Journal of Clinical Virology 120 (2019) 38–43

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

# Determination of anti-p52 IgM and anti-gB IgG by ELISA as a novel diagnostic tool for detection of early and late phase of primary human cytomegalovirus infections during pregnancy

Paola Zelini[a], Chiara Fornara[b,c], Milena Furione[d], Antonella Sarasini[d], Julia Klemens[e], Alessia Arossa[a], Arsenio Spinillo[a], Giuseppe Gerna[b], Daniele Lilleri[b,c,*]

[a] Ostetricia e Ginecologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[b] Laboratorio Genetica – Trapiantologia e Malattie cardiovascolari, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[c] Laboratorio Biochimica-Biotecnologie e Diagnostica avanzata, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[d] Virologia Molecolare, Microbiologia e Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[e] Institute for Experimental Immunology, Euroimmun AG, Luebeck, Germany

ARTICLE INFO

*Keywords:*
HCMV primary infection
Pregnancy
IgM antibody
IgG antibody
IgG avidity
Dating of infection

ABSTRACT

*Background:* Dating of primary human cytomegalovirus (HCMV) infection in pregnancy is crucial to define whether infection occurred before or during pregnancy and at which gestational age.
*Objective:* The aim of this study was to identify a diagnostic strategy for determination of early, intermediate and late phase of HCMV primary infection during pregnancy.
*Study design:* Sequential serum samples from 40 pregnant women with defined onset of HCMV primary infection were tested retrospectively for IgM, IgG and IgG avidity against whole HCMV lysate, along with anti-p52 IgM and anti-gB IgG (Euroimmun AG).
*Results:* Anti-HCMV IgM were positive in all samples collected within the first 2 months, then decreased remaining weakly positive in about 40% of samples collected within 6–12 months after infection. Anti-p52 IgM followed similar kinetics but decreased earlier, remaining weakly positive only in 20% of late samples. Anti-HCMV IgG were positive in all samples and showed variable kinetics. Their avidity increased from low levels, observed within 2 months, to intermediate/high levels from 4 months onwards. Anti-gB IgG increased over time following kinetics similar to anti-HCMV IgG avidity. By combining results of anti-HCMV IgM plus IgG avidity, and confirming them with anti-p52 IgM plus anti-gB IgG as second-line assays, the early (within 2–3 months) and late (after 3 months) phases of HCMV infection were satisfactorily defined, whereas the intermediate phase overlapped with the beginning of the late phase.
*Conclusion:* Anti-p52 IgM and anti-gB IgG provide additional tools besides classical anti-HCMV IgM, IgG and IgG avidity in dating HCMV primary infections.

## 1. Background

Human cytomegalovirus (HCMV) infections are a major concern during pregnancy, when the virus can be transmitted from mother to fetus. HCMV infection is the most common congenital cause of impairments in child development [1] with an overall estimated prevalence of 0.7% [2]. The rate of vertical transmission is about 30–40% in pregnant women with primary infections [2,3], and much lower (around 1%) in pregnant women with preconception immunity [2,4]. Moreover, the most severe *sequelae* in congenitally infected newborns are the result of a primary infection occurring during the first trimester of pregnancy [5–7].

Thus, diagnosis or exclusion of primary infection, as well as definition of the gestational age at which maternal infection occurs, are major objectives of diagnostic monitoring during pregnancy. Since symptoms of HCMV infection are mostly mild and non-specific [8], determining infection onset may be difficult, especially in the absence of routine serological monitoring for HCMV during pregnancy. Conventional assays for HCMV IgM and IgG avidity determination alone are not always reliable. HCMV-specific IgG with moderate avidity have

* Corresponding author at: Laboratorio Genetica – Trapiantologia e Malattie cardiovascolari, Fondazione IRCCS Policlinico San Matteo, Viale Golgi 19, 27100 Pavia, Italy.
*E-mail address:* d.lilleri@smatteo.pv.it (D. Lilleri).

https://doi.org/10.1016/j.jcv.2019.09.006
Received 9 May 2019; Received in revised form 12 September 2019; Accepted 13 September 2019

occasionally been found in early stages of primary infection [9] and HCMV-specific IgM may be detected also in subjects with past infection [8]. In order to ameliorate the serological diagnosis of HCMV infection, some authors observed that detection of antibodies against two antigenic domains of envelope glycoprotein B (gB) was useful for differentiation of primary *vs* recurrent infections [10,11]. In addition, the use of recombinant p52 (a non-structural DNA binding protein) was found to improve detection of HCMV-specific IgM [12].

## 2. Objectives

This study aimed to analyze the kinetics of p52 IgM and gB IgG by two novel ELISA based on recombinant antigens, with a view to identifying a diagnostic strategy to define the early, intermediate and late stages of primary HCMV infection during pregnancy. Sequential blood samples from pregnant women with pre-defined onset of primary HCMV infection were analyzed.

## 3. Study design

### 3.1. Pregnant women with primary infection and controls

The population analyzed consisted of 40 pregnant women with mild symptomatic primary HCMV infection, referred to our center between 2011 and 2013. The first available sample was collected after a median time of 31 (range 13–106) days after the estimated onset of primary infection and sequential samples (median: 4; range 2–6 per patient) were obtained monthly for a median follow-up period of 252 (range: 60–464) days after onset. This population was already described in a previous study [13]. Serum samples from 20 HCMV-seropositive pregnant women were collected at 10, 20, 30, 40 weeks of gestation, and serum samples from 20 HCMV-seronegative pregnant women were collected once as controls.

### 3.2. Diagnosis and timing of primary maternal HCMV infection

Diagnosis of primary maternal infection was achieved based on two or more of the following parameters [13]: IgG seroconversion (ETI-CYTOK-G, DiaSorin, Saluggia, Italy), HCMV-specific IgM kinetics (ETI-CYTOK-M, DiaSorin), low IgG avidity index (AI), detection of HCMV DNA in blood [3,8,14,15]. IgM results obtained by the commercial assay were confirmed by an in-house capture ELISA [15]. IgG AI was determined by an in-house ELISA [16]. Appearance of HCMV-related symptoms defined infection onset. Biochemical and hematological signs associated with HCMV infection were carefully reported [8]. Seropositive and seronegative controls were determined by LIAISON CMV IgGII assay (DiaSorin).

### 3.3. Study assays

For the study purposes, anti−HCMV IgG, IgM and IgG AI were determined retrospectively by conventional ELISA based on cell lysate (Euroimmun anti−HCMV ELISA IgG, Euroimmun anti−HCMV ELISA IgM, Euroimmun anti−HCMV ELISA IgG Avidity) and by novel ELISA based on recombinant proteins (Euroimmun anti−HCMV p52 ELISA IgM, Euroimmun anti−HCMV gB ELISA IgG). All the ELISA (Euroimmun AG, Luebeck, Germany) are commercially available except anti−HCMV gB ELISA (which is based on the gB ectodomain and was undergoing CE registration at the time). Results were semi-quantitative (IgM) or quantitative (IgG). IgM levels were expressed as a ratio with respect to an internal calibrator: a ratio of $< 0.8$ was considered negative, $\geq 1.1$ was considered positive and intermediate results were considered borderline. IgG levels were expressed as relative units (RU) determined after interpolation with a calibration curve: values of $< 16$ RU/ml were considered negative, $\geq 22$ RU/ml were considered positive and intermediate values were considered borderline. IgG AI levels were expressed as percentages and deemed low when $< 40\%$ and high when $> 60\%$.

### 3.4. Statistical analysis

Non-linear regression models were used to express the kinetics of IgG and IgM and IgG AI. Fisher's exact test was used to compare the proportion of positive samples with ELISA based on different antigens. Diagnostic algorithms were defined by combining results from i) conventional anti−HCMV IgM and anti−HCMV IgG AI, ii) novel anti-p52 IgM and anti-gB IgG, or iii) a combination of the two algorithms above. Median, interquartile range, and the 10th-90th percentiles per time interval for each result combination were calculated.

## 4. Results

### 4.1. HCMV- and viral antigen-specific antibody response according to study assays in HCMV-seropositive and -seronegative pregnant women

All but one of the HCMV-seronegative controls were negative according to the anti−HCMV ELISA IgM and all controls were negative according to HCMV IgG assay (Fig. 1 A–B). One sample (#6318) was positive at a low level (ratio: 1.45) for IgM only. Seropositive pregnant women were negative for IgM except for sp#018, who showed a low (1.42) and borderline IgM ratio, and subject sp#023, who showed a low (1.15) IgM ratio (Fig. 1A). All 20 HCMV-seropositive pregnant women were positive according to anti−HCMV IgG (Fig. 1B) and showed high AIs (Fig. 1C).

Anti-p52 IgM were negative in all controls tested (Fig. 1D) bar sp#023, who was weakly positive (ratio: 1.19), also for anti−HCMV IgM, while anti-gB IgG were negative in seronegative women and positive in seropositive women except for sp#009, who was borderline at 30 gestation weeks, and sp#011, who scored negative at three time points (Fig. 1E).

Conventional anti−HCMV ELISA and novel ELISA based on recombinant proteins showed overlapping results in control subjects, although anti-gB IgG were repeatedly negative in sequential samples collected from one otherwise seropositive pregnant woman (sp#11).

### 4.2. Kinetics of the HCMV- and antigen-specific antibody response to primary HCMV infection during pregnancy as determined by study assays

Anti−HCMV IgM were positive in all 54 samples collected within the first 60 days after infection onset (Fig. 2A and Fig. S1A). IgM ratio decreased in all but one patient (p#032, who showed an initial increase in the second month before subsequent decrease, Fig. S1A), but were still positive in 20/45 (44%) samples collected after 180 days and in 6/16 samples (38%) collected after 360 days.

Anti−HCMV IgG were positive in all samples and showed variable kinetics (increasing, decreasing or stable values) during follow-up (Fig. 2B and Fig. S1B). IgG AI was low in almost all samples (49/54; 91%) collected within 60 days, then increased to intermediate or high levels in all but one samples collected after 120 days (Fig. 2C and Fig. S1C).

The anti-p52 IgM followed kinetics similar to anti−HCMV IgM (Fig. 2D and Fig. S1D), although they became negative earlier: anti-p52 IgM were positive in 46/54 samples (85% *vs* 100%; $p < 0.01$ *vs* anti−HCMV IgM) collected within the first 60 days and were still positive in 9/45 samples (20% *vs* 44%; $p = 0.02$ *vs* anti−HCMV IgM) and 3/16 samples (19% *vs* 38%; $p = 0.43$ *vs* anti−HCMV IgM) collected after 180 and 360 days, respectively.

As observed also for anti−HCMV IgM, patient p#032 showed an initial increase of anti-p52 IgM in the second month after infection (before subsequent decrease), whereas p#054 showed a longer persistence at high levels and a delayed decrease of anti-p52 IgM (Fig. S1D).

Contrary to anti−HCMV IgG, anti-gB IgG increased over time after

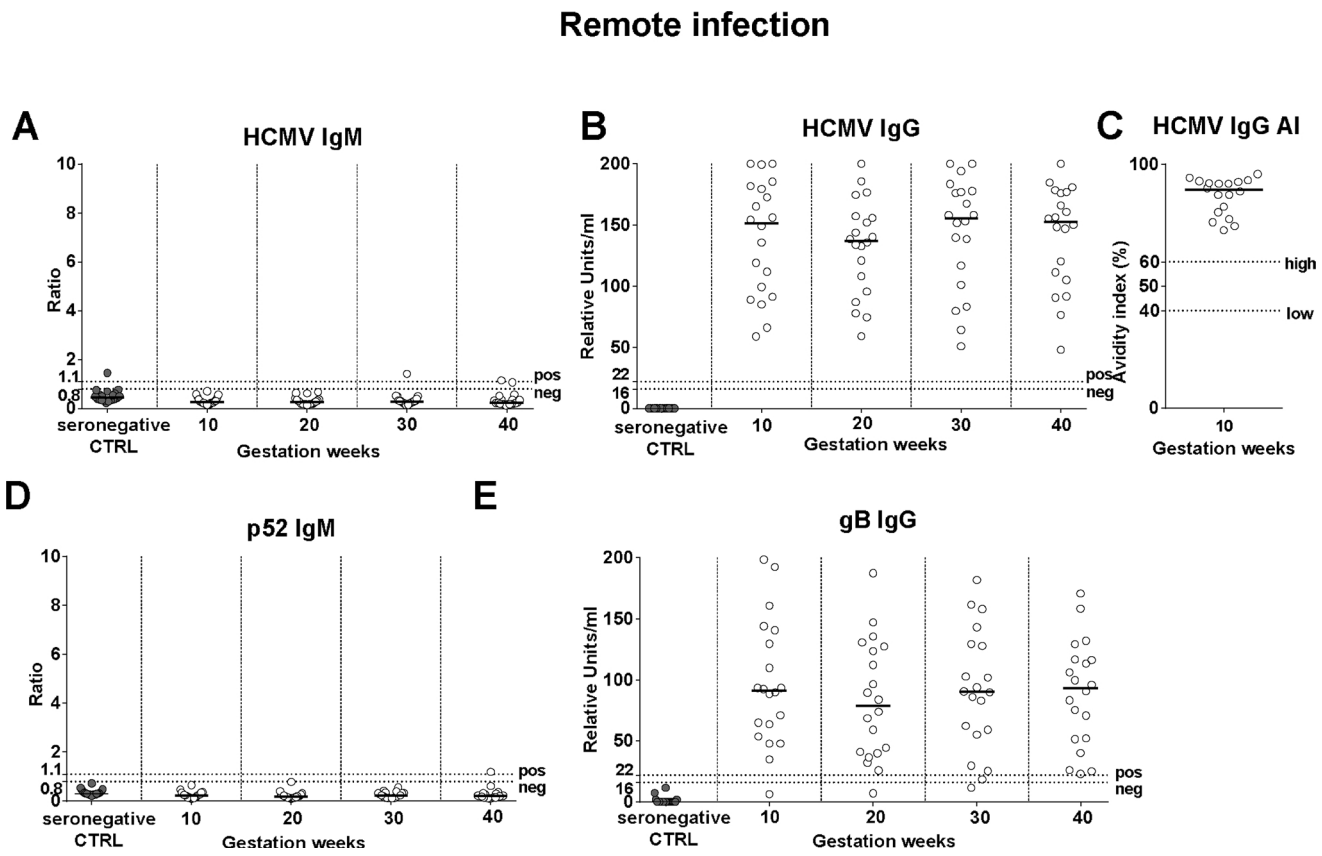


**Fig. 1.** Levels of (A) IgM, (B) IgG and (C) IgG avidity index (AI) by conventional ELISA using whole HCMV lysate and (D) IgM and (E) IgG by novel ELISA using recombinant p52 (IgM) or gB (IgG) in 20 HCMV seropositive pregnant women at different gestation weeks and 20 HCMV-seronegative pregnant women (CTRL).

primary infection (Fig. 2E and Fig. S1E): only 13/54 samples (24%; $p < 0.01$ *vs* anti−HCMV IgG) collected within the first 60 days were positive, whereas all samples collected after 180 days were positive.

Cases with a typical antibody response are shown in Fig. 3. In p#016 (Fig. 3A), anti−HCMV IgG remained stable while IgM decreased rapidly: anti-p52 IgM were negative 3 months after infection while anti−HCMV IgM became negative later. Anti-gB IgG and anti−HCMV IgG AI followed similar kinetics, becoming either positive or

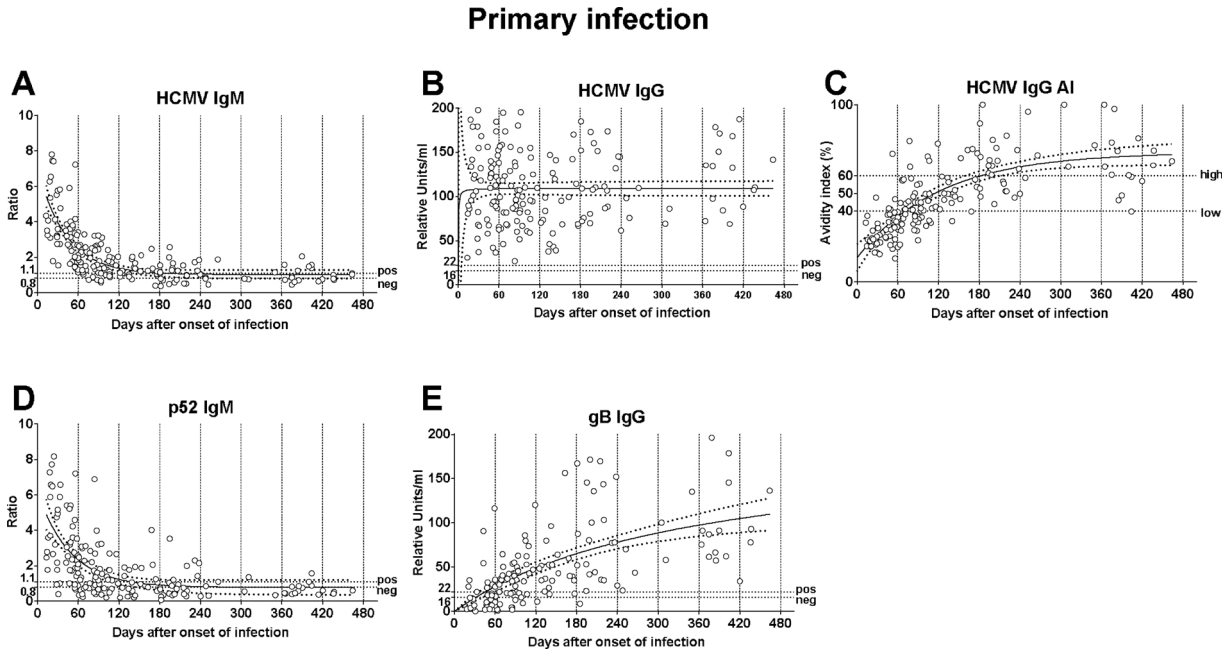


**Fig. 2.** Kinetics of antibody levels in 40 pregnant women with mildly symptomatic HCMV primary infection. (A) IgM, (B) IgG, (C) IgG avidity index (AI) determined by conventional ELISA using whole HCMV lysate; (D) IgM, (E) IgG determined by novel ELISA using recombinant p52 (IgM) or gB (IgG). Regression curves (solid lines) are shown with their 95% confidence interval (dotted lines).

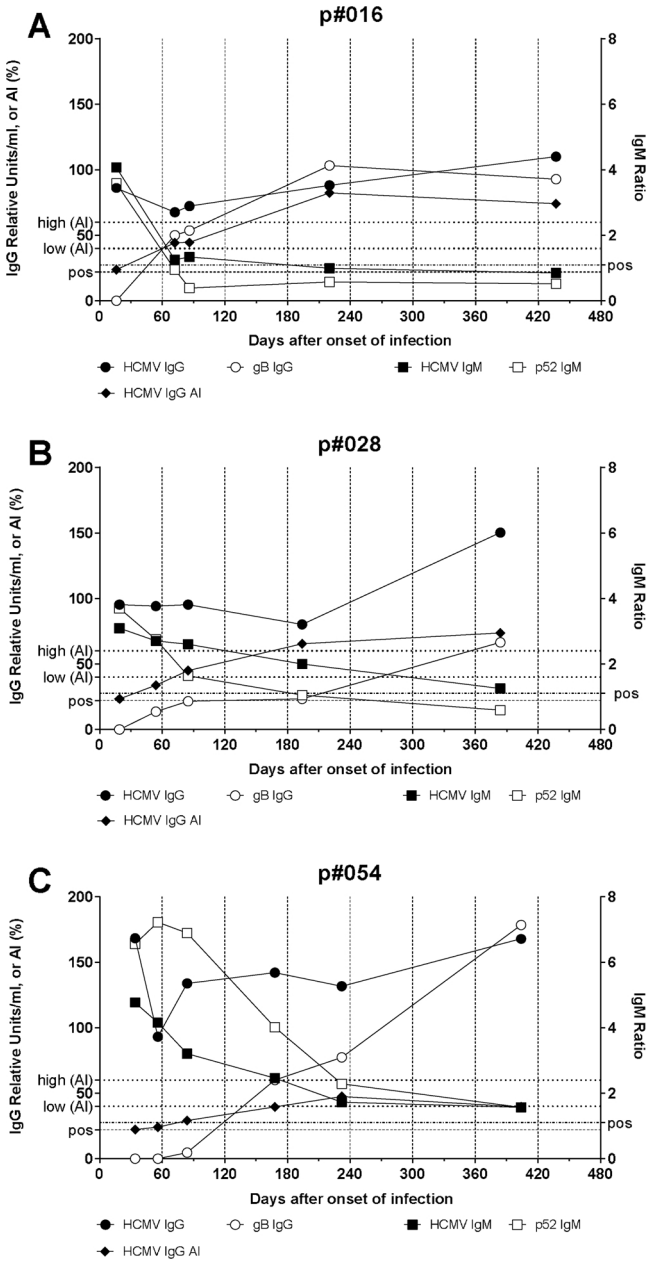


**Fig. 3.** Kinetics of antibody levels in individual patients. In p#016 (A) both anti – HCMV and anti-p52 IgM become negative at early time points, whereas in p#028 (B) anti – HCMV IgM only and in p#054 (C) both IgM are still detectable one year after infection. AI, Avidity Index.

intermediate 2 months after infection and increasing until 7 months after infection. In p#028 (Fig. 3B), anti – HCMV IgG increased after 6 months, while IgM decreased more slowly than in p#016: anti-p52 IgM became negative 6 months after infection, while anti – HCMV IgM were still positive at a low level one year after infection. Anti-gB IgG were borderline 3 months after infection and became positive at 6 months, then increased until one year. Finally, in p#054 (Fig. 3C), HCMV IgG dipped before returning to levels observed in the first sample, while both HCMV and p52 IgM decreased at a very slow rate and were both still positive one year after infection. Also IgG AI showed slow kinetics, remaining at low or intermediate levels 6–12 months after infection. Conversely, anti-gB IgG increased more sharply. They were positive at 6 months after infection and continued to increase until one year.

Anti-p52 IgM appear more suitable than HCMV IgM for defining the early phase of infection, while gB IgG generally increase over time in a similar way to HCMV IgG AI. HCMV IgG (already positive in the first samples tested) does not seem to be helpful in determining the infection phase.

### *4.3. Combination of conventional HCMV IgM and HCMV IgG AI assays or novel p52 IgM and gB IgG assays to detect different stages of HCMV primary infection*

In an attempt to determine three distinct stages of HCMV infection (early, intermediate and late), results of conventional and novel ELISA were combined. Samples with borderline results were excluded from this analysis.

By using conventional HCMV IgM and HCMV IgG AI assays, the three stages were defined as follows: i) early: positive IgM and low AI; ii) intermediate: positive IgM and intermediate/high AI or negative IgM and low AI; and iii) late: negative IgM and intermediate/high AI. Samples with assay results corresponding to each of the three combinations were collected at three time intervals from onset of infection. The $10^{th}$ to $90^{th}$ percentiles of the time intervals for each result combination were calculated.

According to conventional assays, the time intervals of the three infection stages were as follows: early, within 3 months after onset of infection; intermediate, 2–10 months after onset; and late, > 3 months after onset (Fig. 4A).

By using novel p52 IgM and gB IgG, the three stages were defined as follows: i) early, *i.e.* positive IgM and negative IgG: within 2 months after onset of infection; ii) intermediate, *i.e.* positive IgM and positive IgG or negative IgM and negative IgG: 1.5–8 months after onset (it is noteworthy that in 7 samples from 4 patients, p52 IgM were already negative and gB IgG not yet positive); and iii) late, *i.e.* negative IgM and positive IgG: > 2.5 months after onset (Fig. 4B).

Finally, when the results of the two algorithms were combined and novel ELISA confirmed results of conventional ELISA (*i.e.* the two algorithms were concordant in defining the three phases, as observed in 84/136 -62%- samples), results were as follows: i) early: within 2.5 months; ii) intermediate: 2–8 months after onset; and iii) late: > 3 months after onset (Fig. 4C).

Confirming conventional ELISA results with novel ELISA may improve dating of infection by reducing the number of outliers. However, while the early and late stages are well-defined, the intermediate stage overlaps with the beginning of the late stage.

## 5. Discussion

We analyzed the kinetics of p52 IgM and gB IgG after HCMV primary infection by novel ELISA based on recombinant antigens, compared with conventional ELISA for detection of HCMV IgM, IgG and IgG avidity (using whole HCMV lysate). The aim was to define a diagnostic strategy to detect different phases of HCMV primary infection during pregnancy.

It is well-known that detection of HCMV IgM *per se* is not sufficient to diagnose an acute primary infection. The HCMV IgM may be detected also in subjects with past infection as a consequence of HCMV reactivation/reinfection or cross-reactivity or for other reasons. Moreover, HCMV IgM may persist at low levels for months [8,17]. Although p52 and HCMV IgM followed similar kinetics, p52 IgM persistence was shorter, becoming borderline/undetectable earlier than the HCMV IgM. One drawback of this is that in some cases (15% of samples), p52 IgM may already be negative in the first two months after primary infection (when HCMV IgM are usually still positive).

In this study, HCMV IgG kinetics were not helpful in defining the onset of primary infection. On the contrary, gB IgG, which increased slowly over time, appeared more useful in dating the onset of primary infection. The kinetics of gB IgG observed in this study differ from the those of gB IgG observed in the same population using an ELISA developed in-house. The latter, also based on the gB ectodomain [18],

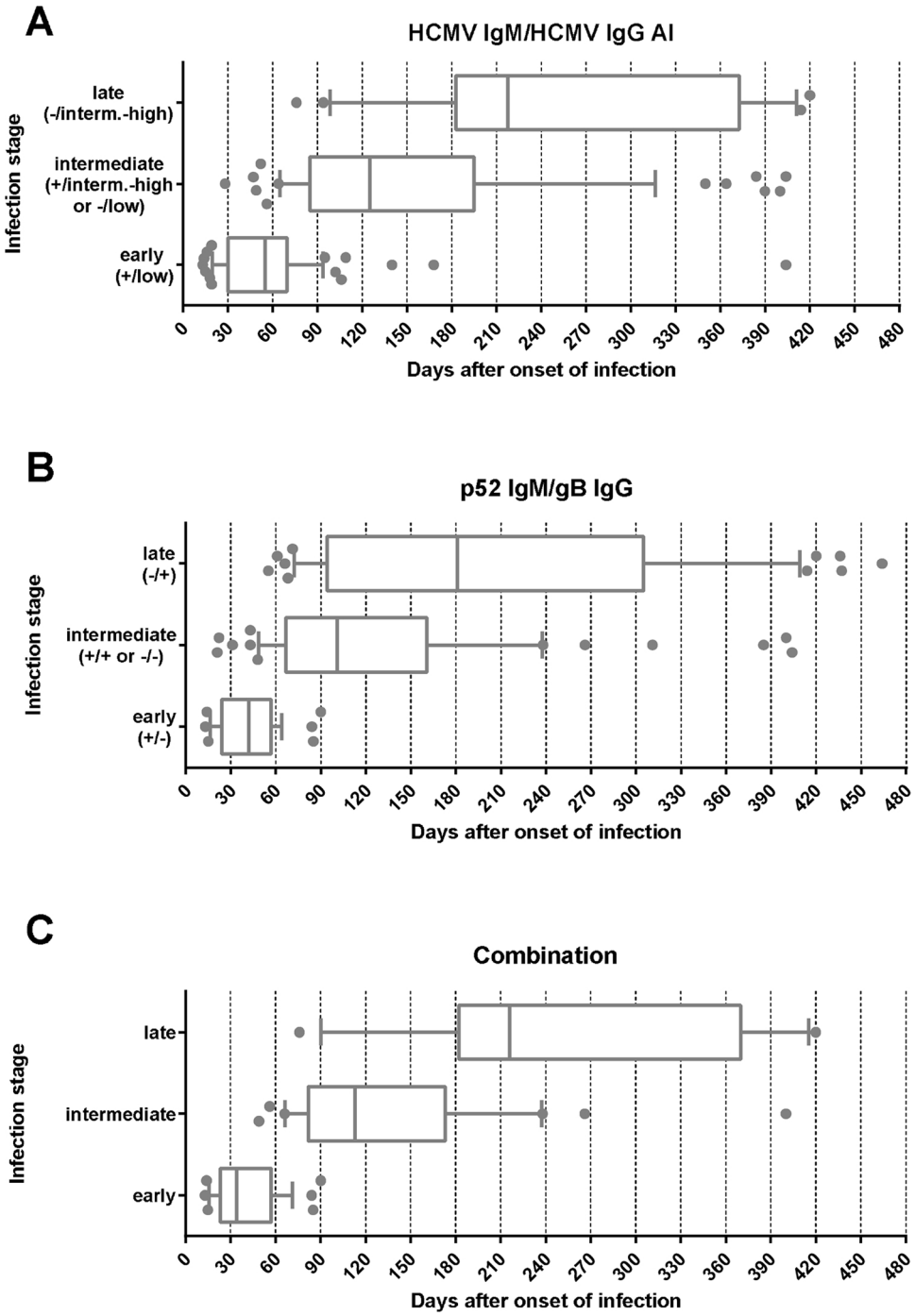


**Fig. 4.** Definition of three time intervals (early, intermediate and late) after HCMV primary infection according to timing algorithms based on (A) anti − HCMV IgM and anti − HCMV IgG avidity index (AI), (B) anti-p52 IgM and anti-gB, (C) combination of results of the two algorithms (*i.e.* the two algorithms were concordant in defining the three phases, as observed in 84/136 -62%- samples). Boxes represent interquartile time (with median) and whiskers represent the $10^{th}$ and $90^{th}$ percentile time interval. Grey dots represent outliers.

showed the early appearance of gB IgG [13]. In the Euroimmun assay, gB is directly attached to the solid phase, whereas in the in-house assay, gB is captured by a specific monoclonal antibody. Thus, the structure of gB (and the epitopes exposed) might be different in the two assays. However, the fact that the Euroimmun gB IgG antibody assay was mostly negative in the first two months after infection, and was found to be negative also in some otherwise seropositive controls, would indicate that it is unsuitable for determining HCMV serostatus but may be considered a second-line assay.

The combination of conventional anti − HCMV IgM with IgG AI, or novel p52 IgM with the anti-gB IgG was useful in defining three stages of primary infection (early, intermediate and late). According to the $10^{th}$-$90^{th}$ percentiles of their relevant time intervals, the early stage (mostly within 2 months after infection) and the late stage (after 6–12 months) were clearly identified, whereas the intermediate stage partly overlapped with the late stage (mostly within 3–6 months). This overlap was also observed with other diagnostic or in-house assays [13].

However, outliers were observed due to IgM persistence and/or slow maturation of IgG AI found in some individuals. This suggests that

some later infections should actually be defined as earlier, whereas a rapid maturation of IgG AI may mean that some earlier infections should actually be considered as intermediate or late. Bearing this in mind, dating of primary HCMV infection should not be based on the evaluation of a single sample, but on the analysis of the kinetics of different serological parameters as determined from sequential blood samples [8].

Novel second-line serological assays, such as the novel ELISA with recombinant antigens, (or the ELISA for HCMV glycoprotein complexes or neutralizing antibody assays [13]), provide additional tools to confirm or re-evaluate results obtained with conventional assays. Although the numbers here are too small to draw general conclusions, we observed that by using novel ELISA to confirm the dating of primary infection provided by classical assays, we reduced the overlap between the infection stages as well as the number of outliers indicating a late infection as early or *vice versa*.

When results are discordant, additional analyses, such as determination of HCMV DNA in blood and assessment of HCMV-specific T-cell immunity, could help in defining infection stage [19,20].

In conclusion, p52 IgM and gB IgG assays provide additional information in relation to dating the onset of primary infection in pregnancy. The results of this study indicate that the combination of novel and conventional ELISA helps to differentiate stages of primary HCMV infection.

## Funding

This work was supported by the Ministero della Salute, Fondazione IRCCS Policlinico San Matteo, Ricerca Corrente (grant numbers 8053615 and8069614). Assay kits were provided free of charge by Euroimmun AG.

## Ethical statement

All participants gave their written informed consent for the use of their stored samples for studies related to HCMV infection.

## Author contribution

**Paola Zelini**: performed tests, and analyzed data; **Chiara Fornara:** collected samples and data; **Milena Furione and Antonella Sarasini**: performed diagnosis and timing of primary infection and revised the manuscript; **Julia Klemens**: designed the study and revised the manuscript; **Alessia Arossa and Arsenio Spinillo**: enrolled the patients; **Giuseppe Gerna**: revised the manuscript; **Daniele Lilleri**: designed the study, analyzed data and wrote the manuscript.

## Declaration of Competing Interest

Julia Klemens is employee of Euroimmun. The other authors declare no conflict of interest.

## Acknowledgments

We thank Valentina Brambati for technical assistance, Daniela Sartori for editing the manuscript, and Sheila McVeigh for revision of the English.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.09.006.

## References

[1] M.J. Cannon, K.F. Davis, Washing our hands of the congenital cytomegalovirus disease epidemic, BMC Public Health 5 (2005) 70, https://doi.org/10.1186/1471-2458-5-70.
[2] A. Kenneson, M.J. Cannon, Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection, Rev. Med. Virol. 17 (2007) 253–276, https://doi.org/10.1002/rmv.535.
[3] M.G. Revello, E. Fabbri, M. Furione, M. Zavattoni, D. Lilleri, B. Tassis, Role of prenatal diagnosis and counseling in the management of 735 pregnancies complicated by primary human cytomegalovirus infection: a 20-year experience, J. Clin. Virol. 50 (2011) 303–307, https://doi.org/10.1016/j.jcv.2010.12.012.
[4] K.B. Fowler, S. Stagno, R.F. Pass, Maternal immunity and prevention of congenital cytomegalovirus infection, JAMA 289 (2003) 1008–1011, https://doi.org/10.1001/jama.289.8.1008.
[5] R.F. Pass, K.B. Fowler, S.B. Boppana, W.J. Britt, S. Stagno, Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome, J. Clin. Virol. 35 (2006) 216–220, https://doi.org/10.1016/j.jcv.2005.09.015.
[6] O. Picone, C. Vauloup-Fellous, A.G. Cordier, S. Guitton, M.V. Senat, F. Fuchs, A series of 238 cytomegalovirus primary infections during pregnancy: description and outcome, Prenat. Diagn. 33 (2013) 751–758, https://doi.org/10.1002/pd.4118.
[7] M. Zavattoni, M. Rustico, B. Tassis, G. Lombardi, M. Furione, A. Piralla, Risk of congenital disease in 46 infected fetuses according to gestational age of primary human cytomegalovirus infection in the mother, J. Med. Virol. 88 (2016) 120–126, https://doi.org/10.1002/jmv.24313.
[8] M.G. Revello, G. Gerna, Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant, Clin. Microbiol. Rev. 15 (2002) 680–715, https://doi.org/10.1128/CMR.15.4.680-715.2002.
[9] M. Bodéus, S. Feyder, P. Goubau, Avidity of IgG antibodies distinguishes primary from non-primary cytomegalovirus infection in pregnant women, Clin. Diagn. Virol. 9 (1998) 9–16.
[10] K. Schoppel, B. Kropff, C. Schmidt, R. Vornhagen, M. Mach, The humoral immune response against human cytomegalovirus is characterized by a delayed synthesis of glycoprotein-specific antibodies, J. Infect. Dis. 175 (1997) 533–544.
[11] M. Rothe, S. Pepperl-Klindworth, D. Lang, R. Vornhagen, W. Hinderer, K. Weise, H.H. Sonneborn, B. Plachter, An antigen fragment encompassing the AD2 domains of glycoprotein B from two different strains is sufficient for differentiation of primary vs. Recurrent human cytomegalovirus infection by ELISA, J. Med. Virol. 65 (2001) 719–729.
[12] R. Vornhagen, B. Plachter, W. Hinderer, T.H. The, J. Van Zanten, L. Matter, C.A. Schmidt, H.H. Sonneborn, G. Jahn, Early serodiagnosis of acute human cytomegalovirus infection by enzyme-linked immunosorbent assay using recombinant antigens, J. Clin. Microbiol. 32 (1994) 981–986.
[13] D. Lilleri, G. Gerna, M. Furione, M. Zavattoni, A. Spinillo, Neutralizing and ELISA IgG antibodies to human cytomegalovirus glycoprotein complexes may help date the onset of primary infection in pregnancy, J. Clin. Virol. 81 (2016) 16–24, https://doi.org/10.1016/j.jcv.2016.05.007.
[14] G. Gerna, P. Vitulo, F. Rovida, D. Lilleri, C. Pellegrini, T. Oggionni, Impact of human metapneumovirus and human cytomegalovirus versus other respiratory viruses on the lower respiratory tract infections of lung transplant recipients, J. Med. Virol. 78 (3) (2006) 408–416, https://doi.org/10.1002/jmv.20555 Erratum in: J Med Virol. 80 (10) (2008) 1869.
[15] M.G. Revello, E. Percivalle, M. Zannino, V. Rossi, G. Gerna, Development and evaluation of a capture ELISA for IgM antibody to the human cytomegalovirus major DNA binding protein, J. Virol. Methods 35 (3) (1991) 315–329, https://doi.org/10.1016/0166-0934(91)90073-9.
[16] M.G. Revello, E. Genini, G. Gorini, C. Klersy, A. Piralla, G. Gerna, Comparative evaluation of eight commercial human cytomegalovirus IgG avidity assays, J. Clin. Virol. 48 (4) (2010) 255–259, https://doi.org/10.1016/j.jcv.2010.05.004.
[17] T. Lazzarotto, B. Guerra, L. Gabrielli, M. Lanari, M.P. Landini, Update on the prevention, diagnosis and management of cytomegalovirus infection during pregnancy, Clin. Microbiol. Infect. 17 (9) (2011) 1285–1293, https://doi.org/10.1111/j.1469-0691.2011.03564.x.
[18] C. Carlson, W.J. Britt, T. Compton, Expression, purification, and characterization of a soluble form of human cytomegalovirus glycoprotein B, Virology 239 (1) (1997) 198–205, https://doi.org/10.1006/viro.1997.8892.
[19] M.G. Revello, M. Furione, V. Rognoni, A. Arossa, G. Gerna, Cytomegalovirus DNAemia in pregnant women, J. Clin. Virol. 61 (4) (2014) 590–592, https://doi.org/10.1016/j.jcv.2014.10.002.
[20] C. Fornara, M. Furione, A. Arossa, G. Gerna, D. Lilleri, Comparative magnitude and kinetics of human cytomegalovirus-specific $CD4^+$ and $CD8^+$ T-cell responses in pregnant women with primary versus remote infection and in transmitting versus non-transmitting mothers: its utility for dating primary infection in pregnancy, J. Med. Virol. 88 (7) (2016) 1238–1246, https://doi.org/10.1002/jmv.24449.