

Epidemiology of cytomegalovirus infection in pregnant women living in the Greater Romagna Area, Italy

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Summary

Background. Aim of this study was to assess the incidence of *Cytomegalovirus* (CMV) infection in pregnant women living in Romagna area, in North East Italy to implement the best management of this infection.

Materials and Methods. In 2012, 23,727 serological tests for CMV IgG and IgM antibodies were performed in the Microbiology Unit, the Hub Laboratory of the Greater Romagna Area: 6931 were pregnant women.

Results and Conclusions. 179 subjects were positive for CMV IgM antibodies: 82 were not pregnant; 97 were IgM positive during pregnancy or in the course of a pre-conception evaluation. The detected incidence of the CMV infection in pregnancy (calculated at 1.40%) actually validates the literature data. This study's findings clearly underline the usefulness of testing the CMV specific immune response in the pre-conception period or as early as possible during pregnancy.

Introduction

Cytomegalovirus (CMV) is a herpes virus. and causes inter-human transmitted infections that are usually benign and self-limited to

immunocompetent hosts. After a primary infection, the virus remains latent in the body inside blood mononuclear cells (CD14+) and bone marrow progenitors (CD34+ and CD33+) (4).

Data from the literature show a seroprevalence of about 70-80% in the adult population of Italy (1).

Humans are believed to be the only reservoir for human CMV and the transmission is thought to usually take place by direct human contact, while indirect transmission is considered to be less common.

During pregnancy, primary infection may occur *in utero* with maternal-fetal transmission in 30-40% of cases; in cases of secondary infection (reactivation or re-infection) the probability of vertical transmission is reduced to 0.5-2%.

Incidence of primary infection during pregnancy is estimated to be 1-2%, while incidence of seroconversions increases in pregnant women who have frequent contact with children especially younger than three, compared to a population with a lower frequency of contact with young children.

It is known that congenital infection can cause serious sequelae in the fetus and newborn, especially if the vertical transmission occurs during the first trimester of gestation .

Only 10-15% of the total of congenitally infected infants suffer from symptoms at birth; 85-90% of transplacental infected newborns do not show any symptoms at birth, but 8-15% of these children will present late signs of infection such as hearing problems.

According to the Guidelines of the National Guidelines System (SNLG) for normal pregnancy (http://www.snlg-iss.it/cms/files/LG_Gravidanza.pdf), there is no clinic-laboratory evidence of the need to advise and perform serological screening for CMV on all pregnant women (6).

The Microbiology Operating Unit (O.U.) of AUSL (the Local Unit of the National Health Service) Romagna includes the whole Forlì-Cesena, Ravenna and Rimini territory with about 1.3 million inhabitants and, therefore, the cases reported in our study can be considered as an epidemiological snapshot of CMV disease throughout this population. This opportunity to collect patients and biological samples, together with the standardization and unification of the diagnostic methods used for serological tests as well as the availability of a 5 years online database for laboratory activity, provide a solid basis to conduct an assessment of CMV infection in pregnant women in Romagna. The goal of identifying epidemiological data is to decide what actions are most appropriate for the best management of CMV infection in pregnancy.

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Materials and Methods

Population examined

In the study period (January-December 2012) we considered all the patients tested for CMV IgG and IgM who had been sent to the

Microbiology O.U.; in particular, we examined the female population of childbearing age (15-52 years). Since serological screening for CMV in pregnancy is not offered by the National Health Service (NHS), we evaluated the representativeness of our population screened for antibodies to CMV by comparing this group (female patients of childbearing age screened for CMV) with the number of pregnant women tested for antibodies to *Toxoplasma gondii* in the same period of time.

Test and tools

The tests were performed by automatic analyzer operating in chemiluminescence LIAISON® (DiaSorin, Saluggia, Italy) using reactive LIAISON® CMVigG and LIAISON® CMVigG II (a new version of the kit was available), and LIAISON® CMVigM and LIAISON® CMVigMII (always a new version of the kit). LIAISON® CMVigG LIAISON® CMVigG and II are both quantitative tests, with IgG expressed in IU/mL for the first test (reported in WHO standard) and in U/mL for version II (relative to internal standard). For LIAISON® CMVigG results <0.4 are considered non-reactive and responsive if >0.6, while for LIAISON® CMVigG II results <12.0 are considered non-reactive and responsive if >14.0. For IgM: LIAISON® CMVigM results <15 AU/mL are considered nonreactive and responsive if >30 AU/mL while LIAISON® CMVigG II considers nonreactive results <18.0 U/mL and responsive if >22.0 U/mL.

IgM positivity was confirmed with the Biomerieux VIDAS® (Biomerieux, Marcy-l'Étoile, France) instrument and CMV IgM kit (ELFA technique) (3,5).

For patients whose pregnancy was certified and signed the exemption code, if the results were both IgG and IgM positive, the avidity test for CMV IgG (CMV IgG avidity LIAISON®) was always performed on automated equipment (2).

Results

In the study period, the Microbiology O.U. performed 23,727 serological tests for both CMV IgG and CMV IgM; 11,985 were women of childbearing age (15-52 years); 6931 of them proved to be pregnant (58% of the test subjects) therefore, tests were required for maternal-fetal infection prevention, while for 5054 women (*i.e.* 42%), tests were prescribed for diagnostic purposes. In January-December 2012, this population of pregnant women (6931 women) was found to be 55.52% of the population of pregnant women that has been tested for antibodies to *Toxoplasma gondii* in the same period (equivalent to 12,484 persons): that is more than half of pregnant women underwent CMV testing, despite the fact that determining CMV immune status in pregnancy is not a free service provided by NHS.

Out of the 11,985 screened women 179 were identified and confirmed as positive for IgM (equal to 1.49% of the total population of surveyed women): of these, 97 were pregnant women (1.40% of pregnant tested women) and 82 were non-pregnant (equal to 1.62%) (Figure 1).

Anamnestic-epidemiological evaluation performed on 97 pregnant women with positive results for IgM allowed this population to be divided as shown in Figure 2, in detail: i) for 16 (17%) positivity for IgM had already been determined and documented before 2012; ii) for 6 (6%) seroconversion was documented with conversion from a previous seronegative status (IgG and IgM a seropositive one (IgG and IgM)); iii) for 75 (77%), we had no serum samples before.

Because of the simultaneous presence of CMV IgG and IgM in these 75 women, they were tested for IgG avidity as well. The results of these tests were: 69 cases of high avidity, 3 cases of moderate avidity, and 3 cases low avidity.

For the 69 women with high avidity antibodies to CMV, gestational age is shown in Figure 3: 7 of these women were under the care of Centers of Assisted Reproduction so they were tested before pregnancy,

while 52 women were found to be within the first trimester of gestation, so it seems likely that the infection may have been contracted before conception.

Table 1 details the six cases with documented seroconversion. The results obtained with the reagents and CMVigG CMVigM are shown in italics, while the results obtained with reactive and CMVigGII CMVigMII are reported in regular font style.

Table 2 summarizes the 6 cases of moderate/low avidity.

Discussion and Conclusions

The national guidelines and the current legislation (Presidential Decree 245 of 10/09/1998) do not provide for routine, mandatory serological screening for CMV infection during pregnancy, therefore, a correct assessment of infection incidence is impossible because data is

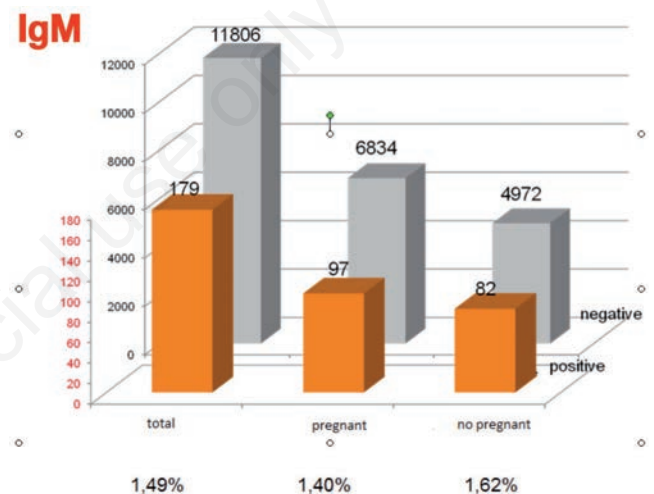


Figure 1. Cytomegalovirus IgM specific antibodies results in the different groups included in the study.

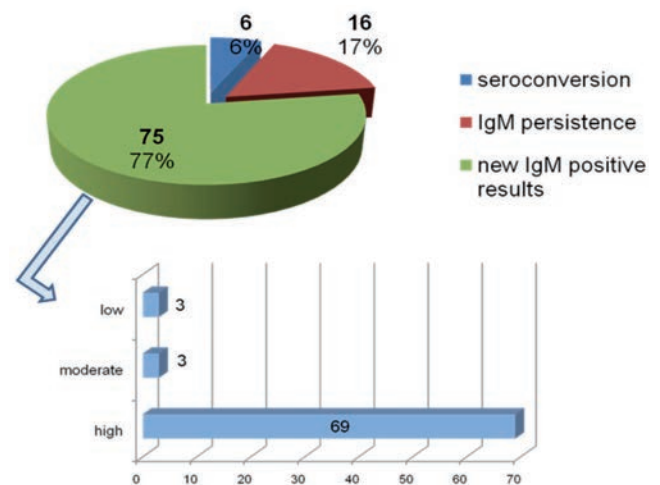


Figure 2. Cytomegalovirus IgM specific antibodies results in pregnancy: seroconversion, persistence and new positive results.

not available on the overall number of pregnant women. The population studied in this survey shows an incidence of IgM specific positivity equal to 1.40% according to the data in the literature, but thanks to additional diagnostic tests (IgG avidity tests) and the availability of historical data relating to the immune status of individuals, this percentage may be found to be lower. The finding of high IgG avidity in the pre-conception period and in the first trimester of pregnancy among the population of pregnant women in Romagna allows to rule out, with a reasonable degree of confidence, an infection acquired after conception for the 59 women found to be IgG and IgM anti-CMV positive.

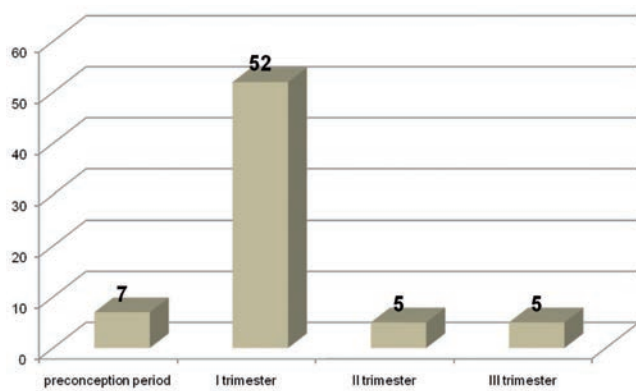


Figure 3. Cytomegalovirus IgG avidity: high index in gestational age.

Table 1. Cytomegalovirus (CMV) seroconversion in pregnancy.

Name	Age	Weeks of pregnancy	CMV IgG	CMV IgM	Avidity
BE	35	19	5	5	0.04
		24	42	34	
BM	31	18	5	5	0.06
		24	38	44	
PA	33	21	5	5	
		27	29	101	
VG	35	18	0.2	8	0.06
		27	7.2	81	
PE	34	26	0.2	8	
		32	1.1	84	
CE	31	28	0.2	8	0.25
		37	4.2	59	

Avidity index: low <0.200; moderate 0.200-0.300; high >0.300.

Table 2. Cytomegalovirus (CMV) low and moderate avidity in pregnancy.

Name	Age	Weeks of pregnancy	CMV IgG	CMV IgM	Avidity
CIR	28	11	4.7	50	0.13
CJ	24	12	59	96	0.08
FS	22	13	5.4	64	0.08
AA	28	00	69	57	0.26
CD	27	00	90	23	0.24
CR	26	7	107	31	0.25

Avidity index: low <0.200; moderate 0.200-0.300; high >0.300.

Thus, based on these considerations, the potentially true number of cases of infection in pregnancy amounts to 20 cases (with an incidence of 0.29%). This total of 20 includes 10 cases of high IgG avidity pregnancies with the test performed after the first quarter and, therefore, the time between acquisition of cytomegalic infection and pregnancy cannot be estimated with a good degree of confidence; 4 cases in which low/moderate avidity was found (two of the six cases of low/moderate avidity were not considered because test were mandated by Centers for Assisted Reproduction performed in the pre-conception period) while the last 6 were documented cases of seroconversion.

To date, there are no proven therapeutic measures that are effective against an infection contracted during pregnancy. Given the low incidence of cytomegalic infection in the population studied, we can assume the recommendations set out in Physiological Pregnancy Guideline to be justified as they recommend the determination of CMV immune status exclusively for particular categories of pregnant women, such as those with professional contact with children, those who have kindergarten children in the household, those with flu-like symptoms or in those cases where morphological alterations during fetal ultrasound check is documented. However, knowing the risk of acquiring post-conceptual transmission is really important for the proper post-natal follow-up (up to age 6-9 years) of newborns with potential transmission cytomegalic infection during intra uterine life. Only in this way can neonatologist identify in a timely manner the clinical manifestations related to CMV infection (including mainly chorioretinitis and late deafness), and thus be able to quickly implement all the necessary therapeutic interventions. Obviously if serological screening for the CMV immune status is performed, this diagnostic practice is recommended in the pre-conception stage or, in the case of unplanned pregnancy, at the earliest stages of pregnancy, when in case of IgG and IgM positivity the IgG avidity test can help date the time of infection. Ofcourse, in case of serological results compatible with past infection (IgG positive and IgM negative) there is no need for additional testing.

For pregnant women who are susceptible to infection, the only possible prophylactic measure is hygiene instruction and engaging in behavior that can reduce the risk of acquiring the infection (1).

Furthermore, if the pregnant woman is seronegative (and the test is performed within 12 weeks of pregnancy), the working group on maternal-fetal infections of our local health unit in Romagna has proposed to repeat the test 1 month before the birth. While not fully adhering to the National Guidelines, this strategy enables a speedy and assured identification of seroconversion during pregnancy with its increased risk of vertical transmission to fetus.

The aim is the inclusion of at risk infants in the postnatal follow-up program as early as possible (because the identification of the infection occurs in prenatal). Another matter of considerable and essential importance for the proper management of the daily problem of CMV infection in pregnant women is the online availability of earlier serological databases. We wish also to emphasize the importance of running the tests at the same laboratory so that the serological profile can be correctly assessed and to ensure the availability of the serum samples which should be stored in a biological *bank* at least until the end of each pregnancy.

References

1. Gruppo multidisciplinare Malattie infettive in ostetricia-ginecologia e neonatologia. Percorsi diagnostici-assistenziali in Ostetricia-Ginecologia e Neonatologia. Citomegalovirus. Aprile 2012. Available from: http://www.neonatologia.it/upload/725_CITOMEGALOVIRUS_Aprile_2012.pdf

2. Kanengisser-Pines B, Hazan Y, Pines G, Appelman Z. High cytomegalovirus IgG avidity is a reliable indicator of past infection in patients with positive IgM detected during the first trimester of pregnancy. *J Perinat Med* 2009; 37: 15-8.
3. Macè M, Sissoeff L, Rudent A, Grangeot-Keros L. Serological testing algorithm for the diagnosis of primary CMV infection in pregnant women. *Prenat Diagn* 2004; 24: 861-3.
4. Mocarski ES, Shenk T. Cytomegalovirus. In: *Fields virology*. 5th ed. Knipe DM, Howley PM, eds. Philadelphia: Lippincott Williams and Wilkins; 2007. pp 2701-72.
5. Lazzarotto T, Guerra B, Lanari M, et al. New advances in the diagnosis of congenital cytomegalovirus infection. *J Clin Virol* 2008; 41: 192-7.
6. SNLG-ISS. Linea guida Gravidanza Fisiologica. Available from: http://www.snlg-iss.it/cms/files/LG_Gravidanza.pdf.

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