Evaluation of a Cytomegalovirus Glycoprotein B Recombinant Enzyme Immunoassay To Discriminate between a Recent and a Past Infection

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RESULTS

Human cytomegalovirus (CMV) is the most common cause of viral intrauterine infection. Symptomatic infection and fetal damage are mostly due to maternal primary infections. It is therefore important to differentiate primary from recurrent or persistent CMV infection in pregnant females. Serological diagnosis is easy in cases of seroconversion, but the discovery of immunoglobulin M (IgM) antibodies in the first serum sample obtained during pregnancy does not allow the diagnosis of a recent CMV primary infection. CMV-specific IgM antibodies can persist for months after primary infection (7) or reappear during recurrences (13). In some cases, specific IgM may also be due to a heterotypical immune response caused by an intercurrent infection (11, 12). No “gold standard” assay and no reference test exist for the detection of primary CMV infection.

The aim of this study was to compare the performance of the commercially available Vidas IgG avidity assay (bioMérieux, Marcy l’Etoile, France) with an EIA based on the use of a recombinant CMV glycoprotein B (gB) (Biotest). The combination of these two methods based on different approaches appears to be a simple system that can be used to improve serological diagnosis and to avoid unnecessary amniocentesis.

Fetal damage following cytomegalovirus (CMV) intrauterine infection is mostly linked to primary infection. To differentiate primary infection from nonprimary infection, immunoglobulin M (IgM) tests are not reliable enough, and measurement of the IgG avidity appears to be the method that is the most widely used at present. In the present study the performance of the Vidas (bioMérieux) avidity assay was compared with that of a new enzyme immunoassay based on the use of a recombinant CMV glycoprotein B protein (Biotest).

MATERIALS AND METHODS

CMV-specific IgG and IgM serology. All serum specimens were tested for CMV-specific IgG and IgM by microparticle ELISAs (Assym: Abbott Diagnostics, Wiesbaden-Delkenheim, Germany). For the IgM test, the procedure and the interpretation were as recommended by the manufacturer. The result was negative when the index value was ≤0.399, equivocal when the index value was >0.400 and ≤0.499, and positive when the index value was ≥0.500. For the IgG test we added a grey area to the manufacturer’s recommendations. The result, quantified in arbitrary units (AU), was negative when it was <15 AU, equivocal when it was ≥15 and <30 AU, and positive when it was ≥30 AU.

CMV-specific IgG avidity. The IgG avidity was measured by the Vidas method (bioMérieux), an automated enzyme-linked fluorescent immunoassay that enables the quantitative measurement of CMV-specific IgG. For the determination of avidity, two Vidas CMV-specific IgG tests are used. One test serves as a reference test. In the second test, the wash buffer is replaced by a buffer containing 6 M urea. The avidity index (AI) is determined by calculating the ratio of the relative fluorescence values obtained with the reference test and with the 6 M urea test. The procedure and the interpretation were as recommended by the manufacturer. The recommended diagnostic threshold (DT) for exclusion of a recent infection (within the past 3 months) was an avidity index (AI) ≥80%. A comparative study of the performances of the Vidas method and an in-house denaturation assay was described previously (1).

Patients. Sera were classified into four groups according to the serology of the patients. Group 1 comprised 80 pregnant women (80 serum specimens) not infected with CMV. The criteria were the absence of CMV-specific IgG and IgM. Group 2 comprised 56 pregnant women (93 serum specimens) with a documented recent CMV seroconversion. The criteria were the appearance of CMV-specific IgG together with an IgM response in a previously seronegative patient. Group 3 comprised 80 pregnant women (80 serum specimens) classified as having a past CMV infection. The criteria were the absence of CMV-specific IgM together with the presence of CMV-specific IgG. For 17 of these women, previous serology performed in our laboratory indicated that they had been infected with CMV for more than 1 year.

Group 4 comprised 50 women (50 serum specimens) with CMV-specific IgG and a positive or equivocal IgM result during pregnancy but without a documented seroconversion.

Details of the tests and the calculation of the results are described in Materials and Methods. A cutoff value of 0.250 for the Vidas IgG avidity assay was recommended by the manufacturer. The recommended diagnostic threshold (DT) for exclusion of a recent CMV infection (within the past 3 months) was an avidity index (AI) ≥80%.

RESULTS

Patients. Sera were classified into four groups according to the serology of the patients. Group 1 comprised 80 pregnant women (80 serum specimens) not infected with CMV. The criteria were the absence of CMV-specific IgG and IgM. Group 2 comprised 56 pregnant women (93 serum specimens) with a documented recent CMV seroconversion. The criteria were the appearance of CMV-specific IgG together with an IgM response in a previously seronegative patient. Group 3 comprised 80 pregnant women (80 serum specimens) classified as having a past CMV infection. The criteria were the absence of CMV-specific IgM together with the presence of CMV-specific IgG. For 17 of these women, previous serology performed in our laboratory indicated that they had been infected with CMV for more than 1 year.

Group 4 comprised 50 women (50 serum specimens) with CMV-specific IgG and a positive or equivocal IgM result during pregnancy but without a documented seroconversion.

The CMV-specific gB indirect enzyme immunoassay (gB-EIA) is based on the determination of the IgG antibody response to the CMV glycoprotein B (gB) protein (gB-EIA; Biotest). The combination of these two methods based on different approaches appears to be a simple system that can be used to improve serological diagnosis and to avoid unnecessary amniocentesis.
gB-EIA. The range of optical densities (ODs) observed in this group was 0.032 to 0.166, with a mean value of 0.054.

Results for group 2 patients. Group 2 comprised 56 pregnant women (93 serum specimens) with recent CMV seroconversion. When only the result for the first CMV IgG-positive serum specimen available from each woman is taken into account, according to the manufacturer’s recommendations, 50 patients were negative by the gB-EIA (OD range, 0.044 to 0.287; mean and median ODs, 0.078 and 0.066, respectively). Six women were positive (OD values, 0.311, 0.314, 0.375, 0.616, 0.619, and 0.620, respectively).

The results obtained for the 93 serum specimens by the gB-EIA are shown in Fig. 1. The distribution of the OD values according to the delay in time between the time of testing of the sample and the time that the last sample was CMV IgG and IgM negative is shown (solid line). The dashed line is the DT for exclusion of a recent infection (within the past 3 months) by the CMV gB-EIA.

![FIG. 1. Results of the CMV gB-EIA for group 2 patients (CMV seroconversion; 93 serum specimens from 56 pregnant women). The distribution of the OD values according to the delay in time between the time of testing of the sample and the time that the last sample was CMV IgG and IgM negative is shown (solid line). The dashed line is the DT for exclusion of a recent infection (within the past 3 months) by the CMV gB-EIA.](image1)

![FIG. 2. Correlation of the results obtained by the CMV gB-EIA and the Vidas avidity assay for group 2 patients (CMV seroconversion; 49 serum specimens from 49 pregnant women). The dashed line indicates the DT for exclusion of a recent infection (within the past 3 months) by the CMV gB-EIA. The solid line indicates the DT for exclusion of a recent infection by the Vidas assay.](image2)
gB-EIA according to the delay in time after the last IgG- and IgM-negative sample are summarized in Fig. 1. It appears that to exclude a CMV infection within the past 3 months the DT to be used is an OD/H11350 = 1.000.

The correlation between the Vidas assay and the gB-EIA for the 49 serum specimens tested by both methods is shown in Fig. 2. The capacity of detection of a recent infection was 100% by both the Vidas assay (DT, AI/H11350 = 80%) and the gB-EIA (DT, OD/H11350 = 1.000).

Results for group 3 patients. Among the 80 women included in group 3 (past CMV infection), according to the manufacturer's recommendation, 64 were positive by the gB-EIA (OD range, 0.335 to 3.523; mean and median ODs, 2.076 and 2.393, respectively). Sixteen women were negative by the gB-EIA (OD range, 0.057 to 0.248; mean and median ODs, 0.110 and 0.081, respectively). However, only 53 women presented an OD/H11350 = 1.000, the DT to be used to exclude a recent infection. The IgG AI was measured for all these patients. It was high (AI/H11350 ≥ 80%) for 67 of them.

The correlation between the Vidas assay and the gB-EIA for these 80 serum specimens is shown in Fig. 3. By use of a DT of an OD/H11350 ≥ 1.000 for the gB-EIA, the gB-EIA was able to exclude a recent infection in 21 women (42%). By use of the recommended DT for the Vidas assay (AI/H11350 ≥ 80%), the Vidas assay was able to exclude a recent infection in 14 women (28%). A recent infection could be excluded in 23 women (46%) if both criteria were combined: AI/H11350 ≥ 80% (Vidas) and OD/H11350 ≥ 1.000 (gB-EIA).

DISCUSSION

After primary infection with CMV an immediate seroconversion to positivity for antibodies directed against phosphoproteins and nonstructural proteins is found. In contrast, for IgG seroconversion to positivity for antiglycoprotein antibodies, a clear delay of 50 to 120 days is observed (15). On the basis of this observation, an EIA based on the use of the recombinant CMV gB protein was proposed as an additional marker for differentiation between primary and nonprimary CMV infection. The aim of this study was to compare the performance of this new EIA with that of the commercially available Vidas IgG avidity assay.

As no gold standard assay and no reference test exist for CMV serology, the only way to evaluate a serological assay correctly was to select groups of patients with unambiguous serologies.
The results obtained for patients with documented seroconversion to positivity for antibodies to CMV suggest that an OD \( \geq 1.000 \) is the DT to be used in the gB-EIA to exclude seroconversion within the past 3 months (capacity of detection, 100%). The capacity of detection was also 100% for the Vidas avidity assay when the recommended DT of an AI \( \geq 80\% \) was used.

The prevalence of anti-gB antibodies was 80% in patients with past CMV infection when the manufacturer’s recommendations were used. However, as a DT of an OD \( \geq 1.000 \) must be used to exclude a recent infection, the capacity of the gB-EIA to exclude a recent infection was 66% (53 of 80 women had ODs \( \geq 1.000 \)). The capacity of the Vidas assay to exclude a recent infection was 84%. More interestingly, it appears that use of the combination of the criterion for the IgG avidity assay and the criterion for the gB-EIA could improve the capacity to exclude a recent infection. Indeed, among the 80 women with past infections, 75 presented at least one of the required criteria (OD \( \geq 1.000 \) or AI \( \geq 80\% \)). The capacity to exclude a recent infection was 94% when these two assays, based on different approaches, were combined.

The practical use of the IgG avidity assay and the gB-EIA in combination was evaluated with a group of 50 women who were CMV IgM positive but who had no documented seroconversion. Among these patients, the gB-EIA was able to exclude a recent infection in 21 women (42%) and the Vidas assay was able to exclude a recent infection in 14 women (28%). By combining both criteria (OD and AI), a recent infection could be excluded in 23 patients (46%).

As congenital infection during primary maternal infection results in significantly more sequelae than recurrent CMV infection (16), it is important to distinguish between primary and nonprimary CMV infections. The use of IgM seropositivity to determine which patients should be enrolled for invasive diagnostic procedures may cause an unacceptable number of unnecessary hazardous procedures to be performed (3).

The IgG avidity and the presence of anti-gB antibodies appear to be complementary parameters. Indeed, a low level of maturation of IgG avidity may be observed in some patients or the patient may remain negative for anti-gB antibodies for a long period. Finally, the gB-EIA could be useful for patients with low anti-CMV IgG titers, in whom IgG avidity cannot be measured. Use of both assays will provide a simple system to improve the serological diagnosis and avoid unnecessary amniocentesis. However, larger studies are needed in order to determine precisely the exclusion and detection abilities of the gB-EIA and to evaluate the benefit of the use of a combination of two such assays.

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REFERENCES


