



**HAL**  
open science

# Positive predictive values of CMV-IgM and importance of CMV-IgG avidity testing in detecting primary infection in three different clinical settings. A French retrospective cohort study

Claire Périllaud-Dubois, Elise Bouthry, Abir Jadoui, Ay-Ling Leng, Anne-Marie Roque-Afonso, Christelle Vauloup-Fellous

## ► To cite this version:

Claire Périllaud-Dubois, Elise Bouthry, Abir Jadoui, Ay-Ling Leng, Anne-Marie Roque-Afonso, et al.. Positive predictive values of CMV-IgM and importance of CMV-IgG avidity testing in detecting primary infection in three different clinical settings. A French retrospective cohort study. *Journal of Clinical Virology*, 2020, 132, pp.104641. 10.1016/j.jcv.2020.104641 . inserm-03227598v2

**HAL Id: inserm-03227598**

**<https://inserm.hal.science/inserm-03227598v2>**

Submitted on 25 May 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Manuscript Number: JCV-D-20-01378R1

Title: Positive predictive values of CMV-IgM and importance of CMV-IgG avidity testing in detecting primary infection in three different clinical settings. A French retrospective cohort study.

Article Type: Full length article

Keywords: CMV; serology; IgM; avidity; positive predictive value; pregnancy

Corresponding Author: Dr. Claire Périllaud Dubois, PharmD

Corresponding Author's Institution: Paul Brousse Hospital

First Author: Claire Périllaud Dubois, PharmD

Order of Authors: Claire Périllaud Dubois, PharmD; Elise Bouthry; Abir Jadoui; Ay-Ling Leng; Anne-Marie Roque-Afonso; Christelle Vauloup-Fellous

Abstract: Background: Diagnosis of Cytomegalovirus (CMV) primary infection during pregnancy or in immunocompetent patients relies on serology with detection of specific CMV-IgG and IgM. In case of positive CMV-IgM in pregnant women, CMV-IgG avidity is now widely recommended, but in general population it is not currently performed.

Objective: In this study, we aimed to determine CMV-IgM positive predictive values (PPV) in different clinical settings.

Material and methods: We conducted a retrospective study on positive CMV-IgM in our virology laboratory from 2013 to 2019, in three clinical groups: screening in non-symptomatic pregnant women (group 1), pregnant women with ultrasound (US) abnormalities (group 2) and patients (general population) with clinical signs suggestive of CMV primary infection (group 3). CMV-IgG avidity had been performed in all cases allowing to evaluate PPV of positive CMV-IgM to diagnose CMV primary-infection in each group.

Results: Between 2013 and 2019, 6,859 serum samples were found positive for CMV-IgM and had been tested for CMV-IgG avidity, with 6,560 sera for group 1, 30 for group 2 and 269 for group 3. Overall, low avidity confirming primary infection was observed respectively in 16.4% for group 1, 36.7% for group 2, and 35.3% for group 3. CMV-IgM PPV was significantly lower in group 1 compared to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

Discussion: Our observations highlight the major importance of including CMV-IgG avidity in the diagnostic algorithm, whatever the clinical situation (for immunocompetent patients), to confirm or exclude a recent CMV primary infection in case of positive CMV-IgM.



**Ms. Ref. No.: JCV-D-20-01378**

**Title: Positive predictive values of CMV-IgM to detect primary infection in three different clinical settings. A French retrospective cohort study.**

**Journal of Clinical Virology**

*Highlights (mandatory)*

*Highlights consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See the following website for more information*

<https://clicktime.symantec.com/3SqMupnFFd243aiwUqgdd6H2?u=http%3A%2F%2Fwww.elsevier.com%2Fhighlights>

- In case of positive CMV-IgM, CMV-IgG avidity is essential to diagnose recent primary infection.
- Positive predictive value of CMV-IgM is 16.4% if systematic screening during pregnancy.
- Positive predictive value of CMV-IgM is 36.7% in case of US abnormalities during pregnancy.
- Positive predictive value of CMV-IgM is 35.3% in case of clinical signs in general population.

*Reviewer #1: The authors evaluate the predictive utility of anti-CMV IgG avidity testing retrospectively in three clinical settings. (1) non-symptomatic pregnant women, (2) pregnant women with ultrasound anomalies and (3) general population with clinical signs suggestive of CMV.*

*The manuscript is well written and coherent and the difficulties in serological diagnoses of recent CMV infection and the impact on, and distress it can cause, during pregnancy is already documented in the literature; this manuscript adds the utility of testing in the general population with clinical symptoms as a comparator.*

**Minor comments:**

*There is no other diagnosis of recent CMV infection or outcome of pregnancy information detailed, how the assay changed over time (if at all) is not commented upon, was a control / reference included to ensure longitudinal comparability within the laboratory?*

*Outcome of pregnancy was not always investigated in our population and correlation between gestational age at CMV infection and outcome has already been widely described.*

*All laboratories in France are subjected national accreditation (ISO 15189 - COFRAC), which implies the use of CE approved assays and annual subscription (and success) to external quality controls. This ensures optimal longitudinal confidence in their results. Moreover, in the past seven years, no major innovation occurred in routine serologic diagnosis of CMV infection and assays used have well-known performances. Assays' references are not mentioned in the manuscript but are available.*

*Line 32: Is further information or review of the sonographic findings to establish consistency with CMV infection?*

*We detailed briefly US abnormalities in the "Material and methods section". In the modified version, we added more details on cerebral US findings. **Lines 87-93:***

*"head perimeter < 5th centile, ventriculomegaly, hyperechogenic ventricular wall, cerebellar and brain calcifications, enlargement of pericerebral spaces, periventricular and subependymal cysts, candlestick, porencephaly, hyperechogenic periventricular halo, candlestick, abnormal gyration, corpus callosum hypoplasia."*

*Line 33: Can the clinical symptoms suggestive of CMV infection for group 3 patients be listed or described, also as there is information on the clinical symptoms is it possible to estimate the time post-infection the samples were taken?*

These information were added in the text. **Lines 95-97:**

**“Mainly fever, headache, flu-like syndrome (fever + rhinitis + myalgia), arthralgia, myalgia, fatigue and/or hepatitis. Samples were collected 5 to 30 days after onset of symptoms.”**

*Line 198-199: The algorithm predicted 7/30 primary CMV infections and 3/30 had high avidity but gave birth to an infected child. Is this a reflection on the testing or the progression of infection dependent upon the gestational age?*

Indeed, US abnormalities may be observed late in pregnancy (explaining high avidity at that time) but result of an infection in the first trimester.

*Were any of group 1 neonates infected?*

For this study this information was not systematically collected and is therefore not available.

*The authors comment on the age variations for the general population group 3, how does this relate to the ages in the other groups, was any age stratification done for groups 1 and 2?*

Both other groups only include pregnant women between 17 and 46 years old. Age stratifications were therefore not relevant for these groups.

*For Figure 2 please convert commas to decimal points.*

We converted commas to decimal points as required.

*Reviewer #4: The authors retrospectively analyzed results of 6857 serum samples that tested positive for CMV IgM. The samples were divided into three groups: 6560 samples from routine screening of pregnant women (group 1), 30 pregnant women with abnormalities observed on ultrasound (group 2), and 297 samples from non-pregnant immunocompetent individuals showing signs of CMV infection (group 3). Using CMV IgG avidity testing to follow-up all of the CMV IgM-positive samples and basing all conclusions on the avidity testing result, recent CMV infection was confirmed in only 16.5% of group 1, 36.7% of group 2, and 35.3% of group 3. The authors strongly support the concept that all CMV IgM-positive samples must be tested by CMV IgG avidity in order to determine the true CMV infection status.*

**Major Comments:**

1. *Adding mention of CMV IgG avidity testing in the title of this paper would more effectively represent the study. Perhaps something like this "Positive predictive values of CMV-IgM and importance of CMV IgG avidity testing in detecting primary infection in three different clinical settings, A French retrospective cohort study."*

We thank the reviewer for his interesting comment and changed the title as suggested.

**Lines 1-2:**

“Positive predictive values of CMV-IgM and importance of CMV IgG avidity testing in detecting primary infection in three different clinical settings. A French retrospective cohort study.”

2. In the Materials and Methods Section:

a. Line 78: "CMV serology (IgG, IgM+/-IgG avidity)" is noted. The "IgM+/-IgG avidity" notation is confusing. Clarify when IgG avidity testing is done.

We clarified our purpose. CMV IgG avidity is performed in case of positive IgG. Actually, IgG avidity could not be performed if no CMV IgG.

**Line 79:**

“CMV serology (IgG, IgM and IgG avidity in case of positive IgG)”

b. Line 86: Give a few examples of the "symptoms suggestive of CMV primary infection" that were used to select patients for this group.

These information were added in the text:

**Lines 93-95:**

“Mainly fever, headache, flu-like syndrome (fever + rhinitis + myalgia), arthralgia, myalgia, fatigue and/or hepatitis.”

c. Line 95-96: It appears that low avidity values obtained with the LXL as a screening test were then confirmed by performing the VIDAS avidity test. Based on this, the reader assumes that two avidity tests are needed. Explain this further. Why were the two avidity tests needed? Also, discuss discrepancies between the two avidity tests. This is the only place in the manuscript that the dual avidity testing is mentioned. More information is definitely needed in order to give the reader the full picture of what is involved with avidity testing in the authors' algorithm and would be of considerable importance to someone considering adding CMV-IgG avidity testing.

Our strategy is based on previous published findings (C. Vauloup-Fellous, M. Berth, F. Heskia, J.-M. Dugua, et L. Grangeot-Keros, Re-evaluation of the VIDAS® cytomegalovirus (CMV) IgG avidity assay: Determination of new cut-off values based on the study of kinetics of CMV-IgG maturation, *J. Clin. Virol.* Ref 19). Two avidity tests are useful only if the first screening test gives a result below 0.4. In that case, confirmation/exclusion of primary infection is only based on the VIDAS assay. This strategy is now detailed in the manuscript.

**Lines 102-117.**

d. Line 99: Indicate how far apart the two consecutive samples were to be collected.

**Line 110:**

“Negative CMV-IgG/positive CMV-IgM on two consecutive samples 21 days apart”

e. Line 104: Add the information expected for the CMV-IgG avidity test for this profile.

Added in the text. **Lines 116-117**

2. Line 112: A total of 6,857 serum samples is shown. However, Figure 1 shows 6,859 samples. Reconcile these numbers.

We corrected in abstract and in the text: 6859

**Line 37 and line 124:**

3. *Add to the limitations of the study: There are known to be technical issues with IgM-specific testing. This should be mentioned. It is possible that some of the CMV-IgM positive results were due simply to false positives. This has been shown to occur due to high levels of analyte specific IgG, to presence of rheumatoid factors, and to other miscellaneous technical factors involved with methods that must include some sort of process to separate CMV IgG from CMV IgM. Manufacturers' stated sensitivity and specificity for CMV IgM detection may not be truly representative. Likewise, the avidity tests are not likely 100% sensitive or 100% specific.*

We added this suggestion in discussion. **Lines 178-182:**

“Moreover, there are known to be technical issues with IgM-specific testing. Indeed, false positive CMV-IgM results were shown to occur due to high levels of analyte specific IgG, to presence of rheumatoid factors, and to other miscellaneous technical factors involved with methods that must include some sort of process to separate CMV IgG from CMV IgM.”

**Minor Comments:**

1. *Lines 2-5 of the Abstract. These sentences are awkward and a bit confusing. Revise for clarity.*

**Lines 26-28:**

Sentences revised

2. *Line 46: Replace the word "first" with "most frequent"*

**Line 47:**

We changed with “most frequent” as suggested.

3. *Line 56: Change "of" to "for"*

**Line 57:**

We corrected with “for”.

4. *Line 68: Change "a" to "at"*

**Line 69:**

We corrected with “at”.

5. *Lines 199-203: Overall, when positive CMV-IgM is detected at that moment (clarify: does this mean late in pregnancy?). Also, change the word "strengths" to "suggests".*

We made the following changes in the text:

**Lines 215-216:**

“Overall, when positive CMV-IgM is detected when US abnormalities are observed (possibly late in pregnancy), it suggests responsibility of CMV in 47% (14/30) cases without ruling out the possibility of CMV congenital infection following maternal non primary infection if the mother is CMV-IgG positive and CMV-IgM is negative.”

6. *Line 207: Change "detection of CMV-IgM is" to "positive CMV-IgM results are" and in line 208 change "case of clinical" to "cases with clinical"*

**Lines 223-224:**

“Remarkably, positive CMV-IgM results are most often considered as indicative of a recent CMV virus infection in case with clinical signs”

7. *Lines 212-216: Divide this lengthy sentence by inserting a period in Line 214 after the references shown [20,21]. Then change "and that is case" to "Also, in cases"*

We made the changes as suggested.

**Lines 230-231:**

“Our results suggest that positive CMV-IgM is less correlated with CMV recent primary infection in young people, and are consistent with the understanding that CMV-IgM can be produced throughout life as a result of CMV non primary infection or polyclonal stimulation of the immune system [20,21]. Also, in cases of positive CMV-IgM, older people may be more likely to have a primary infection than younger people.”

8. *Line 217: Change "Some limitations of our analysis is" to "One limitation of our study is" and in line 218 change "require to come to hospital" to "require the patient to come to the hospital"*

We modified this sentence as suggested.

**Lines 233-234:**

“One limitation of our study is that, although very frequent, CMV primary infection is most often a mild infection that does not require the patient to come to the hospital.”

9. *Line 220: Change "severest symptomatic cases come to hospital and are therefore" to "The patients with the severest symptoms come to the hospital and are the type"*

We modified as suggested.

**Lines 235-236:**

“Consequently, diagnosis of CMV primary infection is probably mostly done by general practitioners and only the patients with the severest symptoms come to the hospital and are therefore included in our data.”

10. *Figure 2 legend: (A), (B), (C), and (D) are mentioned in the legend. However, these markings were not found in the Figure. Check this out.*

We corrected this in the Figure 2.

11. *If possible, check the entire manuscript for errors in English grammar and punctuation.*

We made corrections **Line 55** (peri-conceptionnal) and **Line 175** (CMV primary infection).



Positive predictive values of CMV-IgM and importance of CMV-IgG avidity testing in detecting primary infection in three different clinical settings. A French retrospective cohort study.

Claire Périllaud-Dubois <sup>1,2,3,4</sup>, Elise Bouthry <sup>1,2</sup>, Abir Jadoui <sup>1</sup>, Ay-Ling Leng <sup>1</sup>, Anne-Marie Roque-Afonso <sup>1,4,5</sup>, Christelle Vauloup-Fellous <sup>1,2,4,5</sup>

<sup>1</sup> AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie, , 94804 Villejuif, France

<sup>2</sup> Groupe de Recherche sur les Infections pendant la Grossesse (GRIG), France

<sup>3</sup> INSERM UMR1137, IAME, 75018 Paris, France

<sup>4</sup> Université Paris-Saclay, 94804 Villejuif, France

<sup>5</sup> INSERM U1193, 94804 Villejuif, France

Corresponding author: Dr Claire Périllaud-Dubois

AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie

12-14 avenue Paul Vaillant Couturier

94804 Villejuif, France

+33145593721

claire.perillaud-dubois@inserm.fr

Keywords: CMV; serology; IgM; avidity; PPV; positive predictive value; pregnancy; ultrasound abnormalities; clinical signs

Word count: 2443

## Abstract

Background: Diagnosis of Cytomegalovirus (CMV) primary infection during pregnancy or in immunocompetent patients relies on serology with detection of specific CMV-IgG and IgM. In case of positive CMV-IgM in pregnant women, CMV-IgG avidity is now widely recommended, but in general population it is not currently performed.

Objective: In this study, we aimed to determine CMV-IgM positive predictive values (PPV) in different clinical settings.

Material and methods: We conducted a retrospective study on positive CMV-IgM in our virology laboratory from 2013 to 2019, in three clinical groups: screening in non-symptomatic pregnant women (group 1), pregnant women with ultrasound (US) abnormalities (group 2) and patients (general population) with clinical signs suggestive of CMV primary infection (group 3). CMV-IgG avidity had been performed in all cases allowing to evaluate PPV of positive CMV-IgM to diagnose CMV primary-infection in each group.

Results: Between 2013 and 2019, 6,859 serum samples were found positive for CMV-IgM and had been tested for CMV-IgG avidity, with 6,560 sera for group 1, 30 for group 2 and 269 for group 3. Overall, low avidity confirming primary infection was observed respectively in 16.4% for group 1, 36.7% for group 2, and 35.3% for group 3. CMV-IgM PPV was significantly lower in group 1 compared to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

Discussion: Our observations highlight the major importance of including CMV-IgG avidity in the diagnostic algorithm, whatever the clinical situation (for immunocompetent patients), to confirm or exclude a recent CMV primary infection in case of positive CMV-IgM.

Word count: 247

*Highlights:*

- In case of positive CMV-IgM, CMV-IgG avidity is essential to diagnose recent primary infection.
- Positive predictive value of CMV-IgM is 16.4% if systematic screening during pregnancy.
- Positive predictive value of CMV-IgM is 36.7% in case of US abnormalities during pregnancy.
- Positive predictive value of CMV-IgM is 35.3% in case of clinical signs in general population.

1 Positive predictive values of CMV-IgM and importance of CMV-IgG avidity  
2 testing in detecting primary infection in three different clinical settings. A  
3 French retrospective cohort study.

4

5 Claire Périllaud-Dubois <sup>1,2,3,4</sup>, Elise Bouthry <sup>1,2</sup>, Abir Jadoui <sup>1</sup>, Ay-Ling Leng <sup>1</sup>, Anne-Marie Roque-  
6 Afonso <sup>1,4,5</sup>, Christelle Vauloup-Fellous <sup>1,2,4,5</sup>

7

8 <sup>1</sup> AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie, , 94804 Villejuif, France

9 <sup>2</sup> Groupe de Recherche sur les Infections pendant la Grossesse (GRIG), France

10 <sup>3</sup> INSERM UMR1137, IAME, 75018 Paris, France

11 <sup>4</sup> Université Paris-Saclay, 94804 Villejuif, France

12 <sup>5</sup> INSERM U1193, 94804 Villejuif, France

13

14 Corresponding author: Dr Claire Périllaud-Dubois

15 AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie

16 12-14 avenue Paul Vaillant Couturier

17 94804 Villejuif, France

18 +33145593721

19 claire.perillaud-dubois@inserm.fr

20 Keywords: CMV; serology; IgM; avidity; PPV; positive predictive value; pregnancy; ultrasound  
21 abnormalities; clinical signs

22 Word count: 2443

23

24 **Abstract**

25 Background: Diagnosis of Cytomegalovirus (CMV) primary infection during pregnancy or in  
26 immunocompetent patients relies on serology with detection of specific CMV-IgG and IgM. In case of  
27 positive CMV-IgM in pregnant women, CMV-IgG avidity is now widely recommended, but in general  
28 population it is not currently performed.

29 Objective: In this study, we aimed to determine CMV-IgM positive predictive values (PPV) in  
30 different clinical settings.

31 Material and methods: We conducted a retrospective study on positive CMV-IgM in our virology  
32 laboratory from 2013 to 2019, in three clinical groups: screening in non-symptomatic pregnant women  
33 (group 1), pregnant women with ultrasound (US) abnormalities (group 2) and patients (general  
34 population) with clinical signs suggestive of CMV primary infection (group 3). CMV-IgG avidity had  
35 been performed in all cases allowing to evaluate PPV of positive CMV-IgM to diagnose CMV  
36 primary-infection in each group.

37 Results: Between 2013 and 2019, 6,859 serum samples were found positive for CMV-IgM and had  
38 been tested for CMV-IgG avidity, with 6,560 sera for group 1, 30 for group 2 and 269 for group 3.  
39 Overall, low avidity confirming primary infection was observed respectively in 16.4% for group 1,  
40 36.7% for group 2, and 35.3% for group 3. CMV-IgM PPV was significantly lower in group 1  
41 compared to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

42 Discussion: Our observations highlight the major importance of including CMV-IgG avidity in the  
43 diagnostic algorithm, whatever the clinical situation (for immunocompetent patients), to confirm or  
44 exclude a recent CMV primary infection in case of positive CMV-IgM.

45

## 46 **Introduction**

47 Cytomegalovirus (CMV) is the most frequent worldwide cause of congenital viral infection with a  
48 prevalence estimated between 0.5 and 1% of all live births. Congenital CMV is a major cause of

49 sensorineural hearing loss and mental retardation [1-3]. CMV transmission to the fetus can occur after  
50 primary or secondary maternal CMV infection, with approximately the same proportion of symptoms  
51 and sequelae in both situations [4-6]. At birth, 13% of congenitally infected neonates are symptomatic  
52 with CMV-specific symptoms including growth restriction, microcephaly, ventriculomegaly,  
53 chorioretinitis, sensorineural hearing loss, hepatitis, thrombocytopenia and a purpuric skin eruption [2-  
54 7]. Risk of long term sequelae is higher if CMV transmission occurs in the first or second trimester of  
55 pregnancy or during peri-conceptual period [8-10]. In immunocompetent patients, CMV primary  
56 infection is often asymptomatic. When symptomatic (8 to 10% of cases), primary infection is usually  
57 responsible for a mild disease. Signs most frequently reported are: isolated fever, asthenia,  
58 mononucleosis syndrome with cervical lymphadenopathy and/or cytolytic hepatitis [11].

59 Diagnosis of CMV primary infection during pregnancy mainly relies on serology: detection of specific  
60 CMV-IgG and IgM, associated with CMV-IgG avidity in case of positive CMV-IgM [12]. However,  
61 in immunocompetent patients not pregnant, CMV-IgG avidity is usually not performed in case of  
62 positive CMV-IgM. Reported clinical performances of commercial immunoassays for CMV IgM are  
63 sensitivity >90% and specificity >96% and for CMV IgG avidity, specificity and sensitivity are  
64 comprised between 90 and 100% depending on the assay [13-16]. CMV-IgM can possibly indicate an  
65 acute or a recent infection but can also be due to other causes: long-term persisting IgM, cross-  
66 reaction, secondary CMV infection or nonspecific stimulation of the immune system. Consequently,  
67 diagnosis of primary infection cannot rely only on a positive IgM test result. CMV-IgG avidity  
68 measurement is an essential tool to confirm or exclude CMV primary-infection. CMV-IgG are initially  
69 of low avidity, but will mature to high avidity at 2-4 months after primary infection [12,17,18].

70 The main issue with CMV serology is that CMV-IgG avidity is not available in all laboratories and  
71 that clinicians still too often rely on positive CMV-IgM result to diagnose CMV primary-infection. In  
72 our retrospective cohort study, we aim to determine and compare CMV-IgM positive predictive value  
73 (PPV) to diagnose CMV primary infection depending on the clinical situation: systematic screening

74 during pregnancy, presence of ultrasound abnormalities (US) during pregnancy and clinical signs  
75 suggestive of CMV primary infection in general population (immunocompetent patients).

76

## 77 **Material and methods**

### 78 *Sample collection:*

79 In our hospital virology laboratory, CMV serology (IgG, IgM and IgG avidity in case of positive IgG)  
80 is performed either:

81 - in non-symptomatic pregnant women during first trimester of pregnancy (systematic  
82 screening) and followed in one of the two maternities in Paris South Hospitals, or in pregnant  
83 women referred to our laboratory because of positive CMV-IgM detected in one of the  
84 laboratories part of our network (systematic screening in other centers) (group 1);

85 - in non-symptomatic pregnant women referred to our pluridisciplinary prenatal center for US  
86 abnormalities (not initially screened at beginning of pregnancy) (group 2). Sonographic  
87 findings were mostly intrauterine growth retardation (IUGR) and cerebral abnormalities (head  
88 perimeter < 5th centile, ventriculomegaly, hyperechogenic ventricular wall, cerebellar and  
89 brain calcifications, enlargement of pericerebral spaces, periventricular and subependymal  
90 cysts, candlestick, porencephaly, hyperechogenic periventricular halo, candlestick, abnormal  
91 gyration, and/or corpus callosum hypoplasia);

92 - in immunocompetent patients (general population: adults and children) in case of clinical  
93 symptoms suggestive of CMV primary infection (group 3), mainly fever, headache, flu-like  
94 syndrome (fever + rhinitis + myalgia), arthralgia, myalgia, fatigue and/or hepatitis. Samples  
95 were collected 5 to 30 days after onset of symptoms.

96 All CMV serologic results performed in one of these contexts in our laboratory between January 2013  
97 and December 2019 were retrospectively analysed. In case a patient had several samples, only the

98 most informative (usually the first one) was kept for analysis. Serologies performed in the context of  
99 transplantation or in immunocompromised patients were not included in this study.

100

101 *Serology assays:*

102 CMV-IgG and CMV-IgM were measured with LIAISON XL (LXL, DiaSorin<sup>®</sup>, Saluggia, Italy). In  
103 case of positive CMV-IgM, our strategy is based on previous published findings [19]. In a few words,  
104 we first-line perform LXL IgG avidity as a screening test. An index > 0.40 allows to exclude a recent  
105 CMV primary infection. Below LXL 0.40 index threshold, a second assay is used: VIDAS  
106 (bioMérieux<sup>®</sup>, Craonne, France) CMV-IgG avidity. In this case, confirmation/exclusion of primary  
107 infection (more/less than 3 months before sample collection) is only based on the VIDAS assay result  
108 (cutoffs used are those recommended by manufacturer: 0.4-0.65). Results allowed us to classify  
109 serological profiles as follows:

- 110 - Negative CMV-IgG/positive CMV-IgM on two consecutive samples 21 days apart: non-  
111 specific IgM;
- 112 - Positive CMV-IgG/positive CMV-IgM/high CMV-IgG avidity (LXL > 0.4, or LXL < 0.4 and  
113 VIDAS > 0.65) : recent CMV primary infection excluded;
- 114 - Positive CMV-IgG/positive CMV-IgM/low CMV-IgG avidity (LXL < 0.4 and VIDAS <  
115 0.40): recent CMV primary infection confirmed;
- 116 - Positive CMV-IgG/positive CMV-IgM/moderate CMV-IgG avidity (LXL < 0.4 and VIDAS >  
117 0.40 but < 0.65): recent CMV primary infection not excluded.

118

119 *Statistical analysis:*



120 For the three groups, CMV-IgM PPV to diagnose recent CMV primary infection were calculated with  
121 95% confidence intervals. We calculated p-values with Chi-2 Pearson tests.

122

## 123 **Results**

124 Between 2013 and 2019, 6,859 serum samples were tested positive for CMV-IgM in our laboratory:

125 - 6,560 /6,859 (95.64%) were collected in pregnant women during systematic screening (group  
126 1)

127 - 30/6,859 (0.44%) were collected in pregnant women with US abnormalities (median  
128 gestational age 25 weeks of gestation (WG); range: 12-36 WG) (group 2).

129 - 269/6,859 (3.92%) were collected from immunocompetent patients with clinical symptoms of  
130 CMV primary infection (group 3)

131 All 6,859 serum samples CMV-IgM positive had been tested for CMV-IgG avidity at time of  
132 diagnosis.

### 133 *Systematic screening during pregnancy (group 1)*

134 CMV-IgG avidity was high in 5,486/6,560 (83.6%) cases allowing to exclude recent CMV primary  
135 infection (< 3 months). A total of 1,074/6,560 samples collected from pregnant women for systematic  
136 screening had low or moderate CMV-IgG avidity index and were considered as confirmed or not  
137 excluded recent CMV primary infections (**Figure 1 and Figure 2**). Therefore, PPV of CMV-IgM for  
138 systematic screening during pregnancy to predict a recent primary infection was 16.4% (95% CI =  
139 15.5 – 17.3%).

### 140 *Ultrasound abnormalities during pregnancy (group 2)*

141 CMV-IgG avidity was high in 18/30 (60.0%) cases allowing to exclude recent CMV primary  
142 infection. A total of 11/30 samples collected from pregnant women addressed for US had low or

143 intermediate CMV-IgG avidity index and were considered as confirmed or not excluded recent CMV  
144 primary infections (**Figure 1 and Figure 2**). Therefore, PPV of CMV-IgM in case of US  
145 abnormalities during pregnancy to predict a recent primary infection was 36.7% (95% CI = 19.5 –  
146 53.9%).

147 *Immunocompetent patients with clinical symptoms of CMV primary infection (group 3)*

148 CMV-IgG avidity was high in 174/269 (64.7%) cases allowing to exclude recent CMV primary  
149 infection (< 3 months). A total of 95/269 samples collected from patients with symptoms of acute  
150 CMV primary infection had low or intermediate CMV-IgG avidity index and were considered as  
151 confirmed or not excluded recent CMV primary infections (**Figure 1 and Figure 2**). Therefore, PPV  
152 of CMV-IgM for immunocompetent patients with clinical signs to predict a recent primary infection  
153 was 35.3% (95% CI = 29.6 – 41.0%).

154 We determined CMV-IgM PPV for these 269 patients according to their age (**Figure 3**):

- 155 - less than 10 years old (n=71): CMV-IgG avidity was low or moderate in 21/71 patients  
156 resulting in a PPV of 29.6% (95%CI= 19.0 – 40.5%) (p=0.50)
- 157 - 10 to 20 years old (n=27): CMV-IgG avidity was low or moderate in 3/27 patients resulting in  
158 a PPV of 11.1% (95%CI= 0.0 – 23.0%) (p=0.01)
- 159 - 20 to 40 years old (n=69): CMV-IgG avidity was low or moderate in 31/69 patients resulting  
160 in a PPV of 44.9% (95%CI= 33.2 – 56.6%) (p=0.20)
- 161 - more than 40 years old (n=102): CMV-IgG avidity was low or moderate in 40/102 patients  
162 resulting in a PPV of 39.2% (95%CI= 29.7 – 48.7%) (p=0.90)

163

164 Overall, when positive CMV-IgM are observed, recent CMV primary infection is only confirmed in  
165 respectively 16.4% cases for systematic screening during pregnancy (group 1), 36.7% in case of  
166 ultrasound abnormalities (group 2) and 35.3% in case of clinical signs in general population (group 3).

167 CMV-IgM PPV was significantly lower in group 1 comparing to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

168 CMV-IgM PPV was not statistically different between groups 2 and 3 ( $p>0.90$ ).

169

## 170 **Discussion**

171 Even if formal diagnosis of CMV primary infection is achieved with CMV-IgG  
172 seroconversion, as the “first” seronegative serum is usually not available, documentation of this  
173 seroconversion is rare. Usually, whatever the clinical situation, screening or clinical symptoms, only  
174 one serum specimen is available. The transient CMV-IgM positivity has long been used as a  
175 diagnostic marker for CMV primary infection, but it is well known that is not invariably indicative of  
176 primary infection. Indeed, specific or non-specific CMV-IgM can also be present in many other  
177 situations including infections with other pathogens associated with random polyclonal B cell  
178 stimulation or CMV non primary infection [20,21]. Moreover, there are known to be technical issues  
179 with IgM-specific testing. Indeed, false positive CMV-IgM results were shown to occur due to high  
180 levels of analyte specific IgG, to presence of rheumatoid factors, and to other miscellaneous technical  
181 factors involved with methods that must include some sort of process to separate CMV IgG from  
182 CMV IgM. In these cases, CMV-IgG avidity is essential and in contrast to IgM, low-avidity IgG is  
183 present only with primary infection, increasing over 3 to 4 months to high avidity [22]. CMV-IgG  
184 avidity has thus gained diagnostic importance in identifying primary CMV infection, and several  
185 commercial CMV-IgG avidity tests are currently available. Their performances to confirm a CMV  
186 primary infection were reported to range from 83 to 100%, and to exclude a CMV primary infection  
187 from 71 to 100% [12,16,19,23-28]. Even if not perfect, several groups have reported substantial  
188 improvements in identification of at-risk pregnancies using diagnostic algorithms including CMV-IgG  
189 avidity [7,15,18,22,29-31]. Indeed, it truly improves accuracy of CMV primary infection diagnosis as  
190 in the specific situation of systematic screening, PPV of positive CMV-IgM is quite poor [18,31].  
191 Indeed, in our analysis, if we calculated PPV considering confirmed recent primary infection, PPV is  
192 only of 9.7% (95%CI= 9.0 – 10.4%). However, from a practical point of view, we calculated PPV

193 considering both confirmed and not excluded recent CMV primary infection as these will probably be  
194 managed like confirmed recent primary infection regarding regular ultrasound follow up. It allows  
195 PPV to reach 16.4%.

196         Usefulness of serologic testing for CMV during pregnancy has been questioned since  
197 congenital malformations can occur even following maternal secondary infections [6]. Although  
198 screening is not recommended by any public health system because of its cost/benefit ratio, it is  
199 widely adopted by many general practitioners and obstetricians in our area. Such screening provides  
200 an opportunity to identify seronegative women who can be counselled about using appropriate  
201 hygienic measures to prevent primary infection, especially in relation to their behavior with children,  
202 who are a major source of infection. Furthermore, screening aims to diagnose CMV primary infection  
203 early in pregnancy allowing women to be referred to Reference Centers for appropriate management  
204 (close ultrasonography, amniocentesis and/or neonatal diagnosis...) [32]. Some authors consider that  
205 screening is not justified because of its economic cost, the imperfect nature of congenital infection  
206 prognostic criteria, the risk of spontaneous abortions induced by invasive tests such as amniocentesis,  
207 and the few data concerning effective treatments during pregnancy. However, it is obvious that if US  
208 are observed, and prenatal diagnosis of CMV congenital infection discussed, maternal CMV serology  
209 should always be performed. It is particularly important especially in regions where seroprevalence is  
210 around 50%, as in France, or lower because its first aim is to confirm maternal infection to CMV  
211 (whatever the date of this infection) and allows to definitively exclude congenital CMV if the pregnant  
212 woman is seronegative. If positive, this CMV serology usually performed late in pregnancy, might be  
213 challenging to interpret. Our results show that positive CMV-IgM at time of US indicate a recent  
214 infection in 23% cases (7/30) and that additionally, 3/30 women had high avidity but gave birth to an  
215 infected child. Overall, when positive CMV-IgM is detected when US abnormalities are observed  
216 (possibly late in pregnancy), it suggests responsibility of CMV in 47% (14/30) cases without ruling  
217 out the possibility of CMV congenital infection following maternal non primary infection if the  
218 mother is CMV-IgG positive and CMV-IgM is negative. However, final diagnosis will of course be  
219 obtained with CMV PCR in amniotic fluid and/or in urine in the neonate at birth.

220

221           If CMV-IgG avidity is widely used in pregnant women, far less literature is available on the  
222 usefulness of CMV-IgG avidity in general population (immunocompetent patients), and in our area it  
223 is clearly not usually performed if the patient is not pregnant. Remarkably, positive CMV-IgM results  
224 are most often considered as indicative of a recent CMV virus infection in case with clinical signs, but  
225 they are in fact truly related to a CMV primary infection in only 27.1% cases (73/269) (low CMV IgG  
226 avidity) and possibly related in 35.3% cases (low/moderate CMV IgG avidity). Interestingly, PPV was  
227 significantly lower for patients between 10 to 20 years old (11.1% vs 29.6-44.9%) (p=0.01). Our  
228 results suggest that positive CMV-IgM is less correlated with CMV recent primary infection in young  
229 people, and are consistent with the understanding that CMV-IgM can be produced throughout life as a  
230 result of CMV non primary infection or polyclonal stimulation of the immune system [20,21]. Also, in  
231 cases of positive CMV-IgM, older people may be more likely to have a primary infection than  
232 younger people. These also suggest that whatever the age of the patient, CMV-IgG avidity is essential  
233 for accurate diagnosis. One limitation of our study is that, although very frequent, CMV primary  
234 infection is most often a mild infection that does not require the patient to come to the hospital.  
235 Consequently, general practitioners probably mostly do diagnosis of CMV primary infection and only  
236 the patients with the severest symptoms come to the hospital and are therefore included in our data.  
237 Moreover, it is possible that older people are more likely to have a severe infection compared to young  
238 patients. Nevertheless, for current practice, all clinicians should be aware that, whatever the age of the  
239 patient, and given the non-specific symptoms of CMV primary infection, even in case of positive  
240 CMV-IgM, confirmation by avidity avoids misdiagnosis in more than 60% cases.

241           Our observations are consistent with the preexisting ones [33-37] and highlight the major  
242 importance of including CMV-IgG avidity in the diagnostic algorithm, whatever the clinical situation  
243 (for immunocompetent patients), to confirm or exclude a CMV primary infection in case of positive  
244 CMV-IgM.

245

246 **Sample CRediT author statement:**

247 **Claire Périllaud-Dubois:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

248 **Elise Bouthry:** Writing- Reviewing and Editing. **Abir Jadoui and Ay Ling Leng:** Investigation.

249 **Anne-Marie Roque-Afonso:** Writing- Reviewing and Editing. **Christelle Vauloup-Fellous:**

250 Conceptualization, Writing- Reviewing and Editing, Supervision.

251

252 **Declarations of Interest:** none.

253

254 **Funding sources:** none.

255

256

257 **References**

258

- 259 [1] A. Kenneson et M. J. Cannon, Review and meta-analysis of the epidemiology of congenital  
260 cytomegalovirus (CMV) infection, *Rev. Med. Virol.*, p. 253-276, 2007, doi: 10.1002/rmv.535.
- 261 [2] S. C. Dollard, S. D. Grosse, et D. S. Ross, New estimates of the prevalence of neurological and  
262 sensory sequelae and mortality associated with congenital cytomegalovirus infection, *Rev.*  
263 *Med. Virol.*, p. 355-363, 2007, doi: 10.1002/rmv.544.
- 264 [3] S. Stagno *et al.*, Primary cytomegalovirus infection in pregnancy. Incidence, transmission to  
265 fetus, and clinical outcome, *JAMA*, p. 1904-1908, 1986.
- 266 [4] S. A. Ross *et al.*, Hearing loss in children with congenital cytomegalovirus infection born to  
267 mothers with preexisting immunity, *J. Pediatr.*, p. 332-336, 2006, doi:  
268 10.1016/j.jpeds.2005.09.030.
- 269 [5] M. Leruez-Ville *et al.*, Risk Factors for Congenital Cytomegalovirus Infection Following Primary  
270 and Nonprimary Maternal Infection: A Prospective Neonatal Screening Study Using Polymerase  
271 Chain Reaction in Saliva, *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, p. 398-404, 2017, doi:  
272 10.1093/cid/cix337.
- 273 [6] J. J. C. de Vries, E. W. van Zwet, F. W. Dekker, A. C. M. Kroes, P. H. Verkerk, et A. C. T. M.  
274 Vossen, The apparent paradox of maternal seropositivity as a risk factor for congenital  
275 cytomegalovirus infection: a population-based prediction model: Maternal seropositivity as a  
276 risk factor for cCMV, *Rev. Med. Virol.*, p. 241-249, 2013, doi: 10.1002/rmv.1744.
- 277 [7] M. G. Revello et G. Gerna, Diagnosis and Management of Human Cytomegalovirus Infection in  
278 the Mother, Fetus, and Newborn Infant, *Clin. Microbiol. Rev.*, p. 680-715, 2002, doi:  
279 10.1128/CMR.15.4.680-715.2002.
- 280 [8] O. Picone *et al.*, A series of 238 cytomegalovirus primary infections during pregnancy:  
281 description and outcome: Outcome of maternal CMV infection during pregnancy, *Prenat.*  
282 *Diagn.*, p. 751-758, 2013, doi: 10.1002/pd.4118.
- 283 [9] G. Enders, A. Daiminger, U. Bäder, S. Exler, et M. Enders, Intrauterine transmission and clinical  
284 outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational  
285 age, *J. Clin. Virol.*, p. 244-246, 2011, doi: 10.1016/j.jcv.2011.07.005.
- 286 [10] V. Faure-Bardon *et al.*, Sequelae of congenital cytomegalovirus (cCMV) following maternal  
287 primary infection are limited to those acquired in the first trimester of pregnancy, *Clin. Infect.*  
288 *Dis. Off. Publ. Infect. Dis. Soc. Am.*, 2018, doi: 10.1093/cid/ciy1128.
- 289 [11] T. G. Wreghitt, E. L. Teare, O. Sule, R. Devi, et P. Rice, Cytomegalovirus Infection in  
290 Immunocompetent Patients, *Clin. Infect. Dis.*, p. 1603-1606, 2003, doi: 10.1086/379711.
- 291 [12] C. Vauloup-Fellous, T. Lazzarotto, M. G. Revello, et L. Grangeot-Keros, Clinical evaluation of the  
292 Roche Elecsys CMV IgG Avidity assay, *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin.*  
293 *Microbiol.*, p. 1365-1369, 2014, doi: 10.1007/s10096-014-2080-4.
- 294 [13] C. Busse, A. Strubel, et P. Schnitzler, Combination of native and recombinant cytomegalovirus  
295 antigens in a new ELISA for detection of CMV-specific antibodies, *J. Clin. Virol.*, p. 137-141,  
296 2008, doi: 10.1016/j.jcv.2008.05.011.
- 297 [14] M. G. Revello, C. Fornara, A. Arossa, P. Zelini, et D. Lilleri, Role of human cytomegalovirus  
298 (HCMV)-specific antibody in HCMV-infected pregnant women, *Early Hum. Dev.*, p. S32-S34,  
299 2014, doi: 10.1016/S0378-3782(14)70011-8.
- 300 [15] M. Macé, L. Sissoeff, A. Rudent, et L. Grangeot-Keros, A serological testing algorithm for the  
301 diagnosis of primary CMV infection in pregnant women: DIAGNOSIS OF CMV PRIMARY  
302 INFECTION IN PREGNANT WOMEN, *Prenat. Diagn.*, p. 861-863, 2004, doi: 10.1002/pd.1001.
- 303 [16] M. L. Delforge, L. Desomberg, et I. Montesinos, Evaluation of the new LIAISON<sup>®</sup> CMV IgG, IgM  
304 and IgG Avidity II assays, *J. Clin. Virol.*, p. 42-45, 2015, doi: 10.1016/j.jcv.2015.09.002.

- 305 [17] S. C. Dollard, S. A. S. Staras, M. M. Amin, D. S. Schmid, et M. J. Cannon, National Prevalence  
306 Estimates for Cytomegalovirus IgM and IgG Avidity and Association between High IgM Antibody  
307 Titer and Low IgG Avidity, *Clin. Vaccine Immunol.*, p. 1895-1899, 2011, doi: 10.1128/CVI.05228-  
308 11.
- 309 [18] M. Leruez-Ville, Y. Sellier, L. J. Salomon, J. J. Stirnemann, F. Jacquemard, et Y. Ville, Prediction of  
310 Fetal Infection in Cases With Cytomegalovirus Immunoglobulin M in the First Trimester of  
311 Pregnancy: A Retrospective Cohort, *Clin. Infect. Dis.*, p. 1428-1435, 2013, doi:  
312 10.1093/cid/cit059.
- 313 [19] C. Vauloup-Fellous, M. Berth, F. Heskia, J.-M. Dugua, et L. Grangeot-Keros, Re-evaluation of the  
314 VIDAS® cytomegalovirus (CMV) IgG avidity assay: Determination of new cut-off values based on  
315 the study of kinetics of CMV-IgG maturation, *J. Clin. Virol.*, p. 118-123, 2013, doi:  
316 10.1016/j.jcv.2012.10.017.
- 317 [20] S. L. Nielsen, I. Sørensen, et H. K. Andersen, Kinetics of specific immunoglobulins M, E, A, and G  
318 in congenital, primary, and secondary cytomegalovirus infection studied by antibody-capture  
319 enzyme-linked immunosorbent assay, *J. Clin. Microbiol.*, p. 654-661, 1988.
- 320 [21] R. F. Pass, S. Stagno, W. J. Britt, et C. A. Alford, Specific cell-mediated immunity and the natural  
321 history of congenital infection with cytomegalovirus, *J. Infect. Dis.*, p. 953-961, 1983.
- 322 [22] T. Lazzarotto, B. Guerra, M. Lanari, L. Gabrielli, et M. P. Landini, New advances in the diagnosis  
323 of congenital cytomegalovirus infection, *J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*, p.  
324 192-197, 2008, doi: 10.1016/j.jcv.2007.10.015.
- 325 [23] T. Lazzarotto, S. Brojanac, G. T. Maine, et M. P. Landini, Search for cytomegalovirus-specific  
326 immunoglobulin M: comparison between a new western blot, conventional western blot, and  
327 nine commercially available assays., *Clin. Diagn. Lab. Immunol.*, p. 483-486, 1997, doi:  
328 10.1128/CDLI.4.4.483-486.1997.
- 329 [24] T. Lazzarotto *et al.*, Anticytomegalovirus (anti-CMV) immunoglobulin G avidity in identification  
330 of pregnant women at risk of transmitting congenital CMV infection, *Clin. Diagn. Lab. Immunol.*,  
331 p. 127-129, 1999.
- 332 [25] M. Bodéus, D. Beulné, et P. Goubau, Ability of Three IgG-Avidity Assays to Exclude Recent  
333 Cytomegalovirus Infection, *Eur. J. Clin. Microbiol. Infect. Dis.*, p. 0248-0252, 2001, doi:  
334 10.1007/s100960100484.
- 335 [26] M. G. Revello, G. Gorini, et G. Gerna, Clinical Evaluation of a Chemiluminescence Immunoassay  
336 for Determination of Immunoglobulin G Avidity to Human Cytomegalovirus, *Clin. Diagn. Lab.  
337 Immunol.*, p. 801-805, 2004, doi: 10.1128/CDLI.11.4.801-805.2004.
- 338 [27] K. Lagrou, M. Bodeus, M. Van Ranst, et P. Goubau, Evaluation of the New Architect  
339 Cytomegalovirus Immunoglobulin M (IgM), IgG, and IgG Avidity Assays, *J. Clin. Microbiol.*, p.  
340 1695-1699, 2009, doi: 10.1128/JCM.02172-08.
- 341 [28] M. E. Guisasola, Belé. Ramos, J. C. Sanz, I. García-Bermejo, et F. De Ory Manchón, Comparison  
342 of IgG avidity assays in the confirmation of the diagnosis of cytomegalovirus primary infection:  
343 IGG AVIDITY ASSAYS FOR DIAGNOSIS OF CMV INFECTION, *APMIS*, p. 991-993, 2010, doi:  
344 10.1111/j.1600-0463.2010.02682.x.
- 345 [29] T. Lazzarotto *et al.*, Maternal IgG avidity and IgM detected by blot as diagnostic tools to identify  
346 pregnant women at risk of transmitting cytomegalovirus, *Viral Immunol.*, p. 137-141, 2000, doi:  
347 10.1089/vim.2000.13.137.
- 348 [30] S. C. Munro *et al.*, Diagnosis of and screening for cytomegalovirus infection in pregnant women,  
349 *J. Clin. Microbiol.*, p. 4713-4718, 2005, doi: 10.1128/JCM.43.9.4713-4718.2005.
- 350 [31] C. Vauloup-Fellous *et al.*, Does hygiene counseling have an impact on the rate of CMV primary  
351 infection during pregnancy? Results of a 3-year prospective study in a French hospital, *J. Clin.  
352 Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*, p. S49-53, 2009, doi: 10.1016/j.jcv.2009.09.003.



- 353 [32] W. D. Rawlinson *et al.*, Congenital cytomegalovirus infection in pregnancy and the neonate:  
354 consensus recommendations for prevention, diagnosis, and therapy, *Lancet Infect. Dis.*, p.  
355 e177-e188, 2017, doi: 10.1016/S1473-3099(17)30143-3.
- 356 [33] M. De Paschale, C. Agrappi, M. T. Manco, et P. Clerici, Positive predictive value of anti-HCMV  
357 IgM as an index of primary infection, *J. Virol. Methods*, p. 121-125, 2010, doi:  
358 10.1016/j.jviromet.2010.05.001.
- 359 [34] M. Yoshida *et al.*, Can measurement of maternal anti-cytomegalovirus immunoglobulin-M  
360 antibody levels be used to screen for cytomegalovirus infection in embryos and fetuses?, *J.*  
361 *Obstet. Gynaecol. Res.*, p. 166-169, 2013, doi: 10.1111/j.1447-0756.2012.01900.x.
- 362 [35] A. Goncé *et al.*, Maternal IgM antibody status in confirmed fetal cytomegalovirus infection  
363 detected by sonographic signs, *Prenat. Diagn.*, p. 817-821, 2012, doi: 10.1002/pd.3907.
- 364 [36] Y. Torii *et al.*, Serological screening of immunoglobulin M and immunoglobulin G during  
365 pregnancy for predicting congenital cytomegalovirus infection, *BMC Pregnancy Childbirth*, p.  
366 205, 2019, doi: 10.1186/s12884-019-2360-1.
- 367 [37] Toriyabe K. *et al.*, Anticytomegalovirus IgM titer for congenital infection in first-trimester  
368 pregnancy with primary infection, *J. Obstet. Gynaecol. Res.*, p. 1555-1556, 2018, doi:  
369 10.1111/jog.13762.
- 370

1 Positive predictive values of CMV-IgM **and importance of CMV-IgG avidity**  
2 **testing in detecting** primary infection in three different clinical settings. A  
3 French retrospective cohort study.

4

5 Claire Périllaud-Dubois <sup>1,2,3,4</sup>, Elise Bouthry <sup>1,2</sup>, Abir Jadoui <sup>1</sup>, Ay-Ling Leng <sup>1</sup>, Anne-Marie Roque-  
6 Afonso <sup>1,4,5</sup>, Christelle Vauloup-Fellous <sup>1,2,4,5</sup>

7

8 <sup>1</sup> AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie, , 94804 Villejuif, France

9 <sup>2</sup> Groupe de Recherche sur les Infections pendant la Grossesse (GRIG), France

10 <sup>3</sup> INSERM UMR1137, IAME, 75018 Paris, France

11 <sup>4</sup> Université Paris-Saclay, 94804 Villejuif, France

12 <sup>5</sup> INSERM U1193, 94804 Villejuif, France

13

14 Corresponding author: Dr Claire Périllaud-Dubois

15 AP-HP.Université Paris-Saclay, Hôpital Paul-Brousse, Service de Virologie

16 12-14 avenue Paul Vaillant Couturier

17 94804 Villejuif, France

18 +33145593721

19 claire.perillaud-dubois@inserm.fr

20 Keywords: CMV; serology; IgM; avidity; PPV; positive predictive value; pregnancy; ultrasound  
21 abnormalities; clinical signs

22 Word count: 2443

23

24 **Abstract**

25 Background: Diagnosis of Cytomegalovirus (CMV) primary infection during pregnancy or in  
26 immunocompetent patients relies on serology with detection of specific CMV-IgG and IgM. In case of  
27 positive CMV-IgM in pregnant women, CMV-IgG avidity is now widely recommended, but in general  
28 population it is not currently performed.

29 Objective: In this study, we aimed to determine CMV-IgM positive predictive values (PPV) in  
30 different clinical settings.

31 Material and methods: We conducted a retrospective study on positive CMV-IgM in our virology  
32 laboratory from 2013 to 2019, in three clinical groups: screening in non-symptomatic pregnant women  
33 (group 1), pregnant women with ultrasound (US) abnormalities (group 2) and patients (general  
34 population) with clinical signs suggestive of CMV primary infection (group 3). CMV-IgG avidity had  
35 been performed in all cases allowing to evaluate PPV of positive CMV-IgM to diagnose CMV  
36 primary-infection in each group.

37 Results: Between 2013 and 2019, 6,859 serum samples were found positive for CMV-IgM and had  
38 been tested for CMV-IgG avidity, with 6,560 sera for group 1, 30 for group 2 and 269 for group 3.  
39 Overall, low avidity confirming primary infection was observed respectively in 16.4% for group 1,  
40 36.7% for group 2, and 35.3% for group 3. CMV-IgM PPV was significantly lower in group 1  
41 compared to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

42 Discussion: Our observations highlight the major importance of including CMV-IgG avidity in the  
43 diagnostic algorithm, whatever the clinical situation (for immunocompetent patients), to confirm or  
44 exclude a recent CMV primary infection in case of positive CMV-IgM.

45

## 46 **Introduction**

47 Cytomegalovirus (CMV) is the most frequent worldwide cause of congenital viral infection with a  
48 prevalence estimated between 0.5 and 1% of all live births. Congenital CMV is a major cause of

49 sensorineural hearing loss and mental retardation [1-3]. CMV transmission to the fetus can occur after  
50 primary or secondary maternal CMV infection, with approximately the same proportion of symptoms  
51 and sequelae in both situations [4-6]. At birth, 13% of congenitally infected neonates are symptomatic  
52 with CMV-specific symptoms including growth restriction, microcephaly, ventriculomegaly,  
53 chorioretinitis, sensorineural hearing loss, hepatitis, thrombocytopenia and a purpuric skin eruption [2-  
54 7]. Risk of long term sequelae is higher if CMV transmission occurs in the first or second trimester of  
55 pregnancy or during **peri-conceptional** period [8-10]. In immunocompetent patients, CMV primary  
56 infection is often asymptomatic. When symptomatic (8 to 10% of cases), primary infection is usually  
57 responsible **for** a mild disease. Signs most frequently reported are: isolated fever, asthenia,  
58 mononucleosis syndrome with cervical lymphadenopathy and/or cytolytic hepatitis [11].

59 Diagnosis of CMV primary infection during pregnancy mainly relies on serology: detection of specific  
60 CMV-IgG and IgM, associated with CMV-IgG avidity in case of positive CMV-IgM [12]. However,  
61 in immunocompetent patients not pregnant, CMV-IgG avidity is usually not performed in case of  
62 positive CMV-IgM. Reported clinical performances of commercial immunoassays for CMV IgM are  
63 sensitivity >90% and specificity >96% and for CMV IgG avidity, specificity and sensitivity are  
64 comprised between 90 and 100% depending on the assay [13-16]. CMV-IgM can possibly indicate an  
65 acute or a recent infection but can also be due to other causes: long-term persisting IgM, cross-  
66 reaction, secondary CMV infection or nonspecific stimulation of the immune system. Consequently,  
67 diagnosis of primary infection cannot rely only on a positive IgM test result. CMV-IgG avidity  
68 measurement is an essential tool to confirm or exclude CMV primary-infection. CMV-IgG are initially  
69 of low avidity, but will mature to high avidity **at** 2-4 months after primary infection [12,17,18].

70 The main issue with CMV serology is that CMV-IgG avidity is not available in all laboratories and  
71 that clinicians still too often rely on positive CMV-IgM result to diagnose CMV primary-infection. In  
72 our retrospective cohort study, we aim to determine and compare CMV-IgM positive predictive value  
73 (PPV) to diagnose CMV primary infection depending on the clinical situation: systematic screening

74 during pregnancy, presence of ultrasound abnormalities (US) during pregnancy and clinical signs  
75 suggestive of CMV primary infection in general population (immunocompetent patients).

76

## 77 **Material and methods**

### 78 *Sample collection:*

79 In our hospital virology laboratory, CMV serology (IgG, IgM and IgG avidity in case of positive IgG)  
80 is performed either:

81 - in non-symptomatic pregnant women during first trimester of pregnancy (systematic  
82 screening) and followed in one of the two maternities in Paris South Hospitals, or in pregnant  
83 women referred to our laboratory because of positive CMV-IgM detected in one of the  
84 laboratories part of our network (systematic screening in other centers) (group 1);

85 - in non-symptomatic pregnant women referred to our pluridisciplinary prenatal center for US  
86 **abnormalities** (not initially screened at beginning of pregnancy) (group 2). **Sonographic**  
87 **findings were mostly intrauterine growth retardation (IUGR) and cerebral abnormalities (head**  
88 **perimeter < 5th centile, ventriculomegaly, hyperechogenic ventricular wall, cerebellar and**  
89 **brain calcifications, enlargement of pericerebral spaces, periventricular and subependymal**  
90 **cysts, candlestick, porencephaly, hyperechogenic periventricular halo, candlestick, abnormal**  
91 **gyration, and/or corpus callosum hypoplasia);**

92 - in immunocompetent patients (general population: adults and children) in case of clinical  
93 symptoms suggestive of CMV primary infection (group 3), mainly **fever, headache, flu-like**  
94 **syndrome (fever + rhinitis + myalgia), arthralgia, myalgia, fatigue and/or hepatitis. Samples**  
95 **were collected 5 to 30 days after onset of symptoms.**

96 All CMV serologic results performed in one of these contexts in our laboratory between January 2013  
97 and December 2019 were retrospectively analysed. In case a patient had several samples, only the

98 most informative (usually the first one) was kept for analysis. Serologies performed in the context of  
99 transplantation or in immunocompromised patients were not included in this study.

100

101 *Serology assays:*

102 CMV-IgG and CMV-IgM were measured with LIAISON XL (LXL, DiaSorin<sup>®</sup>, Saluggia, Italy). In  
103 case of positive CMV-IgM, our strategy is based on previous published findings [19]. In a few words,  
104 we first-line perform LXL IgG avidity as a screening test. An index > 0.40 allows to exclude a recent  
105 CMV primary infection. Below LXL 0.40 index threshold, a second assay is used: VIDAS  
106 (bioMérieux<sup>®</sup>, Craonne, France) CMV-IgG avidity. In this case, confirmation/exclusion of primary  
107 infection (more/less than 3 months before sample collection) is only based on the VIDAS assay result  
108 (cutoffs used are those recommended by manufacturer: 0.4-0.65). Results allowed us to classify  
109 serological profiles as follows:

- 110 - Negative CMV-IgG/positive CMV-IgM on two consecutive samples 21 days apart: non-  
111 specific IgM;
- 112 - Positive CMV-IgG/positive CMV-IgM/high CMV-IgG avidity (LXL > 0.4, or LXL < 0.4 and  
113 VIDAS > 0.65) : recent CMV primary infection excluded;
- 114 - Positive CMV-IgG/positive CMV-IgM/low CMV-IgG avidity (LXL < 0.4 and VIDAS <  
115 0.40): recent CMV primary infection confirmed;
- 116 - Positive CMV-IgG/positive CMV-IgM/moderate CMV-IgG avidity (LXL < 0.4 and VIDAS >  
117 0.40 but < 0.65): recent CMV primary infection not excluded.

118

119 *Statistical analysis:*

120 For the three groups, CMV-IgM PPV to diagnose recent CMV primary infection were calculated with  
121 95% confidence intervals. We calculated p-values with Chi-2 Pearson tests.

122

## 123 **Results**

124 Between 2013 and 2019, 6,859 serum samples were tested positive for CMV-IgM in our laboratory:

125 - 6,560 /6,859 (95.64%) were collected in pregnant women during systematic screening (group  
126 1)

127 - 30/6,859 (0.44%) were collected in pregnant women with US abnormalities (median  
128 gestational age 25 weeks of gestation (WG); range: 12-36 WG) (group 2).

129 - 269/6,859 (3.92%) were collected from immunocompetent patients with clinical symptoms of  
130 CMV primary infection (group 3)

131 All 6,859 serum samples CMV-IgM positive had been tested for CMV-IgG avidity at time of  
132 diagnosis.

### 133 *Systematic screening during pregnancy (group 1)*

134 CMV-IgG avidity was high in 5,486/6,560 (83.6%) cases allowing to exclude recent CMV primary  
135 infection (< 3 months). A total of 1,074/6,560 samples collected from pregnant women for systematic  
136 screening had low or moderate CMV-IgG avidity index and were considered as confirmed or not  
137 excluded recent CMV primary infections (**Figure 1 and Figure 2**). Therefore, PPV of CMV-IgM for  
138 systematic screening during pregnancy to predict a recent primary infection was 16.4% (95% CI =  
139 15.5 – 17.3%).

### 140 *Ultrasound abnormalities during pregnancy (group 2)*

141 CMV-IgG avidity was high in 18/30 (60.0%) cases allowing to exclude recent CMV primary  
142 infection. A total of 11/30 samples collected from pregnant women addressed for US had low or

143 intermediate CMV-IgG avidity index and were considered as confirmed or not excluded recent CMV  
144 primary infections (**Figure 1 and Figure 2**). Therefore, PPV of CMV-IgM in case of US  
145 abnormalities during pregnancy to predict a recent primary infection was 36.7% (95% CI = 19.5 –  
146 53.9%).

147 *Immunocompetent patients with clinical symptoms of CMV primary infection (group 3)*

148 CMV-IgG avidity was high in 174/269 (64.7%) cases allowing to exclude recent CMV primary  
149 infection (< 3 months). A total of 95/269 samples collected from patients with symptoms of acute  
150 CMV primary infection had low or intermediate CMV-IgG avidity index and were considered as  
151 confirmed or not excluded recent CMV primary infections (**Figure 1 and Figure 2**). Therefore, PPV  
152 of CMV-IgM for immunocompetent patients with clinical signs to predict a recent primary infection  
153 was 35.3% (95% CI = 29.6 – 41.0%).

154 We determined CMV-IgM PPV for these 269 patients according to their age (**Figure 3**):

- 155 - less than 10 years old (n=71): CMV-IgG avidity was low or moderate in 21/71 patients  
156 resulting in a PPV of 29.6% (95%CI= 19.0 – 40.5%) (p=0.50)
- 157 - 10 to 20 years old (n=27): CMV-IgG avidity was low or moderate in 3/27 patients resulting in  
158 a PPV of 11.1% (95%CI= 0.0 – 23.0%) (p=0.01)
- 159 - 20 to 40 years old (n=69): CMV-IgG avidity was low or moderate in 31/69 patients resulting  
160 in a PPV of 44.9% (95%CI= 33.2 – 56.6%) (p=0.20)
- 161 - more than 40 years old (n=102): CMV-IgG avidity was low or moderate in 40/102 patients  
162 resulting in a PPV of 39.2% (95%CI= 29.7 – 48.7%) (p=0.90)

163

164 Overall, when positive CMV-IgM are observed, recent CMV primary infection is only confirmed in  
165 respectively 16.4% cases for systematic screening during pregnancy (group 1), 36.7% in case of  
166 ultrasound abnormalities (group 2) and 35.3% in case of clinical signs in general population (group 3).



167 CMV-IgM PPV was significantly lower in group 1 comparing to groups 2 ( $p=0.01$ ) and 3 ( $p<0.001$ ).

168 CMV-IgM PPV was not statistically different between groups 2 and 3 ( $p>0.90$ ).

169

## 170 **Discussion**

171 Even if formal diagnosis of CMV primary infection is achieved with CMV-IgG  
172 seroconversion, as the “first” seronegative serum is usually not available, documentation of this  
173 seroconversion is rare. Usually, whatever the clinical situation, screening or clinical symptoms, only  
174 one serum specimen is available. The transient CMV-IgM positivity has long been used as a  
175 diagnostic marker for **CMV primary infection**, but it is well known that is not invariably indicative of  
176 primary **infection. Indeed, specific** or non-specific CMV-IgM can also be present in many other  
177 situations including infections with other pathogens associated with random polyclonal B cell  
178 stimulation or CMV non primary infection [20,21]. Moreover, **there are known to be technical issues**  
179 **with IgM-specific testing. Indeed, false positive CMV-IgM results were shown to occur due to high**  
180 **levels of analyte specific IgG, to presence of rheumatoid factors, and to other miscellaneous technical**  
181 **factors involved with methods that must include some sort of process to separate CMV IgG from**  
182 **CMV IgM.** In these cases, CMV-IgG avidity is essential and in contrast to IgM, low-avidity IgG is  
183 present only with primary infection, increasing over 3 to 4 months to high avidity [22]. CMV-IgG  
184 avidity has thus gained diagnostic importance in identifying primary CMV infection, and several  
185 commercial CMV-IgG avidity tests are currently available. Their performances to confirm a CMV  
186 primary infection were reported to range from 83 to 100%, and to exclude a CMV primary infection  
187 from 71 to 100% [12,16,19,23-28]. Even if not perfect, several groups have reported substantial  
188 improvements in identification of at-risk pregnancies using diagnostic algorithms including CMV-IgG  
189 avidity [7,15,18,22,29-31]. Indeed, it truly improves accuracy of CMV primary infection diagnosis as  
190 in the specific situation of systematic screening, PPV of positive CMV-IgM is quite poor [18,31].  
191 Indeed, in our analysis, if we calculated PPV considering confirmed recent primary infection, PPV is  
192 only of 9.7% (95%CI= 9.0 – 10.4%). However, from a practical point of view, we calculated PPV

193 considering both confirmed and not excluded recent CMV primary infection as these will probably be  
194 managed like confirmed recent primary infection regarding regular ultrasound follow up. It allows  
195 PPV to reach 16.4%.

196         Usefulness of serologic testing for CMV during pregnancy has been questioned since  
197 congenital malformations can occur even following maternal secondary infections [6]. Although  
198 screening is not recommended by any public health system because of its cost/benefit ratio, it is  
199 widely adopted by many general practitioners and obstetricians in our area. Such screening provides  
200 an opportunity to identify seronegative women who can be counselled about using appropriate  
201 hygienic measures to prevent primary infection, especially in relation to their behavior with children,  
202 who are a major source of infection. Furthermore, screening aims to diagnose CMV primary infection  
203 early in pregnancy allowing women to be referred to Reference Centers for appropriate management  
204 (close ultrasonography, amniocentesis and/or neonatal diagnosis...) [32]. **Some** authors consider that  
205 screening is not justified because of its economic cost, the imperfect nature of congenital infection  
206 prognostic criteria, the risk of spontaneous abortions induced by invasive tests such as amniocentesis,  
207 and the few data concerning effective treatments during **pregnancy**. **However**, it is obvious that if US  
208 are observed, and prenatal diagnosis of CMV congenital infection discussed, maternal CMV serology  
209 **should always be** performed. It is particularly important especially in regions where seroprevalence is  
210 around 50%, as in France, or lower because its first aim is to confirm maternal infection to CMV  
211 (whatever the date of this infection) and allows to definitively exclude congenital CMV if the pregnant  
212 woman is seronegative. If positive, this CMV serology usually performed late in pregnancy, might be  
213 challenging to interpret. Our results show that positive CMV-IgM at time of US indicate a recent  
214 infection in 23% cases (7/30) and that additionally, 3/30 women had high avidity but gave birth to an  
215 infected child. Overall, when positive CMV-IgM is detected **when US abnormalities are observed**  
216 **(possibly late in pregnancy)**, it **suggests** responsibility of CMV in 47% (14/30) cases without ruling  
217 out the possibility of CMV congenital infection following maternal non primary infection if the  
218 mother is CMV-IgG positive and CMV-IgM is negative. However, final diagnosis will of course be  
219 obtained with CMV PCR in amniotic fluid and/or in urine in the neonate at birth.

220

221           If CMV-IgG avidity is widely used in pregnant women, far less literature is available on the  
222 usefulness of CMV-IgG avidity in general population (immunocompetent patients), and in our area it  
223 is clearly not usually performed if the patient is not pregnant. Remarkably, **positive CMV-IgM results**  
224 **are** most often considered as indicative of a recent CMV virus infection in case **with** clinical signs, but  
225 they are in fact truly related to a CMV primary infection in only 27.1% cases (73/269) (low CMV IgG  
226 avidity) and possibly related in 35.3% cases (low/moderate CMV IgG avidity). Interestingly, PPV was  
227 significantly lower for patients between 10 to 20 years old (11.1% vs 29.6-44.9%) (p=0.01). Our  
228 results suggest that positive CMV-IgM is less correlated with CMV recent primary infection in young  
229 people, and are consistent with the understanding that CMV-IgM can be produced throughout life as a  
230 result of CMV non primary infection or polyclonal stimulation of the immune system [20,21]. **Also, in**  
231 **cases** of positive CMV-IgM, older people may be more likely to have a primary infection than  
232 younger people. These also suggest that whatever the age of the patient, CMV-IgG avidity is essential  
233 for accurate diagnosis. **One limitation of our study** is that, although very frequent, CMV primary  
234 infection is most often a mild infection that does not require **the patient to come to the hospital.**  
235 Consequently, **general practitioners probably mostly do diagnosis of CMV primary infection and only**  
236 **the patients with the severest symptoms come to the hospital and are therefore included in our data.**  
237 Moreover, it is possible that older people are more likely to have a severe infection compared to young  
238 patients. Nevertheless, for current practice, all clinicians should be aware that, whatever the age of the  
239 patient, and given the non-specific symptoms of CMV primary infection, even in case of positive  
240 CMV-IgM, confirmation by avidity avoids misdiagnosis in more than 60% cases.

241           Our observations are consistent with the preexisting ones [33-37] and highlight the major  
242 importance of including CMV-IgG avidity in the diagnostic algorithm, whatever the clinical situation  
243 (for immunocompetent patients), to confirm or exclude a CMV primary infection in case of positive  
244 CMV-IgM.

245

246 **Sample CRediT author statement:**

247 **Claire Périllaud-Dubois:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

248 **Elise Bouthry:** Writing- Reviewing and Editing. **Abir Jadoui and Ay Ling Leng:** Investigation.

249 **Anne-Marie Roque-Afonso:** Writing- Reviewing and Editing. **Christelle Vauloup-Fellous:**

250 Conceptualization, Writing- Reviewing and Editing, Supervision.

251

252 **Declarations of Interest:** none.

253

254 **Funding sources:** none.

255

256

257

259

- 260 [1] A. Kenneson et M. J. Cannon, Review and meta-analysis of the epidemiology of congenital  
261 cytomegalovirus (CMV) infection, *Rev. Med. Virol.*, p. 253-276, 2007, doi: 10.1002/rmv.535.
- 262 [2] S. C. Dollard, S. D. Grosse, et D. S. Ross, New estimates of the prevalence of neurological and  
263 sensory sequelae and mortality associated with congenital cytomegalovirus infection, *Rev.*  
264 *Med. Virol.*, p. 355-363, 2007, doi: 10.1002/rmv.544.
- 265 [3] S. Stagno *et al.*, Primary cytomegalovirus infection in pregnancy. Incidence, transmission to  
266 fetus, and clinical outcome, *JAMA*, p. 1904-1908, 1986.
- 267 [4] S. A. Ross *et al.*, Hearing loss in children with congenital cytomegalovirus infection born to  
268 mothers with preexisting immunity, *J. Pediatr.*, p. 332-336, 2006, doi:  
269 10.1016/j.jpeds.2005.09.030.
- 270 [5] M. Leruez-Ville *et al.*, Risk Factors for Congenital Cytomegalovirus Infection Following Primary  
271 and Nonprimary Maternal Infection: A Prospective Neonatal Screening Study Using Polymerase  
272 Chain Reaction in Saliva, *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, p. 398-404, 2017, doi:  
273 10.1093/cid/cix337.
- 274 [6] J. J. C. de Vries, E. W. van Zwet, F. W. Dekker, A. C. M. Kroes, P. H. Verkerk, et A. C. T. M.  
275 Vossen, The apparent paradox of maternal seropositivity as a risk factor for congenital  
276 cytomegalovirus infection: a population-based prediction model: Maternal seropositivity as a  
277 risk factor for cCMV, *Rev. Med. Virol.*, p. 241-249, 2013, doi: 10.1002/rmv.1744.
- 278 [7] M. G. Revello et G. Gerna, Diagnosis and Management of Human Cytomegalovirus Infection in  
279 the Mother, Fetus, and Newborn Infant, *Clin. Microbiol. Rev.*, p. 680-715, 2002, doi:  
280 10.1128/CMR.15.4.680-715.2002.
- 281 [8] O. Picone *et al.*, A series of 238 cytomegalovirus primary infections during pregnancy:  
282 description and outcome: Outcome of maternal CMV infection during pregnancy, *Prenat.*  
283 *Diagn.*, p. 751-758, 2013, doi: 10.1002/pd.4118.
- 284 [9] G. Enders, A. Daiminger, U. Bäder, S. Exler, et M. Enders, Intrauterine transmission and clinical  
285 outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational  
286 age, *J. Clin. Virol.*, p. 244-246, 2011, doi: 10.1016/j.jcv.2011.07.005.
- 287 [10] V. Faure-Bardon *et al.*, Sequelae of congenital cytomegalovirus (cCMV) following maternal  
288 primary infection are limited to those acquired in the first trimester of pregnancy, *Clin. Infect.*  
289 *Dis. Off. Publ. Infect. Dis. Soc. Am.*, 2018, doi: 10.1093/cid/ciy1128.
- 290 [11] T. G. Wreghitt, E. L. Teare, O. Sule, R. Devi, et P. Rice, Cytomegalovirus Infection in  
291 Immunocompetent Patients, *Clin. Infect. Dis.*, p. 1603-1606, 2003, doi: 10.1086/379711.
- 292 [12] C. Vauloup-Fellous, T. Lazzarotto, M. G. Revello, et L. Grangeot-Keros, Clinical evaluation of the  
293 Roche Elecsys CMV IgG Avidity assay, *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin.*  
294 *Microbiol.*, p. 1365-1369, 2014, doi: 10.1007/s10096-014-2080-4.
- 295 [13] C. Busse, A. Strubel, et P. Schnitzler, Combination of native and recombinant cytomegalovirus  
296 antigens in a new ELISA for detection of CMV-specific antibodies, *J. Clin. Virol.*, p. 137-141,  
297 2008, doi: 10.1016/j.jcv.2008.05.011.
- 298 [14] M. G. Revello, C. Fornara, A. Arossa, P. Zelini, et D. Lilleri, Role of human cytomegalovirus  
299 (HCMV)-specific antibody in HCMV-infected pregnant women, *Early Hum. Dev.*, p. S32-S34,  
300 2014, doi: 10.1016/S0378-3782(14)70011-8.
- 301 [15] M. Macé, L. Sissoeff, A. Rudent, et L. Grangeot-Keros, A serological testing algorithm for the  
302 diagnosis of primary CMV infection in pregnant women: DIAGNOSIS OF CMV PRIMARY  
303 INFECTION IN PREGNANT WOMEN, *Prenat. Diagn.*, p. 861-863, 2004, doi: 10.1002/pd.1001.
- 304 [16] M. L. Delforge, L. Desomberg, et I. Montesinos, Evaluation of the new LIAISON<sup>®</sup> CMV IgG, IgM  
305 and IgG Avidity II assays, *J. Clin. Virol.*, p. 42-45, 2015, doi: 10.1016/j.jcv.2015.09.002.

- 306 [17] S. C. Dollard, S. A. S. Staras, M. M. Amin, D. S. Schmid, et M. J. Cannon, National Prevalence  
307 Estimates for Cytomegalovirus IgM and IgG Avidity and Association between High IgM Antibody  
308 Titer and Low IgG Avidity, *Clin. Vaccine Immunol.*, p. 1895-1899, 2011, doi: 10.1128/CVI.05228-  
309 11.
- 310 [18] M. Leruez-Ville, Y. Sellier, L. J. Salomon, J. J. Stirnemann, F. Jacquemard, et Y. Ville, Prediction of  
311 Fetal Infection in Cases With Cytomegalovirus Immunoglobulin M in the First Trimester of  
312 Pregnancy: A Retrospective Cohort, *Clin. Infect. Dis.*, p. 1428-1435, 2013, doi:  
313 10.1093/cid/cit059.
- 314 [19] C. Vauloup-Fellous, M. Berth, F. Heskia, J.-M. Dugua, et L. Grangeot-Keros, Re-evaluation of the  
315 VIDAS® cytomegalovirus (CMV) IgG avidity assay: Determination of new cut-off values based on  
316 the study of kinetics of CMV-IgG maturation, *J. Clin. Virol.*, p. 118-123, 2013, doi:  
317 10.1016/j.jcv.2012.10.017.
- 318 [20] S. L. Nielsen, I. Sørensen, et H. K. Andersen, Kinetics of specific immunoglobulins M, E, A, and G  
319 in congenital, primary, and secondary cytomegalovirus infection studied by antibody-capture  
320 enzyme-linked immunosorbent assay, *J. Clin. Microbiol.*, p. 654-661, 1988.
- 321 [21] R. F. Pass, S. Stagno, W. J. Britt, et C. A. Alford, Specific cell-mediated immunity and the natural  
322 history of congenital infection with cytomegalovirus, *J. Infect. Dis.*, p. 953-961, 1983.
- 323 [22] T. Lazzarotto, B. Guerra, M. Lanari, L. Gabrielli, et M. P. Landini, New advances in the diagnosis  
324 of congenital cytomegalovirus infection, *J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*, p.  
325 192-197, 2008, doi: 10.1016/j.jcv.2007.10.015.
- 326 [23] T. Lazzarotto, S. Brojanac, G. T. Maine, et M. P. Landini, Search for cytomegalovirus-specific  
327 immunoglobulin M: comparison between a new western blot, conventional western blot, and  
328 nine commercially available assays., *Clin. Diagn. Lab. Immunol.*, p. 483-486, 1997, doi:  
329 10.1128/CDLI.4.4.483-486.1997.
- 330 [24] T. Lazzarotto *et al.*, Anticytomegalovirus (anti-CMV) immunoglobulin G avidity in identification  
331 of pregnant women at risk of transmitting congenital CMV infection, *Clin. Diagn. Lab. Immunol.*,  
332 p. 127-129, 1999.
- 333 [25] M. Bodéus, D. Beulné, et P. Goubau, Ability of Three IgG-Avidity Assays to Exclude Recent  
334 Cytomegalovirus Infection, *Eur. J. Clin. Microbiol. Infect. Dis.*, p. 0248-0252, 2001, doi:  
335 10.1007/s100960100484.
- 336 [26] M. G. Revello, G. Gorini, et G. Gerna, Clinical Evaluation of a Chemiluminescence Immunoassay  
337 for Determination of Immunoglobulin G Avidity to Human Cytomegalovirus, *Clin. Diagn. Lab.  
338 Immunol.*, p. 801-805, 2004, doi: 10.1128/CDLI.11.4.801-805.2004.
- 339 [27] K. Lagrou, M. Bodeus, M. Van Ranst, et P. Goubau, Evaluation of the New Architect  
340 Cytomegalovirus Immunoglobulin M (IgM), IgG, and IgG Avidity Assays, *J. Clin. Microbiol.*, p.  
341 1695-1699, 2009, doi: 10.1128/JCM.02172-08.
- 342 [28] M. E. Guisasola, Belé. Ramos, J. C. Sanz, I. García-Bermejo, et F. De Ory Manchón, Comparison  
343 of IgG avidity assays in the confirmation of the diagnosis of cytomegalovirus primary infection:  
344 IGG AVIDITY ASSAYS FOR DIAGNOSIS OF CMV INFECTION, *APMIS*, p. 991-993, 2010, doi:  
345 10.1111/j.1600-0463.2010.02682.x.
- 346 [29] T. Lazzarotto *et al.*, Maternal IgG avidity and IgM detected by blot as diagnostic tools to identify  
347 pregnant women at risk of transmitting cytomegalovirus, *Viral Immunol.*, p. 137-141, 2000, doi:  
348 10.1089/vim.2000.13.137.
- 349 [30] S. C. Munro *et al.*, Diagnosis of and screening for cytomegalovirus infection in pregnant women,  
350 *J. Clin. Microbiol.*, p. 4713-4718, 2005, doi: 10.1128/JCM.43.9.4713-4718.2005.
- 351 [31] C. Vauloup-Fellous *et al.*, Does hygiene counseling have an impact on the rate of CMV primary  
352 infection during pregnancy? Results of a 3-year prospective study in a French hospital, *J. Clin.  
353 Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*, p. S49-53, 2009, doi: 10.1016/j.jcv.2009.09.003.

- 354 [32] W. D. Rawlinson *et al.*, Congenital cytomegalovirus infection in pregnancy and the neonate:  
355 consensus recommendations for prevention, diagnosis, and therapy, *Lancet Infect. Dis.*, p.  
356 e177-e188, 2017, doi: 10.1016/S1473-3099(17)30143-3.
- 357 [33] M. De Paschale, C. Agrappi, M. T. Manco, et P. Clerici, Positive predictive value of anti-HCMV  
358 IgM as an index of primary infection, *J. Virol. Methods*, p. 121-125, 2010, doi:  
359 10.1016/j.jviromet.2010.05.001.
- 360 [34] M. Yoshida *et al.*, Can measurement of maternal anti-cytomegalovirus immunoglobulin-M  
361 antibody levels be used to screen for cytomegalovirus infection in embryos and fetuses?, *J.*  
362 *Obstet. Gynaecol. Res.*, p. 166-169, 2013, doi: 10.1111/j.1447-0756.2012.01900.x.
- 363 [35] A. Goncé *et al.*, Maternal IgM antibody status in confirmed fetal cytomegalovirus infection  
364 detected by sonographic signs, *Prenat. Diagn.*, p. 817-821, 2012, doi: 10.1002/pd.3907.
- 365 [36] Y. Torii *et al.*, Serological screening of immunoglobulin M and immunoglobulin G during  
366 pregnancy for predicting congenital cytomegalovirus infection, *BMC Pregnancy Childbirth*, p.  
367 205, 2019, doi: 10.1186/s12884-019-2360-1.
- 368 [37] Toriyabe K. *et al.*, Anticytomegalovirus IgM titer for congenital infection in first-trimester  
369 pregnancy with primary infection, *J. Obstet. Gynaecol. Res.*, p. 1555-1556, 2018, doi:  
370 10.1111/jog.13762.  
371

1 **Figure 1: Study population flowchart.**

2 Serums with positive CMV-IgM were classified according to CMV-IgG avidity index: CMV-IgG  
3 negative or equivocal and no seroconversion on a second sample after 15 days = **non specific CMV**;-  
4 positive CMV-IgG and high CMV-IgG avidity index = **recent primary infection excluded** (PI>3  
5 months) (black box); CMV-IgG positive and low avidity index = **recent primary infection**  
6 **confirmed** (PI<3 months) (light grey box); CMV-IgG positive and intermediate avidity index =  
7 **recent primary infection not excluded** (PI<3 months?) (dark grey box).

8 Positive predictive values (PPV) were calculated for every clinical situation.

9 PI: primary infection – PPV: positive predictive value

10

11 **Figure 2: Final diagnosis in each clinical setting in case of positive CMV-IgM.**

12 (A) high avidity => recent CMV primary-infection (PI) excluded. (B): low avidity => recent PI  
13 confirmed. (C) moderate avidity: recent PI not excluded. (D): no seroconversion => nonspecific  
14 CMV-IgM. Systematic screening during pregnancy = group 1 (white bars); US abnormalities = group  
15 2 (grey bars); clinical signs in general population = group 3 (black bars).

16 US: ultrasound

17

18 **Figure 3: PPV of positive CMV-IgM depending on patients' age in general population (group 3).**

19 PI: primary infection

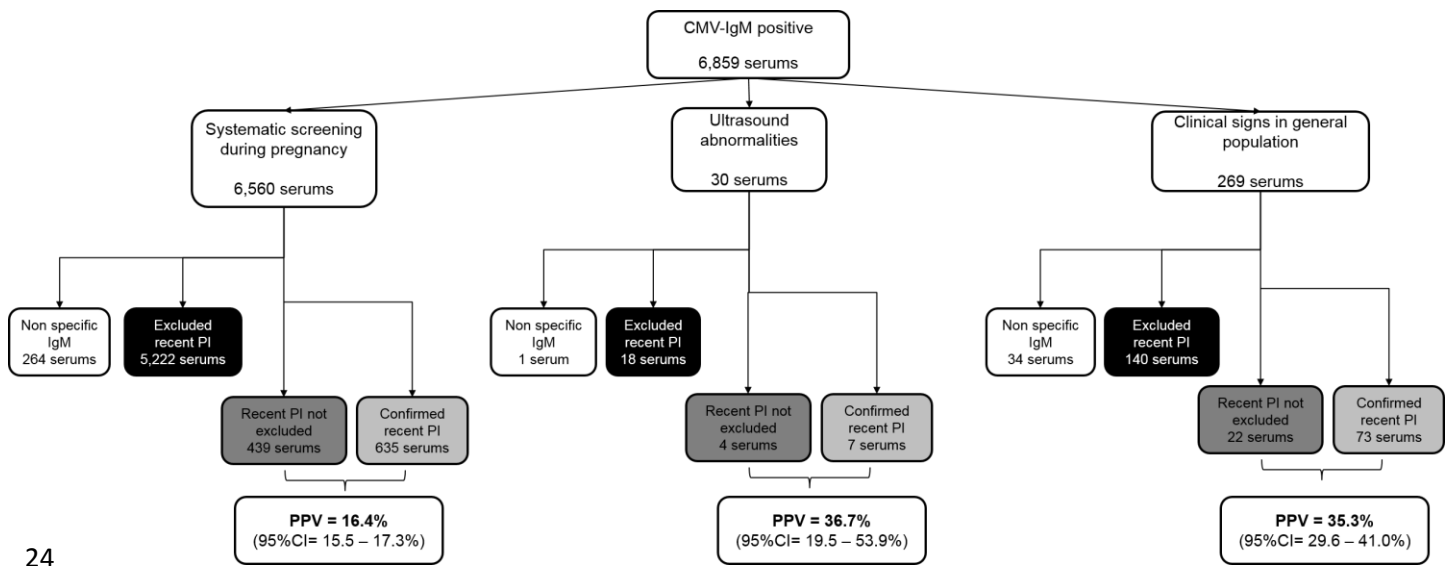
20

21



22 **Figure 1: Study population flowchart.**

23



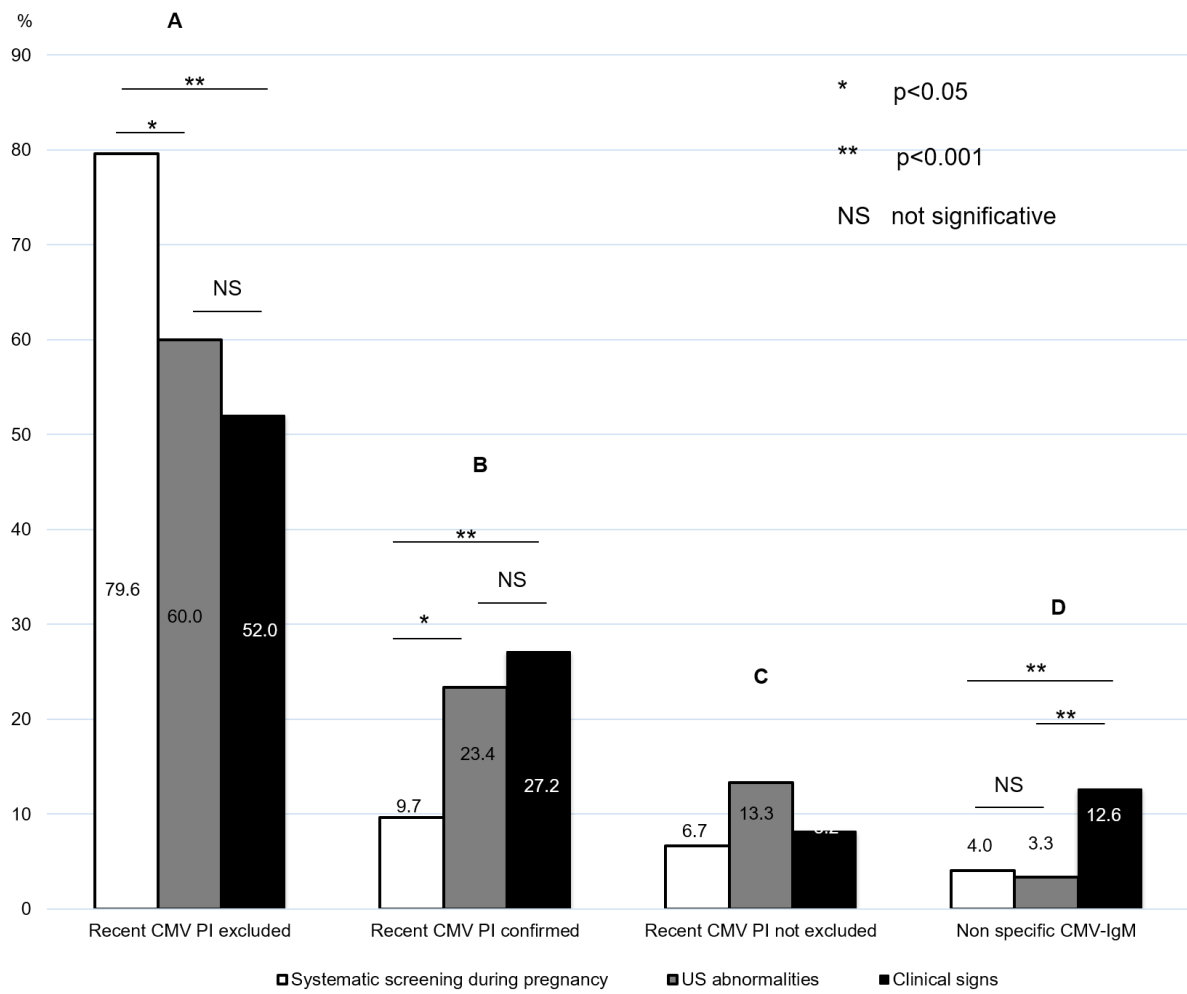
24

25

26

27 **Figure 2: Final diagnosis in each clinical setting in case of positive CMV-IgM.**

28



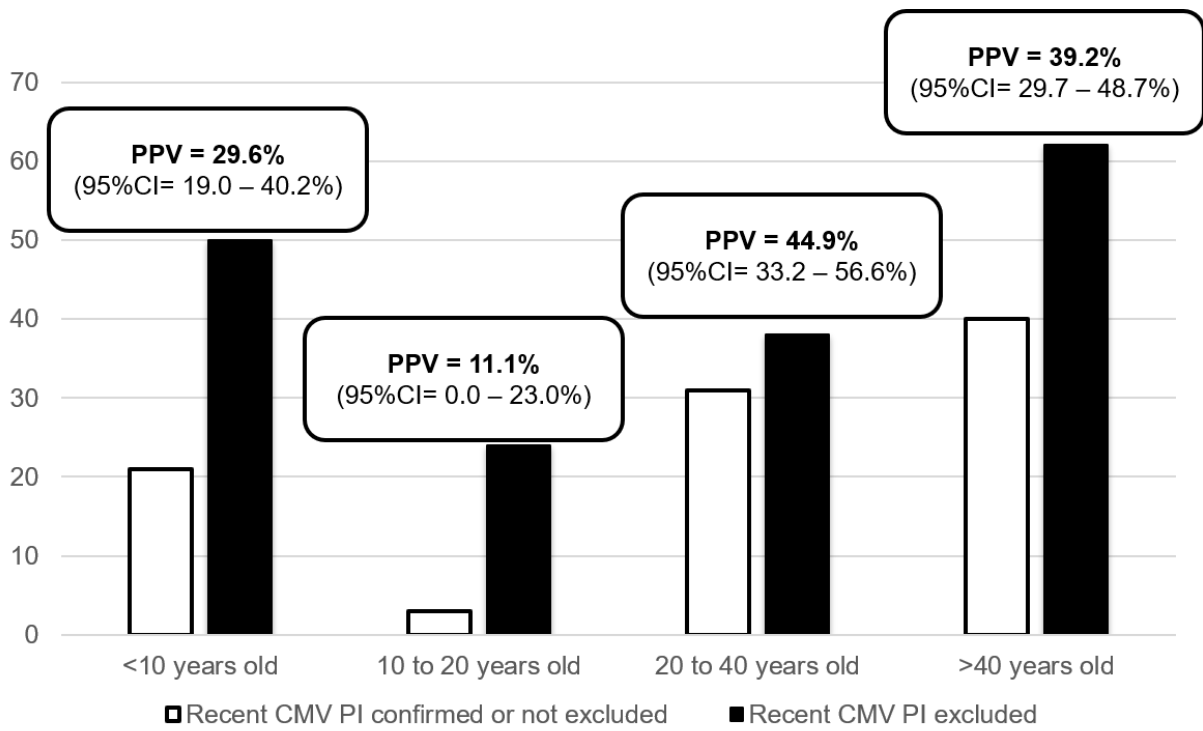
29

30

31

32

33 **Figure 3: PPV of positive CMV-IgM depending on patients' age in general population (group 3).**



34

35

36

**Sample CRediT author statement:**

**Claire Périllaud-Dubois:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

**Elise Bouthry:** Writing- Reviewing and Editing. **Abir Jadoui and Ay Ling Leng:** Investigation.

**Anne-Marie Roque-Afonso:** Writing- Reviewing and Editing. **Christelle Vauloup-Fellous:** Conceptualization, Writing- Reviewing and Editing, Supervision.

**Declarations of Interest:** none.

**Funding sources:** none.