MAJOR ARTICLE

Prediction of Fetal Infection in Cases With Cytomegalovirus Immunoglobulin M in the First Trimester of Pregnancy: A Retrospective Cohort

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Background. Interpretation of positive cytomegalovirus (CMV) immunoglobulin M (IgM) in the first trimester of pregnancy is ill-defined. We aimed to quantify the risk of fetal transmission in women with positive CMV IgM in the first trimester.

Methods. A retrospective cohort of women (2009–2011) was tested for CMV immunoglobulin G (IgG) and IgM before 14 weeks of gestation. IgG avidity was tested with 2 assays (LIAISON and VIDAS). CMV polymerase chain reaction (PCR) was done in maternal serum, amniotic fluid, or neonatal urine at birth.

Results. A total of 4931 consecutive women were screened; 201 presented with positive or equivocal IgM and with high, intermediate, or low IgG avidity in 58.7%, 18.9%, and 22.3%, respectively. In 72 women with low or intermediate avidity, fetal transmission was 23.6%. In multivariate analysis, positive CMV PCR in maternal serum, decreasing avidity index with both LIAISON and VIDAS, and low IgG titers were all associated with fetal transmission (odds ratio [OR], 12.38 [95% confidence interval {CI}, 1.77–86.33], $P = .011$; OR, 0.16 [95% CI, .03–.95], $P = .044$; OR, 0.54 [95% CI, .11–.88], $P = .028$; and OR, 0.27 [95% CI, .29–.84], $P = .010$, respectively).

Conclusion. This study demonstrates a significant association between the risk of vertical transmission and the avidity index combined with CMV PCR in maternal serum or IgG titers. This allows calculation of incremental risk of fetal transmission upon which informed choice can be based and could lead to a better pickup rate of fetal infection while decreasing unnecessary invasive procedures.

Keywords. cytomegalovirus; CMV IgG avidity; fetal transmission; CMV IgM.

Cytomegalovirus (CMV) infection occurs in 0.7% of live births [1] with 15%–20% of infected children developing long-term disability including hearing loss and cognitive deficit [2]. The most severe cases of congenital CMV infection are the consequence of primary CMV infection during pregnancy [3, 4]. Accurate identification of primary infection in pregnancy and accurate evaluation of the risk of fetal transmission is important for antenatal management and could also allow for the possibility of prenatal treatment [5, 6].

In our center, information on congenital CMV infection and on the risk of primary infection in pregnancy as well as its prevention is routinely explained at registration in order to follow national recommendations [7]. As a result, women electing to know their serological status are offered immunoglobulin M (IgM) and immunoglobulin G (IgG) assessment. In cases with positive IgM and IgG, the CMV IgG avidity

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index is used to discriminate between primary infection during or prior to pregnancy based on the correlation between increased binding avidity of maternal IgG to CMV antigens over time [8–12]. When CMV IgG avidity is low or moderate, the women are classified as having a high suspicion of primary infection and are informed that there is a risk of fetal transmission of 30%–40% [2]. Amniocentesis after 20–21 weeks of gestation and/or fetal ultrasound monitoring are usually recommended in these cases [13, 14]. However, this may lead to patients undergoing unnecessary invasive procedures or even to a decision of termination of pregnancy on the sole basis of a high risk of fetal infection.

We built a retrospective cohort of women who had CMV serology testing in the first trimester of pregnancy in our institution. The main objective of this retrospective study was to identify virological markers that could help to accurately predict the risk of fetal infection in order to better counsel women with primary infection either during or prior to the first trimester of pregnancy and to help them to decide on the need for an invasive procedure.

MATERIALS AND METHODS

Population

The primary exposure variable to enter this retrospective cohort was to have been tested for CMV IgG and IgM at registration in the first trimester of pregnancy between September 2009 and December 2011. We identified the cases through the virology laboratory database. For women presenting with positive or equivocal IgM, the obstetrical file was reviewed to get information on the outcome of pregnancy (antenatal ultrasound data, CMV testing, clinical and imaging evaluation of the baby at birth).

According to French laws, an ethics statement from an institutional review board was not required for this work. However, all women gave written consent for the result of their screening tests to be used anonymously for research purposes. Moreover, all women who had amniocentesis for CMV prenatal diagnosis gave a written consent.

The standardized protocol used for CMV infection screening in our institution is described in Figure 1. One of the specificities of this algorithm is the use of 2 IgG avidity assays in parallel as well as that of CMV polymerase chain reaction (PCR) in maternal serum.

Methods

All laboratory tests were done prospectively but data were analyzed retrospectively.

CMV serology was performed using LIAISON CMV IgG and LIAISON CMV IgM. When IgG was positive (>0.6 IU/mL) and IgM was equivocal (≥15 arbitrary units [AU]/mL and <30 AU/mL) or positive (≥30 AU/mL), CMV IgG avidity was tested. The LIAISON CMV IgG Avidity assay and the VIDAS CMV IgG Avidity assay were used in parallel when enough serum was available and the results were interpreted as recommended by the manufacturer. According to the manufacturer, a LIAISON DiaSorin avidity index <0.200 indicates a primary infection within the last 3 months, an index >0.300 excludes a primary infection in the last 3 months, and an index ranging from 0.200 to 0.300 is considered as intermediate. Similarly, a VIDAS CMV IgG avidity index <0.20 indicates a primary infection in the last 3 months, an index >0.80 excludes a primary infection in the last 3 months, and an index ranging from 0.20 to 0.80 is considered as intermediate. When at least 1 of the 2 avidity tests results was in favor of a primary infection dating more than 3 months back, the woman was reassured; in other cases CMV PCR was performed in maternal serum and amniocentesis for prenatal diagnosis was advised to be performed after 20 weeks of gestation for CMV PCR.

CMV PCR in amniotic fluid and in maternal serum was performed after automated DNA extraction with MagNaPure LC using the total nucleic acid extraction kit (Roche Diagnostic, Meylan, France) followed by amplification using an in-house CMV PCR assay [15, 16]. Follow-up of infected fetuses consisted of targeted ultrasound examination every fortnight and fetal brain magnetic resonance imaging (MRI) was offered at 33 weeks. Termination of pregnancy was offered in cases with cerebral anomalies [17]. Infected babies were followed up with serial perinatal and pediatric examination and auditory brainstem response, as well as ultrasound and MRI of the neonatal brain.

Statistical Methods

Qualitative data were compared by means of χ^2 test or Fisher exact test as appropriate. Student t test and Mann-Whitney U test were used for parametric and nonparametric variables or unequal standard deviations, respectively. Concordance between the 2 avidity techniques was assessed based on the percentage of concordance and κ statistic. In case of possible associations, a logistic regression model was used to investigate the association between outcome (fetal infection at the time of amniocentesis or at birth) and each of the possible explanatory parameter or possible confounders (gestational age at testing, maternal IgM levels, maternal IgG levels, maternal positive CMV PCR, avidity index by LIAISON × 10, and avidity index by VIDAS × 10). Multivariate logistic regression models were performed including all variables with a $P < .2$ on univariate analysis. To avoid overfitting, we aimed at obtaining multivariate models with at least 1 explanatory variable per 8–10 events, as suggested by the study of Vittinghoff et al [18]. Only models with variables statistically significant were considered.

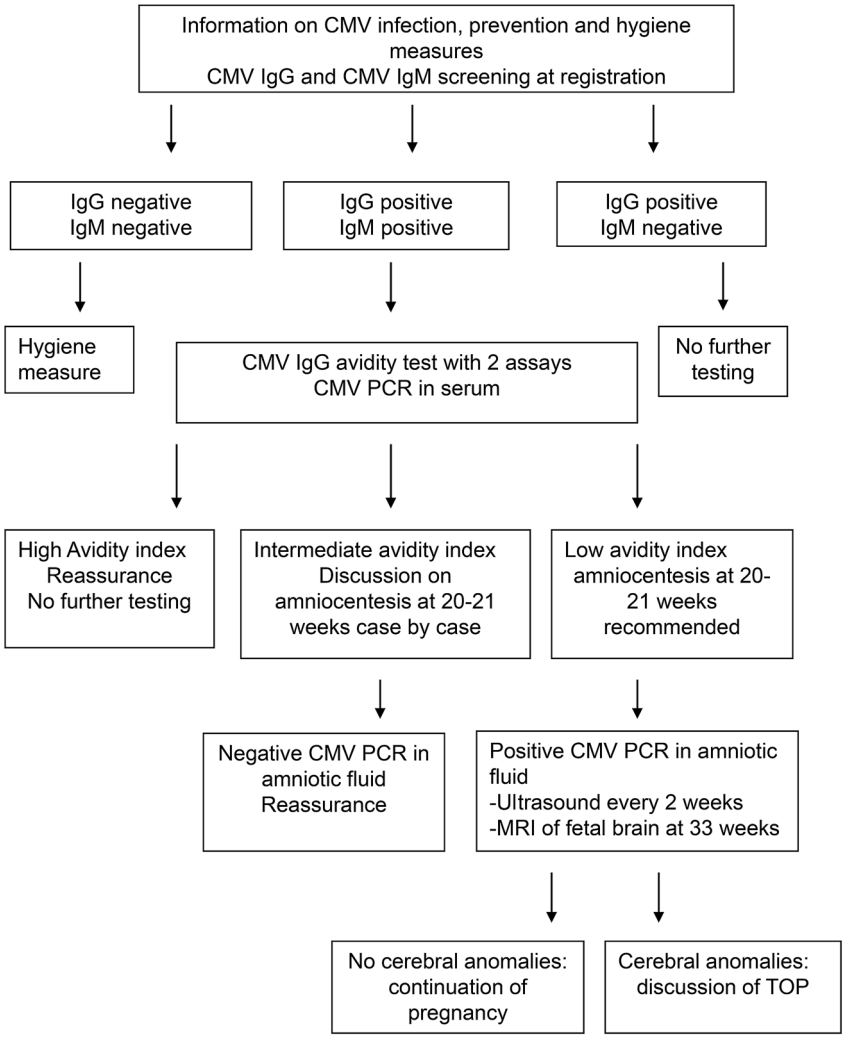

Figure 1. Standardized protocol for cytomegalovirus infection screening in first trimester of pregnancy in the Necker institution. Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; TOP, termination of pregnancy.

Because avidity index values are <1, for univariate and multivariate analysis we used avidity index values ×10 in order to obtain clinically meaningful odds ratios (ORs).

All tests were 2-tailed and a *P* value <.05 was considered statistically significant.

Because each missing piece of data was unrelated to its value or to the value of other variables, data were considered to be missing at random.

RESULTS

A total of 4931 pregnant women were screened for CMV infection during the study period and 201 (4.1%) had positive or equivocal CMV IgM with positive IgG prior to 14 weeks of gestation at a median of 11 weeks (range, 9–12 weeks) (Figure 2). Within this group, 183 women had an avidity test done with the 2 assays and 18 had only 1 of the 2 avidity assays (VIDAS, n = 11; LIAISON, n = 7).

One hundred eighteen of these 201 women (58.7%) had a high avidity index with at least 1 of the 2 assays and could be reassured immediately. However, 83 (41.3%) women had either a low or intermediate avidity test including 45 (22.3%) and 38 (18.9%) with a low or intermediate avidity index, respectively. CMV PCR was done in the maternal serum of 126 of the 201 including 40 of the 83 women with low or intermediate avidity index. The median CMV DNA load in maternal serum with positive CMV PCR was 800 copies/mL (range, 110–56 000 copies/mL) (2.90 log copies/mL [range, 2.04–4.74]).

Details on the 83 women with low or intermediate avidity index are shown in Figure 2, leaving 72 cases for analysis in

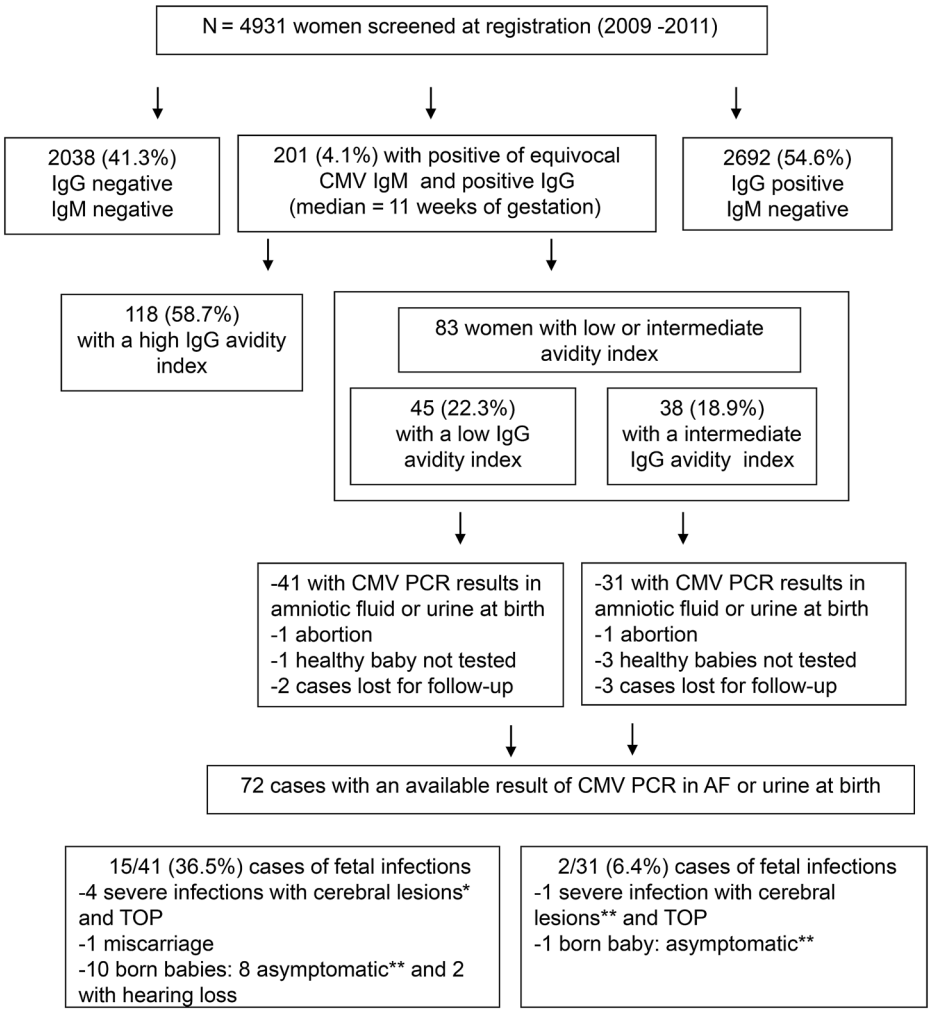

Figure 2. Result of serological cytomegalovirus screening in the retrospective cohort. *Cerebral anomalies in ultrasound, antenatal cerebral magnetic resonance imaging (MRI), and anatomopathology after termination. **Asymptomatic at birth: no clinical symptoms, no anomalies on neonatal MRI, and normal hearing test. Abbreviations: AF, amniotic fluid; CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; TOP, termination of pregnancy.

this group. Seventeen (23.6%) of these 72 cases with a CMV PCR results available tested positive for CMV PCR in amniotic fluid and/or in neonatal samples proving congenital infection. Of these 17 cases, 36.5% (15/41) and 6.4% (2/31) were in the group of women with a low or intermediate avidity result, respectively ($P = .002$). The outcome of congenital infections included termination of pregnancy for severe cerebral lesions in 6 cases, 9 asymptomatic newborns in 9 cases, and profound bilateral and unilateral hearing loss in 1 case each.

Factors Associated With Fetal Transmission in the Group of 72 Women With a Low or Intermediate Avidity Test in the First Trimester

Table 1 describes the variables studied in these 72 women. Maternal serum was obtained in the first trimester at a similar gestational age in both groups (transmitting vs not transmitting), IgM levels were higher, and IgG levels and IgG avidity values with both assays were lower in women who transmitted the virus. CMV PCR in maternal sera was more often positive in these women.

Results of univariate analysis with 6 variables are shown in Table 2. IgM levels and gestational age were the only 2 variables that were not associated with fetal transmission.

Given the number of events, we aimed to build up multivariate models with at least 2 explanatory variables. There were no significant models with 3 variables. Ten bivariate models were tested with the 5 variables showing a P value <.2 in univariate analysis. Only 3 bivariate models showed significant association of both variables with fetal transmission (Table 2).

Table 1. Virological Variables by Cytomegalovirus Fetal Transmission Status in the Population of 72 Women

Variable	Overall (N = 72)	CMV Fetal Infection (n = 17)	No Fetal Infection (n = 55)	*P* Value
Gestational age				
Median (IQR)	11.0 (9.0–12.0)	11.0 (9.0–12.0)	11.0 (9.0–12.0)	**.85**[a]
No.	72	17	55	
LIAISON IgM levels in maternal serum				
Median (IQR)	75.5 (57.5–90.0)	84.5 (75.0–105.0)	72.5 (50.0–88.0)	**.048**[a]
No.	48	12	36	
LIAISON IgG levels in maternal serum				
Median (IQR)	4.40 (2.6–7.3)	2.1 (1.1–3.2)	5.3 (3.9–7.7)	**<.001**[a]
No.	49	12	37	
LIAISON avidity index value				
Median (IQR)	0.164 (0.110–0.240)	0.107 (0.082–0.145)	0.182 (0.125–0.243)	**.001**[a]
No.	56	12	44	
VIDAS avidity index value				
Median (IQR)	0.330 (0.230–0.515)	0.190 (0.125–0.245)	0.445 (0.305–0.570)	**<.001**[a]
No.	64	16	48	
CMV PCR in maternal serum, No. (%)				
Positive	15/40 (37.5%)	8/10 (80%)	7/30 (23.3%)	**.0024**[b]
No.	58	12	46	

P values in boldface indicate significance.

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; PCR, polymerase chain reaction.

[a] Mann-Whitney *U* test.

[b] Fisher exact test.

Table 2. Factors Associated With Fetal Transmission in Univariate and Bivariate Analysis (Group of 72 Women With Low or Intermediate Avidity Index and Documentation of Fetal Infection)

Variable	β	SE	OR	95% CI	*P* Value	AIC	R^2
Univariate analysis							
Gestational age	0.01	0.11	1.01	.81–1.27	.900		
LIAISON IgM levels in maternal serum	0.01	0.09	1.01	.99–1.03	.155		
LIAISON IgG levels in maternal serum	0.64	0.22	0.53	.34–.81	***.004***		
LIAISON avidity index value ×10	–1.91	0.70	0.14	.04–.59	***.007***		
VIDAS avidity index value ×10	–1.47	0.42	0.23	.09–.53	***.001***		
Positive CMV PCR in maternal serum	2.57	0.90	13.14	2.24–76.80	***.004***		
Bivariate analysis							
LIAISON IgG levels in maternal serum	–0.69	0.27	0.49	.29–.84	***.010***		
LIAISON avidity index value ×10	–2.04	0.88	0.13	.02–.73	***.021***		
Constant	4.70	1.75	…	…	…	***26.8***	***0.53***
Positive CMV PCR in maternal serum	2.51	0.99	12.38	1.77–86.33	***.011***		
LIAISON avidity index value ×10	–1.83	0.91	0.16	.03–.95	***.044***		
Constant	0.37	1.38	…	…	…	***32.6***	***0.39***
Positive CMV PCR in maternal serum	2.15	1.03	8.59	1.13–65.10	***.037***		
VIDAS avidity index value × 10	–1.18	0.54	0.31	.11–.88	***.028***		
Constant	1.46	1.62	…	…	…	***31.0***	***0.43***

Values in boldface indicate significance.

Abbreviations: AIC, Akaike criterion; β, estimate; CI, confidence interval; CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; OR, odds ratio; PCR, polymerase chain reaction; SE, standard error.

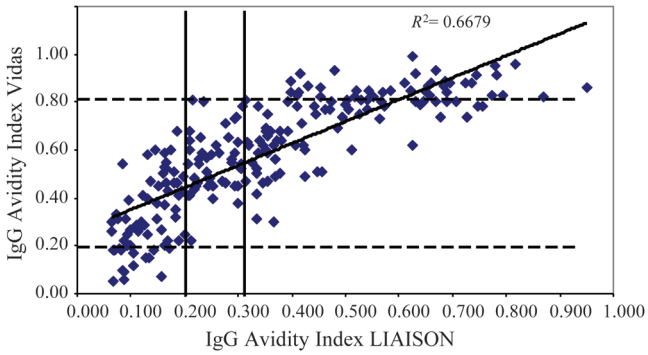

Figure 3. Comparison of avidity index values results obtained with the 2 avidity assays (LIAISON and VIDAS) in 236 serum samples. Abbreviation: IgG, immunoglobulin G.

Comparison of the 2 CMV IgG Avidity Assays

Avidity test was done in parallel with the 2 commercial avidity assays in 166 of the 201 women (236 sera some woman had sequential sera). Avidity index values were roughly correlated ($r^2 = 0.667$; Figure 3), but there was only a 49.5% concordance between avidity test interpretations (Table 3). The κ statistic was 0.306 (95% confidence interval [CI], .237–.375), demonstrating fair agreement.

Relationship Between IgM Levels and IgG Avidity Values in the Population of 236 Sera With Positive IgM and Avidity Results Available With the 2 Tests

Median IgM value was lower (50 AU/mL [range, 15–250 AU/mL]) in the group of 123 sera that had a high avidity index with at least 1 assay than in the group of 113 sera with intermediate or low avidity index (71 AU/mL [range, 22–250 AU/mL], $P < .0001$). There was an inverse statistical correlation between CMV IgM levels and CMV avidity index. For each 1-unit decrease of CMV IgM, there was a 1.1-fold increase of having a high LIAISON avidity index (OR, 0.97 [95% CI, .95–.98], $P = .001$) and a high VIDAS avidity (OR, 0.97 [95% CI, .94–.99]), $P = .027$). However, the IgM levels were overlapping in the 2 groups (high avidity and low or intermediate one), and no threshold value of IgM levels could be established to differentiate between the 2 groups with a good predictive value (area under the receiver operating characteristic curve, 0.75 and 0.72, respectively).

Table 3. Comparison of Immunoglobulin G Avidity Index Interpretation (Low, Intermediate, High) With the 2 Commercial Assays in 236 Sera From Women With Cytomegalovirus Immunoglobulin M Positive or Equivocal Results

	LIAISON DiaSorin		
IgG Avidity Index (N = 236)	Low	Intermediate	High
Vidas bioMérieux			
Low	22	0	0
Intermediate	49	42	68
High	0	2	53

κ = 0.306 (95% confidence interval, .237–.375); 49.5% of concordant avidity test interpretation; 50.5% of discordant avidity test interpretation.

Abbreviation: IgG, immunoglobulin G.

DISCUSSION

The incidence of positive IgM in pregnant women who enrolled before 12 weeks of gestation (201/4931 [4.1%]) was in agreement with previously published data [19, 20]. A large proportion (58.7%) could be immediately reassured by a high IgG avidity index as demonstrated elsewhere [21, 22]. However, the proportion with a suspicion of recent primary infection (41.3%) among women with positive IgM was higher than reported in the literature, ranging from 14% to 22% [20, 23]. This could reflect either a recruitment bias or a different performance of CMV IgM test used in these studies in terms of specificity or sensitivity. The rate of fetal transmission was 36.5% in the group of women with a suspicion of primary infection in the first trimester of pregnancy (women with low avidity index) and it was 6.4% in the group of women with a suspicion of periconceptional infection (women with intermediate avidity index). These transmission rates are similar to those described in the literature. [24, 25]. The inverse relationship between IgM antibody levels and IgG avidity index has also been previously underlined [23, 26]. However, using a algorithm based on IgM levels alone without avidity testing would be misleading as IgM levels are overlapping in the 2 groups of high and low/intermediate avidity, with extreme values found in both groups.

CMV IgG avidity tests are not well standardized and a recent comparative study reported only moderate agreements between kits when testing a panel of sequential sera collected in pregnant women with primary infection [27]. In our study, the interpretation of avidity test (low, intermediate, high) was concordant with the 2 assays only in 49% of the sera. This discrepancy is 2-fold. Fewer sera (44%) reached high avidity status with the VIDAS than with the LIAISON assay, suggesting that the cutoff value used in the former is too high. This has already been pointed out by other groups [28–30]. In addition, fewer sera (31%) displayed a low avidity with the VIDAS than with the LIAISON assay, as has been reported previously [27].

We have shown that there is a continuous increased risk of vertical transmission with decreasing avidity even within low or intermediate values. In addition, the risk of vertical transmission is further increased with either low IgG titers or a

positive PCR in maternal serum. There are few data available on the kinetics of CMV DNA in serum or plasma of primary infected women, but it is likely to be found within 1–2 months of infection [31]. Therefore, the added value of these associations is likely to reflect a better identification of true infections during pregnancy as opposed to periconceptional or anteconceptional infections with a very low risk of transmission [24, 25]. Another explanation for the predictive value of decreasing avidity index could be that intrauterine transmission could be facilitated when CMV IgG avidity takes longer to mature. Antibody affinity maturation has been shown to be critical for production of high levels of neutralizing antibodies, which are known to protect from fetal transmission [32], suggesting that a defect in affinity maturation might play a role in intrauterine transmission [32]. To test this hypothesis further, it would be interesting to study avidity maturation in sequential sera of pregnant women with primary infection in the first trimester of pregnancy.

Irrespective of the explanation for this association, this variation in the fetal transmission risk according to the index value and the presence or absence of a positive CMV PCR in maternal serum or high IgG titers can be used as a tool to calculate incremental risk of fetal infection.

Strengths of the study include the large sample of women tested and the standardized protocol used for CMV infection screening. Limitations of the study include missing virological data, as the serum sample was too small to achieve all analysis in some women, and clinical missing data as 13% (11/83) of cases with low or intermediate avidity index did not have CMV PCR in amniotic fluid or in urine at birth. However, data were missing at random. We are currently testing the 3 significant models prospectively; however, the number of cases seen prospectively since then is not sufficient to assess any significant change in choice or strategy.

CONCLUSIONS

In our hands, the LIAISON assay was the most effective to exclude a recent primary infection and to reassure up to 60% of women presenting with positive IgM in the first trimester of pregnancy. In the subgroup of women with a low or intermediate avidity, both IgG avidity index values (LIAISON and VIDAS) allow for an accurate prediction of the risk of transmission when used in combination with either the result of CMV PCR in maternal serum or the IgG titers. This strategy may further reduce both the use of invasive procedures and the proportion of women faced with the anxiety of a late diagnosis of fetal infection.

Notes

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Author contributions. M. L.-V., L. J. S., and Y. V. conceived and designed the experiments. Y. S., L. J. S., J. J. S., F. J., and Y. V. counseled the women in the obstetric and fetal medicine department. M. L.-V. and Y. S. coordinated the CMV serology testing and the CMV PCR tests in the virology laboratory. M. L.-V., L. J. S., J. J. S., F. J., and Y. V. were responsible for study design, data interpretation, and manuscript preparation. All authors participated in data interpretation and performed a critical revision of manuscript. L. J. S. was responsible for statistical analysis. All authors approved the final version of the paper.

Potential conflicts of interest. M. L.-V. has received travel grants and honoraria for speaking or participation at meetings or for reviewing scientific projects from DiaSorin and from bioMérieux. Y. V. has received consulting fees as a clinical advisor for Sequenom. All other authors report no potential conflicts.

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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