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

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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.

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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
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- 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).

***Title Page (including author details, affiliations and word count)**

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

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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 ($p<0.001$).

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Word count: 247

***Highlights (for review)**

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- In case of positive CMV-IgM, CMV-IgG avidity is essential to diagnose recent primary infection.
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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.

Introduction

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

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

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

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

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

Material and methods

Sample collection:

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

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

- in non-symptomatic pregnant women referred to our pluridisciplinary prenatal center for US abnormalities (not initially screened at beginning of pregnancy) (group 2). Sonographic findings were mostly intrauterine growth retardation (IUGR) and cerebral abnormalities (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, and/or corpus callosum hypoplasia);

- in immunocompetent patients (general population: adults and children) in case of clinical symptoms suggestive of CMV primary infection (group 3), 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.

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

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

Serology assays:

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

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

Statistical analysis:

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

Results

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

- 6,560 /6,859 (95.64%) were collected in pregnant women during systematic screening (group 1)
- 30/6,859 (0.44%) were collected in pregnant women with US abnormalities (median gestational age 25 weeks of gestation (WG); range: 12-36 WG) (group 2).
- 269/6,859 (3.92%) were collected from immunocompetent patients with clinical symptoms of CMV primary infection (group 3)

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

Systematic screening during pregnancy (group 1)

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

Ultrasound abnormalities during pregnancy (group 2)

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

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

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

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

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

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

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

CMV-IgM PPV was significantly lower in group 1 comparing to groups 2 ($p=0.01$) and 3 ($p<0.001$). CMV-IgM PPV was not statistically different between groups 2 and 3 ($p>0.90$).

Discussion

Even if formal diagnosis of CMV primary infection is achieved with CMV-IgG seroconversion, as the "first" seronegative serum is usually not available, documentation of this seroconversion is rare. Usually, whatever the clinical situation, screening or clinical symptoms, only one serum specimen is available. The transient CMV-IgM positivity has long been used as a diagnostic marker for CMV primary infection, but it is well known that is not invariably indicative of primary infection. Indeed, specific or non-specific CMV-IgM can also be present in many other situations including infections with other pathogens associated with random polyclonal B cell stimulation or CMV non primary infection [20,21]. 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. In these cases, CMV-IgG avidity is essential and in contrast to IgM, low-avidity IgG is present only with primary infection, increasing over 3 to 4 months to high avidity [22]. CMV-IgG avidity has thus gained diagnostic importance in identifying primary CMV infection, and several commercial CMV-IgG avidity tests are currently available. Their performances to confirm a CMV primary infection were reported to range from 83 to 100%, and to exclude a CMV primary infection from 71 to 100% [12,16,19,23-28]. Even if not perfect, several groups have reported substantial improvements in identification of at-risk pregnancies using diagnostic algorithms including CMV-IgG avidity [7,15,18,22,29-31]. Indeed, it truly improves accuracy of CMV primary infection diagnosis as in the specific situation of systematic screening, PPV of positive CMV-IgM is quite poor [18,31]. Indeed, in our analysis, if we calculated PPV considering confirmed recent primary infection, PPV is only of 9.7% (95%CI= 9.0 – 10.4%). However, from a practical point of view, we calculated PPV

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

Usefulness of serologic testing for CMV during pregnancy has been questioned since congenital malformations can occur even following maternal secondary infections [6]. Although screening is not recommended by any public health system because of its cost/benefit ratio, it is widely adopted by many general practitioners and obstetricians in our area. Such screening provides an opportunity to identify seronegative women who can be counselled about using appropriate hygienic measures to prevent primary infection, especially in relation to their behavior with children, who are a major source of infection. Furthermore, screening aims to diagnose CMV primary infection early in pregnancy allowing women to be referred to Reference Centers for appropriate management (close ultrasonography, amniocentesis and/or neonatal diagnosis…) [32]. Some authors consider that screening is not justified because of its economic cost, the imperfect nature of congenital infection prognostic criteria, the risk of spontaneous abortions induced by invasive tests such as amniocentesis, and the few data concerning effective treatments during pregnancy. However, it is obvious that if US are observed, and prenatal diagnosis of CMV congenital infection discussed, maternal CMV serology should always be performed. It is particularly important especially in regions where seroprevalence is around 50%, as in France, or lower because its first aim is to confirm maternal infection to CMV (whatever the date of this infection) and allows to definitively exclude congenital CMV if the pregnant woman is seronegative. If positive, this CMV serology usually performed late in pregnancy, might be challenging to interpret. Our results show that positive CMV-IgM at time of US indicate a recent infection in 23% cases (7/30) and that additionally, 3/30 women had high avidity but gave birth to an infected child. 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. However, final diagnosis will of course be obtained with CMV PCR in amniotic fluid and/or in urine in the neonate at birth.

If CMV-IgG avidity is widely used in pregnant women, far less literature is available on the usefulness of CMV-IgG avidity in general population (immunocompetent patients), and in our area it is clearly not usually performed if the patient is not pregnant. Remarkably, positive CMV-IgM results are most often considered as indicative of a recent CMV virus infection in case with clinical signs, but they are in fact truly related to a CMV primary infection in only 27.1% cases (73/269) (low CMV IgG avidity) and possibly related in 35.3% cases (low/moderate CMV IgG avidity). Interestingly, PPV was significantly lower for patients between 10 to 20 years old (11.1% vs 29.6-44.9%) (p=0.01). 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. These also suggest that whatever the age of the patient, CMV-IgG avidity is essential for accurate diagnosis. 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. Consequently, general practitioners probably mostly do diagnosis of CMV primary infection and only the patients with the severest symptoms come to the hospital and are therefore included in our data. Moreover, it is possible that older people are more likely to have a severe infection compared to young patients. Nevertheless, for current practice, all clinicians should be aware that, whatever the age of the patient, and given the non-specific symptoms of CMV primary infection, even in case of positive CMV-IgM, confirmation by avidity avoids misdiagnosis in more than 60% cases.

Our observations are consistent with the preexisting ones [33-37] and 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 CMV primary infection in case of positive CMV-IgM.

Sample CRediT author statement:

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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.

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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.

Introduction

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

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

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

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

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

Material and methods

Sample collection:

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

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

- in non-symptomatic pregnant women referred to our pluridisciplinary prenatal center for US abnormalities (not initially screened at beginning of pregnancy) (group 2). Sonographic findings were mostly intrauterine growth retardation (IUGR) and cerebral abnormalities (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, and/or corpus callosum hypoplasia);

- in immunocompetent patients (general population: adults and children) in case of clinical symptoms suggestive of CMV primary infection (group 3), 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.

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

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

Serology assays:

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

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

Statistical analysis:

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

Results

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

- 6,560 /6,859 (95.64%) were collected in pregnant women during systematic screening (group 1)
- 30/6,859 (0.44%) were collected in pregnant women with US abnormalities (median gestational age 25 weeks of gestation (WG); range: 12-36 WG) (group 2).
- 269/6,859 (3.92%) were collected from immunocompetent patients with clinical symptoms of CMV primary infection (group 3)

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

Systematic screening during pregnancy (group 1)

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

Ultrasound abnormalities during pregnancy (group 2)

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

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

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

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

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

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

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

CMV-IgM PPV was significantly lower in group 1 comparing to groups 2 ($p=0.01$) and 3 ($p<0.001$). CMV-IgM PPV was not statistically different between groups 2 and 3 ($p>0.90$).

Discussion

Even if formal diagnosis of CMV primary infection is achieved with CMV-IgG seroconversion, as the "first" seronegative serum is usually not available, documentation of this seroconversion is rare. Usually, whatever the clinical situation, screening or clinical symptoms, only one serum specimen is available. The transient CMV-IgM positivity has long been used as a diagnostic marker for CMV primary infection, but it is well known that is not invariably indicative of primary infection. Indeed, specific or non-specific CMV-IgM can also be present in many other situations including infections with other pathogens associated with random polyclonal B cell stimulation or CMV non primary infection [20,21]. 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. In these cases, CMV-IgG avidity is essential and in contrast to IgM, low-avidity IgG is present only with primary infection, increasing over 3 to 4 months to high avidity [22]. CMV-IgG avidity has thus gained diagnostic importance in identifying primary CMV infection, and several commercial CMV-IgG avidity tests are currently available. Their performances to confirm a CMV primary infection were reported to range from 83 to 100%, and to exclude a CMV primary infection from 71 to 100% [12,16,19,23-28]. Even if not perfect, several groups have reported substantial improvements in identification of at-risk pregnancies using diagnostic algorithms including CMV-IgG avidity [7,15,18,22,29-31]. Indeed, it truly improves accuracy of CMV primary infection diagnosis as in the specific situation of systematic screening, PPV of positive CMV-IgM is quite poor [18,31]. Indeed, in our analysis, if we calculated PPV considering confirmed recent primary infection, PPV is only of 9.7% (95%CI= 9.0 – 10.4%). However, from a practical point of view, we calculated PPV

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

Usefulness of serologic testing for CMV during pregnancy has been questioned since congenital malformations can occur even following maternal secondary infections [6]. Although screening is not recommended by any public health system because of its cost/benefit ratio, it is widely adopted by many general practitioners and obstetricians in our area. Such screening provides an opportunity to identify seronegative women who can be counselled about using appropriate hygienic measures to prevent primary infection, especially in relation to their behavior with children, who are a major source of infection. Furthermore, screening aims to diagnose CMV primary infection early in pregnancy allowing women to be referred to Reference Centers for appropriate management (close ultrasonography, amniocentesis and/or neonatal diagnosis…) [32]. Some authors consider that screening is not justified because of its economic cost, the imperfect nature of congenital infection prognostic criteria, the risk of spontaneous abortions induced by invasive tests such as amniocentesis, and the few data concerning effective treatments during pregnancy. However, it is obvious that if US are observed, and prenatal diagnosis of CMV congenital infection discussed, maternal CMV serology should always be performed. It is particularly important especially in regions where seroprevalence is around 50%, as in France, or lower because its first aim is to confirm maternal infection to CMV (whatever the date of this infection) and allows to definitively exclude congenital CMV if the pregnant woman is seronegative. If positive, this CMV serology usually performed late in pregnancy, might be challenging to interpret. Our results show that positive CMV-IgM at time of US indicate a recent infection in 23% cases (7/30) and that additionally, 3/30 women had high avidity but gave birth to an infected child. 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. However, final diagnosis will of course be obtained with CMV PCR in amniotic fluid and/or in urine in the neonate at birth.

If CMV-IgG avidity is widely used in pregnant women, far less literature is available on the usefulness of CMV-IgG avidity in general population (immunocompetent patients), and in our area it is clearly not usually performed if the patient is not pregnant. Remarkably, positive CMV-IgM results are most often considered as indicative of a recent CMV virus infection in case with clinical signs, but they are in fact truly related to a CMV primary infection in only 27.1% cases (73/269) (low CMV IgG avidity) and possibly related in 35.3% cases (low/moderate CMV IgG avidity). Interestingly, PPV was significantly lower for patients between 10 to 20 years old (11.1% vs 29.6-44.9%) (p=0.01). 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. These also suggest that whatever the age of the patient, CMV-IgG avidity is essential for accurate diagnosis. 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. Consequently, general practitioners probably mostly do diagnosis of CMV primary infection and only the patients with the severest symptoms come to the hospital and are therefore included in our data. Moreover, it is possible that older people are more likely to have a severe infection compared to young patients. Nevertheless, for current practice, all clinicians should be aware that, whatever the age of the patient, and given the non-specific symptoms of CMV primary infection, even in case of positive CMV-IgM, confirmation by avidity avoids misdiagnosis in more than 60% cases.

Our observations are consistent with the preexisting ones [33-37] and 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 CMV primary infection in case of positive CMV-IgM.

Sample CRediT author statement:

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Figure 1: Study population flowchart.

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

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

PI: primary infection – PPV: positive predictive value

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

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

US: ultrasound

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

PI: primary infection

Figure 1: Study population flowchart.

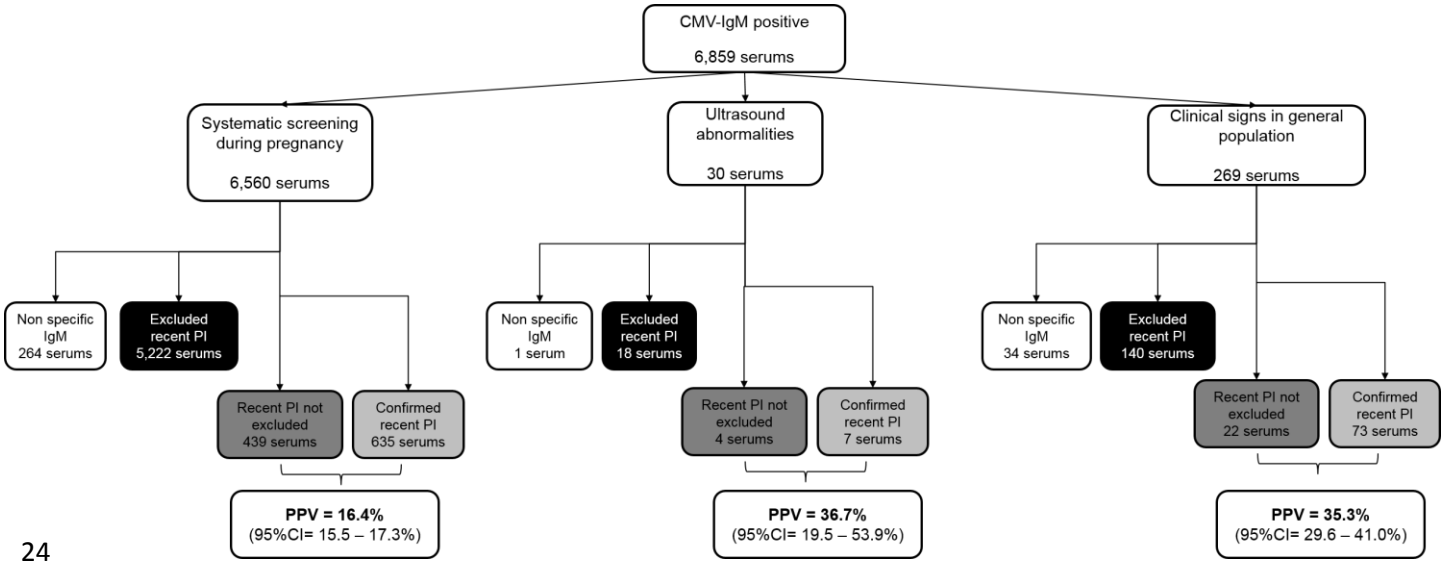


Figure 2: Final diagnosis in each clinical setting in case of positive CMV-IgM.

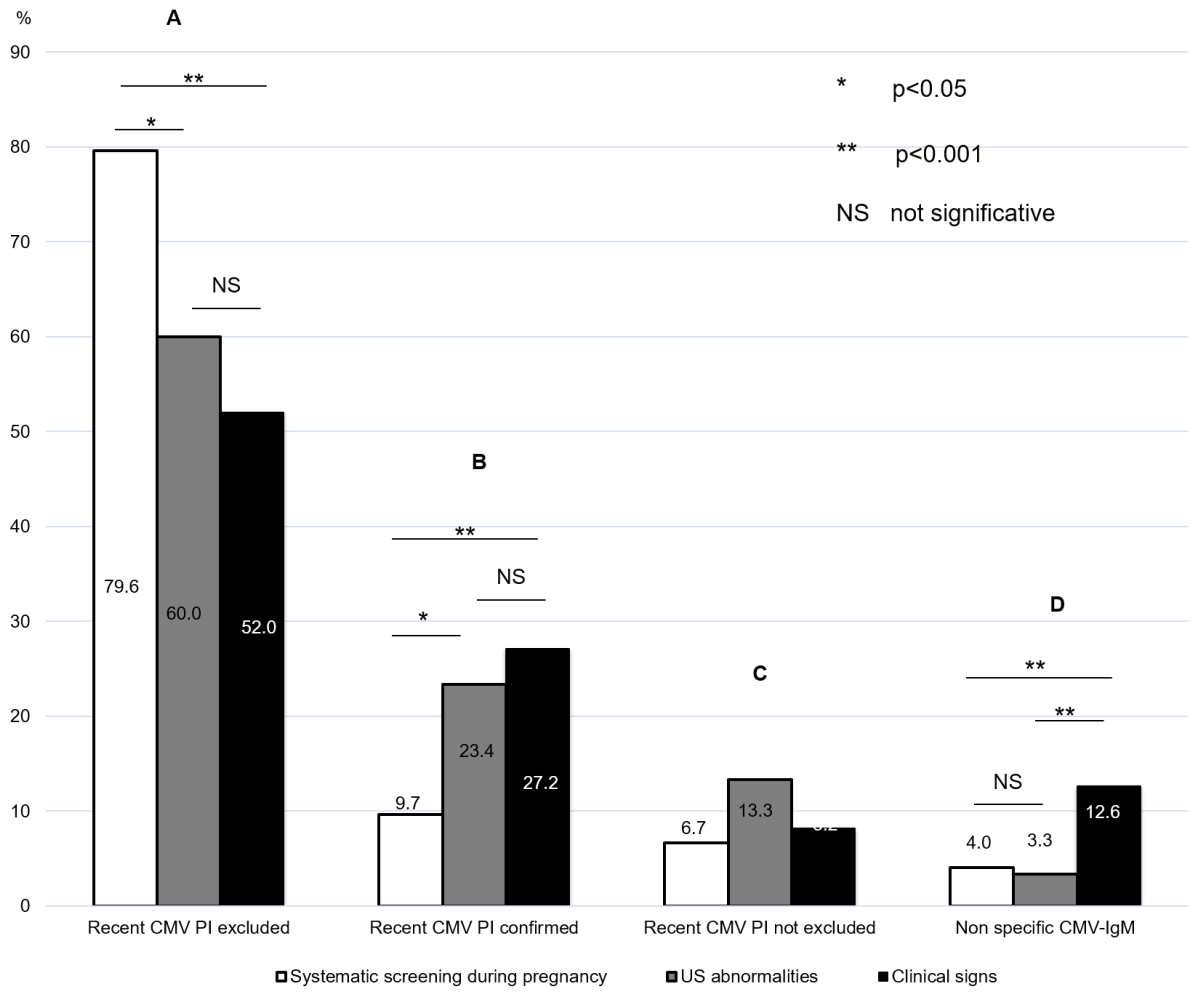
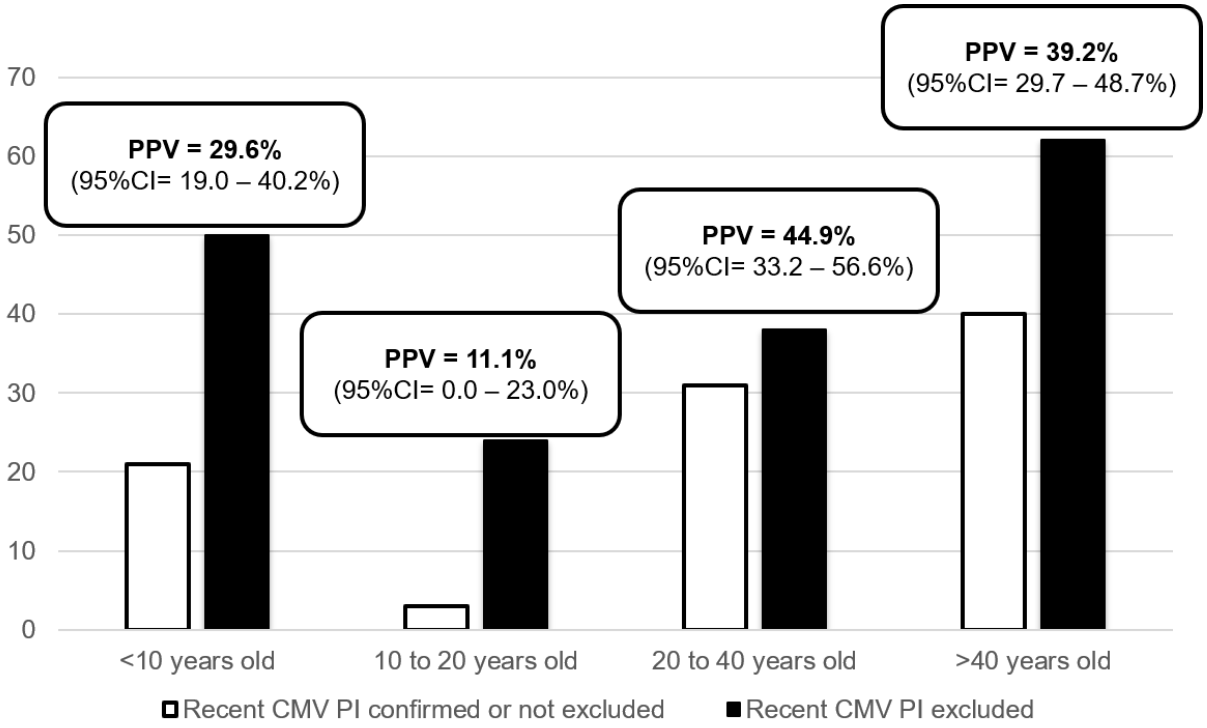


Figure 3: PPV of positive CMV-IgM depending on patients' age in general population (group 3).



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