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BRIEF REPORT

Prevalence and Risk Factors of Cytomegalovirus Among Men Having Sex With Men Enrolled in a Pre-Exposure Prophylaxis Study

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Risk factors for cytomegalovirus (CMV) acquisition in men having sex with men remain unclear. Seroprevalence, incidence, risk factors and shedding of CMV were analyzed among participants enrolled in the HIV pre-exposure prophylaxis IPERGAY-ANRS trial. Among the 417 participants tested, 382 were seropositive at baseline (prevalence of 91.6%; 95%CI[88.5–94.1]) and 10/35 seroconverted during the study (incidence of 17.1 per 100 person-years; 95%CI[8.2–31.3]). A high number of sexual partners was independently associated with CMV seroprevalence. Shedding among CMV-seroconverters was reported for 6/9 and 2/9 at the oral and anal levels, respectively. Our data supports transmission of CMV during sexual contacts.

Study part of the ANRS-IPERGAY Clinical trial. ClinicalTrials.gov number, NCT01473472.

Keywords. behavior; CMV; MSM; risk-factors; seroconversion.

Cytomegalovirus (CMV) seroconversion usually occurs during childhood and seroprevalence increases with age [1]. CMV has been linked to sexual transmission in men having sex with men (MSM) with and without HIV [2, 3]. Salivary shedding of CMV is usually reported in people with advanced HIV stages [4]. Nonetheless, CMV oral shedding is also reported in people without HIV, although the clinical significance and role in transmission is not elucidated [5, 6]. CMV intermittent shedding in semen has been shown in MSM with HIV [7]. Anal shedding of CMV, although suspected, is not usually reported [6]. This potential shedding of CMV in various biological fluids may contribute to its transmission.

We aimed to determine prevalence, incidence and risk-factors for CMV infection among high-risk MSM participants in a pre-exposure prophylaxis (PrEP) clinical trial (ANRS-IPERGAY; NCT01473472) [8, 9] and explore anal shedding of CMV DNA as potential mode of transmission in MSM.

METHOD

The ANRS-IPERGAY study was a trial of PrEP for HIV seronegative highly exposed MSM which took place between February 2012 and June 2016 [8, 9]. The study protocol allowed for sampling serum, stored at −80°C at each study trial visit: baseline, months 1, 2 and every two months for all participants. Throat and rectal swabs were also collected and stored at −80°C, at inclusion (D0) and every 6 months. Participants completed online questionnaires every 2 months on alcohol and recreational drug use, and sexual behavior at their last sexual encounter and over the previous 2 months. All participants in both the randomized controlled phase and the open-labelled extension were eligible to this study.

The study was carried out in accordance with Good Clinical Practices, the ANRS Ethical Chart for Research and the Declaration of Helsinki. The protocol was approved by national ethics committees in France (Comité de Protection des Personnes de Paris Ile-de-France-I). All participants provided written informed consent.

We performed a retrospective assessment of IgG CMV serology (Abbott ARCHITECT™ cytomegalovirus IgM/IgG, France) of participants with available serum at D0 and at each subsequent visit for those negative at D0. The IgM CMV was performed longitudinally in participants with incident infection. The reactive threshold for IgG was ≥6 AU/mL and for IgM an index >1. CMV DNA quantification was performed on anal swab among D0 seropositive participants and on throat/anal swabs among seroconverters using the Cobas 6800 system (Roche, Meylan, France) with a threshold of 24.5 UI/mL. Sexually transmitted infections (STI) screened at baseline and every 6 months were: syphilis serology and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* PCR in urine samples, throat and anal swabs. Serological status for

Received 28 May 2024; editorial decision 08 July 2024; accepted 16 July 2024; published online 18 July 2024

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https://doi.org/10.1093/ofid/ofae400

Table 1. Characteristics of the ANRS IPERGAY Trial Participants According to CMV Serostatus at Baseline

Characteristic	Total n = 417	CMV − n = 35	CMV + n = 382	*P* Value
Center at inclusion				
Paris	207/417 (50)	15/35 (43)	192/382 (50)	.41
Others	210/417 (50)	20/35 (57)	190/382 (50)	
Age in years	34.8 [29.1 ; 42.5]	33.1 [24.3–42.4]	35.0 [29.2–42.6]	
≤ 30	122/417 (29)	15/35 (43)	107/382 (28)	.27
> 30	295/417 (71)	20/35 (57)	275/382 (72)	.07
Ethnic origin				
Caucasian	382/417 (92)	32/35 (91)	350/382 (92)	1.00
Other	35/417 (8)	3/35 (9)	32/382 (8)	
Education Level				
Postsecondary[a]	296/412 (72)	27/34 (79)	269/378 (71)	.31
Other[b]	116/412 (28)	7/34 (21)	109/378 (29)	
Circumcision	84/417 (20)	7/35 (20)	77/382 (20)	.99
Sexual risk factors at baseline[c]				
Number of partners in the past 2 m	8.3 [5.0; 16.7]	**5.0 [2.5–7.0]**	**8.3 [5.0–16.7]**	**.0001**
Number of sexual intercourses in the past 4 wks	10.0 [5.0; 16.0]	**4.0 [2.0–10.0]**	**10.0 [6.0–18.0]**	**.0001**
Most recent sexual act				
Oral sex	50/402 (12)	5/33 (15)	45/369 (12)	.66
Insertive anal sex	132/402 (33)	12/33 (36)	120/369 (33)	.31
Receptive anal sex	220/402 (55)	16/33 (48)	204/369 (55)	
Including condomless receptive anal sex	*149/220 (68)*	*9/33 (56)*	*140/369 (69)*	
Bacterial STIs diagnosed at screening[d]				
All bacterial STIs	115/417 (28)	8/35 (23)	107/382 (28)	.52
Only oral STIs (*Neisseria gonorrhoeae, Chlamydia trachomatis*)	48/417 (12%)	4/35 (11%)	44/382 (12%)	1.00
Hepatitis B virus status[e]				
Susceptible	87/415 (21)	7/35 (20)	80/380 (21)	.87
Immune from natural infection or vaccination	328/415 (79)	28/35 (80)	300/380 (79)	
Hepatitis A virus status[e]				
Susceptible	206/415 (50)	19/35 (54)	187/380 (49)	.57
Immune from natural infection or vaccination	209/415 (50)	16/35 (46)	193/380 (51)	
Positive herpes simplex virus-1 serology[e]	271/384 (71)	**18/35 (51)**	**253/349 (72)**	**.01**
Positive herpes simplex virus-2 serology[e]	150/385 (39)	**8/35 (23)**	**142/350 (41)**	**.05**
Positive human herpes virus-8 serology[e]	100/417 (24)	**2/35 (6)**	**98/382 (26)**	**.009**
Use of recreational drugs[f] in the last 12 m	197/410 (48)	**10/34 (29)**	**187/376 (50)**	**.03**

Data are expressed as n (%), median (IQR), or n/N (%).

Values in bold indicate statistically significant *P*-values ($P < .05$).

[a]Postbaccalaureate certificate achieved at the end of high school.

[b]No education beyond baccalaureate.

[c]All types of sexual acts are included (insertive or receptive anal sex and oral sex).

[d]Bacterial Sexually Transmitted Infections includes syphilis serological testing by the rapid plasma reagin method confirmed by the use of a treponema-specific assay; *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were detected by PCR with urine samples and throat and anal swabs.

[e]The status of hepatitis B virus, hepatitis A virus, herpes simplex virus 1 and 2 and HHV-8 were tested by serological assays. Individuals vaccinated for hepatitis B were excluded.

[f]Recreational drugs including ecstasy, crack, cocaine, crystal (methamphetamine), speed (amphetamine), GHB/GBL, LSD, mephedrone and heroin.

HAV, HBV, HSV-1 and 2 and HHV-8 were assessed at baseline [8, 10, 11].

Values are presented as median and interquartile range (IQR) for continuous variables and as numbers and percentages for qualitative variables. Baseline characteristics were compared between CMV seronegative and seropositive participants using a Student's t-test or an exact Wilcoxon test for continuous characteristics and a Chi-Square test or a Fisher's exact test for qualitative characteristics, as appropriate. Univariate and multivariate ($\alpha < 0.05$) logistic regression models were used to study the association between baseline risk-factors and CMV seropositivity at D0. Risk-factors imputed in the univariate model were: center (Paris *vs* other); age, ethnicity, circumcision, level of education, last sex act, condom use at the last sex act, number of partners (past 2 months), number of sex acts (past month), chemsex use (taking a psychoactive product during sexual intercourse), recreational drug use, bacterial STI and serological status for hepatitis and herpes viruses. Significant independent risk factors in the univariate analysis were imputed in the multivariate analysis. All *P* values and confidence intervals were two-sided. All analyses

Table 2. Univariate and Multivariate Analysis of Baseline Demographic and Behavioral Characteristics Associated With CMV Baseline Seropositivity

		Univariate Analysis					Multivariate Analysis N = 380 (346 Events)			
Baseline Characteristics and CMV Baseline Seropositivity	Sample Size	No. Events	*P* Value	Odds Ratio	Lower OR	Upper OR	*P* Value	Odds Ratio	Lower OR	Upper OR
Number of partners in the past 2 m ↑5	409	375	**<.0001**	**1.99**	**1.38**	**2.87**	**.001**	**1.86**	**1.28**	**2.69**
Herpes simplex virus-2 serology[a] (positive *vs* negative)	385	350	**.045**	**2.30**	**1.02**	**5.22**	.177	1.79	0.77	4.17
Use of recreational drugs during the last sexual act (chemsex)[b] (yes *vs* no)	410	376	**.027**	**2.37**	**1.11**	**5.10**	.051	2.19	1.00	4.78

A multivariate analysis was performed using the significant univariate risk factors for baseline seropositivity ($\alpha < 0.05$).

Values in bold indicate statistically significant *P*-values ($P < .05$).

[a]The statues of hepatitis A virus, herpes simplex virus 1 and 2 and cytomegalovirus statues were tested by serological assays.

[b]Recreational drugs including ecstasy, crack, cocaine, crystal (methamphetamine), speed (amphetamine), GHB/GBL, LSD, mephedrone and heroin.

were conducted with SAS version 9.4 [SAS Institute Inc. 2013. SAS® 9.4 Statements: Reference. Cary, NC: SAS Institute Inc.].

RESULTS

Of 429 participants included in the trial, 11 withdrew their consent to further use of the samples and one sample was missing at D0. CMV seroprevalence (IgG) at D0 was 91.6% (n = 382/417, 95%CI [88.5%–94.1%]). Patient characteristics according to the CMV status are described in Table 1. To identify demographic and behavioral factors associated with CMV infection, we used baseline characteristics to perfom both univariate and multivariate analysis (Table 2). In the multivariate analysis, a high number of sexual partners in the past 2 months was independently associated with baseline CMV seropositivity (OR = 1.86 [1.28–2.69]; $P = .001$).

Ten individuals (out of 35) seroconverted during the study (28.6% 95%CI = [14.6–46.3]), with a median time of 6 months (IQR[1–8]), for an estimated incidence of 17.1 per 100 person-years (95%CI = [8.2–31.4]). In 7/10, IgM was concomitant with IgG seroconversion and did not last more than 4 months in most cases. (Supplementary Figure 1).

Among the 382 participants seropositive at D0, CMV DNA was detected in 7/321 (2%) participants with available anal swabs (3 detectable under the cut-off and 4 with low levels of 37.1, 52.7, 58.1 and 3060 UI/ml, respectively). Among seroconverters, CMV DNA was detected within 6 months of seroconversion in throat swabs among 6/9 cases (66.7% ; median 589 UI/ml IQR[56–1170]) and in rectal swabs among 2/9 cases (22.2% ; 37.4 and 111 UI/ml, respectively). One participant among the seroconverters did not provide any swab for analysis.

DISCUSSION

We report a CMV seroprevalence of 92% and an incidence of 17 per 100 person-years in a large cohort of MSM enrolled in a trial of on demand oral PrEP for HIV-1 prevention. A high number of sexual partners was independently associated with CMV seroprevalence.

A previous study described prevalence of 98% and 80% in MSM with and without HIV, respectively [2]. Robain *et al.* reported an annual incidence of CMV in HIV infected MSM of 28 per 100 person-years, they also demonstrated that CMV seroconversion was associated with condomless intercourse and recommended condom use against CMV transmission in people with HIV [12]. Seroprevalence of CMV is higher among people with HIV due to more frequent risk factors of exposure [13]. In a study on HHV8, CMV was presented as a marker of unsafe sexual practices in young MSM without HIV [14]. Our study suggests that rather than HIV itself being a risk factor for CMV infection, it is the shared sexual behaviors associated with HIV risk that may explain the higher prevalence of CMV among both HIV-positive individuals and HIV-negative MSM. We observed an association between partner count in the past two months and CMV seropositivity mirroring findings for HHV8 [11] in the same trial. Use of recreational drug in the past year was associated with CMV seropositivity in univariate analysis and showed a trend towards significance in multivariate analysis. Chemsex was also associated with HHV8 transmission as previously reported [11]. It has been reported that the number of partners and chemsex use are surrogate markers of unsafe practices in the IPERGAY study [8, 15].

CMV and other Herpesviridae (HSV1, HSV2, HHV8) that share modes of transmission could be co-transmitted during oral or anal intercourse in people with high number of partners [11]. Compared with seronegative individuals, CMV-seropositive participants were more frequently seropositive for HSV1, HSV2 and HHV8 suggesting the similar routes of transmission. Saliva is a mode of transmission demonstrated for HHV8 [11] during deep kissing, oral-penile/oral-anal sex, or with the use of saliva as a lubricant during anal-penile sex. CMV could be transmitted through intermittent or persistent shedding in oral or genital biological fluids, during the weeks after primary

infection or in asymptomatic infected individuals. Oro-pharyngeal CMV shedding in our study was frequent in participant who seroconverted (66.7%), around the time of seroconversion. A review of CMV shedding in biological fluids reported that oral shedding is infrequently documented among adult populations. Nonetheless, it is reported ranging from 0% to 14% with a higher frequency of oral shedding in population with CMV risk factors such as individuals attending STI clinics, MSM and women with congenitally infected children [6]. Although detection of CMV DNA in digestive explants and stools is common practice, and the digestive tropism of CMV is known, our data showed very limited anal excretion of CMV, suggesting unlikely anal transmission as the main route [16]. Conversely, in the EVARIST and CCTG592 cohorts of MSM with HIV, CMV shedding in the seminal plasma was frequent with high viral load [7, 17]. The main limitation of our study is that it was not designed to assess CMV excretion by frequent and repeated sampling of mucosal fluids. IgM seroconversion was frequent among seroconverters and did not last more than 4 months in most cases. IgM CMV serology is a valuable test for detecting recent CMV seroconversion with reported sensitivity over 90% [18]. However, its utility is limited because IgM levels decline rapidly.

To conclude, our study adds to the body of evidence linking CMV, akin to other herpes viruses, with sexual behavior [1, 12, 14], underscoring the various potential modes of transmission, including salivary.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. Designed the study: CD, JMM. Managed the participants" care: EC, GP, JMM. Performed the measurement: MM, AG. Analyzed the data: NL, VF, IC, LM. Wrote the manuscript: SC, JMM, CD. All authors provided critical review and feedback of the manuscript.

Financial support. This work was supported by the National Agency of Research on AIDS and Viral Hepatitis [ANRS] and the Bill & Melinda Gates Foundation. (as part of the IPERGAY trial); ClinicalTrials.gov number, NCT01473472.

Patients consent statement. The study was carried out in accordance with Good Clinical Practices, the ANRS Ethical Chart for Research and the Declaration of Helsinki. The protocol was approved by national ethics committees in France (Comité de Protection des Personnes de Paris Ile-de-France-I). All participants provided written informed consent.

Prior presentation at meetings. This study was not presented elsewhere.

Data availability. Datasets are available upon request from corresponding author.

Potential conflicts of interest. All authors: No reported conflicts.

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