Primary human cytomegalovirus (HCMV) infection occurring in pregnant women within 3 months before (preconceptional) or within 4 weeks after (periconceptional) the last menstrual period represents an as-yet-undefined risk to the fetus. One (9.1%) of 11 newborns born to 12 women with preconceptional infection was subclinically infected (1 aborted fetus was not examined for infection). Of 20 pregnancies in women with periconceptional infection, 7 were terminated before 12 weeks of gestation (aborted fetus was not examined), 1 was terminated at 23 weeks after prenatal diagnosis of congenital infection, and 12 continued to term. Of those 12, 3 resulted in newborns who were congenitally infected. Thus, in the periconceptional group, intrauterine transmission occurred in 4 (30.8%) of 13 pregnancies for which the virologic outcome was known. One newborn was symptomatic at birth, and disseminated HCMV infection was diagnosed in an aborted fetus. Periconceptional primary HCMV infection seems to bear a higher risk of unfavorable outcome than preconceptional infection, and counseling should be adjusted accordingly.

CONCISE COMMUNICATION

Diagnosis and Outcome of Preconceptional and Periconceptional Primary Human Cytomegalovirus Infections

Maria Grazia Revello, Maurizio Zavattoni, Milena Furione, Daniele Lilleri, Giovanna Gorini, and Giuseppe Gerna

Primary human cytomegalovirus (HCMV) infection occurring in pregnant women within 3 months before (preconceptional) or within 4 weeks after (periconceptional) the last menstrual period represents an as-yet-undefined risk to the fetus. One (9.1%) of 11 newborns born to 12 women with preconceptional infection was subclinically infected (1 aborted fetus was not examined for infection). Of 20 pregnancies in women with periconceptional infection, 7 were terminated before 12 weeks of gestation (aborted fetus was not examined), 1 was terminated at 23 weeks after prenatal diagnosis of congenital infection, and 12 continued to term. Of those 12, 3 resulted in newborns who were congenitally infected. Thus, in the periconceptional group, intrauterine transmission occurred in 4 (30.8%) of 13 pregnancies for which the virologic outcome was known. One newborn was symptomatic at birth, and disseminated HCMV infection was diagnosed in an aborted fetus. Periconceptional primary HCMV infection seems to bear a higher risk of unfavorable outcome than preconceptional infection, and counseling should be adjusted accordingly.

It is well known that human cytomegalovirus (HCMV) can be transmitted to 40%–50% of fetuses following primary infection in the mother [1]. Moreover, 10%–15% of newborns with congenital HCMV infection will be symptomatic at birth, and ~15% of asymptomatic congenitally infected babies will develop long-term sequelae [1]. However, the risk of intrauterine transmission and the outcomes of pregnancies complicated by primary HCMV infection occurring before or at approximately the time of conception are not known. The principal reasons for this are the absence, in most countries, of serologic screening programs carried out during pregnancy; the difficulty in obtaining a definite diagnosis of primary HCMV infection; and the difficulty in dating the onset of primary HCMV infection [2]. The main objective of the present study was to investigate the risk to the fetus following maternal preconceptional or periconceptional primary HCMV infection, in view of improving counseling and pregnancy management.

Subjects, Materials, and Methods

Subjects and diagnostic criteria. The records of 182 pregnant women examined during 1992–2000 who received a definite diagnosis of acute/recent primary HCMV infection and for whom the outcome of pregnancy was known were reviewed to identify women in whom infection occurred before or near conception. For this purpose, infections that occurred within 3 months before the last menstrual period (LMP) were considered preconceptional, and infections that occurred within 4 weeks after the LMP (±2 weeks after or before the presumed time of conception) were considered periconceptional. The timing of primary HCMV infection was based on the following 4 criteria: decreasing titers of HCMV-specific IgM antibody, increasing levels of IgG antibody avidity, detection of HCMV and HCMV products in blood, and presence of clinical symptoms and/or abnormal laboratory findings [2].

Serologic and virologic tests. Virus-specific IgG and IgM antibody levels were determined by ELISAs developed in the laboratory [3, 4]. The level of IgM antibody was expressed by calculation of the IgM ratio, which is the ratio of the optical density of the test serum to the optical density of the cutoff control serum. The same ELISA for IgG detection was used to determine IgG avidity according to a procedure reported elsewhere [5], with minor modifications [2]. In brief, test serum was added to wells coated with HCMV antigen and control antigen. After 1 h of incubation, wells were washed with 6 M urea solution for 15 min at room temperature to remove low-avidity IgG. Residual antigen-bound IgG was then detected by the addition of peroxidase-conjugated anti–human IgG and the chromogen substrate solution. An IgG avidity index (AI) was calculated by multiplication of the ratio of absorbance in the presence of urea to the absorbance in the absence of urea by 100. AIs <35% were mostly found for women with recent (primary) infections (acquired within <3 months), AIs of 35%–50%
indicated intermediate infection, and AIs ≥51% were associated with past (remote) infection (acquired ≥6 months before) [2].

The presence of HCMV products in blood was determined by quantitation of antigenemia and DNAemia as reported elsewhere [6, 7]. For early HCMV isolation and identification, clinical specimens (urine and amniotic fluid) were inoculated onto confluent monolayers of human fibroblasts that were stained with a monoclonal antibody to the major HCMV immediate early protein p72 [8].

**Prenatal diagnosis.** Prenatal diagnosis of congenital HCMV infection was done by virus isolation [8] and viral DNA detection by polymerase chain reaction (PCR) [9, 10] in amniotic fluid samples. In addition, antigenemia [7] and DNAemia [10] were quantitated in fetal blood samples, and virus-specific IgM [4] was determined in fetal serum samples obtained after 20 weeks of gestation.

**Statistical analysis.** Data were compared between groups by use of the Mann-Whitney rank sum test. Differences in distribution were evaluated by the χ² test.

**Results**

**Diagnosis and timing of primary HCMV infection.** Overall, 32 women in whom primary HCMV infection occurred before the LMP or at approximately the time of conception were retrospectively identified. For 12 women, primary HCMV infection occurred 2–11 weeks (median, 4 weeks) before the LMP (preconceptional infection), and for 20 women, infection occurred 1–4 weeks (median, 2 weeks) after the LMP (periconceptional infection). Precise dating of the infection was possible because all women recalled the onset of symptoms or had documented abnormal laboratory findings. One or more of the following symptoms were reported by each subject: fever (53.1%), asthenia (50%), headache (30%), upper respiratory symptoms (30%), and myalgia and malaise (25%). In addition, elevated liver enzyme levels were recorded for 13.3% of the women. At referral, the median time of gestation was 7.5 weeks (range, 6–14 weeks) in the preconceptional group and 8 weeks (range, 5–15 weeks) in the periconceptional group (P > .05).

Eleven (91.7%) of 12 and 20 of 20 women in the preconceptional and periconceptional groups, respectively, had positive results of testing for HCMV-specific IgM. Median IgM ratio values were significantly higher (4.8 [range, 1.6–15]) among women with periconceptional infection vs. 2.6 [range, <1–4] among women with preconceptional infection; P = .0014) and median AIs were significantly lower (21.5% [range, 0%–37%] vs. 36% [range, 22%–46%]; P = .0002) among women with periconceptional infection. Similarly, the rate of HCMV positivity in blood was significantly higher (P < .05) among women with periconceptional infection than among women with preconceptional infection. In fact, DNAemia was detected in 12 (92.3%) of 13 women and pp65 antigenemia in 5 (45.4%) of 11 women with periconceptional infection who were tested, whereas DNAemia was detected in 3 (37.5%) of 8 women and pp64 antigenemia in 0 of 8 women with preconceptional infection who were tested.

The kinetics of HCMV-specific IgM antibody levels and IgG avidity were determined in 129 and 82 sequential serum samples collected from the 12 women with preconceptional and the 20 women with periconceptional infections (median, 4 samples/subject [range, 2–9 samples] and 4.5 samples/subject [range, 2–8 samples], respectively) (figure 1). Subjects with periconceptional infection were followed up for 135.5 days (range, 32–235 days), and subjects with periconceptional infection were followed up for 174.5 days (range, 14–309 days), respectively. The mean IgM levels and IgG A1 values observed at 0–4 weeks after referral in serum samples obtained from women with periconceptional infection were similar to those observed 8–12 weeks after referral in serum samples from women with periconceptional infection.

**Outcomes of pregnancies in women with preconceptional infection.** In the group of 12 women with preconceptional infection, 1 woman terminated her pregnancy at 11 weeks of gestation (aborted fetus was not examined), 2 women underwent testing for prenatal diagnosis of HCMV infection (with negative results; 3 procedures were performed in all), and for 11 women the pregnancy continued to term (table 1). Overall, 10 (90.9%) of the 11 delivered infants were uninfected, and 1 newborn (9.1%) was subclinically infected.

The mother of the infected newborn recalled having experienced fever, asthenia, headache, and upper respiratory symptoms 8 weeks before her LMP. A prenatal diagnostic procedure done at 18 weeks of gestation, while the woman still had positive results of testing for viral DNAemia, yielded negative results. However, at birth, the virus was isolated from the newborn’s urine, and viral DNA was detected in the newborn’s blood, as were low levels of HCMV-specific IgM. Tests for both DNAemia and IgM still yielded positive results at age 6 months, although no symptoms developed.

**Outcome of pregnancies in women with periconceptional infection.** As shown in table 1, of the 20 women with periconceptional HCMV infection, 5 elected to terminate their pregnancies within 12 weeks of gestation, and 1 elected to terminate the pregnancy at 23 weeks of gestation (after congenital infection had been diagnosed prenatally; see below); 2 women had spontaneous abortions at 7 weeks of gestation (products of conception were not examined for HCMV), and in 12 women, the pregnancy continued to term.

Eight women underwent prenatal diagnosis at weeks 17–23 of gestation (median, 20 weeks), and 11 diagnostic procedures were performed (3 women underwent 2 procedures 5–6 weeks apart). The results of testing for congenital HCMV infection were negative for 7 fetuses and positive for 1 fetus. The latter pregnancy was terminated at 23 weeks of gestation, and generalized HCMV infection was diagnosed at fetal autopsy.

Nine of the 12 newborns examined at birth were free of HCMV infection (including the 7 who were examined during fetal life), and 3 newborns were congenitally infected (table 1). All infected newborns also tested positive for HCMV-specific IgM. One of these newborns presented with high-frequency fever, asthenia, headache, and upper respiratory symptoms 8 weeks before her LMP.
action tremors, hyperkalemia, hypoglycemia, and developing neuromuscular deficits of the left arm at 7 months that were still present at age 15 months. The other 2 newborns were asymptomatic at birth. Thus, in the periconceptional group, overall intrauterine transmission occurred in 4 (30.8%) of 13 pregnancies (1 of which was terminated, and 3 of which continued to term) for which the virologic outcome was known.

Discussion

To our knowledge, the risk associated with primary HCMV infection that is acquired shortly before the LMP or at approximately the time of conception has not yet been defined. The issue is delicate, because it affects both counseling and decision-making. In addition, our finding that as many as 32 (17.6%) of 182 pregnant women examined acquired primary HCMV infection either before or at approximately the time of conception reveals that this is a common event.

For the purpose of this study, the timing of primary HCMV infection was the most crucial issue. Very strict inclusion criteria were adopted, therefore, and recalled clinical symptoms were used to indicate the time of onset of HCMV infection only when they were in keeping with serologic and virologic findings. The clinical features of spontaneous HCMV mononucleosis have been well known for a long time [11]. However, it is recognized that, in immunocompetent hosts, most primary HCMV infections go unnoticed [1, 2]. On the other hand, pregnant women seem to be an exception to this rule. In fact, in the presence of virologic findings suggestive of primary HCMV infection, careful questioning by experienced personnel can help patients to recall ≥1 symptoms in >60% of cases [2].

In this study, we observed 1 case (9.1%) of congenital infection in 11 pregnancies for which the virologic outcome was known that occurred in women with documented preconceptional primary HCMV infection. This newborn was subclinically infected at birth, although the results of prenatal diagnostic procedures done at 18 weeks of gestation were negative. Explanations for these discrepant results include the possibility that (1) the antenatal procedure was performed too early during pregnancy, (2) delayed transplacental transmission of the infection occurred, or (3) iatrogenic transmission occurred. The reliability of prenatal diagnosis results is greatest when procedures are done at ≥121 weeks of gestation [12, 13]. However, we have observed contrasting results [9, 10]. As for the second possibility, it has been suggested that allowing a 6- to 8-week interval between the onset of maternal infection and prenatal diagnostic procedures reduces the risk of false-negative results [12, 14]. However, this does not apply to the present case, in which ≥6 months elapsed between maternal infection and fetal sampling. Keeping in mind that ~20% of immunocompetent subjects who have documented primary HCMV infection still have positive results of testing for DNAemia 6 months after the onset of primary infection [6, 15], a waiting period of at least 6 months before conception could be reasonably recom-
Table 1. Outcomes of 32 pregnancies complicated by preconceptional or periconceptional primary human cytomegalovirus (HCMV) infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preconceptional</th>
<th>Periconceptional</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>33 (22–35)</td>
<td>30 (22–37)</td>
</tr>
<tr>
<td>Outcome of pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of women</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Weeks of gestation, median</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Voluntary abortion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of women</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Weeks of gestation, median</td>
<td>11 (NA)</td>
<td>&lt;12 (&lt;12–23)</td>
</tr>
<tr>
<td>Delivery at term</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Prenatal diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of infected fetuses/no. examined</td>
<td>0/2</td>
<td>1/8</td>
</tr>
<tr>
<td>No. of diagnostic procedures done</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Weeks of gestation at which procedures were done, median (range)</td>
<td>18 (15–22)</td>
<td>20 (15–23)</td>
</tr>
<tr>
<td>Overall prevalence of congenital infection, no. of infected fetuses and newborns/no. examined (%)</td>
<td>1/11 (9.1)</td>
<td>4/13 (30.7)</td>
</tr>
</tbody>
</table>

Note: NA, not applicable.

* Five pregnancies were terminated at <12 weeks of gestation, and 1 was terminated at 23 weeks of gestation, after prenatal diagnosis of congenital infection was made.

* At birth, 3 of 12 newborns were found to be congenitally infected; 1 fetus was aborted at 23 weeks of gestation, after prenatal diagnosis of congenital infection.

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References