% CI: .12–.84],  $P = .021$ ) [10].

However, the same RCT [9] showed that, although treatment efficacy was good overall, it was better when the primary infection occurred in the first trimester than in the periconception period. On reanalysis of the data, we attributed the difference to the time elapsed from maternal infection to start of the medication. Data on the interval from maternal infection to fetal viremia are limited. Current guidelines recommend that amniocentesis be performed 6–8 weeks after the assumed time of infection [4, 11, 12]. Accordingly, we revised the protocol for valaciclovir treatment in pregnant women infected with primary CMV.

The aim of the present study was to evaluate the efficacy of the new protocol to prevent CMV transmission compared to the original data.

## METHODS

Pregnant women with primary early CMV infection treated with valaciclovir from January 2020 to December 2022, who

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met the revised criteria of our previous RCT study, were identified retrospectively from the electronic fetomaternal database of the same tertiary medical center. All had serological evidence of a primary cytomegalovirus infection acquired during the periconception period (4 weeks before and up to 3 weeks after the recorded date of the last menstruation) or the first trimester of pregnancy. Primary CMV infection was defined as specific immunoglobulin M (IgM) only with immunoglobulin G (IgG) that become positive on repeated assay performed later, or specific IgM with low IgG avidity that increased on a second repeated assay. All women had normal renal function.

The main change from the original protocol was the timing of valaciclovir administration. Treatment was initiated up to 9 weeks from the assumed time of infection in women infected in the periconception period or up to 8 weeks from the assumed time of maternal infection and before 18 weeks of pregnancy in women infected in the first trimester. The time of maternal infection was determined by the serology results. Avidity was assessed with the VIDAS CMV Avidity II panel (BioMérieux; Marcy l'Étoile, France). Using the data of Vauloup-Fellous et al [13], Figure 1, "VIDAS CMV-IgG avidity index following seroconversion," we used visual linear interpolation to draw the best approximate straight line to determine the time of infection in relation to the avidity results.

Treated women agreed to undergo amniocentesis at 20–22 weeks of pregnancy. Valaciclovir was given orally, 8 grams/day in 2–4 doses until the day of the amniocentesis. Follow-up of renal function was recommended every 2–3 weeks. Adherence was assessed by asking the women about any missed doses at every clinical visit and/or telephone visit.

The primary endpoint of the study was the rate of CMV-positive polymerase chain reaction (PCR) tests of amniotic fluid.

Women with early primary CMV infection who arrived to our clinic out of the time frame of the revised protocol were excluded from the study as were women who refused to take the medication, who started treatment but used <50% of the prescribed medication, and who terminated the pregnancy early after the first clinic visit.

Findings were compared with the data of women in the control arm of the original RCT who were diagnosed with primary CMV infection in the periconception period or the first trimester and started treatment with placebo at up to 16 weeks of pregnancy [9].

The Ethics Committee of Rabin Medical Center approved the study.

### Statistical Methods

Baseline characteristics were summarized by mean and standard deviation. Findings between women who started treatment in the periconception period or during the first trimester were

compared using Fisher exact test and independent *t* test. Odds ratios and 95% confidence intervals were calculated for the primary outcome for each subgroup and compared with the placebo arm in our previous study [9]. Statistical analyses were conducted using SPSS ver. 27 (IBM Corp. Armonk, New York, USA), Rversion 4.2, and WINPEPI programs [14].

## RESULTS

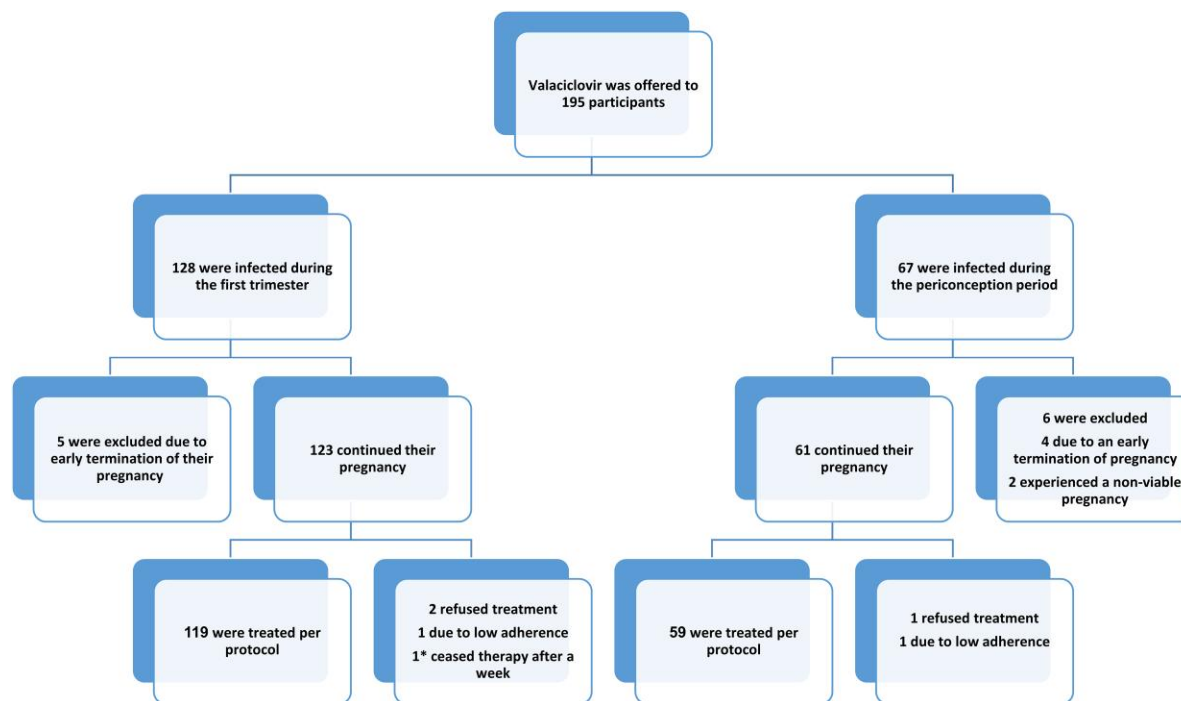
From January 2020 to July 2022, valaciclovir was offered according to the revised protocol to 195 pregnant women with primary CMV infection for the prevention of fetal infection. Seventeen women were excluded from the analysis because they did not take the medication at all (*n* = 3), decided to terminate the pregnancy before starting the medication (*n* = 9), had an early spontaneous abortion (*n* = 2), used <50% of the medication due to severe vomiting (*n* = 2), or stopped treatment after 1 week due to transient renal failure (1 woman with a single kidney) (Figure 1).

The remaining 178 women took valaciclovir until the scheduled date of amniocentesis, including 59 infected in the periconception period and 119 in the first trimester (Table 1). Amniocentesis was performed in 176 women. Two women infected in the first trimester refused amniocentesis, but they were included in the analysis (as negative PCR) because the urine of their newborn babies was negative for the virus.

Among the whole cohort, amniocentesis was positive for CMV in 14 of the 178 patients (7.9%) compared to 14 of the 47 patients (30%) in the historical placebo group (*P* < .001). Patients infected in the first trimester accounted for all cases of vertical CMV transmission in the cohort. Nevertheless, the rate of positive results in this subgroup (14/119, 11.8%) was still significantly lower than that of the historical controls infected in the first trimester in the original RCT (11/23, 47.8%; OR 0.15 [95% CI: .05–.45] *P* < .001). None of the amniocenteses performed in the 59 patients infected in the periconception period was positive for CMV compared with 3 of 24 (13%) in the placebo group (OR 0.000 [95% CI: 0–.97], *P* = .02).

Out of 195 women who were offered the treatment with the revised protocol, 9 decided to terminate the pregnancy before starting the medication, and 2 experienced an early spontaneous abortion; hence, 184 women continued with their pregnancy. The results of intent to treat analysis included 3 women who refused therapy, 2 with a very low adherence due to severe vomiting, and 1 with a single kidney who ceased therapy after a week. In the periconception group, only 1/61 (1.64%) was infected compared to the controls of 3/24 (12.50%) (OR 0.12 [95% CI: .000–1.59], *P* = .066). In the first trimester group, 16/123 (13.01%) were infected compared with the controls of 11/23 (47.83%) (OR 0.16 [95% CI: .060–.490], *P* < .001).

In accordance with the protocol revision, the mean interval from the time of assumed maternal infection to treatment



**Figure 1.** Trial profile. \*Transient renal failure: a woman with single kidney.

**Table 1. Study Population of Women Treated With Valaciclovir, by Period of CMV Infection**

N	Periconception		First Trimester		<i>P</i>
	n = 59		n = 119		
Age, years mean SD	30.47	3.73	31.42	4.38	.157
Parity (%)	...	...	...	...	.932
0	7	6.5	14	11.8	...
1	43	82.6	84	70.6	...
2	6	8.7	14	11.8	...
≥3	2	3.4	7	5.8	...
GA days at therapy initiation, mean SD	54.88	12.34	93.05	19.23	<.001
Days to treatment initiation, mean SD	46.98	12.32	39.08	9.75	<.001
GW at amniocentesis, mean SD	21.28	0.59	21.44	0.55	.086
Amniocentesis positive <sup>a</sup> (%)	0	0%	14	11.8	.014

Abbreviations: CMV, cytomegalovirus; GA, gestational age; GW, gestational week.

<sup>a</sup>In 2 cases negative status was determined by other indications.

initiation was shorter in the present study than in the patients treated with valaciclovir using the original protocol [9]: for women infected in the periconception period,  $46.98 \pm 12.32$  days versus  $60.58 \pm 19.29$  ( $P < .001$ ); for women infected in the first trimester,  $39.08 \pm 9.75$  versus  $43.84 \pm 14.16$  days ( $P = .15$ ). In the periconception infection group, the interval between the assumed maternal infection in the historical control of the placebo group was  $66.5 \pm 18$  days versus  $46.98 \pm 12$  in the present study,  $P < .001$ .

The self-reported adherence rate in the present cohort was  $>90\%$  in all patients. Side effects were rare. A transient increase in creatinine level was detected in 1 patient in whom treatment was stopped for 4 days and then resumed after creatinine normalization.

## DISCUSSION

Currently, the absence of an approved CMV vaccine and the difficulties in implementing behavioral hygiene changes for primary prevention of maternal infection make secondary prevention by valaciclovir the main method for decreasing the risk of congenital CMV infections.

Our randomized controlled study published in 2020 was the first to evaluate the efficacy of valaciclovir in decreasing the rate of viral transmission to the fetus after early maternal infection [9]. However, the results were statistically significant only for women infected in the first trimester. For women infected with primary CMV in the periconception period, treatment did not significantly modify the rate of vertical transmission. This discrepancy was presumed to be due to the later initiation of treatment after primary maternal infection: mean 60 days in the periconception subgroup compared to 43 days in the first-trimester subgroup ( $P < .001$ ). Further analysis of the data revealed that within the first-trimester subgroup, treatment was initiated significantly later in the 5 fetuses that were infected than in the fetuses that were not (mean 75 vs 50 days after

maternal infection,  $P = .0047$ ) [9]. In the *Lancet* publication [9], 3 women in the periconception group who transmitted the virus to the fetus were treated with valaciclovir. The time interval between primary infection and initiation of treatment was 10, 12, and 14 weeks, and duration of treatment was 8, 6, and 6 weeks, respectively.

These data emphasize the importance of time of initiation of valaciclovir treatment in relation to time of onset of maternal infection. More information is needed on the temporal sequence of events, from maternal CMV infection to maternal viremia to infection of the fetus. Transplacental transmission may occur via cell-to-cell spread in the maternal decidua, where invasive fetal cytotrophoblasts remodel the uterine vasculature and closely interact with maternal cells, and/or transfer from the maternal blood in the intervillous space to fetal cells within the chorionic villi, crossing the syncytiotrophoblast cell layer [15]. This process is believed to span around 6–8 weeks according to studies of the timing of amniocentesis for CMV detection. Ender et al [4] found that amniocentesis sensitivity was about twice as high when the interval between maternal seroconversion and amniocentesis was  $>8$  weeks (90.9%) compared to  $\leq 8$  weeks (45.5%). Therefore, to optimize efficacy, we initiated treatment at 8 weeks from infection in women infected during the first-trimester, extending it to 9 weeks in women infected in the periconception period in whom we assumed a lower and slower viral transmission (Supplementary Table 1).

The other concern related to the time of maternal infection was the interpretation of the serological results. The best available method is based on the data of Vauloup-Fellous et al [13]. Using their Figure 1, “VIDAS CMV-IgG avidity index following seroconversion,” we used visual linear interpolation to draw the best approximate straight line to determine the time of infection in relation to the avidity results.

The main finding of the present study was the efficacy of valaciclovir treatment in women infected in the periconception period, none of whom tested positive for a fetal infection on amniocentesis. The only difference between the present cohort and the comparative placebo arm in our previous study was the shorter mean time from assumed maternal infection to initiation of valaciclovir treatment (46.98 vs 66.50 days,  $P < .001$ ).

In women infected in the first trimester, the fetal transmission rate was significantly lower than in the placebo controls but similar to that of the valaciclovir-treated arm in the original study (12% in both). The time to treatment in this subgroup was also shorter than in the original study, but the difference was small and not statistically significant (39 vs 43 days,  $P = .15$ ). We assume the higher transmission rate in the first-trimester subgroup than in the periconception subgroup was probably related to the variable time of fetal viral invasion, which may range from 4 to 8 weeks after maternal infection and the difficulty in accurately determining the time of maternal infection.

The main limitations of this study are the retrospective design and the use of a historical placebo control group. Nevertheless, the placebo group was very similar to the treated group in the present study in terms of main inclusion criteria and method used to calculate timing of early primary maternal CMV infection. Although treatment was initiated earlier in the revised protocol, this did not affect the results of the placebo group. We believed that as the data on the use of valaciclovir to prevent fetal infection is based on 2 published studies with similar results so far [9, 10], it was not ethical to form another placebo control study. An additional limitation of this study is the method used to determine the time of maternal infection. We primarily used the avidity results of the Vidas assay, which has a lower accuracy for low avidity. Nonetheless, at present, this is the best method we have.

In conclusion, this study provides additional evidence of the efficacy of valaciclovir in the prevention of vertical transmission of cytomegalovirus after early primary maternal infection. The revised protocol dramatically improved the results in women infected in the periconception period compared to the original protocol. For better implementation of this strategy of secondary prevention, early screening of CMV serology in seronegative women is needed, including frequent serological follow-up in the first 3 months of pregnancy.

#### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

#### Notes

**Potential conflicts of interest.** The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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