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NOTES

Anticytomegalovirus (Anti-CMV) Immunoglobulin G Avidity in Identification of Pregnant Women at Risk of Transmitting Congenital CMV Infection

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In this work, we show that the determination of the anticytomegalovirus antibody avidity carried out before week 18 of gestation is a helpful tool to identify women for enrollment in prenatal diagnosis. This procedure can identify all pregnant women who will give birth to an infected newborn.

Congenital cytomegalovirus (CMV) infection is the leading cause of congenital viral infection in developed countries, occurring in approximately 1% of all live births (1, 3, 4, 9, 18). Transmission of CMV to the fetus follows approximately 40% of primary maternal infections, whereas approximately 0.5% of women who are seropositive before pregnancy deliver infected infants (17). In addition, symptomatic infections and debilitating sequelae are rare in congenitally infected children born to women with preexisting immunity to CMV (5).

Diagnosis of primary CMV infection in immunocompetent adults is accomplished by serological methods (3). CMV-specific immunoglobulin M (IgM) is a sensitive indicator of an ongoing or recent infection. However, it is not a specific indicator of primary infection, as it is often produced during nonprimary infections (2, 10, 15). Another serological procedure useful in identifying primary infections is the determination of IgG avidity (6, 8, 11, 13).

In this work, we determined the avidity index (AI) of anti-CMV antibody in 76 pregnant women at risk of transmitting CMV to their offspring as well as in 20 pregnant women at no risk of transmitting CMV. All the women went through prenatal diagnoses, and pregnancy outcomes were monitored.

Between January 1994 and May 1998, 76 pregnant women were enrolled in the prenatal diagnosis program for CMV infection either because of a seroconversion for CMV during the first trimester of pregnancy ($n = 15$) or because of the presence of CMV-specific IgM ($n = 61$).

Twenty pregnant women at no risk of fetal CMV transmission (as determined by a negative IgM test) but who had amniotic fluid (AF) and fetal blood taken for fetal karyotype assessment constituted a control group. Twenty milliliters of AF was collected by amniocentesis at 21 to 23 weeks' gestation with informed consent from all women. CMV DNA was individually extracted from 3 to 6 aliquots of AF (100 μl each), and PCR was carried out as described in detail previously (12). AF was considered positive if at least one of the aliquots was positive.

Congenital CMV infection in a newborn was determined by CMV isolation (7) from urine or saliva during the first week of life.

The determination of IgG avidity was carried out using a commercial kit (Cytomegalovirus IgG avidity EIA WELL; RADIM, Rome, Italy).

As shown in Table 1, we obtained blood samples from 76 pregnant women during the early phase of pregnancy (6 to 18 weeks' gestation; mean, 13.6 weeks). Anti-CMV antibody avidity was determined, and in only six cases it was not possible because of an IgG level that was too low. Of these women, one with congenital hypogammaglobulinemia who was found to be PCR positive in AF testing decided to interrupt her pregnancy. No sign of CMV infection was found in the fetus. The other five women continued their pregnancies, and no congenitally infected newborn was documented. In the remaining 70 samples, the AI was determined, and the women were classified into one of the following three groups on the basis of the result obtained: low AI (38 cases), moderate AI (6 cases), and high AI (26 cases). Of the 38 women who showed a low avidity, 17 had the viral genome in the AF (45%) as detected by PCR. Thirteen women (seven who seroconverted and six who were IgM positive) continued their pregnancies, and the virus was isolated from six newborns (four asymptomatic and two symptomatic). Four women (one who seroconverted and three who were IgM positive) decided to interrupt their pregnancies, and all four fetuses showed the presence of CMV in multiple organs (data not shown). A detailed analysis of the results obtained on prenatal diagnoses has been recently published (12).

Among the six women with moderate avidity, two had the viral genome in the AF (33%). All of them decided to continue their pregnancies, and no congenital infection was documented in their newborns. Finally, of 26 women with high AI, 3 had the viral genome in the AF (11%). All of them continued their pregnancies and no congenital infection was documented in their newborns.

Later during pregnancy (at the time of amniocentesis, i.e., after 21 to 23 weeks' gestation), another blood sample was obtained from the same 76 pregnant women, and the AI was

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TABLE 1. Avidity index of anti-CMV IgG at two different gestation times in relation to prenatal diagnosis carried out by PCR in amniotic fluid and pregnancy outcomes in pregnant women at risk of transmitting a CMV infection and in a control population

Classification of AI of:	*n*	No. of PCR-positive prenatal diagnoses of amniotic fluid	No. of infected fetuses or newborns	SNS (%)[a]	SPE (%)[b]	PPV (%)[c]	NPV (%)[d]
Women at risk							
After 6–18 weeks of gestation				100	57.5	26.3	100
Low	38	17	10				
Moderate	6	2	0				
High	26	3	0				
Not determinable[e]	6	1	0				
At time of amniocentesis (21–23 weeks)				60	60.6	18.8	90.9
Low	32	12	6				
Moderate	6	3	1				
High	32	7	3				
Not determinable[e]	6	1	0				
Control population							
High (IgM negative)	20	0	0				

[a] SNS, sensitivity (true positives/total infected fetuses or newborns × 100).
[b] SPE, specificity (true negatives/total uninfected fetuses or newborns × 100).
[c] PPV, positive predictive value (true positives/true positives + false positives × 100).
[d] NPV, negative predictive value (true negatives/true negatives + false negatives × 100).
[e] Not determinable because of anti-CMV IgG levels too low to determine AI.

redetermined. As shown in the table, the six samples with IgG levels too low to allow the determination of AI at the first testing were again found to have low anti-CMV IgG levels at the time of amniocentesis, and the AI could not be determined. All six women had CMV-specific IgM, and one was excreting the virus in urine. One of them suffered from congenital hypogammaglobulinemia, and in four cases the presence of CMV-specific IgM was not confirmed by blot, suggesting a false IgM positivity at the first testing. No explanation for the observed delay in IgG development for the other IgM-positive woman was found.

Of the 38 women who had anti-CMV antibody with low avidity at the first testing, 32 (84%) still had a low AI at the time of amniocentesis. The other six had reached either a moderate or a high avidity. Among the 32 women who still had a low AI at the time of amniocentesis, 6 (19%) had a congenitally infected fetus or newborn. Among the 6 women with a moderate avidity and the 32 with a high avidity at the time of amniocentesis, one and three congenitally infected fetuses or newborns, respectively, were documented.

We also determined the anti-CMV IgG avidity of 20 pregnant women who had amniocentesis carried out due to genetic problems. The AI was higher than 60% for all of them (data not shown). PCR carried out on their AFs gave negative results, and no congenitally infected newborns were found. From the data presented, we calculated the sensitivity and specificity and the positive and negative predictive values of avidity determination with regard to the outcome of pregnancy (Table 1).

The main finding of this article is that if the determination of the anti-CMV antibody avidity is carried out early enough (before 18 weeks), it can identify all pregnant women who will transmit congenital CMV infection to their offspring. Furthermore, if the determination of AI is carried out later on during pregnancy, 60% of the women who will transmit the infection still have antibody with low avidity, while the others have developed a moderate or high avidity.

Interestingly, PCR detected viral DNA in the AF of 17 of 76 women, and in only 10 of the samples did we document a congenital infection in fetuses or newborns. This is probably due to the high sensitivity of the procedure, which detects a viral load so low as to be cleared by the fetal defenses, and is consistent with other published data (14, 16). Quantitative PCR is in progress to verify this hypothesis.

In conclusion, the early determination of anti-CMV antibody avidity is a helpful tool to identify a subgroup of IgM-positive women to enroll in prenatal diagnosis.

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