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Low-level positive results in the Liaison CMV IgG II assay may misclassify pregnant woman as immune

Daniel Hitz, Simone Exler, Anja Daiminger, Uwe Bartelt, Martin Enders *

Laboratory Prof. Gisela Enders and colleagues, MVZ, Stuttgart, Germany

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ABSTRACT

The aim of this study is to report on the specificity in the low-positive range of the Liaison CMV IgG II assay for determination of cytomegalovirus immune status in pregnancy. Sera with test results between 12.0 and 40.0 U/mL were retested with the Enzygnost Anti-CMV/IgG assay. Enzygnost-negative samples were analyzed by the Serion ELISA classic Cytomegalovirus IgG assay and, if positive or equivocal, also with the Mikrogen *recom*Line CMV IgG assay. A total of 12,117 sera were tested with the Liaison assay. Sixty sera were equivocal (12.0–13.9 U/mL), and 400 of 4295 positive sera were low-positive (14.0–40.0 U/mL). Based on consensus, at least 14% of the low-positives and 1.3% of all Liaison-positives can be considered as misclassified. The proportion of misclassified sera increased with lower Liaison IgG results. We suggest that the range for equivocal results in the Liaison assay should be revised.

1. Background

The most common infectious cause of neurodevelopmental delay and sensorineural hearing loss in early childhood is congenital cytomegalovirus (cCMV) infection (Britt, 2018). Primary and nonprimary infections may result in intrauterine viral transmission (Saldan et al., 2017). However, the risk for cCMV disease and permanent severe sequelae is highest following primary CMV infection around conception and during the first trimester (Enders et al., 2011; Faure-Bardon et al., 2018). A German guideline for diagnosis of pregnancy-associated viral infections currently recommends antibody testing in early pregnancy to identify CMV seronegative women, who have the highest risk for cCMV infection (Leruez-Ville et al., 2017; Modrow and Huzly, 2014; Mussi-Pinhata et al., 2018). To reduce the risk of maternal primary CMV infection, hygiene counseling should be offered to susceptible (CMV seronegative) pregnant women (Revello et al., 2015). To avoid miscounseling, CMV IgG assays that are used to screen pregnant women have to be highly specific. Unfortunately, available information on test specificity of commercial immunoassays is limited.

We recently changed our CMV IgG routine testing from the Enzygnost Anti-CMV/IgG test (Enzygnost) to the Liaison CMV IgG II test (Liaison). The verification of the Liaison assay showed that requirements for intra-assay precision, interassay precision, linearity, and accuracy were all fulfilled. However, a comparison of both assays, using sera in the negative to the low-positive range of the Enzygnost assay (OD < 0.6; $n = 277$), showed that 1% (2/192) of Enzygnost-negative (OD <0.1) and 31% (7/22) of Enzygnost-equivocal (OD 0.1–0.2) sera were positive in the Liaison (≥14.0 U/mL; range: 14–27 U/mL). Therefore, we decided to retest blood samples from pregnant women with equivocal (12.0–13.9 U/mL) and positive (14.0–40.0 U/mL) results in the Liaison test with additional IgG immunoassays. The aim of the study is to report our observations on the specificity on sera with a low-positive (≤40.0 U/mL) result in the Liaison assay.

2. Material and methods

Detection of CMV IgG antibodies was performed by Liaison CMV IgG II (DiaSorin, Sallugia, Italy), Enzygnost Anti-CMV/IgG (Siemens, Marburg, Germany), Serion ELISA classic/Cytomegalovirus IgG (Virion/Serion, Würzburg, Germany) (Serion), and *recom*Line CMV IgG (Mikrogen, Neuried, Germany) (Mikrogen). All EIAs and the Mikrogen line immunoassay were performed according to the manufacturers' instructions.

The study period extends to the first 4 months after we implemented the Liaison assay in routine diagnosis in April 2018. Serum samples from pregnant women were sent to our laboratory for determination of CMV immune status. Sera with test results between 12.0 and 40.0 U/mL in the Liaison were also tested with the Enzygnost assay. Discordant sera (Enzygnost-negative) were further analyzed with the Serion assay and, if positive or equivocal, investigated by the Mikrogen line immunoassay to further corroborate the test results. Sera with concordantly negative results in the Enzygnost and Serion tests were considered IgG negative. Sera with discordant results (Enzygnost-negative

* Corresponding author. Tel.: +49-711-6357-120; fax: +49-711-6357-202.
E-mail address: menders@labor-enders.de (M. Enders).

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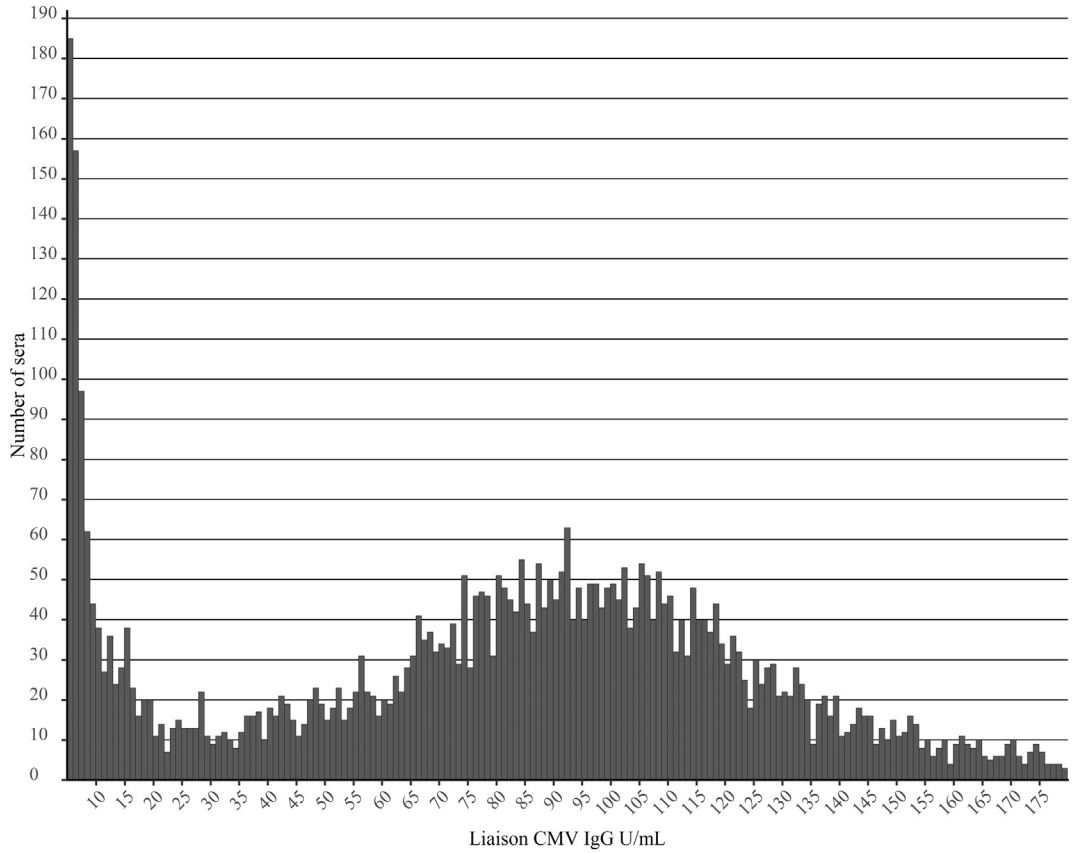

Fig. 1. Distribution of Liaison CMV IgG II results (n=4770). Number of sera for each value in the Liaison CMV IgG assay range. Sera with <5 U/mL (n=7152) and >180 U/mL (n=195) are not shown.

and Serion-equivocal or -positive) but a negative Mikrogen line immunoassay were also considered CMV IgG negative. Confidence intervals were calculated as exact Clopper–Pearson confidence intervals.

3. Results

In the first 4 months after test implementation, a total of 12,117 blood samples were analyzed by the Liaison assay with negative (<12.0 U/mL), equivocal (12.0–13.9 U/mL), or positive results (≥14.0 U/mL) in 64.1% (7762/12,117), 0.5% (60/12,117), and 35.4% (4295/12,117), respectively. For the whole study population, the distribution of IgG values in the assay range (5.0–180.0 U/mL) is shown in Fig. 1.

The 460 sera with Liaison results between 12.0 and 40.0 U/mL showed negative (OD < 0.1), equivocal (OD 0.1–0.2), and positive (OD >0.2) Enzygnost results in 28.5% (131/460), 12.0% (55/460), and 59.5% (274/460), respectively. Details are shown in Table 1.

A total of 107 of 131 (86.1%) Enzygnost-negative samples had enough leftover material to be retested with the Serion assay (Fig. 2) and showed negative (<25 PEIU/mL), equivocal (25–40 PEIU/mL), and positive (>40 PEIU/mL) results in 87.8% (94/107), 10.3% (11/107), and 1.9% (2/107), respectively, as shown in Table 2. A total of 56 of 68 (82.4%) Liaison low-positive samples were negative with the Serion and Enzygnost assay. Based on these results, at least 14% (56/400) of Liaison low-positives and 1.3% (56/4295) of all Liaison-positives can be considered as misclassified. The 13 Enzygnost-negative samples that were either positive (42 and 44 PEIU/mL) or equivocal in the Serion assay tested negative by the Mikrogen line immunoassay (Fig. 2). Therefore, we considered Enzygnost-negative results as true negatives. Fig. 3 shows the proportion of low-positive samples that are misclassified as positive in the Liaison assay. This proportion increased with lower Liaison IgG results.

4. Discussion

At least 1.3% (56/4295) of all Liaison-positive samples in the study period were tested negative in 2 additional IgG assays (Enzygnost and

Table 1
Comparison of the Liaison CMV IgG II and the Enzygnost Anti-CMV/ IgG results.

Liaison CMV IgG [U/mL]	Enzygnost CMV IgG [OD]			
	Positive (>0.2)	Equivocal (0.1 < = OD < = 0.2)	Negative (<0.1)	Total
12.0–13.9[a]	6	7	47	60
14.0-15.9	14	12	40	66
16.0–17.9	11	11	16	38
18.0–19.9	15	10	15	40
20.0–21.9	16	4	5	25
22.0–23.9	14	3	3	20
24.0–25.9	23	5	0	28
26.0–27.9	24	0	2	26
28.0–29.9	30	0	3	33
30.0–31.9	19	1	0	20
32.0–33.9	21	1	0	22
34.0–35.9	18	1	0	19
36.0–37.9	32	0	0	32
38.0–40.0	31	0	0	31
Total	274	55	131	460

[a] Equivocal range.

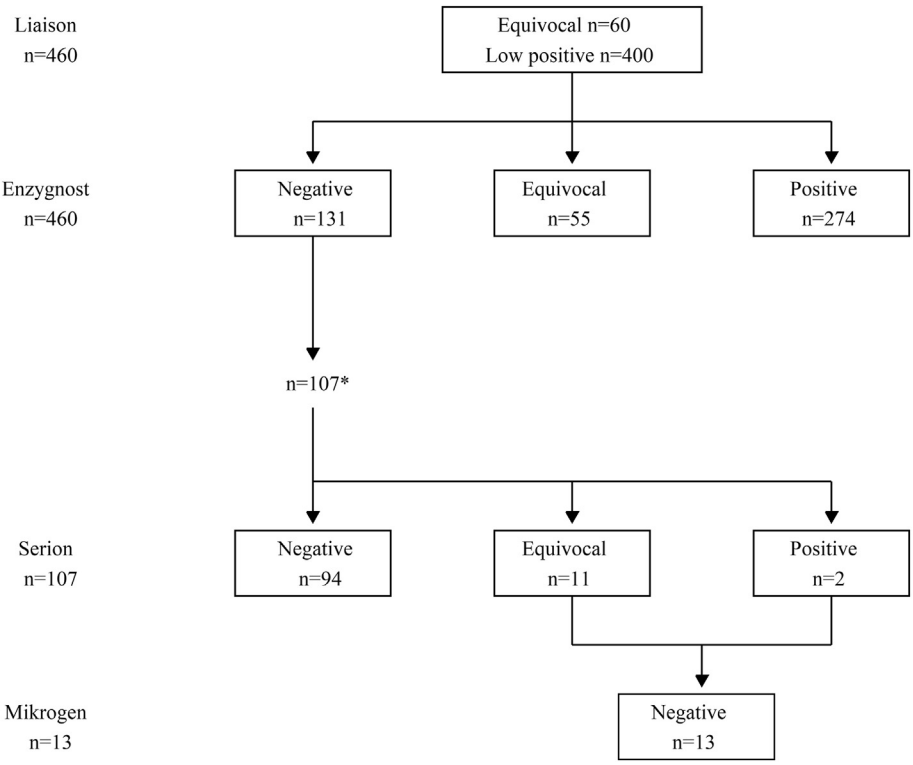

Fig. 2. Flow diagram of investigated sera. * In 107 of 131 sera enough leftover material was available for further testing.

Serion). Since there is no gold standard for CMV serology testing, we used agreement via consensus. Retesting of discordant samples (Enzygnost-negative, Liaison/Serion-positive) with a line immunoassay (Mikrogen) confirmed the negative Enzygnost results. Our findings are in agreement with the results of an Italian study in which 1.6% of the positive results in the Liaison assay were found to be negative in further testing (line immunoassay, EIA, NT test, and T-cell assay) (Furione et al., 2018). Half of the samples in their study with results lower than 30 U/mL were found to be negative at retest, and the probability of a misclassification got higher with lower IgG levels. This trend was also found in our study, where no sera were misclassified at Liaison levels >29.9 U/mL (Fig. 3). In our study, 30% (84/276) of the sera with low-positive results <30 U/mL and 49% (71/144) of the sera with low-positive results <20 U/mL were found to be negative at retest (Table 1). Furione et al. found 1 false-positive sample with a Liaison CMV IgG result of 41.2 U/mL (Furione et al., 2018), which was higher than our upper limit of 40.0 U/mL for retesting. Nevertheless, based on our results, we assume that the probability of a misclassification for Liaison results >40.0 U/mL is very low (Fig. 3). A limitation of our study is that only discordant samples in both confirmatory EIAs (Enzygnost, Serion) were retested with the line immunoassay. Furthermore, the study only investigates pregnant women, and it remains open if our observations also apply to other populations. In addition, no T-cell tests were performed to evaluate the cellular immune status in women with discordant serological results (Abate et al., 2013; Sester et al., 2003).

It appears that the cutoff values in the Liaison test were set to increase sensitivity and minimize the risk of false-negative results at the expense of specificity. This might be suitable for CMV screening of solid organ donors. In antenatal care, however, nonimmune women classified as CMV seropositive receive inadequate counseling on their individual risk of CMV infection for herself and the fetus and on preventive hygiene measures (Modrow and Huzly, 2014). The latter were shown to be effective to reduce the risk of primary CMV infection in nonimmune pregnant women (Revello et al., 2015). Moreover, pregnant women in high-risk occupations (e.g., day care) with false-positive IgG results are not restricted from work or sent on leave as required in some countries by legislation and guidelines on maternity protection (Joseph et al., 2006). If an obligatory screening is implemented in Germany, a false-positive rate of 1.3% would mean that at least 10,238 women (787,500 liveborn infants in 2018) are misclassified and would receive inadequate management (Statistisches Bundesamt, 2019).

Table 2
Comparison of Liaison CMV IgG II and Serion ELISA classic/Cytomegalovirus CMV IgG test results in Enzygnost CMV IgG negative sera ($n = 107$).

Liaison CMV IgG [U/mL]	Serion CMV IgG [PEIU/mL]			Total
	Positive (>40)	Equivocal (25–40)	Negative (<25)	
12.0–13.9[a]	0	1	38	39
14.0–40.0	2	10	56	68
Total	2	11	94	107

[a] Equivocal range.

5. Conclusion

On the basis of our results, we suggest that for determination of CMV immune status in pregnant woman, the range for equivocal results in the Liaison assay should be revised.

Declaration of interests

None.

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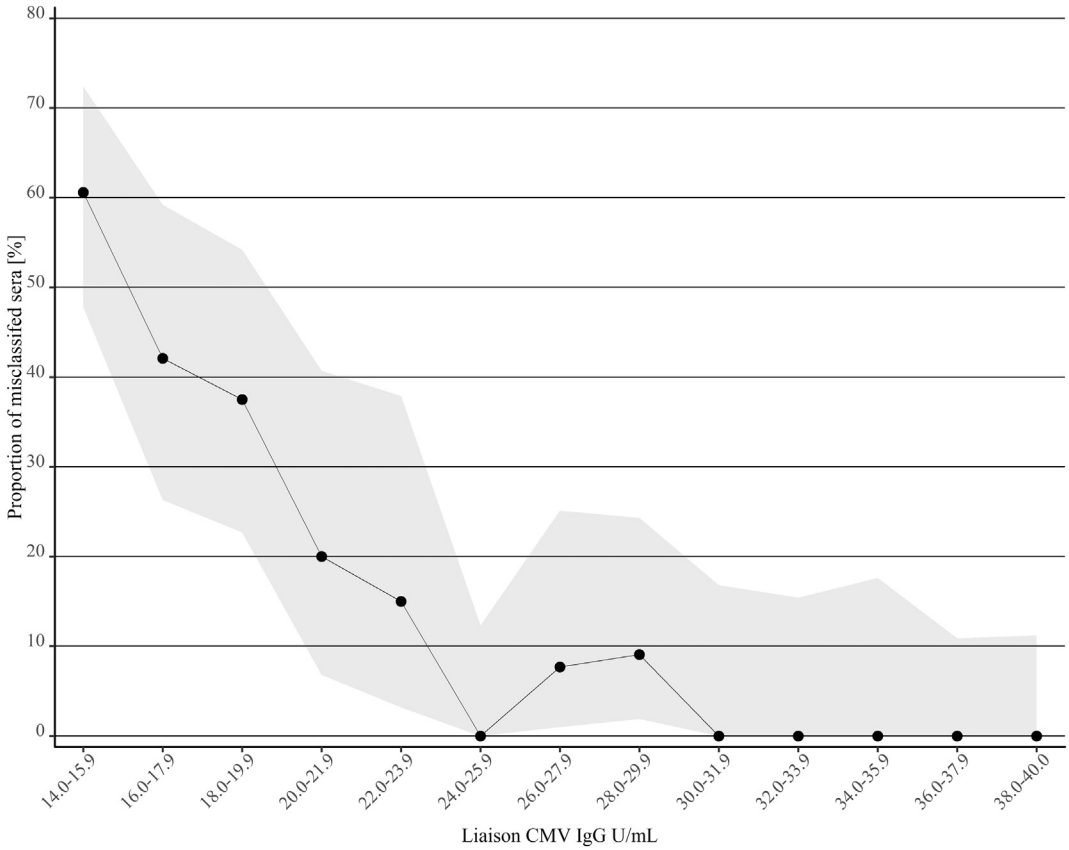

Fig. 3. Proportion of misclassified sera. Proportion of sera misclassified as positive by the Liaison assay (Enzygnost negative) at different IgG levels. Grey area indicates 95% confidence interval.

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