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HIV risk associated with serum medroxyprogesterone acetate (MPA) levels among women in East and southern Africa: a case-control study

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Author contributions

RH and JMB conceived the study. RH wrote the first draft of the manuscript. RH, RS, and MP performed statistical analyses. JMB and JRL were awarded grant to fund the study. DWE and SWB oversaw laboratory technicians that performed analyses of progestin quantification. NM led site teams that collected the data. All authors contributed critical revisions to the analysis and interpretation and reviewed the final manuscript draft.

Conflicts of Interest

JMB is on an Advisory Committee of Gilead Sciences. All other authors declare no conflicts of interest.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Abstract

Background: Some observational studies have found increased HIV risk associated with self-reported use of injectable depot medroxyprogesterone acetate (DMPA). Testing blood samples for MPA, the progestin in DMPA, permits validation of self-reported data and exploration of whether potential HIV risk is correlated with MPA levels, which are highest soon after injection.

Methods: We conducted a case-control study testing archived serum from women who participated in three longitudinal studies of HIV prevention in East and southern Africa. Case samples, from women who acquired HIV, were from visits that occurred at or immediately prior to the first evidence of HIV infection. Secondary analyses restricted to case samples collected within 15 and 30 days of the estimated date of HIV infection. Matched control samples were from women who remained HIV-uninfected. We used multivariable conditional logistic regression to compare exogenous hormone levels, quantified through mass spectrometry, among cases and controls.

Results: When restricted to cases with samples collected within ≤15 days of estimated date of HIV infection, MPA detection was more frequent among women who acquired HIV (adjusted odds ratio [AOR]=2.75, 95% confidence interval [CI] 1.22-6.19). In this subset, the increase in HIV risk was only among samples with MPA detected at a low level of 0.02-0.50ng/ml: 36.7% of cases and 9.4% of controls, AOR=6.03, 95% CI 2.50-14.54.

Conclusions: Detection of MPA at low levels close to the estimated time of HIV acquisition was significantly more frequent among women who acquired HIV. Studies are needed that explore biological mechanisms elicited by any MPA level and HIV risk.

Keywords

hormonal contraception; DMPA; HIV; women; Africa

Introduction

The availability of safe and effective reversible contraception is a top priority in women's health globally; greater contraceptive choice affords women greater control in planning their families and maintaining their health and the health of their children (1, 2). In many settings where the predominance of contracepting women use injectable hormonal methods, women also face a high risk of HIV, and a recent update to the World Health Organization (WHO) Medical Eligibility Criteria acknowledges the potential for injectable contraceptives to increase HIV risk (3-5). The data supporting this recommendation are from multiple observational analyses and meta-analyses, some, but not all, of which demonstrate increased HIV risk with the use of intramuscularly-administered depot medroxyprogesterone acetate (DMPA) (6-8). The risk estimates in these studies could be spuriously derived from inaccurate measurement of self-reported contraceptive use (9) and/or sexual behavior (10, 11), or they could represent true behaviorally and/or biologically driven increases in HIV risk.

Biologic studies have generated many hypotheses about mechanisms by which DMPA, or its progestin, MPA that circulates as a measurable metabolite in blood, could enhance HIV susceptibility (12). Pharmacokinetic properties of DMPA include an early peak in serum with steep declines during the first 30 days and less steep declines during the subsequent 60 days that enable maintenance of levels above thresholds needed for contraceptive efficacy (13). Notably, there is large inter-individual variation in peak MPA levels (14). One mechanistic hypothesis about the potential for DMPA to increase HIV susceptibility in women relates to the high amounts of MPA in serum immediately following administration and that this amount is sufficient to elicit inflammatory responses that increase HIV susceptibility (12, 15).

Given uncertainty in the accuracy of self-reported contraceptive data and hypotheses for HIV susceptibility related to hormonal quantities, particularly MPA levels, the objective of our study was to use state-of-the-art laboratory techniques to measure progestin levels in women's serum and relate these levels to HIV seroconversion using a case-control study design. In a rapidly expanding field, this study is novel as it relates incident HIV infection with laboratory-defined measures of contraceptive exposure.

Methods

Population.

Women were participating in three prospective HIV prevention studies that took place from 2004-2013 in 7 African countries (South Africa, Rwanda, Zambia, Kenya, Uganda, Tanzania, and Botswana). All women were HIV-negative members of HIV serodiscordant couples. Their male partners living with HIV were ART naïve at enrollment, underwent 6-monthly CD4+ T cell count and HIV RNA quantification during the studies, and were referred to ART as soon as they were eligible based on the national guidelines of the time.

The Partners in Prevention HSV/HIV Transmission Study (Clinicaltrials.gov # NCT00194519) was a placebo-controlled randomized trial to evaluate the efficacy of daily acyclovir for HIV prevention when used by people co-infected with HIV and HSV-2. The primary findings included that daily acyclovir had no effect on HIV transmission (16). The Couples Observational Study was a longitudinal cohort study to identify immune correlates of HIV infection (17). In this study, as well as the Partners in Prevention Study, women underwent quarterly HIV testing. In the Partners PrEP Study (Clinicaltrials.gov #NCT00557245), a placebo-controlled double-blind, randomized trial to evaluate daily, oral tenofovir-based pre-exposure prophylaxis (PrEP) for HIV prevention, women attended monthly study visits for HIV testing and study drug refills. The Partners PrEP Study demonstrated 75% efficacy overall and 66% efficacy among women for co-formulated emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) and 67% efficacy overall and 71% efficacy among women for single agent TDF (18). In all studies, quarterly interviewer-administered questionnaires captured self-reported data on sexual behavior and contraceptive use. At enrollment and when clinically indicated, genital exams were conducted and endocervical swab samples were collected from women for screening for *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Trichomonas vaginalis* with the GenProbe Aptima Combo2 nucleic acid amplification assay (San Diego, California). Blood samples were collected from women at quarterly visits and in the event of seroconversion and archived at −80°C.Contraception was promoted through family planning counseling at all sites but use of contraception was not a study requirement. Participants could access contraceptives at research sites or by referral.

Study design.

We conducted a case-control study with sampling based on HIV seroconversion status and defined hormone exposure using quantified levels of specified progestins for all cases and controls. Case samples were from all women who seroconverted in the trials and these were matched in a 1:4 ratio with control samples from women who did not acquire HIV. To identify women for the control group, we applied a validated risk scoring tool to each case participant and frequency matched controls to cases based on the risk score (19) and study arm (active or placebo, Figure 1). The risk score, which incorporates age, children within the partnership, occurrence of condomless sex, male circumcision status, and the HIV viral load of the partner living with HIV, all from the time of study enrollment, is a stronger predictor of HIV transmission than any of its individual factors or other common factors associated with HIV seroconversion. To avoid over-matching, we limited matching to these two factors

(risk score and study arm) and considered other potential confounders for inclusion in our final models.

Up to 2 samples per woman were analyzed – a sample from the visit with the first evidence of HIV infection (i.e., sometimes after the estimated date of HIV acquisition) and the visit immediately prior to the first evidence of HIV infection. For controls, up to 2 samples from sequential visits that occurred at the same time of HIV detection in the cases were selected for testing to ensure that case and control samples were collected after approximately the same duration of study follow up. Control samples were excluded from selection if the HIV-infected male partner had already initiated ART to reflect the lack of seroconversion in these studies when partners were using ART with suppressed viremia (18, 20).

HIV outcome.

HIV seroconversions were defined by rapid HIV antibody tests conducted at study sites at each study visit and positive results were confirmed by plasma HIV RNA quantification. Stored samples from seroconverters that were collected at enrollment into the parent study were tested for HIV RNA. If RNA was not detected in the enrollment sample, women were determined to have incident HIV infection and included as cases. If HIV RNA was detected at enrollment, women were classified as HIV-infected at enrollment and excluded from this study. For all incident infections, archived samples from study visits immediately prior to seroconversion were tested retrospectively for HIV RNA to determine whether the first evidence of infection was the same day as when the woman tested positive at the study site or at an earlier time point. The date of first evidence of infection was determined to be the earliest date when HIV RNA was detected (which could be as late as the day when rapid HIV antibody tests were first positive or at an earlier time point). If the first quantifiable HIV RNA occurred on the same day as the first antibody positivity, we estimated the date of HIV infection to be the midpoint between the day of the last antibody negative test and the day when serology and RNA were first positive. If the first evidence of infection (e.g. date sample drawn with quantifiable HIV RNA) was at a visit prior to the first antibody positive date, we estimated the date of infection to have been 17 days prior to the date of first RNA positive test result (21, 22).

Measurement of exogenous hormones.

A validated, high-performance liquid chromatography-heated electrospray ionization-tandem triple quadrupole mass spectrometry (LC-MS/MS) assay was used to simultaneously quantify the amount of four progestins in each sample (23, 24). Progestins included MPA and levonorgestrel (LNG), used in oral contraceptives and implants; norethindrone (NET), used in another injectable known as NET-EN; and etonogestrel (ENG), used in a contraceptive implant. Additionally, exogenous ethynyl estradiol (EE2) and endogenous progesterone (P4) and estradiol (E2) were measured in the same assay.

Statistical analysis.

We used conditional logistic regression to determine the association of each exogenous hormone with case-control status. In our primary analysis, we used the exogenous hormone levels from case and control samples collected: 1) at the visit with the first evidence of HIV

infection (or equivalent for controls) and 2) the visit immediately prior to the first evidence of infection. To reduce the amount of time elapsing between the estimated date of infection and sample collection, we conducted secondary analyses where we restricted the cases to those with samples collected 1) ≤30 days and 2) ≤15 days before or after the estimated infection date. In these secondary analyses, case samples were compared to control samples collected at the visit comparable to the case visit that occurred immediately prior to the first evidence of infection.

Samples with quantities less than the lower limit of quantification (LLQ) were assigned a value equal to half the LLQ for that particular analyte (LLQ=0.02 ng/mL for LNG, ENG, and MPA; 0.04 ng/mL for NET; 0.01 for E2). For MPA, serum levels were categorized based on quartiles and our understanding of MPA pharmacokinetics (25). Other analytes could only be dichotomized into detected/undetected due to the infrequency of detection. All models included variables to account for the HIV risk score and study arm, which were matching variables. Variables for the woman's age and whether the woman reported any condomless sex during the past month were included in all final models based on *a priori* knowledge of the role for these factors to confound the contraception-HIV relationship. For MPA analyses, other potential confounders such as infection with a curable STI and partner's HIV viral load, were included in models if they contributed to a substantial (>10%) change in the point estimate. Finally, we adjusted the MPA models for E2 levels to determine whether hypoestrogenism, characterized by low levels of E2, may account for any association (26, 27). SAS 9.4 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Ethics.

Protocols for all three prospective studies were approved by the Human Subjects Division at the University of Washington and research ethics committees overseeing each study site. Women provided written informed consent, which included archiving of blood samples for future testing related to HIV.

Results

Participant characteristics.

132 HIV-seroconverting women with available samples (cases) were matched to 525 non-seroconverting women (controls, Figure 1). Of these women, 115 cases had samples available from the visit with the first evidence of infection and 525 controls had sample from the time point comparable to the case time point. 117 cases had samples available at the visit immediately prior to the first evidence of HIV infection and 414 controls had samples from the comparable time point. Baseline characteristics were similar between women with samples available at both time points or only one time point (data not shown). The median age for cases was 27 years (interquartile range [IQR] 24-33) and for controls, the median age was 30 years (IQR 24-37, Table 1). Most women were married to their HIV-infected study partner and had at least one child. Women reported a median of 3 sex acts with their partner in the month prior to study enrolment (IQR 1-4 for cases and 2-6 for controls) and 17.1% of cases and 13.4% of controls were infected with a curable STI at enrollment.

Of the 115 samples available from cases at the study visit with first evidence of HIV infection, the median time between sample collection and estimated date of HIV infection was 17 days (IQR=15-46 days, Table 1). Using the set of case samples drawn at the visit prior to the first evidence of infection, which incorporates samples drawn before and after the estimated date of infection, the median time between sample collection and estimated date of HIV infection was 45 days prior to estimated date of infection (IQR=29-67 days before, 103/117 [88.0%] of samples were before infection). Restricting to samples collected within 30 days of the estimated date of HIV infection resulted in a set of 81 cases collected at a median of 17 days after infection (IQR 14-17 days after infection, 64/81 [79.0%] were after infection). Restricting further to samples collected within 15 days of the estimated date of HIV infection resulted in a set of 32 cases collected at a median of 14 days after infection (IQR 14 days after to 17 days before infection, 20/32 [65.6%] were after infection).

Levels of exogenous hormones.

From samples drawn at the visit with the first evidence of infection, MPA alone was detected in 29.5% of cases and 22.7% of controls and most frequently in low amounts (0.02-0.50 ng/ml) (Table 2). When detected, the median quantity of MPA was 0.41 (IQR 0.15-1.03) overall. LNG alone was detected in 11.8% of cases (n=10 samples) and 8.7% of controls (n=35 samples) with a median quantity of 0.86 ng/ml (IQR 0.33-2.56) when detected. NET alone was detected in 2.6% of cases (n=2 samples) and 3.2% of controls (n=12 samples) with a median quantity of 0.83 ng/ml, (IQR 0.23-2.42) when detected. ENG alone was detected in 2.6% of cases (n=2 samples) and 3.2% of controls (n=12 samples), with a median of 0.25 ng/ml, (IQR 0.18-0.36) when detected. Using the set of cases and controls drawn from the visit immediately prior to the visit with first evidence of infection, distributions and frequencies of progestin detection were very similar.

Association between exogenous hormone in serum and HIV.

The frequency of MPA detection was not significantly different between cases and controls, both for samples from the visit with the first evidence of infection (adjusted OR=1.31, 95% CI 0.81-2.14) and samples from the visit prior to the first evidence of infection (adjusted OR = 1.33, 95% CI 0.79-2.23, Table 3). However, when cases were restricted to those with a sample tested within 30 days of estimated HIV infection and further to those with a sample within 15 days of HIV infection, there was an association of MPA detection and HIV risk with point estimates of 1.75 (95% CI 0.97-3.14) and 2.75 (95% CI 1.22-6.19), respectively. With regards to MPA quantity, there was no indication of a dose-response pattern between increasing MPA levels and odds of HIV acquisition. Only the lowest levels of detectable MPA (0.02-0.50 ng/ml relative to no detectable MPA) were associated with elevated HIV risk: adjusted OR=1.67, 95% CI 0.94-2.98 among case samples collected from the first evidence of infection; adjusted OR=2.16, 95% CI 1.17-3.99 among case samples collected from the visit prior to the first evidence of infection; adjusted OR=3.24, 95% CI 1.63-6.46 for cases within 30 days of HIV infection; adjusted OR=6.03, 95% CI 2.50-14.54 for cases within 15 days of HIV infection. Adding E2 to the models did not change the results substantially. No other progestin was associated with HIV risk.

Discussion

In this case-control study, we found some evidence that seroconverting women were more likely to have MPA detected than a matched set of non-seroconverting women. The association was statistically significant when we limited our analysis to samples drawn closest to the estimated day of HIV infection. Notably, only low serum levels of MPA (0.02-0.50 ng/ml), but not higher levels, were associated with increased risk of HIV relative to samples with MPA undetected. Recent work, including from the cohorts in this study and others, has demonstrated that self-reported data on hormonal contraceptive use may inaccurately reflect serum levels of exogenous hormones, with up to 30% of women having exogenous hormones detected while reporting no hormonal contraceptive use (9, 24, 28). By using serum MPA levels, this study provides objective evidence relating MPA to HIV risk beyond that of prior studies that have relied on self-reported contraceptive use data.

Our findings are somewhat contrary to hypotheses about possible mechanisms by which DMPA could increase HIV vulnerability. Pharmacokinetic studies have demonstrated that injectable contraceptives have high serum levels following injection that taper off until levels fall below the threshold for contraceptive efficacy and subsequent injection is required. Hypotheses about HIV risk have suggested that risk may be greatest when levels of MPA are highest and could potentially wane as MPA levels reduce (12, 29, 30). Our data could be interpreted as suggesting that small levels of MPA in serum could increase risk. The association between low MPA levels and HIV risk may support hypotheses about DMPA and HIV susceptibility that are based on the high glucocorticoid binding affinity of MPA, which is unique to MPA as a synthetic progestin and may not be dose dependent (31). In *in vitro* studies, MPA can stimulate and inhibit transcription of target genes observed through changes in cytokines and chemokines, suggesting that a high dose may not be required to elicit mechanisms that increase HIV vulnerability (12). However, further research is needed to explore these hypotheses in order to better understand the potential clinical and/or programmatic implications of these findings.

A lower dose subcutaneously-administered form of DMPA (DMPA-SC) available in a self-injection device has been demonstrated to have higher continuation rates than the standard intramuscularly injected DMPA (32, 33). Relative to intramuscular DMPA, it has been postulated that the lower dose (104 mg in DMPA-SC relative to the 150 mg delivered through intramuscular DMPA) could result in lower maximum concentration (C_{max}) values of MPA and differential effects on biologic mechanisms that might enhance susceptibility to HIV during the first 30 days after administration (34). While our results have limitations, they would suggest that a lower MPA C_{max} may not be the most important feature to overcome in efforts to decrease HIV risk and support the need for head-to-head comparison studies of MPA formulations to determine whether HIV risk is different based on the route of administration or amount of MPA delivered (34).

Current epidemiologic data do not raise concerns about the impact of oral contraceptives on HIV risk and little is known about HIV risk with other contraceptive methods, including NET-EN and hormonal implants that contain LNG or ENG (6). It is reassuring that we do not see any trend for other progestins (LNG, NET, or ENG) to be more frequently detected

in seroconverting versus non-seroconverting women. However, our ability to draw conclusions about these hormones is limited by the infrequent use of contraceptive methods containing these progestins in sub-Saharan Africa.

This is a unique study of serum MPA levels drawn from a large cohort of women and we carefully considered ways to account for misclassification in our analysis. Nonetheless, the study has limitations. Our selected case samples were a mix of those collected before and after the estimated date of HIV infection and we lacked data on the dates when DMPA injections were administered. Given the early marked peak and subsequent decline of serum MPA levels following DMPA injection, serum MPA measured in samples collected on the day with the first evidence of infection are likely different from the level on the day when HIV infection actually occurred. Our analyses restricting case samples to those closest to the estimated date of infection help to focus on data with potentially fewer misclassifications but these subsets contain small numbers of cases and samples were drawn after the estimated date of infection on average, raising the possibility for serum levels on the date of infection to have been greater than we measured. We lacked samples with MPA levels that are near the expected C_{max} levels and the infrequent sampling scheme for these clinical trials does not allow us to evaluate peak MPA levels or cumulative MPA exposure among cases compared with controls. Thus, our results should be used to generate new hypotheses about mechanisms related to DMPA and HIV susceptibility that can be explored in well-powered clinical studies and incorporate laboratory measurement of MPA levels that are sampled at a range of times following injection.

DMPA is the most widely used contraceptive in East and southern Africa, where over half the burden of HIV is carried by heterosexual women (3). Understanding mechanisms by which intramuscular DMPA may potentially increase HIV susceptibility, including biological and behavioral mechanisms and with varying quantities of MPA, is a priority for women's health and is needed to drive development of novel methods that will not increase HIV vulnerability. WHO closely monitors the development of novel data pertaining to the question of hormonal contraception and HIV risk and continues to call for higher quality data to permit more definitive conclusions and recommendations. Incorporating quantitative measurements of hormonal exposure into contraceptive studies will lead to better accuracy in exposure measurement and permit disaggregation into low, medium, and high hormonal levels to look for dose-response trends. These efforts will further our understanding of current contraceptive methods and can be integrated into efforts to develop novel methods and expansion of the contraceptive method mix.

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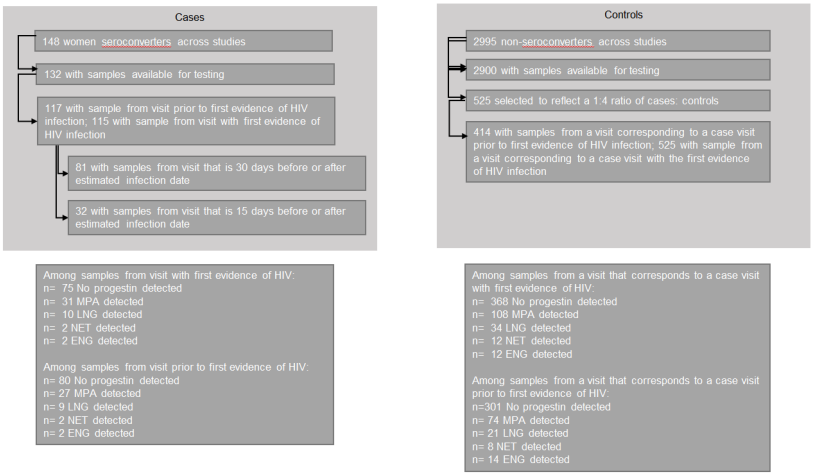

Figure 1.
Selection criteria for cases and controls

Table 1.

Characteristics of HIV negative women with HIV positive partners included for exogenous hormone quantification, Median (IQR) or n (%)

	Visit with first evidence of HIV infection in cases (and corresponding control visit)		**Visit prior to first evidence of HIV infection in cases (and corresponding control visit)**		**From either the visit with first evidence of HIV infection or the visit prior**	
	Cases	**Controls**	**Cases**	**Controls**	**Cases with sample within 30-day window of estimated infection**	**Cases with sample within 15-day window of estimated infection**
Number of women	115	525	117	414	81	32
Age, yrs	27.0 (24.0-33.0)	30.0 (24.0-37.0)	27.0 (24.0-33.0)	30.0 (24.0-37.0)	28.0 (25.0-35.0)	28.0 (25.5-35.0)
Number of children	2 (1-4)	2 (1-4)	2 (1-4)	2 (1-4)	2 (1-4)	3 (2-4)
Partnership duration, yrs	5.0 (1.8-9.0)	6.3 (3.3-13.2)	4.3 (1.7-9.0)	6.3 (3.5-13.4)	5.0 (1.7-9.5)	4.3 (1.3-9.0)
Married to study partner	107 (93.0)	476 (90.7)	109 (93.2)	381 (92.0)	80 (98.8)	31 (96.9)
Number of sex acts with study partner, past month	3 (1-4)	3 (2-6)	3 (1-5)	3 (2-6)	3 (2-4)	4 (2-5)
Any unprotected sex with study partner, past month	36 (31.6)	81 (15.7)	26 (23.2)	66 (16.1)	27 (34.2)	13 (40.6)
Infected with GC, CT or TV	7 (17.1)	17 (13.4)	11 (29.7)	9 (9.2)	9 (22.0)	5 (23.8)
Study						
Partners PrEP Study	59 (51.3)	249 (47.4)	53 (45.3)	206 (49.8)	55 (67.9)	31 (96.9)
Partners HSV/HIV Transmission Study	46 (40.0)	235 (44.8)	51 (43.6)	181 (43.7)	19 (23.5)	1 (3.1)
Couples Observational Study	10 (8.7)	41 (7.8)	13 (11.1)	27 (6.5)	7 (8.6)	0
Days between sample collection and estimated date of HIV infection in cases	Median: 17 days before infection (IQR 15-46 days)	N/A	Median: 45 days before infection (IQR 29-67 days before infection)	N/A	Median: 17 days after infection (IQR 14-17 days after infection)	Median: 14 days after infection (IQR 14 days after to 17 days before infection)

GC: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; TV: *Trichomonas vaginalis*

Table 2.

Frequency of exogenous hormone detection among cases and controls (overall, restricted to 30-day window, restricted to 15-day window)

	Visit with first evidence of HIV infection (and corresponding control visit)		Visit prior to first evidence of HIV infection (and corresponding control visit)		From either the visit with first evidence to HIV infection or the visit prior	
	Cases	Controls	Cases	Controls	Cases within 30-day window of estimated infection	Cases within 15-day window of estimated infection
MPA, Total samples *	105	476	107	375	75	30
<0.02 ng/ml (undetectable)	74 (70.5)	368 (77.3)	80 (74.8)	301 (80.3)	50 (66.7)	18 (60.0)
0.02-0.10 ng/ml	10 (9.5)	20 (4.2)	8 (7.5)	14 (3.7)	10 (13.3)	4 (13.3)
0.11-0.20 ng/ml	4 (3.8)	8 (1.7)	5 (4.7)	6 (1.6)	3 (4.0)	2 (6.7)
0.21-0.50 ng/ml	7 (6.7)	30 (6.3)	7 (6.5)	15 (4.0)	6 (8.0)	5 (16.7)
0.51-1.19 ng/ml	7 (6.7)	23 (4.8)	2 (1.9)	23 (6.1)	4 (5.3)	1 (3.3)
1.20-1.49 ng/ml	1 (1.0)	11 (2.3)	4 (3.7)	4 (1.1)	1 (1.3)	0 (0)
≥1.50 ng/ml	2 (1.9)	16 (3.4)	1 (0.9)	12 (3.2)	1 (1.3)	0 (0)
LNG, Total samples *	85	404	89	322	58	21
<0.02 ng/ml (undetectable)	75 (88.2)	369 (91.3)	80 (89.9)	301 (93.5)	50 (86.2)	18 (85.7)
Detected	10 (11.8)	35 (8.7)	9 (10.1)	21 (6.5)	8 (13.8)	3 (14.3)
NET, Total samples *	77	381	82	309	50	18
<0.04 ng/ml (undetectable)	75 (97.4)	369 (96.8)	80 (97.6)	301 (97.4)	50 (100.0)	18 (100.0)
Detected	2 (2.6)	12 (3.2)	2 (2.4)	8 (2.6)	0 (0.0)	0 (0.0)
ENG, Total samples *	77	381	82	315	52	19
<0.02 ng/ml (undetectable)	75 (97.4)	369 (96.8)	80 (97.6)	301 (95.6)	50 (96.2)	18 (94.7)
Detected	2 (2.6)	12 (3.2)	2 (2.4)	14 (4.4)	2 (3.8)	1 (5.3)

* Total Ns for each progestin reflect the number of samples without another progestin detected. Numbers are n (%) of the total samples except when giving the number of total samples for each progestin.

Table 3.

Association of serum exogenous progestins and HIV acquisition

MPA quantity	All cases using sample from the visit with the first evidence of infection		All cases using sample from the visit prior to first evidence of infection		Cases with sample within 30-day window of estimated infection		Cases with sample within 15-day window of estimated infection	
	OR* (95% CI) p-value	Adjusted** OR (95% CI) p-value	OR* (95% CI) p-value	Adjusted** OR (95% CI) p-value	OR* (95% CI) p-value	Adjusted** OR (95% CI) p-value	OR* (95% CI) p-value	Adjusted** OR (95% CI) p-value
Any level of MPA detected	1.42 (0.89-2.28) p=0.1	1.31 (0.81-2.14) p=0.3	1.40 (0.84-2.32) p=0.2	1.33 (0.79-2.23) p=0.3	1.74 (0.99-3.04) p=0.05	1.75 (0.97-3.14) p=0.06	2.69 (1.23-5.85) p=0.01	2.75 (1.22-6.19) p=0.01
MPA 0.02-0.50 ng/ml	1.82 (1.04-3.18) p=0.04	1.67 (0.94-2.98) p=0.08	2.18 (1.19-3.99) p=0.01	2.16 (1.17-3.99) p=0.01	2.87 (1.50-5.52) p=0.002	3.24 (1.63-6.46) p=0.001	5.23 (2.28-12.03) p<0.0001	6.03 (2.50-14.54) p<0.0001
MPA >0.50 ng/ml	0.97 (0.47-2.02) p=0.9	0.93 (0.44-1.98) p=0.9	0.69 (0.30-1.60) p=0.4	0.63 (0.27-1.49) p=0.3	0.77 (0.30-1.94) p=0.6	0.69 (0.26-1.79) p=0.4	0.42 (0.05-3.30) p=0.4	0.38 (0.04-3.18) p=0.4
Any level of LNG detected	1.40 (0.65-3.00) p=0.4	1.33 (0.60-2.94) p=0.5	1.72 (0.76-3.92) p=0.2	1.71 (0.73-3.97) p=0.2	2.17 (0.90-5.23) p=0.09	2.23 (0.88-5.62) p=0.09	2.26 (0.60-8.42) p=0.2	1.84 (0.45-7.52) p=0.4
Any level of NET detected	0.87 (0.19-4.05) p=0.9	0.84 (0.18-3.95) p=0.8	0.87 (0.19-3.93) p=0.9	0.39 (0.07-2.23) p=0.3	0.65 (0.00-3.37) p=0.7^	0.82 (0.00-4.26) p=0.9^	2.19 (0.00-12.07) p=0.9^	2.91 (0.00-16.44) p=0.9^
Any level of ENG detected	0.82 (0.17-3.85) p=0.8	0.66 (0.14-3.23) p=0.6	0.63 (0.14-2.83) p=0.5	0.63 (0.13-3.00) p=0.6	0.82 (0.18-3.76) p=0.8	0.77 (0.16-3.66) p=0.7	1.01 (0.12-8.42) p=0.9	0.86 (0.10-7.54) p=0.9

Reference group for all is no detectable progestin analyte.

*Adjusted for HIV risk score and study arm.

**Adjusted for HIV risk score, study arm, woman's age, and condom use.

^Exact logistic regression used due to lack of cases with detectable analyte.