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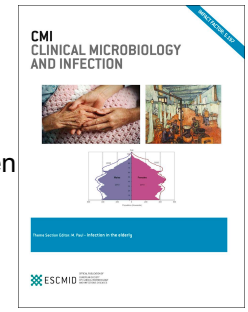


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# Pregnancy outcomes and cytomegalovirus DNAemia in HIV-infected pregnant women with CMV.

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Sir, it is well known that cytomegalovirus (CMV) coinfection affects a large proportion of people with HIV, with a significant impact on disease progression and survival [1]. In HIV-CMV coinfected pregnant women, however, few studies have been conducted: maternal immunosuppression has been linked to a higher risk of CMV infant infection, and CMV DNAemia to higher maternal and infant mortality [2-3]. Overall, little is known about pregnancy outcomes and CMV viremia in CMV-coinfected pregnant women with HIV. In order to further explore this issue, we evaluated the impact of CMV coinfection on pregnancy outcomes in a national cohort of pregnant women with HIV, assessing in a study subsample the prevalence and correlates of CMV DNAemia in HIV-CMV coinfected pregnant women.

Data from the Italian National Program on Surveillance on Antiretroviral Treatment in Pregnancy were used [4], considering all HIV-infected women with known CMV serostatus. We compared the main pregnancy outcomes between women with and without positive serology for CMV, and evaluated plasma CMV-DNA levels in a subset of CMV-positive women who had given specific consent to virologic evaluations and had evaluable plasma samples. The cases were analyzed retrospectively, and no systematic screening for CMV infection was conducted in infants. Ethics approval was obtained from the Ethics Committee of the I.N.M.I. Lazzaro Spallanzani in Rome; ref. deliberations: 578/2001 (September 28, 2001) and 7/2003 (February 26, 2003). Plasma CMV-DNA was quantified with the kPCR PLX® Cytomegalovirus DNA assay (Siemens Healthcare) using the VERSANT™ kPCR Molecular System (Siemens), with a detection limit of 214.6 (2.33 log) IU/ml. Quantitative data were compared by the Mann-Whitney U test and proportions by the chi-square test, with odds ratios (OR) and 95% confidence intervals (CI) calculated. P values below 0.05 were considered significant, using for all analyses the SPSS software, version 22.0 (IBM Corp, Released 2013, Armonk, NY, USA).

As of March 21, 2016, among 2250 pregnancies with available information on CMV serostatus (62% of all cases in the database, including ongoing pregnancies and cases lost to follow up), 1490 were CMV antibody-positive (66.2%). Women with and without positive serology for CMV were similar for age, CD4 counts, CDC-HIV disease stage, parity, antiretroviral treatment experience and treatment status at conception. No differences between the two groups were also found for the main pregnancy outcomes, represented by miscarriage or fetal demise, preterm delivery, low (<2500g) or very low (<1500g) birthweight, intrauterine growth restriction (gender-and gestational age-adjusted Z-score for birthweight <10° percentile), major birth defects, delivery complications, and HIV transmission (Table 1). Among 1126 infants from CMV-positive mothers with available information on clinical status, three neonatal cases of CMV infection were reported.

Among the 1490 women positive for CMV antibodies, 123 (8.3%) had available plasma samples collected during pregnancy, usually (90.2%) after the first trimester, that were evaluated for CMV-DNA quantitation.

None of these women had clinical signs of new viral infection or viral reactivation during pregnancy. Only four of them (3.3%) had positive CMV DNAemia in plasma, all at very low levels (range: 2.35-2.61 log IU/ml). None of these four women had low (<200/mm$^3$) CD4 counts (range: 270-852), and all had normal pregnancy outcomes, with no preterm delivery, low birthweight, birth defects, CMV or HIV transmission reported. Interestingly, all the four mothers had detectable HIV in plasma at third trimester (range: 99-20004 copies/ml).

This study showed that in a large cohort of pregnant women with HIV, roughly two thirds had positive serology for CMV, with no adverse consequences of coinfection on the main pregnancy outcomes. This rate is almost identical to that observed in two different studies conducted among a general population of pregnant women in Northern and Southern Italy, that showed prevalences of positive CMV serology of 68.3% (1925/2817) and 65.9% (595/797), respectively [5,6], suggesting similar CMV prevalence for HIV-negative and HIV-positive pregnant women. We also showed in a nested evaluation that among pregnant women with HIV with positive serology for CMV and no signs of primary infection, a small proportion (4/123, 3.3%) have detectable CMV DNAemia, usually at low levels. This figure is consistent with published data in an HIV-CMV coinfected African population, where rate of detectable CMV in plasma was 4.8% (7/146) [7], suggesting slightly higher rate of CMV DNAemia among pregnant women with HIV compared to the general population. In a previous Italian study on a general population of CMV IgG-positive pregnant women with no evidence of primary infection, 0.5% (4/774) had positive, low-level CMV DNAemia [8]. A similar rate (2/134, 1.4%) was found in an unselected population of Turkish pregnant women [9]. Detectable low-level CMV DNAemia could represent either subclinical viral reactivation or the terminal phase of blood viral clearance after a recent primary infection. It is unknown whether partial immunosuppression in pregnant women with HIV may be responsible for low-level CMV replication and detectable DNAemia, and we were unable to define timing of CMV infection by antibody avidity testing or evaluation of CMV-specific IgM. In any case, asymptomatic maternal CMV DNAemia in a context of relatively preserved CD4 counts seems to represent for pregnant women with HIV an infrequent condition not associated with major clinical consequences.

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**Conflicts of interest:**

None to declare. None of the authors has a commercial or other association, financial interest, activity, relationship or association that might pose a conflict of interest. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Contribution to authorship**

MF designed the study, drafted and finalised the manuscript and was responsible for statistical analysis; MFP and RA performed virological analyses on plasma samples and contributed to manuscript finalisation; AdA, AM, ET, CP, GG, GN, GM, SD, IC, MS and MR substantially contributed to clinical activities, acquisition of data and to critical revision of the manuscript. All the authors gave approval to the final version to be published.

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**Table 1.** Characteristics and pregnancy outcomes according to CMV serostatus.

| *Women's characteristics* | CMV-positive | | CMV-negative | | CMV-positive | CMV-negative | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | N | | | | Median values (IQR) | | |
| Age, years (n: 2245) | 1486 | | 759 | | 33 (29-36) | 33 (28-37) | 0.924 |
| CD4 cell count, cells/mm$^3$ (n: 1453) [b] | 998 | | 455 | | 437 (297-591) | 425 (289-612) | 0.978 |
| | n/N | % | n/N | % | | | |
| At least one previous pregnancy (n: 2218) | 1090/1467 | 74.3 | 551/751 | 73.4 | | | 0.636 |
| History of AIDS-defining events (n: 2220) | 84/1470 | 5.7 | 45/750 | 6.0 | | | 0.785 |
| Antiretroviral-treatment experienced (n: 2185) | 929/1447 | 64.2 | 504/738 | 68.3 | | | 0.057 |
| On treatment at conception (n: 2225) | 777/1475 | 52.7 | 390/750 | 52.0 | | | 0.762 |
| *Pregnancy outcomes* | | | | | OR[c] | OR 95% CI | |
| Miscarriage or fetal demise (n: 1941) | 95/1313 | 7.2 | 56/628 | 8.9 | 0.797 | 0.564-1.125 | 0.196 |
| Preterm delivery (n: 1672) | 234/1152 | 20.3 | 108/520 | 20.8 | 0.972 | 0.753-1.256 | 0.830 |
| Low birthweight (<2500 g) (n: 1576, twins included) | 250/1082 | 23.1 | 101/494 | 20.4 | 1.169 | 0.901-1.517 | 0.239 |
| Very low birthweight (<1500 g) (n: 1576, twins included) | 37/1082 | 3.4 | 11/494 | 2.2 | 1.555 | 0.786-3.074 | 0.205 |
| Small by gestational age (<10$^{th}$ percentile) (n: 1516, singletons only) | 130/1034 | 12.6 | 53/482 | 11.0 | 1.164 | 0.829-1.634 | 0.381 |
| Complications of delivery [d] (n: 1606) | 85/1100 | 7.7 | 37/506 | 7.3 | 1.062 | 0.711-1.586 | 0.771 |
| Major birth defects (n: 1616) | 39/1108 | 3.5 | 17/508 | 3.3 | 1.054 | 0.590-1.881 | 0.860 |
| HIV transmission (n: 1235) | 14/857 | 1.6 | 8/370 | 2.1 | 0.768 | 0.319-1.847 | 0.556 |

OR = odds ratio; CI = Confidence interval; IQR = interquartile range; ARV = antiretroviral therapy.

[a] Mann-Whitney *U* test for quantitative variables and Chi-square test for categorial variables. [b] 2nd trimester. [c] Reference category: CMV-positive. [d] Usually represented by surgical wound infections and fever.