Research Square

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Immunoblot analysis to determine the time of a primary CMV infection in pregnancy

Alfred L Bissinger
Universitätsklinikum Tübingen
Lara Schmid
Universitätsklinikum Tübingen
Markus Hoopmann
Universitätsklinikum Tübingen
Martin Enders
Laboratory Prof. Dr. G. Enders and Colleagues, MVZ, Stuttgart, Germany
Karl Oliver Kagan
oliver.kagan@med.uni-tuebingen.de

Universitätsklinikum Tübingen

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Abstract

Aim

To evaluate the utility of immunoblot analysis for determining the timing of maternal CMV infection in pregnancy.

Methods

Retrospective study including 151 pregnant women referred for suspected primary CMV infection at the University Hospital Tübingen between October 2023–June 2025. CMV-specific IgG, IgM and IgG avidity were measured and a CMV immunoblot assay was carried out. Associations between immunoblot band reactivity and CLIA IgG avidity as gold standard were analyzed by Firth penalized logistic regression and ordinal logistic regression.

Results

Increasing CLIA IgG avidity was associated with a decreasing probability of IgM p150 and IgG IE1, CM2 and p65 reactivity, and with a significantly increasing probability of IgG gB1, gB2 and avidity gB2 band positivity. Among cases with low CLIA IgG avidity (< 15%; n = 56), absence of IgG avidity gB2 achieved the highest detection rate (92.9%) and absence of IgG gB1 the lowest false-positive rate (8.4%).

Conclusion

Immunoblot band patterns correlate systematically with CLIA IgG avidity and can help determine the timing of primary CMV infection in pregnancy. Absent IgG gB1 reactivity provides a specific marker for very recent infection, while absent IgG avidity gB2 offers high sensitivity.

What is already known?

Dating of a primary CMV infection in pregnancy is mainly based on indirect serological tests and remains challenging.

What is new?

Immunoblot antibody analysis can be helpful as a secondary test system.

INTRODUCTION

Congenital cytomegalovirus (cCMV) infection is the most common congenital viral infection worldwide, affecting approximately 0.5–0.7% of all live births, corresponding to an estimated 1.8 million newborns

annually. It is a leading cause of non-genetic sensorineural hearing loss and neurodevelopmental impairment, with long-term sequelae occurring in 10–15% of infected infants and substantially higher rates among those symptomatic at birth [1–4].

Despite its clinical relevance, prenatal diagnosis and risk stratification remain challenging. Accurate dating of maternal primary infection is critical, as only infections occurring in the periconceptional period or first trimester are associated with a high risk of adverse fetal outcome, thereby justifying antenatal treatment with Valaciclovir [5, 6].

Serological testing remains the cornerstone of maternal CMV diagnosis but has important limitations. CMV-specific IgM antibodies lack specificity for recent primary infection, as they may persist for months or arise during viral reactivation, with only approximately 50% of IgM-positive cases representing true primary infection [7, 8]. IgG seroconversion is rarely documented in routine care due to the absence of preconceptional baseline samples, and reliance on single time-point serology frequently results in diagnostic uncertainty. IgG avidity testing has therefore become a key tool for estimating the timing of infection: low avidity indicates recent infection within the previous three months, whereas high avidity effectively excludes primary infection during early pregnancy – especially when serological testing was performed in the first half of pregnancy. However, its diagnostic performance is limited, especially in the intermediate range and later gestation, where maturation kinetics may overlap [9].

Immunoblot-based assays have been proposed as an adjunct to refine temporal classification. By analyzing antigen-specific antibody responses, they may provide additional diagnostic resolution beyond global avidity indices. Previous studies suggest that immunoblot analysis can improve identification of recent infection and increase diagnostic confidence, although its clinical utility remains limited by variability in interpretation and lack of standardization [10]. In a previous study, the presence of gB2-specific antibodies in women with primary CMV infection and Valaciclovir treatment was associated with a lower rate of maternal–fetal transmission [5].

Given the central importance of infection timing in prenatal CMV management and the limitations of current serological approaches, improved diagnostic strategies for precise temporal stratification are needed. The present study therefore aims to evaluate the utility of immunoblot analysis for determining the timing of maternal CMV infection in pregnancy.

METHODS

This was a retrospective study conducted at the Department of Prenatal Medicine, University Hospital Tübingen, Germany, between October 2023 and June 2025. We included pregnant women referred for suspected primary CMV infection or unclear serological findings.

In all cases, CMV-specific IgG and IgM levels as well as IgG avidity were assessed routinely using a chemiluminescent immunoassay (CLIA) conducted by a fully automated platform (Liaison XL, DiaSorin,

Saluggia, Italy). According to the producer, IgG avidity is classified as low, intermediate and high, if the avidity is < 15.0%, between 15.0 and 24.9% and > 25.0%, respectively.

In addition, commercial CMV immunoblot assays (recomLine CMV IgG, IgM, IgG avidity; Mikrogen GmbH, Neuried, Germany) were performed. The immunoblots evaluate antigen-specific antibody responses against CMV non-structural proteins IE1 (reading frame UL 123) and CM2 (UL 44, UL 57), CMV tegument proteins p150 (UL 32) and p65 (UL 83), as well as against CMV membrane glycoproteins gB1 and gB2 (UL 55), allowing detection of phase-specific IgG and IgM antibodies as well as avidity-related patterns. Band reactivity was assessed semi-quantitatively and classified into four categories (0, 1, 2, or 3) according to signal intensity relative to a cut-off control band (= semi-quantitative intensity category 1). Interpretation was based on visual assessment. According to the manufacturer´s recommendations, the IgG avidity p65 and gB1 bands should not be taken into account routinely for immunoblot avidity interpretation due to their very variable kinetics and are therefore marked with an asterisk*.

In clinical practice, pregnant women with a recent periconceptional or first-trimester primary CMV infection are offered antiviral treatment with Valaciclovir (8 g/day). Patients are counselled regarding the off-label use of the drug, potential risks, and the evidence supporting its use according to national and international guidelines [3, 11, 12]. Treatment is continued until amniocentesis is performed at approximately 20 weeks' gestation. Data on fetal development, intrauterine transmission status at amniocentesis, and neonatal outcome were recorded in a standardized perinatal database (Viewpoint, Solingen, Germany).

The study was approved by the ethics committee of the University of Tübingen (approval number: 374/2025BO2). This is a sub-analysis of our previous study that focused on the impact of gB2 antibodies on the risk of maternal-fetal transmission in women with a primary CMV infection and treatment with Valaciclovir [5].

Statistical analysis

CLIA IgG avidity was used as reference standard for the timing of primary CMV infection, and immunoblot results were compared against CLIA IgG avidity. CLIA IgG avidity values were categorized as follows: <10%, 10.0–19.9%, 20.0–29.9%, 30.0–39.9%, and ≥ 40.0%.

First, we assessed the presence of any immunoblot band reactivity (IgG IE1, CM2, p65, p150, gB1, gB2; IgM IE1, CM2, p150, p65, gB1, gB2; and avidity bands IE1, CM2, p65*, p150, gB1*, gB2) and stratified frequencies according to CLIA IgG avidity. Associations between band reactivity and increasing CLIA IgG avidity were analyzed using Firth penalized univariate logistic regression, with IgG avidity modelled as a continuous variable.

Second, we examined band intensity (graded 1–3) among samples with detectable reactivity. Trends in band intensity were analyzed using ordinal logistic regression, with CLIA IgG avidity as a continuous variable.

Third, using the manufacturer-defined CLIA IgG avidity cut-off of < 15% for low avidity, cases were grouped into low (< 15%) and intermediate or high (≥ 15%) avidity categories. The frequency of band reactivity and band intensity were compared between groups using the Fisher's exact and Mann–Whitney U test. Different immunoblot band combinations were used to detect cases with low CLIA IgG avidity, and the results are given as detection and false positive rates (DR and FPR). For this analysis, only the parameters with a significant difference between low and intermediate/high CLIA IgG avidity group were used.

To account for the numerous statistical comparisons, significance level was defined as $p < 0.001$.

RESULTS

The database search identified 151 women referred for suspected primary CMV infection. Median maternal age at presentation was 31.7 years (IQR 29.8–34.6), and median gestational age was 10.7 weeks (IQR 8.9–16.6).

IgG antibodies were present in all cases, and IgM antibodies were detected in 132 (87.4%) women using the CLIA. Median IgG level and IgG avidity were 78.0 IU/mL (IQR 57.7–99.9) and 17.0% (IQR 12.0–28.1), respectively. IgM levels were not assessed quantitatively. IgG avidity was < 10%, 10.0–19.9%, 20.0–29.9%, 30.0–39.9%, and ≥ 40.0% in 25 (16.6%), 61 (40.4%), 33 (21.9%), 14 (9.3%), and 18 (11.9%) cases, respectively. Median avidity values in these groups were 6.8%, 14.8%, 24.8%, 36.1%, and 53.9%. Further details are shown in Table 1.

Table 1
Details about the study population

CLIA IgG avidity class	n	Gestational age in weeks		IgG levels quantitative		CLIA IgG Avidity in %	
		Median	IQR	Median	IQR	Median	IQR
< 10%	25	11.3	8.9–15.3	71.5	46.1–80.9	6.8	5.5–7.7
10.0–19.9%	61	10.3	8.7–14.9	80.8	57.5–101.0	14.8	12.2–16.3
20.0–29.9%	33	10.7	9.1–16.9	79.0	60.2–94.0	24.8	21.2–26.3
30.0–39.9%	14	11.0	9.6–12.7	71.4	63.1–93.4	36.1	32.4–37.1
≥ 40.0%	18	11.9	9.1–20.3	105.0	62.8–131.0	53.9	44.9–64.4
TOTAL	151	10.7	8.9–16.6	78.0	57.7–99.9	17.0	12.0–28.1

Table 2 and both figures show the proportion of immunoblot samples with any band reactivity across CLIA IgG avidity categories. We used Firth penalized univariate logistic regression to examine whether the proportion of immunoblot reactivity changed with increasing CLIA IgG avidity. We observed a lower probability of a positive reaction of the IgM p150, IgG IE1, IgG CM2, and IgG p65 band with increasing CLIA IgG avidity. Furthermore, there was a significant positive association between the probability of a positive IgG gB1 and gB2 band reaction as well as a positive IgG avidity gB2 band reaction and the CLIA IgG avidity (Table 3). In Table 4 and in both figures, the strength of the reaction of the individual bands is shown. Cases with no reaction were not included in the analysis. Increasing CLIA IgG avidity was associated with an increase in IgG p150 and gB1 band intensity as well as an increase in IgG avidity p150 and gB2 band intensity (Table 5).

Table 2
Proportion of immunoblot analysis results with a positive reaction according to the IgG avidity measured by the CLIA system.

		CLIA Avidity		CLIA Avidity		CLIA Avidity		CLIA Avidity		CLIA Avidity	
Immunoblot bands		**< 10.0%**		**10.0–19.9%**		**20.0–29.9%**		**30.0–39.9%**		**≥ 40.0%**	
		TOTAL n = 25		**TOTAL n = 61**		**TOTAL n = 33**		**TOTAL n = 14**		**TOTAL n = 18**	
		Any reaction		**Any reaction**		**Any reaction**		**Any reaction**		**Any reaction**	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
IgM	**IE1**	11	44.0%	7	11.5%	1	3.0%	2	14.3%	0	0.0%
	CM2	23	92.0%	50	82.0%	22	66.7%	10	71.4%	9	50.0%
	p150	25	100.0%	60	98.4%	29	87.9%	13	92.9%	11	61.1%
	p65	15	60.0%	18	29.5%	9	27.3%	3	21.4%	3	16.7%
	gB1	1	4.0%	0	0.0%	1	3.0%	0	0.0%	0	0.0%
	gB2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
IgG	**IE1**	24	96.0%	61	100.0%	31	93.9%	12	85.7%	11	61.1%
	CM2	25	100.0%	60	98.4%	31	93.9%	11	78.6%	13	72.2%
	p150	24	96.0%	57	93.4%	32	97.0%	14	100.0%	18	100.0%
	p65	25	100.0%	59	96.7%	29	87.9%	12	85.7%	12	66.7%
	gB1	10	40.0%	42	68.9%	32	97.0%	12	85.7%	18	100.0%
	gB2	3	12.0%	18	29.5%	22	66.7%	7	50.0%	13	72.2%
Avidity	**IE1**	21	84.0%	55	90.2%	27	81.8%	11	78.6%	10	55.6%
	CM2	23	92.0%	52	85.2%	30	90.9%	10	71.4%	12	66.7%
	p150	18	72.0%	49	80.3%	31	93.9%	13	92.9%	18	100.0%
	p65*	4	16.0%	7	11.5%	1	3.0%	1	7.1%	1	5.6%
	gB1*	0	0.0%	2	3.3%	2	6.1%	0	0.0%	1	5.6%
	gB2	1	4.0%	10	16.4%	19	57.6%	5	35.7%	12	66.7%

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

Table 3

Association between IgG avidity measured by the CLIA system and immunoblot band reactivity: results of Firth penalized logistic regression analysis (odds ratios with 95% confidence intervals).

IgM		**OR**	**95% Confidence Interval**		**p**
	IE1	**0.000**	**0.000**	**0.011**	**0.002**
	CM2	0.024	0.002	0.256	0.002
	p150	0.000	0.000	0.008	< 0.001
	p65	0.015	0.001	0.326	0.007
	gB1	0.002	0.000	33900.000	0.462
	gB2	3.24	0	∞	1.000
IgG	**IE1**	0.000	0.000	0.007	< 0.001
	CM2	0.001	0.000	0.034	< 0.001
	p150	199.000	0.025	1.610E + 06	0.249
	p65	0.002	0.000	0.039	< 0.001
	gB1	1.370E + 07	1.040E + 04	1.810E + 10	< 0.001
	gB2	630.200	34.200	11625.500	< 0.001
Avidity	**IE1**	0.016	0.001	0.194	0.001
	CM2	0.045	0.004	0.568	0.017
	p150	44300.000	48.100	4.080E + 07	0.002
	p65*	0.014	0.000	3.240	0.124
	gB1*	3.250	0.017	613.700	0.659
	gB2	1090.000	59.100	2.000E + 04	< 0.001

Odds ratios (OR) with 95% confidence intervals (CI) were estimated using Firth penalized logistic regression to account for small sample size and potential separation in the data. Each model evaluated the association between IgG avidity and the presence of any reaction for the respective immunoblot band.

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

Table 4

Strength of the immunoblot reaction according to the avidity measured by the CLIA system. Cases with no reaction were not included.

		CLIA IgG avidity < 10.0%	CLIA IgG avidity 10.0–19.9%	CLIA IgG avidity 20.0–29.9%	CLIA IgG avidity 30.0–39.9%	CLIA IgG avidity ≥ 40.0%
		Median strength	Median strength	Median strength	Median strength	Median strength
IgM	**IE1**	1.0	1.0	1.0	1.0	–
	CM2	2.0	2.0	1.0	1.0	1.0
	p150	2.0	2.0	2.0	1.0	1.0
	p65	1.0	1.0	1.0	1.0	1.0
	gB1	1.0	–	1.0	–	–
	gB2	–	–	–	–	–
IgG	**IE1**	2.0	2.0	2.0	1.5	2.0
	CM2	3.0	2.0	2.0	2.0	2.0
	p150	2.0	3.0	3.0	3.0	3.0
	p65	3.0	2.0	2.0	2.0	2.0
	gB1	1.0	1.5	2.0	1.5	2.0
	gB2	1.0	1.0	1.5	2.0	2.0
Avidity	**IE1**	1.0	1.0	1.0	1.0	2.0
	CM2	2.0	1.0	1.0	1.0	1.5
	p150	1.0	2.0	2.0	2.0	3.0
	p65*	2.0	1.0	1.0	1.0	1.0
	gB1*	–	1.0	2.0	–	2.0
	gB2	1.0	1.0	1.0	2.0	2.0

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

Table 5
Ordinal logistic regression analysis of the association between IgG avidity and immunoblot band intensity after exclusion of cases with no reaction in the immunoblot analysis.

		OR	95% Confidence Interval		p
IgM	**IE1**	**0.000**	**0.000**	**1.600E + 07**	**0.275**
	CM2	0.012	0.000	0.353	0.010
	p150	0.002	0.000	0.041	< 0.001
	p65	0.000	0.000	0.826	0.045
	gB1	–	–	–	–
	gB2	–	–	–	–
IgG	**IE1**	0.230	0.018	2.970	0.262
	CM2	0.026	0.002	0.287	0.003
	p150	639.000	28.000	14390.000	< 0.001
	p65	0.021	0.001	0.320	0.005
	gB1	1233.000	60.000	25320.000	< 0.001
	gB2	217.000	7.000	6392.000	0.002
Avidity	**IE1**	71.000	4.000	1229.000	0.003
	CM2	0.590	0.040	8.340	0.696
	p150	4711.000	245.000	90620.000	< 0.001
	p65*	~ 0	–	–	0.134
	gB1*	extreme	–	–	0.924
	gB2	1849.000	28.000	120817.000	< 0.001

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

As the definition of low IgG avidity is test-dependent, we applied the manufacturer's CLIA cut-off of < 15% and examined the immunoblot profile of cases with low and higher avidity (Table 6). In samples with low CLIA IgG avidity (n = 56), a positive reaction in the IgG gB1 and gB2 bands was less common than in those with a higher CLIA IgG avidity (gB1: 48.2% vs. 91.6%; gB2: 16.1% vs. 56.8%). The IgG avidity gB2 band reaction increased from 7.1% to 45.3%.

Table 6

Immunoblot band patterns and intensities stratified by IgG avidity (< 15% vs. ≥15%). In the first column, the immunoblot band is shown, the next two columns show the proportion of cases with any reaction in the group of cases with a CLIA IgG avidity of less than 15% or 15% and more. The next column gives the results of the chi square test. In the following two columns the median strength of the reaction is shown, again stratified according to the CLIA IgG avidity. The last column gives the p values of the Mann–Whitney U test.

Immunoblot bands		**CLIA IgG avidity < 15%**	**CLIA IgG avidity ≥ 15%**	**p**	**CLIA IgG avidity < 15%**	**CLIA IgG avidity ≥ 15%**	**p**
		N = 56 n (%)	**N = 95 n (%)**		**Median strength**	**Median strength**	
IgM	**IE1**	15 (26.8%)	6 (6.3%)	0.001	1.0	1.0	0.277
	CM2	47 (83.9%)	67 (70.5%)	0.079	2.0	1.0	< 0.001
	p150	55 (98.2%)	83 (87.4%)	0.032	2.0	1.0	0.001
	p65	24 (42.9%)	24 (25.3%)	0.030	1.0	1.0	0.092
	gB1	1 (1.8%)	1 (1.1%)	1.000	1.0	1.0	1.000
	gB2	0 (0.0%)	0 (0.0%)	1.000	–	–	–
IgG	**IE1**	55 (98.2%)	84 (88.4%)	0.033	2.0	2.0	0.026
	CM2	55 (98.2%)	85 (89.5%)	0.055	3.0	2.0	0.002
	p150	53 (94.6%)	92 (96.8%)	0.671	2.0	3.0	< 0.001
	p65	55 (98.2%)	82 (86.3%)	0.018	2.0	2.0	< 0.001
	gB1	**27 (48.2%)**	**87 (91.6%)**	**< 0.001**	1.0	2.0	0.053
	gB2	**9 (16.1%)**	**54 (56.8%)**	**< 0.001**	1.0	2.0	0.520
Avidity	**IE1**	48 (85.7%)	76 (80.0%)	0.510	1.0	1.0	0.054
	CM2	48 (85.7%)	79 (83.2%)	0.819	1.0	1.0	0.416
	p150	40 (71.4%)	89 (93.7%)	0.001	1.0	2.0	< 0.001
	p65*	8 (14.3%)	6 (6.3%)	0.145	1.0	1.0	0.126
	gB1*	1 (1.8%)	4 (4.2%)	0.652	1.0	2.0	0.414

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

Immunoblot bands	CLIA IgG avidity < 15%	CLIA IgG avidity ≥ 15%	p	CLIA IgG avidity < 15%	CLIA IgG avidity ≥ 15%	p
	N = 56 n (%)	N = 95 n (%)		Median strength	Median strength	
gB2	4 (7.1%)	43 (45.3%)	< 0.001	1.0	1.0	0.089

*according to the manufacturer, these IgG avidity bands should not be used for clinical purposes.

Table 6 also shows the median intensity of the band reaction in those with a low and a higher CLIA IgG avidity. We found a higher intensity of the IgM CM2 band, a lower intensity of the IgG p150 band as well as a lower intensity of the IgG avidity p150 band in those with a low CLIA IgG avidity.

Finally, we searched for combinations of immunoblot band reactions that can detect low CLIA IgG avidity. The detection and false positive rates of the individual markers are shown in the first columns of Table 7. For the analysis we only used the bands with a significant difference between the low CLIA IgG avidity group and the other cases. The absence of an IgG gB1, IgG gB2, or avidity gB2 reaction was associated with detection rates of 51.8%, 83.9%, and 92.9% and false positive rates of 8.4%, 43.2%, and 54.7%, respectively. None of the combinations of the three bands were associated with a higher detection rate or a lower false positive rate than the best individual marker. Thus, if the focus is on the detection rate, it is best to use the avidity gB2 band, and if the aim is to have a low false positive rate, it is best to focus on the IgG gB1 reaction.

Table 7
Detection (DR) and false positive rate (FPR) of low CLIA IgG avidity (< 15%) by single and combined use of different immunoblot bands. Our study cohort involved 56 women with low IgG avidity and 95 women with intermediate or high avidity. The table also includes the positive and negative predictive value of the single or combined use of the markers in our cohort. We only included the immunoblot bands with a significant difference in the proportion of cases with a positive reaction (Table 6, first columns).

Immunoblot bands	**DR**	**FPR**	**PPV***	**NPV***
	N (%)	**N (%)**	**%**	**%**
IgG gB1 no reaction	29 (51.8)	8 (8.4)	78.4	76.3
IgG gB2 no reaction	47 (83.9)	41 (43.2)	53.4	85.7
IgG avidity gB2 no reaction	52 (92.9)	52 (54.7)	50.0	91.5
IgG gB1 and IgG gB2 no reaction	29 (51.8)	8 (8.4)	78.4	76.3
IgG gB1 and IgG avidity gB2 no reaction	29 (51.8)	8 (8.4)	78.4	76.3
IgG gB2 and IgG avidity gB2 no reaction	47 (83.9)	41 (43.2)	53.4	85.7
IgG gB1 or IgG gB2 no reaction	47 (83.9)	41 (43.2)	53.4	85.7
IgG gB1 or IgG avidity gB2 no reaction	52 (92.9)	52 (54.7)	50.0	91.5
IgG gB2 or IgG avidity gB2 no reaction	52 (92.9)	52 (54.7)	50.0	91.5
IgG gB1, IgG gB2 and IgG avidity gB2 no reaction	29 (51.8)	8 (8.4)	78.4	76.3
IgG gB1, IgG gB2 or IgG avidity gB2 no reaction	52 (92.9)	52 (54.7)	50.0	91.5

DISCUSSION

In this study, we examined immunoblot results in pregnancies with primary CMV infection, stratified by CLIA IgG avidity. The immunoblot bands were assessed in terms of a reactivity and the strength of the reaction. We observed a significant correlation between the CLIA IgG avidity and the reactivity of the IgM p150 antibodies, the IgG IE1, CM2, p65, gB1 and gB2 antibodies as well as the reactivity of IgG avidity gB2 band. In most of these bands, we also observed a correlation between the CLIA IgG avidity and the strength of the reaction. Furthermore, we searched for individual or combinations of immunoblot bands that can detect cases with low CLIA IgG avidity as additional, supportive analysis. In view of a high detection rate, the best indicator was the absence of IgG avidity gB2 band but for an unacceptably high false positive rate of more than 50%. The lowest false positive rate was achieved with the absence of the IgG gB1 band.

In a previous study, we have examined, if the additional assessment of the immunoblot analysis can be helpful in women with a primary CMV infection [5]. In a series of 85 pregnancies (47 periconceptional and 38 first trimester infections), we observed a maternal-fetal transmission in almost 13% of the cases

if there was no reactivity of the IgG gB2 band and in none of the cases if the gB2 antibodies were present.

The diagnostic utility of gB antibodies for dating primary CMV infection has been recognized for more than two decades. Eggers et al. showed that antibodies to gB and gH recombinant epitopes appear on average 13 weeks after seroconversion and that their absence therefore reliably marks an earlier primary infection [13]. The present data are consistent with this kinetics: gB1 reactivity was absent preferentially in samples with low IgG avidity, reflecting an early phase of infection, while both gB1 and gB2 positivity accumulated progressively with rising avidity. The low false-positive rate of absent IgG gB1 (8.4 %) nderlines the specificity value of this single band as a marker for very recent infection.

Absence of IgG gB1 antibodies provides a specific marker for very recent primary CMV infection while circumventing a significant diagnostic limitation: the gB non-responder phenomenon. Among healthy CMV-seropositive blood donors, 18–30% (Rothe et al., J Med Virol 2001; [9] Enders et al., J Clin Virol 2013) are gB non-responders who fail to generate detectable IgG gB2 antibodies despite retaining IgG gB1 antibodies throughout persistent infection [14, 15]. This inherent constraint directly explains the elevated false-positive rate of absent IgG gB2 antibodies in our study. Many samples lacking gB2 reactivity simply reflect this non-responder phenotype rather than a very recent infection. Conversely, the low false-positive rate of absent gB1 reflects the rarity of this antibody pattern in long-standing infection, making it a markedly more reliable marker for recent seroconversion. Therefore, prioritizing IgG gB1 reactivity is advantageous for identifying pregnant women at risk for congenital CMV transmission.

The sequential emergence of immunoblot antibodies observed in our cohort mirrors the antigen-specific kinetics described by Zelini et al. who demonstrated that anti-p52 IgM appears early after primary infection whereas anti-gB IgG rises significantly later, and that the combination of both markers produces conclusive results in 92.8% of initial samples while reducing the number of tests compared with a lysate-based approach [16]. Our findings extend this picture to the immunoblot platform: IgM reactivities against IE1, CM2, p150 and p65 decreased progressively with increasing avidity, consistent with the known gradual waning of IgM after the acute phase, while IgG gB1, gB2 and avidity-gB2 band intensities increased. This bidirectional kinetic signature — declining IgM alongside rising anti-glycoprotein IgG — provides the conceptual rationale for using the combination of IgM antigen pattern and gB-specific IgG reactivity to narrow the window of infection.

In our study we have focused on detecting primary infections with low IgG avidity. However, the relevance of supplementary assays beyond conventional IgG avidity testing is further supported by the frequent occurrence of intermediate avidity results in routine diagnostics. Delforge et al. (2019) evaluated the combination of the Mikrogen recomLine CMV IgG and IgG Avidity line immunoassays in 40 women with a precisely determined onset of primary infection and demonstrated correct dating in 83.1% of sequential samples, with superior sensitivity for infections within 14 weeks compared with the VIDAS ELFA (85.9% vs. 76.9%); crucially, this combination resolved timing in 79% of samples that yielded an intermediate VIDAS avidity result [17].

Enders et al. had similarly shown that the recomBlot, the direct predecessor test format, achieved a positive predictive value of 96.9% for infections within the preceding 14 weeks [10]. Taken together with the present results, these data support the use of the recomLine immunoblot as a confirmatory tool, particularly when the results of the serological avidity test are inconclusive. Lilleri et al. further broadened the toolkit by demonstrating that ELISA IgG responses to distinct HCMV glycoprotein complexes (gH/gL/pUL128L, gH/gL/gO and gB) display differential kinetics and, in combination with conventional avidity testing, define an algorithm for infection timing that correctly classified the phase of primary infection in a high proportion of cases [18].

The well-established limitations of CMV IgM as a stand-alone marker are also reflected in our data. IgM reactivities against all four tegument/regulatory antigens (IE1, CM2, p150, p65) were present across the full avidity spectrum, including in a subset of samples with high CLIA avidity. This observation is concordant with published evidence that IgM can persist for many months after primary infection and may be detectable even in reactivation settings, so that IgM positivity alone carries poor positive predictive value for recent primary infection when not accompanied by avidity testing [7] Importantly, however, IgM reactivities were not universally present even in low-avidity samples in our cohort, meaning that the absence of IgM cannot be used to exclude a very early primary infection — a diagnostic gap that the IgG gB1 absence criterion, with its low false-positive rate, partially addresses.

We acknowledge that our study has several limitations. First, our study is a retrospective study that was carried out in one center. Second, we only used the CLIA IgG avidity test and did not confirm the results with other fully automated ligand avidity tests. Third, although we collected a reasonably large sample of cases, there is still not enough data to assess the development of the strength of the reaction across the avidity classes. And fourth, this study focused on the impact on immunoblot analysis on the timing of the primary infection. For this purpose, we used the CLIA IgG avidity as gold standard. From a clinical point of view, a stratification according to the transmission rate would be more interesting but for this task, a much larger dataset would be necessary.

In conclusion, in our study we have shown how the immunoblot bands change with increasing avidity. The immunoblot analysis can help to assess the timing of a primary CMV infection if the timing of the infection is unclear.

Declarations

Conflict of interest/Funding: none

Competing Interests

Prof. Martin Enders works at Laboratory Prof. Dr. G. Enders and Colleagues, MVZ, Stuttgart, Germany, which is a private laboratory offering CMV testing. Karl Oliver Kagan is the Editor in Chief of Archives of Gynecology and Obstetrics.

Author Contribution

Alfred L Bissinger – Analysis and writingLara Schmid – data collectionMarkus Hoopmann - WritingMartin Enders - WritingKarl Oliver Kagan – Conceptualization, Methodology, Writing

Data Availability

The data that support the findings of this study are available from the researchers, but restrictions apply to the availability of these data, so that they are not publicly available. The data are, however, available upon request.

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Figures

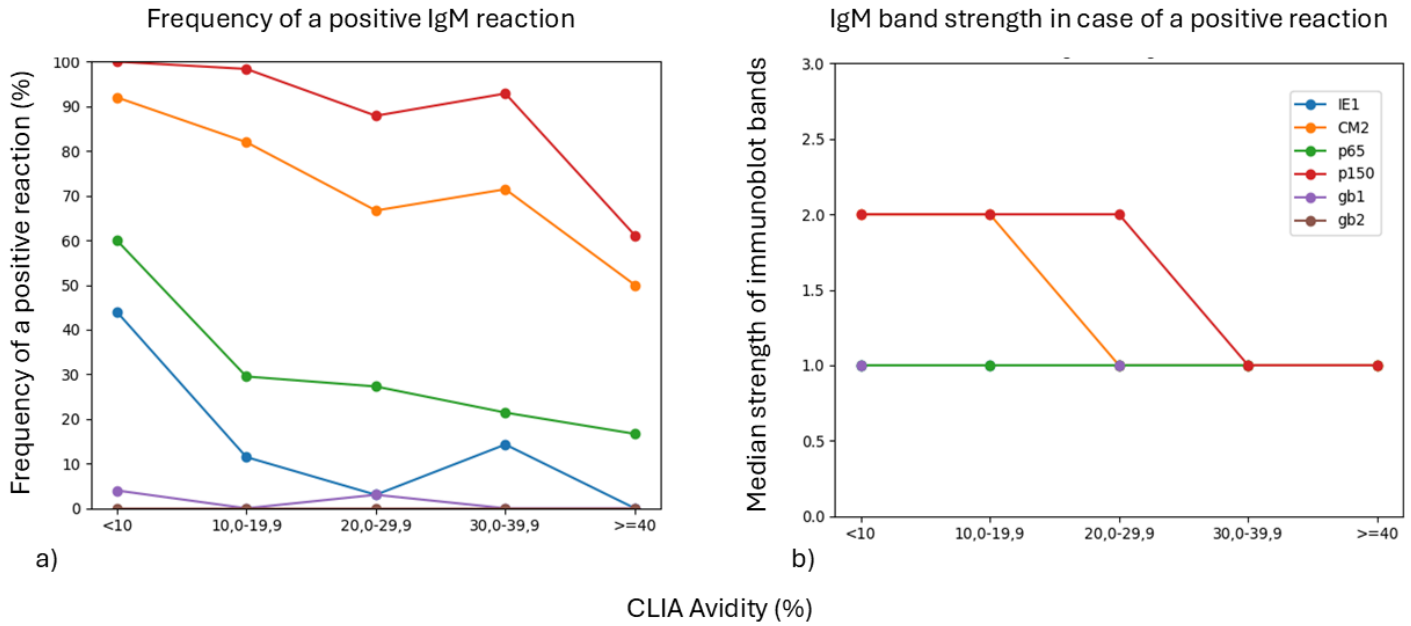


Figure 1

Figure 1

Figure 1a (left) demonstrates the frequency of a positive IgM reaction according to the CLIA IgG avidity. Figure 1b (right) shows the strength of the reaction in case of a positive reaction.

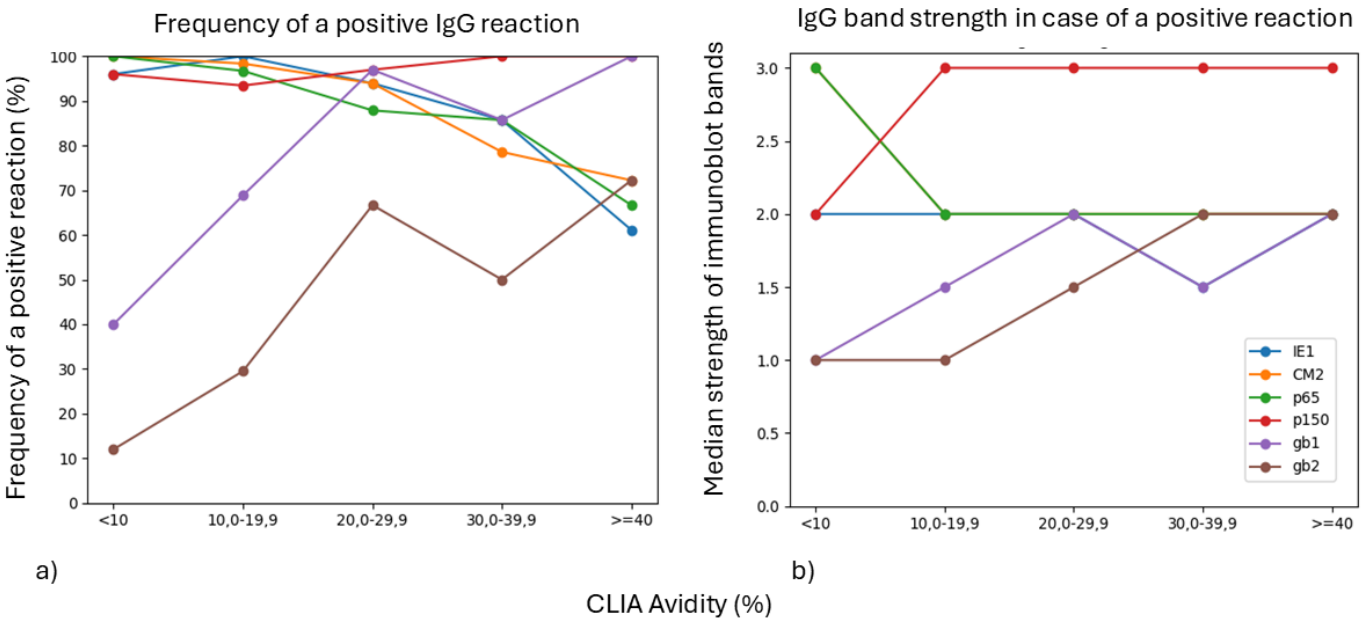


Figure 2

Figure 2

Figure 2a (left) demonstrates the frequency of a positive IgG reaction according to the CLIA IgG avidity. Figure 2b (right) shows the strength of the reaction in case of a positive reaction.