ACQUISITION OF HIV AND GENITAL TRACT INFECTION AMONG
INJECTABLE PROGESTIN CONTRACEPTION USERS IN SOUTH AFRICA:
A PROSPECTIVE COHORT STUDY

by
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I. DISSERTATION ABSTRACT

Background

Effective contraception contributes substantially to reduced risk for maternal mortality. Some observational studies have noted increased HIV acquisition among women using injectable progestin contraceptives (IPC), particularly depot medroxyprogesterone acetate (DMPA) compared to women not using hormonal contraception (HC). Whether DMPA and another common but different IPC, norethisterone enanthate (NET-EN), confer different risk of HIV and genital tract infection remains relatively unexplored.

Methods

We analyzed prospective data from 3,316 South African injectable contraception users enrolled in the VOICE study. Cox proportional hazards models were used to compare DMPA and NET-EN users’ HIV risk, adjusting for demographic and behavioral factors. We used Andersen-Gill proportional hazards models to compare DMPA and NET-EN users’ risk for infection with Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), bacterial vaginosis (BV), and Trichomonas vaginalis.

Results

During 2,734 person-years (py) of follow-up, 207 incident HIV infections were detected (HIV incidence: 7.6/100 py, 95% CI 6.6 to 8.7/100 py). Users of DMPA had higher HIV incidence (8.7/100 py, 95% CI 7.4 to 10.2/100 py), compared to NET-EN users (5.6/100 py, 95% CI 4.3 to 7.2/100 py) (hazard ratio [HR]: 1.58, 95% CI 1.16 to 2.15, p=0.004).
This association persisted in the adjusted model (adjusted HR [aHR] 1.46, 95% CI 1.10 to 1.95, p=0.01). However, DMPA and NET-EN users did not differ in acquisition of CT, NG, or trichomonas. Incident BV was lower among DMPA compared to NET-EN users (aHR 0.86, 95% CI 0.75 to 0.98, p=0.02).

**Conclusions**

In this prospective cohort of South African IPC users, DMPA was associated with a higher risk of HIV acquisition, compared to NET-EN. This observed difference in HIV-1 risk did not appear to be explained by differential risk for genital tract infection. Our findings are the first to propose that BV acquisition may differ between DMPA and NET-EN users. Most observational data suggest no increased risk for HIV-1 or genital tract infection specific to NET-EN, an equally effective IPC. If these findings are confirmed in other datasets with well-characterized contraceptive exposures, consideration should be given to preferential prescription of NET-EN over DMPA in high HIV-1 incidence settings where NET-EN is available.
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VII. LIST OF ACRONYMS

aHR  adjusted hazard ratio
AIDS  acquired immune deficiency syndrome
aIRR  adjusted incidence rate ratio
aOR  adjusted odds ratio
BV  bacterial vaginosis
COC  combined oral contraceptive(s)
CT  *Chlamydia trachomatis*
DMPA  depot medroxyprogesterone acetate
FTC  emtricitabine
HC  hormonal contraception/hormonal contraceptive
HIV-1  human immunodeficiency virus type 1
HR  hazard ratio
HSV-2  herpes simplex virus type 2
IM  intramuscular
IPC  injectable progestin contraceptive(s)
IRB  institutional review board
IRR  incidence rate ratio
MSM  marginal structural model
MTN  Microbicide Trials Network
NET-EN  norethisterone enanthate
NG  *Neisseria gonorrhoeae*
OR  odds ratio
<table>
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<tr>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PrEP</td>
<td>pre-exposure prophylaxis</td>
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<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>RR</td>
<td>risk ratio</td>
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<tr>
<td>SDA</td>
<td>strand displacement amplification</td>
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<tr>
<td>SIV</td>
<td>simian immunodeficiency virus</td>
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<tr>
<td>SHIV</td>
<td>simian/human immunodeficiency virus</td>
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<tr>
<td>STI</td>
<td>sexually transmitted infection</td>
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<tr>
<td>TDF</td>
<td>tenofovir disoproxil fumarate</td>
</tr>
<tr>
<td>TFV</td>
<td>tenofovir</td>
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<td>TV</td>
<td><em>Trichomonas vaginalis</em></td>
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<td>United States National Institutes of Health</td>
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<tr>
<td>VOICE</td>
<td>Vaginal and Oral Interventions to Control the Epidemic (MTN-003)</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1: INTRODUCTION
1.1 BACKGROUND

1.1.1 Sub-Saharan Africa and the burden of HIV-1 infection among women

Although substantial progress in HIV/AIDS prevention and treatment science has occurred in the last decade, the epidemic continues to have a devastating impact on women of reproductive age.\(^1\) This is particularly true in southern Africa, the region most affected by the HIV/AIDS epidemic.\(^2\) South Africa has the world’s highest prevalence of HIV-1 infection.\(^1\) Here, the HIV-1 burden for women is especially grave, with nearly 19% of women aged 15-49 living with HIV-1 infection.\(^3\) Young women between 15-24 years old are considered one of the most-at-risk populations for incident HIV-1, and are as much as eight times as likely to be infected with HIV-1 compared to men.\(^1,3\)

1.1.2 Possible biologic co-factors for HIV-1 acquisition among women

Globally, over half of people living with HIV-1 are girls and women.\(^1\) Heterosexual exposure accounts for an estimated 80% of new infections, and is the primary mode of HIV-1 transmission in sub-Saharan Africa.\(^2\) Worldwide, the majority of all adolescent HIV-1 infections have resulted from heterosexual intercourse.\(^1\) Compared to men, women are especially susceptible to heterosexual HIV-1 transmission, likely due to substantial mucosal exposure to semen.\(^4-6\)

Several other biologic factors have been identified that may increase the risk of incident HIV-1 in women, including unprotected vaginal or anal intercourse with a person known to have or be at risk for HIV-1, injection drug use, bacterial vaginosis (BV), sexually transmitted infections (STI) (particularly those that cause ulceration of the vagina), and pregnancy.\(^7-11\) While increased access to effective contraception has contributed
substantially to advancements in women’s health, some studies have suggested that progestin-based injectable contraception, particularly depot medroxyprogesterone acetate (DMPA), may increase the risk of HIV-1 acquisition, although other studies have not reported this association (Table 1.1).

1.1.3 Biologic plausibility for impact of hormonal contraception on HIV-1 acquisition in women

The possibility that HIV-1 infection risk is increased by use of injectable progestin contraception has biologic plausibility. Some studies have suggested that HC, particularly DMPA, may increase risk of STI other than HIV-1 in women, such as chlamydial and gonorrheal infection. An association between progestin use and genital tract infection (as well as HIV-1) has also been observed in animal models. The administration of exogenous hormones such as DMPA are used in macaque models to enhance vaginal infection with simian immunodeficiency viruses (SIVs) and recombinant SIVs expressing HIV genes (SHIVs). Several theories have been suggested to explain this observed association between exogenous progestin exposure and HIV-1 acquisition. These include the possibility that contraceptive hormones change the response or susceptibility of genital tissue and/or microenvironment to sexually transmitted pathogens, potentially by thinning of genital mucosal surfaces or other structural immune mechanisms.

1.1.4 Injectable progestin-only contraception

Across the world, DMPA is the most commonly used injectable contraceptive. Depot medroxyprogesterone acetate contraceptive injection (known commercially as Depo-Provera CI or Petogen) is a progesterone derivative indicated only for the prevention of
pregnancy. The recommended dose is 150 mg every 3 months (13 weeks) administered by deep, intramuscular (IM) injection in the gluteal or deltoid muscle. While some concerns about its impact on bone mineral density have been raised, overall, DMPA is widely considered a safe and convenient option for women desiring contraception.

Norethisterone enanthate (NET-EN) is another progestin-only injectable contraceptive, but containing a first-generation synthetic progestin. Known commercially as Nuristerate, Noristerat or Norigest, 200 mg of NET-EN administered by IM injection provides contraception for eight weeks. Synthetic progestins are compounds created by chemical synthesis that have progestational activity, but differ structurally from natural progestational hormones.

Both DMPA and NET-EN are highly effective for the prevention of pregnancy. The primary mechanism of contraceptive action is thought to be the inhibition of sperm ascension through cervical mucus. However, contraceptive effect for progestin-only contraception may be based on three mechanisms: 1) typical cyclical changes in cervical mucus are inhibited; 2) gonadotropin formation in the pituitary is temporarily inhibited; and 3) atrophic changes in the endometrium. The Pearl index is a common method of summarizing contraceptive effectiveness, indicating number of method failures (pregnancies) per 100 woman-years of exposure. The Pearl index has been estimated at 0.66 for NET-EN, and 0.44 for DMPA. Other types of injectable contraception, which contain both estrogen and progestin hormones, are commonly used in countries other than South Africa, but are not addressed in this dissertation.

1.1.5 Injectable progestin-only contraception and chlamydial and gonorrheal infection

Both Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections are
common STIs associated with potentially serious adverse reproductive health outcomes, including pelvic pain, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, and perinatal morbidities for women and infants. Some observational findings have suggested a potential adverse effect of injectable progestin contraception on CT and NG acquisition. Baeten and colleagues found that among sex workers in Kenya, DMPA use was associated with a higher risk of chlamydia acquisition compared to those not using any hormonal contraception (aHR 1.6, 95% CI 1.1 to 2.4). Morrison and colleagues also found a higher risk of cervical STI (pooled chlamydial and gonorrheal infection) associated with DMPA use compared to use of no hormonal contraception in Baltimore (aHR 3.6, 95% CI 1.6 to 8.5). However, other analyses have found no clear increased risk of chlamydial or gonorrheal infection in women using injectable progestin contraception. In an analysis of 643 HIV-negative women recruited from contraception clinics in South Africa, statistically non-significant increases were observed for chlamydial (aIRR 1.24, 95% CI 0.80 to 1.94] and gonorrheal infections (aIRR 1.30, 95% CI 0.58 to 2.98) in DMPA users compared to those not using hormonal contraception. That analysis also found no increased risk of chlamydial or gonorrheal infection associated with use of NET-EN compared to no use of hormonal contraception.

1.1.6 Injectable progestin contraception and vaginitis acquisition

Trichomoniasis, bacterial vaginosis (BV), and intermediate vaginal flora (by Nugent score) have been linked to increased risk of HIV-1 acquisition in women. Recent observational findings also suggest that bacterial vaginosis in an HIV-infected woman may increase risk of HIV-1 transmission to a male partner. Additionally, ample evidence has accumulated to associate BV with a number of adverse reproductive health
outcomes for women.\textsuperscript{38-41} Thus, the potential impact of injectable contraceptive type on the incidence of trichomoniasis and BV is an important area for further study.

Some observational studies have noted a link between DMPA use and reduced risk of BV, compared to those not using hormonal contraception.\textsuperscript{14,16,42} While these findings may reflect unmeasured and/or unaddressed confounding, a physiologic explanation is also plausible. As amenorrhea is often associated with prolonged use of DMPA, this may reduce women’s susceptibility to shifts in BV-associated bacteria, via reductions in genital lactoferrin, an important iron source for \textit{Gardnerella vaginalis}.\textsuperscript{43} Compared to data on DMPA, there are limited data on the impact of NET-EN exposure on BV, intermediate vaginal flora classification and HIV-1 acquisition.

Baeten and colleagues found a significantly decreased risk of trichomoniasis among Kenyan sex workers using DMPA compared to those not using HC.\textsuperscript{14} When compared to use of no HC, DMPA (but not NET-EN) was associated with a marginally significant reduction in trichomoniasis acquisition in a cohort of injectable contraception users in South Africa.\textsuperscript{16} To date, no published studies have compared DMPA to NET-EN use directly to investigate incidence of either of these types of vaginitis.

1.1.7 Potential impact of DMPA and NET-EN on immune function

To what extent and by which mechanisms different progestins may affect HIV-1 acquisition at contraceptive doses is currently unknown.\textsuperscript{44} However, some in vitro and in vivo evidence indicates that differential impacts of progestins on the immune response may play a role. Progestin hormones modify transcription of particular genes in target cells via regulation of steroid receptor activity.\textsuperscript{45} In vitro work by Hapgood and
colleagues has demonstrated that medroxyprogesterone acetate (MPA) and norethisterone acetate (NET-A) exhibit different affinities for and actions via glucocorticoid receptors.\textsuperscript{44,46,47} As glucocorticoid receptors regulate gene transcription for many genes involved in immunity, MPA and NET may exert different influences on HIV-1 acquisition via these different influences on immune function modulated by the glucocorticoid receptor.\textsuperscript{44,48} Recent in vitro data also suggests that contraceptive doses of MPA but not NET-A could accelerate CD4+ T cell depletion in HIV-seropositive women.\textsuperscript{44}

While little is known about the effects of norethisterone on immune function,\textsuperscript{44} some in vivo evidence from animals and humans suggests that MPA may alter systemic immune function.\textsuperscript{49} Contraceptive doses of DMPA have been associated with increased recruitment of inflammatory cells (white blood cells, polymorphonuclear leukocytes, and monocytes) in cervicovaginal lavage, when compared to lavage results for women not using hormonal contraception, although this association did not persist in a multivariable model.\textsuperscript{50}

1.1.8 Injectable progestin contraception in South Africa

Both DMPA and NET-EN are approved for use as contraceptives in South Africa and are common in the public sector, but unlike DMPA, NET-EN is not commonly used in the majority of African countries. According to the most recently published South African Demographic Health Survey report,\textsuperscript{51} over half of sexually active South African women (56%) have ever used injectable contraception. In South Africa, injectable contraception is the most commonly used method among women current users of contraception (32.8% in 2003, increased from 30.1% in 1998). Approximately, 13.8% of sexually active South
African women are currently using NET-EN for contraception, compared to 19.0% currently using DMPA.52

1.1.9 Data specific to NET-EN

Kleinschmidt and colleagues investigated whether risk of HIV infection was higher with use of depot medroxyprogesterone acetate (DMPA) or norethisterone enanthate (NET-EN) injections compared to use of non-hormonal or no contraception.53 However, due to its size (N=551, 491 person-years of follow-up), this study had limited power.53 Findings from this study, which was conducted from 1999 through 2001 in South Africa, indicated no significant association between progestin contraceptive use and HIV infection (rate ratio 1.1, 95% CI 0.5 to 2.8; log-rank test, p=0.73). The adjusted hazard ratios for NET-EN and DMPA were 1.76 (95% CI 0.64 to 4.84) and 0.46 (95% CI 0.06 to 3.79), respectively, relative to non-HC use. Due to the limited power of the study and because similar studies had not included young women using NET-EN, the authors recommended that further research be carried out to focus on the use of NET-EN and HIV acquisition in high-risk groups.53

A larger and more recent secondary analysis by Morrison and colleagues on the Carraguard vaginal microbicide trial conducted in South Africa during 2004 to 2007 examined the effect of combined oral contraceptives (COC), DMPA and NET-EN on risk of HIV in women.54 In this analysis, HC did not significantly increase the risk of HIV acquisition (HIV incidence was 2.8, 4.6, 3.5 and 3.4 per 100 woman-years in the COC, DMPA, NET-EN and non-hormonal contraceptive groups, respectively (p=0.09). However, age appeared to modify the effect of HC on HIV acquisition risk (among young women, the adjusted hazard ratios were 1.02 (95% CI 0.46–2.28) for COCs, 1.68
(95% CI 0.96–2.94) for DMPA and 1.36 (95% CI 0.78–2.35) for NET-EN), and their effect estimate did not rule out a moderate increase in HIV risk associated with DMPA use. Unfortunately, neither this analysis nor the one conducted by Kleinschmidt et al was able to adjust for herpes simplex virus type 2 (HSV-2) serostatus in the analysis.53,54

1.1.10 Role of herpes simplex virus type 2

Observational data indicate that HSV-2 infection increases women’s risk for acquisition of HIV-1. Prior studies (comparing DMPA or pooled HC to no HC) have reported either an increased risk of HIV-1 acquisition among HSV-2 seronegative women,55 or no indication of effect modification by HSV-2 status.56-58 Thus, observational data are currently too conflicted to draw any conclusions about a potential interaction between DMPA exposure and HSV-2 infection. Potential interaction between NET-EN use and HSV-2 status remains relatively unexplored.

1.1.11 New disaggregated estimates for NET-EN use and HIV-1 acquisition

An updated systematic review on hormonal contraception and HIV-1 acquisition in women was recently published.13 This review included several previously unpublished estimates for the association of NET-EN use with HIV-1 infection.13 The new estimates specific to NET-EN were previously unpublished because original published analyses included aggregated estimates for use of any injectable progestin method, regardless of type.59 The updated review noted that most results suggested no increased risk with NET-EN use, but that estimates for NET-EN tended to be larger than for DMPA when assessed within the same study.13 However, 95% confidence intervals for these estimates overlapped substantially.13 The systematic review did not specify what methods,
including adjustment strategies, were used to generate these new disaggregated estimates. The new disaggregated estimates were provided to the authors of the updated systematic review via personal communication, and thus did not undergo separate peer review in the context of their original analyses.

1.1.12 Recommendations from the World Health Organization

Following a recent analysis by Heffron et al., which found nearly double the risk of HIV-1 acquisition among women using injectable contraception, a WHO panel of 75 experts reviewed relevant evidence in January 2012 and agreed it did not warrant change in current guidance on the use of hormonal contraception, although the panel was unable to characterize definitively the impact of contraceptive type on HIV-1 acquisition. Because findings regarding risk of HIV-1 acquisition were inconclusive, and several high-quality studies suggested a potential adverse effect of DMPA, WHO recommended women using progestin-only injectable contraception be strongly advised to use condoms and other HIV prevention measures, and commented on the need for further research on the relationship between HC and HIV. The 2012 WHO review of existing evidence concluded that women living with HIV could continue to use all existing HC methods without restriction. As with the expert panel’s analysis of HC and HIV acquisition in women, a clear recommendation was made on the need for further research and an undertaking to keep emerging evidence under close review.

Recommendations from WHO were recently updated and presented at the 20th International AIDS Conference in Melbourne, Australia. The new WHO guidance continues to recommend no restriction on the use of progestin-only injectable contraceptives; however, “women and couples at high risk of HIV infection should be
informed about (and have access to) HIV preventative measures, including male and female condoms.” Although support for a randomized clinical trial was not specifically mentioned, the recommendations did refer to the “need for further research to identify definitive answers” for questions regarding women using injectable progestin contraceptives and vulnerability to HIV infection.62

1.2 OPPORTUNITIES FOR EXPANDING KNOWLEDGE ON HORMONAL CONTRACEPTION AND HIV-1-1 ACQUISITION

1.2.1 Data on NET-EN use and HIV-1 acquisition

The NET-EN injection is a commonly used contraceptive in South Africa, but not in most other African countries. However, to date only five observational studies have focused on the potential association of NET-EN with incident HIV-1 infection.13 A recently published systematic review included one new disaggregated estimate that linked NET-EN with HIV-1 acquisition, compared to use of no HC.13 Previously, this study had reported estimates for HIV-1 acquisition based on aggregate exposure for DMPA and NET-EN use,63 and the new estimate was based on a personal communication to the authors of the systematic review.13 Overall, concerns regarding adequate data and power have predominated, along with the recognition of a current gap in knowledge regarding this question.53,64

1.2.2 Direct comparisons between contraceptive methods

While the comparison of HC use to no HC use may be valuable in trying to isolate a pure biologic (direct) effect of HC on HIV-1 acquisition, it is a comparison potentially
confounded by self-reporting of sensitive behaviors, limiting interpretation of findings and implications to clinical practice. A direct comparison between DMPA and NET-EN potentially may be less subject to such confounding, and have greater utility for the generation of clinical recommendations for women who have already chosen to use injectable contraception. However, the majority of analyses to date on the HC-HIV association have focused on the comparison of HC to no HC use.

1.2.3 Debate and discussion on strategies to address concerns regarding injectable hormonal contraception and HIV-1 risk

The WHO routinely convenes panels of experts to review emerging research findings that could impact the guidelines for medical eligibility for contraceptive use. Additional reviews of data have been conducted that focus on guidance for women at high risk of or living with HIV-1 infection. However, other public discussions and debates are ongoing, particularly to discuss optimal strategies to resolve outstanding questions related to injectable HC and HIV-1 risk. These discussions have included debate regarding methods for observational research and the possibility of conducting a large randomized trial. Recently, a meeting was held to identify the most appropriate methods for analyses of observational data on hormonal contraception and HIV-1 acquisition. Debate continues on the feasibility and cost of a randomized trial to assess the potential relationship between HC and HIV-1 acquisition. However, because access to HC is critically important for the health of reproductive-age women, and studies to date have had analytic limitations, it is important that we continue to analyze existing observational datasets, particularly ones with well-characterized contraceptive use data.
1.2.4 Additional context for the role of contraception in public health

Because it helps women avoid unintended pregnancy and its health risks, contraception is extremely important for women who have or are at risk of HIV-1 infection. Globally, contraception plays a major role in reducing potential maternal deaths. A recent analysis found that even with assumption of a two-fold increased risk of HIV acquisition with use of progestin injectables, the use of all contraception, including injectables, results in increased life expectancy. Thus, it is critical that the field continues to examine the potential risk of HIV-1 acquisition associated with contraceptive methods, to better inform policy decisions related to contraceptive access and individual choices regarding family planning.

1.2.5 Potential policy implications

Observational data suggest potential differences in risk between DMPA and NET-EN, both common among young reproductive age women in the country most affected by the HIV/AIDS epidemic. Additional data are needed to inform considerations for an important and yet unanswered public health question for young women and policy makers in South Africa: despite increased unit cost and greater visit burden associated with a 2-monthly contraceptive injection, should women at risk for HIV-1 infection who wish to use an injectable method of contraception be encouraged to choose NET-EN over DMPA?

Data are needed on the question of relative impact of different types of progestin-based contraception on HIV-1 acquisition. Estimation of impact on incident HIV-1 infection, sexually transmitted genital tract infection, and bacterial vaginosis associated with
contraceptive type would contribute to the known safety profile of these drugs, which could be valuable to the fields of family planning and HIV prevention. Such data could impact risk-benefit analyses of different methods for women choosing contraception, particularly NET-EN injection, which few studies have investigated separately.

1.3 SPECIFIC AIMS

1.3.1 Overall goal of the dissertation

The most recently released guidance from WHO did not clearly characterize the association between injectable contraceptive type and HIV-1 acquisition. However, it is critical that women who choose to use contraception have clear and accurate information on the potential risks associated with both use and non-use of family planning methods. This dissertation uses survival analysis methods to investigate the possibility that different progestin contraceptives confer different risk of HIV-1 and genital tract infection among reproductive age women in South Africa. Throughout the dissertation, direct comparisons are made between DMPA and NET-EN use, with NET-EN use as the comparator group. The longitudinal data on contraception use in the VOICE microbicide and pre-exposure prophylaxis (PrEP) trial\textsuperscript{75} provide an opportunity to evaluate relationships between injectable contraception and HIV/STI acquisition among women in South Africa. New strategies to address the outstanding questions around injectable hormonal contraception use and HIV-1 infection should be undertaken where possible. Here, we undertook the novel strategy of a direct comparison between two injectable progestin methods of contraception to investigate potential associations with HIV-1 and
genital tract infection.

1.3.2 Specific Aim 1

To compare the potential impact of DMPA to NET-EN injection use on incident HIV among South African women. We hypothesized that there would be no difference in risk of incident HIV-1 between the two injectable contraceptive methods.

1.3.3 Specific Aim 2

To compare the potential impact of DMPA to NET-EN injection use on acquisition of a) gonorrhea and b) chlamydia among South African women. Sexually transmitted cervical infections, which have been associated with incident HIV-1, are common in South Africa, and impact of HC on incidence has not been clearly characterized. We hypothesized that there would be no difference in risk of chlamydial or gonorrheal infection between the two injectable contraceptive methods.

1.3.4 Specific Aim 3

To compare the potential impact of DMPA to NET-EN injection on acquisition of two types of vaginal infection among South African women: a) bacterial vaginosis, and b) trichomoniasis. We hypothesized that there would be no difference in acquisition of bacterial vaginosis or trichomonas infection between the two injectable contraceptive methods.

1.3.5 Organization of the dissertation

Chapter 2 includes an analysis of injectable progestin contraceptive type and HIV-1 acquisition in South African women. Chapter 3 investigates whether acquisition of
chlamydia and gonorrhea differ between users of DMPA and NET-EN. Chapter 4 compares DMPA and NET-EN users’ acquisition of several forms of vaginitis. Results from Chapter 2 provide context and rationale for the analyses in Chapters 3 and 4. Chapters 2, 3, and 4 are intended as stand-alone analyses, and thus repeat some material from the dissertation’s introduction. The appendices (I through IV) provide additional details regarding the design and conduct of the VOICE trial, from which this cohort of injectable progestin contraceptive users was drawn.

1.4 STUDY POPULATION

We used prospective data from a cohort of 3,294 injectable contraception users in MTN-003 (also known as VOICE, Vaginal and Oral Interventions to Control the Epidemic). This study was a Phase 2B, five-arm, double-blinded, placebo-controlled, randomized trial evaluating daily use of antiretroviral drugs for prevention of sexual transmission of HIV-1 (see Appendix I). The MTN-003 study was conducted by the Microbicide Trials Network (MTN) and funded by the United States National Institutes of Health (US NIH). At all sites, a local Ethics Committee and National Drug Authority approved the study. Additional approval for analyses in this dissertation was provided by the Johns Hopkins Bloomberg School of Health Institutional Review Board. All participants provided written informed consent for participation.

The VOICE study included 5,029 sexually active HIV-uninfected women, 18-45 years old enrolled by 15 sites in South Africa, Uganda, and Zimbabwe from 2009 to 2011. As originally designed, the study products included oral tenofovir disoproxil fumarate (TDF) tablets, oral emtricitabine/TDF (FTC/TDF) tablets, and topical 1% tenofovir (TFV) gel (see Appendix I). Eligibility criteria required women to be healthy, HIV-uninfected,
sexually active at least once in the past 3 months, non-pregnant (and not intending pregnancy in next 24 months), not currently breastfeeding, and free of abnormal blood chemistry results, urinary and/or reproductive tract infection (Appendix II). Importantly, women were also required to be using effective contraception at time of enrollment, although they were not discontinued from the study if they did not remain on effective contraception, which was defined by the study protocol as oral contraceptive pills, injections, implants, patches, intrauterine contraceptive device, or surgical sterilization of the participant or her partner(s). All participants were provided with condoms and condom use counseling on a monthly basis as part of a standard HIV prevention package implemented across all study sites (Appendix II).

The schedule of study visits and evaluations in VOICE is included in Appendix III. Each participant was intended to have 12-36 months of study product use. The trial was scheduled to continue until 217 incident HIV-1 infections were observed. Study participants were referred to local care providers for HIV management, antiretroviral medications (as clinically indicated), social services and other routine care.

At interim analyses in September and November 2011, the oral TDF tablet and vaginal 1% TFV gel arms were stopped due to findings of futility, although the FTC/TDF arm did continue at the recommendation of the US NIH Vaccine and Prevention Data and Safety Monitoring Board. The trial completed follow-up of participants in August 2012. Results of the primary analysis were released in March 2013, and showed that none of the three active product arms had a lower risk of HIV-1 acquisition, likely because most participants were not fully adherent to study products.76,77
### 1.5 TABLE

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Design and Population</th>
<th>Findings (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulterys 1994</td>
<td>Cohort of 1,524 Rwandan pregnant women</td>
<td>aOR for ever HC use: 1.9 (0.8 to 4.6)</td>
</tr>
<tr>
<td>Kapiga 1998</td>
<td>Cohort of 2,471 Tanzanian women</td>
<td>aHR injectable: 0.30 (0.07 to 1.26)</td>
</tr>
<tr>
<td>Kilmár 1998</td>
<td>Cohort of 340 Thai SW</td>
<td>Crude RR DMPA: 1.5 (0.6-4.0)</td>
</tr>
<tr>
<td>Kiddugavu 2003</td>
<td>Population-based cohort of 5,117 Ugandan women</td>
<td>aIRR injectable: 0.84 (0.41 to 1.72)</td>
</tr>
<tr>
<td>Baeten 2007</td>
<td>Cohort of 1,498 Kenyan SW</td>
<td>aHR DMPA: 1.73 (1.28 to 2.34)</td>
</tr>
<tr>
<td>Myer 2007</td>
<td>Cohort of 4,555 South African women</td>
<td>aIRR DMPA: 0.75 (0.33-1.68)</td>
</tr>
<tr>
<td>Morrison 2007</td>
<td>Cohort of 6,109 Ugandan &amp; Zimbabwean women</td>
<td>aHR DMPA (Cox): 1.25 (0.89 to 1.78)</td>
</tr>
<tr>
<td>Kleinschmidt 2007</td>
<td>Cohort of 634 South African women</td>
<td>aIRR NET-EN: 1.76 (0.64 to 4.84)</td>
</tr>
<tr>
<td>Kumwenda 2008</td>
<td>Cohort of 842 Malawian women, RCT of genital infection treatment</td>
<td>aOR DMPA: 2.84 (1.07 to 7.55)</td>
</tr>
<tr>
<td>Morrison 2010</td>
<td>Cohort of 6,109 Ugandan &amp; Zimbabwean women, reanalysis</td>
<td>aHR DMPA (MSM): 1.48 (1.02 to 2.15)</td>
</tr>
<tr>
<td>Feldblum 2010</td>
<td>7,364 women from 6 African countries, 4 microbicide RCTs</td>
<td>HR injectables: 2.51 (1.12 to 5.60)</td>
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<tr>
<td>Reid 2010</td>
<td>Cohort of 1,358 South African, Zambian, Zimbabwean women</td>
<td>aHR injectables: 0.94 (0.46 to 1.92)</td>
</tr>
<tr>
<td>Heffron 2012</td>
<td>Cohort of immune correlates and RCT of acyclovir, 1,314 HIV serodiscordant couples</td>
<td>aHR injectables (Cox): 2.05 (1.04 to 4.04)</td>
</tr>
<tr>
<td>Morrison 2012</td>
<td>Cohort of 5,567 South African women (Carraguard)</td>
<td>aHR DMPA (Cox): 1.27 (0.93 to 1.73), (MSM): 1.28 (0.92 to 1.78)</td>
</tr>
<tr>
<td>Wand 2012</td>
<td>Cohort of 2,236 South African women (MDP 301)</td>
<td>aHR injectables: 1.72 (1.19 to 2.49)</td>
</tr>
<tr>
<td>McCoy 2013</td>
<td>Cohort of 4,948 South African and Zimbabwean women (MIRA)</td>
<td>aHR DMPA: 1.22 (0.84 to 1.74)</td>
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<tr>
<td>Lutalo 2013</td>
<td>190 Ugandan serodiscordant couples, Rakai cohort</td>
<td>aIRR 1.42 (0.60-3.36)</td>
</tr>
<tr>
<td>Morrison 2014</td>
<td>Meta-analysis of 37,124 women, 18 studies</td>
<td>aHR DMPA: 1.50 (1.24 to 1.83)</td>
</tr>
</tbody>
</table>

Abbreviations: aOR, adjusted odds ratio; aHR, adjusted hazard ratio; aIRR, adjusted incidence rate ratio; Cox, Cox proportional hazards regression analysis; DMPA, depot medroxyprogesterone acetate; HC, hormonal contraceptive; NET-EN, norethisterone enanthate; MSM, marginal structural model; RCT, randomized controlled trial; RR, risk ratio; SW, sex workers. *Re-calculated site-stratified estimates with robust standard errors.
Chapter 2: MANUSCRIPT 1
Risk of HIV-1 acquisition among women using different types of injectable progestin contraception in South Africa: a prospective cohort study

Lisa M. Noguchi, Barbra A. Richardson, Jared M. Baeten, Sharon L. Hillier, Jennifer E. Balkus, Z. Mike Chirenje, Katherine Bunge, Gita Ramjee, Gonasagrie Nair, Thesla Palanee-Phillips, Pearl Selepe, Ariane van der Straten, Urvi M. Parikh, Kaila Gomez, Jeanna M. Piper, D. Heather Watts, Jeanne M. Marrazzo, for the VOICE Study Team

2.1 ABSTRACT

Background Several observational studies have reported increased HIV-1 acquisition among women using depot medroxyprogesterone acetate (DMPA), compared to women not using hormonal contraception. Current WHO guidance emphasizes the need for consistent condom use among users of progestin-only injectable contraception. We aimed to assess whether DMPA and norethisterone enanthate (NET-EN), another common but different injectable progestin contraceptive, confer different risk of HIV-1 acquisition.

Methods Data from 3,141 South African women who used injectable contraception while participating in the VOICE study (MTN-003) were included. Participants were enrolled between 2009 and 2011 and evaluated monthly for contraceptive use and incident HIV-1 infection. We calculated a site-stratified estimate for the difference in incident HIV-1 infection between DMPA and NET-EN users, with adjustment for potential confounding variables (baseline age, marriage/cohabitation, secondary education, and herpes simplex
virus type 2 serostatus, and time-varying combined oral contraceptive use, primary partner’s other sexual partnerships, and condom use).

**Findings** Among 3,141 users of injectable contraception at South African VOICE sites, 1,788 (56.9%) solely used DMPA, 1,097 (34.9%) solely used NET-EN, and 256 (8.2%) used both types of injectable contraception at different points during follow-up. During 2,734 person-years (py) of follow-up, 207 total incident HIV-1 infections were detected (HIV-1 incidence: 7.6/100 py, 95% CI 6.6 to 8.7/100 py). Users of DMPA had a significantly higher risk of HIV-1 acquisition (152 HIV-1 infections/1,744 py; HIV-1 incidence: 8.7/100 py, 95% CI 7.4 to 10.2/100 py), compared to users of NET-EN (55 HIV-1 infections/990 py; HIV-1 incidence: 5.6/100 py, 95% CI 4.3 to 7.2/100 py) (hazard ratio [HR]: 1.58, 95% CI 1.16 to 2.15, p=0.004). This association persisted when adjusted for potential confounding variables (adjusted hazard ratio (aHR): 1.46, 95% CI 1.10 to 1.95, p=0.01). The increased hazard of HIV-1 infection for DMPA compared to NET-EN users persisted in sensitivity analyses restricted to women less than 25 years old, women with sexually transmitted infections at baseline, and in women who used both DMPA and NET-EN during follow-up. Among women seropositive for herpes simplex virus type 2 (HSV-2) at enrollment, the aHR was approximately doubled; for those women HSV-2 seronegative at enrollment, no elevated risk was observed (aHR 2.10 versus 1.12, pinteraction= 0.07).

**Interpretation** Among South African women using injectable contraception in an HIV-1 prevention study, DMPA was associated with higher risk of HIV-1 infection than NET-EN. While HSV-2 serostatus appeared to be an important effect modifier in this analysis, results across observational analyses are currently too variable to inform clinical
guidance for women with or at risk for HSV-2 infection. While modest associations in observational analyses should be interpreted cautiously, these findings suggest that consideration should be given to switching from DMPA to NET-EN in high HIV-1 incidence settings where NET-EN is available.
Risk of HIV-1 acquisition among women using different types of injectable progestin contraception in South Africa: a prospective cohort study

2.2 INTRODUCTION

While overall benefits outweigh potential risks of contraception to women’s health,\textsuperscript{12,89} results of observational studies evaluating the impact of hormonal contraception (HC) on HIV-1 acquisition are mixed.\textsuperscript{13} In light of several studies suggesting increased HIV-1 risk for the injectable progestin depot medroxyprogesterone acetate (DMPA), the World Health Organization (WHO) recommended that “women using progestogen-only injectable contraception should be strongly advised to also always use condoms, male or female, and other HIV preventive measures.”\textsuperscript{60} Additionally, WHO recommended that injectable contraceptive alternatives to DMPA be investigated for epidemiological associations with HIV acquisition in women.\textsuperscript{61} Recently updated WHO guidance acknowledges these mixed findings, and affirms the need for further research.\textsuperscript{62} This uncertainty is especially relevant in sub-Saharan Africa, where HIV-1 incidence among young women is high and injectable contraception is common.\textsuperscript{2,52,90}

In Eastern and Southern Africa, injectable contraceptives are the most popular methods of family planning, accounting for over 40 percent of contraceptive use.\textsuperscript{91} South African women are increasingly using injectable contraception;\textsuperscript{19,51} approximately half of women currently using contraception use injectable progestin methods, although estimates reach nearly 90% in some regions of South Africa.\textsuperscript{51} The two commonly used methods are DMPA 150 mg, a progesterone derivative given every three months,\textsuperscript{20} and norethisterone
enanthate (NET-EN) 200 mg, a first-generation synthetic progestin administered 2-monthly. While more sexually active South African women use DMPA than NET-EN, both are highly effective (97% with typical use), available in the public sector, where most South African women obtain contraception, have the same medical eligibility criteria, and are generally considered exchangeable in policy guidance. However, their pharmacokinetic profiles differ and DMPA users may experience delayed return to ovulation compared to NET-EN users. In clinical practice, DMPA and NET-EN are generally distinguished by their duration of effectiveness, although amenorrhea may be more common with continued DMPA use. To date, no studies disaggregating progestin type have clearly implicated NET-EN in HIV-1 acquisition, including a large individual participant data meta-analysis, although data specific to NET-EN are generally limited. Recent laboratory studies indicating different progestins impact immune cells differently also suggest that HIV-1 risk may differ between these two methods.

A recent systematic review included previously unpublished estimates for HIV-1 risk specific to DMPA and NET-EN compared to no use of HC. In the past, Wand and colleagues had reported measures of association for a pooled exposure category including both DMPA and NET-EN use. The authors of the systematic review, who collected these new disaggregated point estimates via personal communication with study authors, noted that estimates were generally higher for NET-EN than DMPA when evaluated in the same study, although 95% confidence intervals overlapped.
Most analyses on HC and HIV-1 acquisition have employed a comparator group not using HC.\textsuperscript{59} However, an acknowledged limitation of this approach is the inadequate comparison between groups with potentially different HIV-related risk behaviors, including unprotected intercourse and coital frequency.\textsuperscript{64,95} Women in a no hormonal contraceptive comparator group may be using condoms for contraception, or they may even be seeking pregnancy. These differential risks of HIV-1 exposure could confound associations between contraceptive type and HIV-1 acquisition. However, restricting analysis to one delivery system (injection) may address potential behavioral confounding by contraceptive type, and allow a more direct comparison between two HC types of interest. Here, we sought to quantify the potential difference in HIV-1 acquisition for DMPA and NET-EN users.

2.3 METHODS

2.3.1 Study design and participants

We analyzed prospective data from VOICE (MTN-003), a randomized, placebo-controlled trial testing safety and effectiveness of three formulations of tenofovir for prevention of HIV-1 in women (see Appendix I). Participants were recruited from Uganda, Zimbabwe and South Africa (Durban, Johannesburg, and Klerksdorp) from 2009 to 2011 and followed until 2012. Eligibility criteria required participants to be HIV-uninfected, sexually active, non-pregnant, without curable genitourinary infection, and willing to use effective contraception (hormonal method, intrauterine device (IUD), or sterilization) (see Appendix II).\textsuperscript{75} The main findings of the VOICE trial have been
previously reported; HIV-1 acquisition was not significantly reduced in any of the active study arms compared to corresponding placebo.\textsuperscript{76} However, adherence to VOICE study drugs was low.\textsuperscript{76} We restricted the current analysis to participants in South Africa, where both DMPA and NET-EN were used. Figure 2.1 details selection of cohort participants. We hypothesized there would be no difference in HIV-1 acquisition between DMPA and NET-EN users.

2.3.2 Procedures

2.3.3 Laboratory methods

Participants attended monthly visits for study product management, behavioral interviews using standardized questionnaires, documentation of contraceptive use and HIV-1 testing.\textsuperscript{75} Sensitive questions, e.g., sexual practices, were also asked via audio computer-assisted self-interview (ACASI).\textsuperscript{75} All participants were provided condoms and standard risk reduction counseling. Testing for HIV-1 followed a protocol-defined algorithm.\textsuperscript{75} Screening for chlamydia, gonorrhea and trichomonas infection occurred at baseline, annually and when clinically indicated, with treatment provided on-site.\textsuperscript{75} Herpes simplex virus type 2 (HSV-2) status at baseline and exit visits was determined by FOCUS EIA (Focus Technologies, Cypress, CA, USA). Pregnancy status was ascertained with monthly urine hCG testing.\textsuperscript{75}

Participants provided written consent. Institutional Review Boards (IRBs) in Zimbabwe, Uganda, South Africa and the United States approved the VOICE study, with additional approval for this secondary analysis provided by Johns Hopkins Bloomberg School of Public Health IRB.
2.3.4 Independent HIV-1 endpoint adjudication committee

The MTN Endpoint Adjudication Committee provided guidance to the protocol team regarding the determination of HIV-1 endpoints in VOICE, in the event that the final HIV-1 status of one or more study participants was not unequivocal, or if the point at which one or more participants became infected was not clear. Protocol team members did not serve as members of the Endpoint Adjudication Committee. Cases reviewed by the Committee were blinded to study participant identities and associated exposures.

2.3.5 Statistical analysis

The VOICE protocol pre-specified an analysis of contraceptive use and HIV-1 acquisition. The primary comparison of DMPA to NET-EN use in the current analysis was designed following VOICE completion, but preceding primary data analysis. Based on the anticipated number of HIV-1 endpoints in VOICE, we estimated 80-90% power to detect a 50% difference in hazard of HIV-1 acquisition between DMPA and NET-EN users. To compare baseline characteristics of DMPA and NET-EN users, we grouped participants according to their initial post-enrollment injection exposure. Exposure was estimated using injection dates, which were recorded in charts for on-site injections and transcribed from family planning cards for off-site injections. Exposure lengths per injection (17 weeks (DMPA) and ten weeks (NET-EN) were based a priori on WHO guidelines for duration of contraceptive coverage. Exposure was further categorized to distinguish periods where combined oral contraceptive pill (COC) and injectable exposure overlapped, for example, to treat breakthrough vaginal bleeding. We then estimated distinct segments of exposure representing the days that each woman used the contraceptive method. Participant-years of use for each method thus include all segments
of use. Those women who switched injectable progestin methods contributed exposure segments to both methods. Thus, person-time for both injectable types was used in the analysis, including for those who switched types during follow-up. Date of HIV-1 infection was estimated using midpoint between last negative and first confirmed positive test. We excluded 33 participants missing data on injectable contraceptive type and 22 identified by plasma HIV-1 ribonucleic acid (RNA) polymerase chain reaction (PCR) as HIV-infected at enrollment (Figure 2.1).

To describe baseline demographic and behavioral characteristics of the cohort, we used medians, frequencies and percentages. We compared baseline characteristics between participants using DMPA and NET-EN as their first injectable method on study, using the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. We calculated overall HIV-1 incidence rates per 100 person-years of follow-up and 95% confidence intervals, as well as for person-time specific to DMPA and NET-EN exposure.

A Cox proportional hazards model with time-varying exposure was used to compare rates of HIV-1 acquisition between DMPA and NET-EN users. We confirmed that these data met the proportional hazards assumption. Follow-up time was initiated from enrollment until censoring at the estimated date of HIV-1 infection, pregnancy detection, loss to follow-up, or last negative HIV-1 test. Time origin for the model was date of first injection resulting in exposure during follow-up. We adjusted for baseline age, marriage/cohabitation, education, and HSV-2 serostatus as time-fixed variables. Time-varying factors included COC use, condom use (measured by ACASI), and whether a primary partner had other partners (measured by ACASI). Due to overall low adherence
to study products as measured in pharmacokinetic analyses, we did not adjust by trial arm. Potential confounding variables were selected based on clinical relevance. We calculated a site-stratified estimate in the adjusted model, due to variable HIV-1 incidence by site (2.18-10.40/100 person-years [py]). Clustering of variance–covariance estimation methods was used to calculate standard errors allowing for intragroup correlation at the study site level. Application of a marginal structural model (MSM) to address time-dependent confounding was considered; however, intermittent COC use and pregnancy were deemed potential violations of the positivity assumption requisite for MSM validity.97 In addition, time-varying factors theorized to impact both prescription of DMPA versus NET-EN and HIV-1 acquisition did not vary substantially during follow-up.

We conducted three pre-specified sub-group analyses to evaluate robustness of results: women who reported at baseline not using condoms for vaginal sex in the past week, women under age 25, and women with versus without HSV-2 at baseline. The likelihood ratio test was used to assess interaction between injectable type and HSV-2 serostatus in the adjusted model. We tested model sensitivity to censoring at pregnancy detection via an additional model omitting all pregnant person-time, but with allowance for return to observation after pregnancy outcomes (not pre-specified). We also evaluated results in those with baseline chlamydia, gonorrhea or trichomonas infection, as a potentially objective marker for recent unprotected sex (not pre-specified). We repeated the model restricting the analysis to participants who used both injectable methods at different times during follow-up (not pre-specified). Lastly, we examined the unadjusted hazard ratios for DMPA versus NET-EN use in two metropolitan regions: the Durban region and the
Johannesburg/Klerksdorp region (not pre-specified). Analyses were performed using Stata version 13.1. All statistical tests were two-sided with a type I error rate of 0.05.

2.3.6 Role of the funding source

The sponsor (US National Institutes of Health) participated in study design and oversight, results interpretation, and manuscript preparation, and provided for an independent data and safety monitoring board to review VOICE every 6 months. Writing team members had full access to study data. Protocol chairs and sponsor representatives had final responsibility for the decision to submit for publication.

2.4 RESULTS

2.4.1 Participants and study follow-up

Among 3,141 South African injectable contraception users, 1,788 (56.9%) solely used DMPA, 1,097 (34.9%) solely used NET-EN, and 256 (8.2%) used both injectable types at different times during follow-up. As most did not switch injectable type, Table 2.1 compares baseline characteristics of women whose first injectable was DMPA (n=1,927, 61.4%) versus NET-EN (n=1,214, 38.7%). Overall, most women were young and unmarried. Women whose first injectable was DMPA were slightly older, more likely married or cohabitating, more likely parous, and less likely to report multiple partners or a circumcised partner. At baseline, reported condom use at last sex was similar for both injectable contraceptive groups. Median number of sex acts for past week was comparable, but with the distribution weighted toward fewer sex acts among NET-EN users. Women whose first injectable was NET-EN more commonly reported recent anal
sex. While prevalence of curable genitourinary infection was comparable between
groups, baseline seropositivity for HSV-2 was higher for those whose first method on
record was DMPA.

Overall median follow-up time for women was 13.8 months (IQR 11.5-17.0), and was
similar between groups (p=0.27). Women whose first injectable was DMPA more
frequently continued participation until scheduled termination (92.8% versus 89.4%,
p=0.001). Reasons for early termination (n=269) included declined further participation
(n=127, 47.2%), relocation (n=61, 22.7%), inability to contact (n=41, 15.2%), other
reasons (n=35, 13.0%), death (n=3, 1.1%), and investigator decisions (n=1, <1%). Users
of DMPA were less likely to use COCs (alone or for management of breakthrough
bleeding) during follow-up, compared to NET-EN users (14.0 versus 27.5%, p<0.001).
Pregnancy during follow-up was less common among those who initiated injectable use
with DMPA compared to NET-EN (1.7 versus 3.3% ever pregnant, p=0.003). However,
overall pregnancy incidence (0.5% for both methods) was consistent with published rates
for method failure and did not differ during DMPA and NET-EN exposure (p=0.76).

2.4.2 HIV-1 incidence

Overall HIV-1 incidence was 7.57 per 100 py (95% CI 6.61 to 8.68; 207 new HIV-1
infections over 2,733.9 py of follow-up) (Table 2.2). We detected 152 incident HIV-1
infections during 1,744.2 py of DMPA use (8.71/100 py, 95% CI 7.43 to 10.22) and 55
during 989.8 py of NET-EN use (5.56/100 py, 95% CI 4.27 to 7.24). The overall hazard
of HIV-1 infection was higher among DMPA compared to NET-EN users (HR 1.58, 95%
CI 1.16 to 2.15, p=0.004). This association persisted in the multivariable model adjusted
for baseline and time-varying factors, with stratification by study site (aHR 1.46, 95% CI 1.10 to 1.95, p=0.01).

2.4.3 Sub-group analyses (pre-specified)

Among women HSV-2-seronegative at baseline, no significant difference in risk was observed between DMPA and NET-EN users (Table 2.2). However, among women HSV-2-seropositive at baseline, we observed a two-fold higher risk of incident HIV-1 infection in DMPA compared to NET-EN users (aHR 2.10 versus 1.12, p_{interaction} = 0.07).

2.4.4 Sub-group analyses (not pre-specified)

Non-pre-specified sub-group analyses are presented in Table 2.3. Repeating the primary comparison in the whole cohort, but including additional person-time after pregnancy outcomes, did not substantially alter results. Results were also not substantially changed by restricting analysis to those diagnosed with trichomonas, chlamydia or gonorrhea infection at baseline. When we restricted analysis to those who used both DMPA and NET-EN at different times during follow-up; DMPA use was strongly associated with a higher risk of HIV-1 acquisition, compared to NET-EN use in the model adjusted for potential confounders (aHR 6.57, 95% CI 3.25 to 13.28, p<0.001). In the Durban metropolitan region, results were mostly consistent with the primary comparison across sites. However, in the combined Johannesburg/Klerksdorp region, no significant difference in HIV-1 acquisition was noted between DMPA and NET-EN users.
2.5 DISCUSSION

2.5.1 Summary
In this large prospective study of South African women using injectable progestin-only contraception, we found that those using DMPA had an approximately 50% increase in incident HIV-1 infection compared to those using NET-EN; an increased risk persisted after controlling for important demographic and behavioral factors and in the majority of sensitivity analyses. Current WHO recommendations suggest that progestin-only injectable methods have potential to heighten risk of HIV-1 acquisition; our results suggest risk may differ across different progestin-only injectable types.

2.5.2 Context
Our findings cannot address whether DMPA increases risk of HIV-1 acquisition in women compared to no use of HC, nor can they address HIV-1 risk associated with use of combination (estrogen plus progestin) injectable methods. However, these results may offer unique and clinically relevant information for the millions of women at risk for HIV-1 infection who want to use progestin-only injectable contraception, particularly those unable to negotiate condom use and those in areas where non-hormonal alternatives are scarce. The more common comparison of DMPA to no HC is appropriate when estimating the direct effect of DMPA on HIV-1 acquisition, but comparisons between effective methods have greater utility for women desiring to avoid pregnancy, and for contraceptive providers. Moreover, a comparison between different two injectable methods is less likely to be confounded by behavioral differentials than comparisons between women using HC versus those using non-hormonal methods.
Given the high HIV-1 incidence observed overall in this cohort, our findings support WHO recommendations on consistent condom use by couples in which the woman is using injectable progestin contraception, although adherence to this recommendation may be unrealistic. Condom use is frequently outside women’s control, and this persistent inequity itself contributes to demand for injectable contraceptives, which can be used independently of a partner’s knowledge or consent. In this cohort, women not using condoms at baseline had overall lower HIV-1 risk, compared to the whole cohort, which may be related to both risk perception and partner choice. Scale-up is needed for contraceptive alternatives to injectable methods such as implants and non-hormonal methods such as the copper IUD, although it is acknowledged that there are few data estimating impact of these methods on HIV-1 risk. Future development of multi-purpose technologies for prevention of both HIV-1 infection and undesired pregnancy is also needed.

2.5.3 Stratification by HSV-2 serostatus

Among women who were HSV-2 seropositive at baseline, we found DMPA was associated with a two-fold risk of HIV-1 acquisition compared to NET-EN, while this association was not observed among women HSV-2 seronegative at baseline. Our findings contrast with those of prior studies (comparing DMPA or pooled HC to no HC) that have reported either an increased risk of HIV-1 acquisition among HSV-2 seronegative women\textsuperscript{55} or no indication of effect modification by HSV-2 status.\textsuperscript{56-58} Thus, data are currently insufficient to inform clinical guidance specifically for women with known or suspected HSV-2 infection.
2.5.4 Strengths of the analysis

Because analyzing HC and HIV-1 acquisition was a planned objective in VOICE, we prospectively implemented a robust data collection and site monitoring strategy for this cohort, one of the largest to date with regard to person-time and HIV-1 infections. The frequency with which we updated contraceptive exposure status, sexual behavior data, and pregnancy and HIV-1 status data is also high among studies investigating the HC-HIV association. Contraception was offered on-site to all participants, and injection types and dates were directly observed or determined from family planning cards, rather than self-report. Such frequent measurement of documented contraceptive use allows more precise characterization of exposures relative to HIV-1 outcome, reducing potential exposure misclassification. The VOICE design also permitted simultaneous investigation of genital tract infection, and neither prevalent nor incident gonorrhea or chlamydia differed between DMPA and NET-EN users, as might be expected if the observed increase in HIV-1 acquisition were related solely to differences in behavior (condom use, for example).\textsuperscript{98}

2.5.5 Limitations of the analysis

Our results may be consistent with those of recent in vitro analyses suggesting pro-inflammatory effects of DMPA compared to NET-EN.\textsuperscript{44,94} However, ultimately our findings are based on laboratory outcomes that cannot definitively articulate potential mechanisms for the observed difference in HIV-1 acquisition between groups. The primary limitation to this study is possible bias, a significant risk in all observational analyses, which may have limited capacity to reliably detect modest associations.\textsuperscript{99} In this cohort, which did not determine partners’ HIV-1 serostatus, true HIV-1 exposure levels
are unknown and may have differed between injectable groups. The sole inclusion of healthy women participating in research limits generalizability of our results. Social desirability may have affected measurement of self-reported covariates, such as condom use. Pharmacokinetic profiles may differ between injectable progestins and among women, and physiologic effects may outlast detectable drug;\textsuperscript{100} thus, our exposure definitions, which were modeled and not based on pharmacokinetic analyses for exogenous hormones, may not have demarcated the most relevant exposure periods.

While we hypothesized that DMPA and NET-EN users were more similar to each other regarding HIV-1 risk, than to women not using HC, we did observe differences between women in the two contraceptive groups (Table 2.1). First, DMPA users were more likely to continue follow-up until scheduled termination, which may have impacted estimates for comparative risk of HIV-1 acquisition. Some factors, such as partner’s circumcision status, uncertainty about a partner’s other partners or HIV-1 status, and participant’s HSV-2 serostatus might suggest higher HIV-1 risk for DMPA compared to NET-EN users. However, other differences between groups, such as age, extra-primary partnerships, recent anal sex, and knowing a primary partner had other partners or HIV-1, potentially indicate higher HIV-1 risk in NET-EN users, although we did not observe this in our results. Thus, demographic and behavioral differences between DMPA and NET-EN users do not clearly indicate a consistent direction for bias. Of note, some differences between DMPA and NET-EN users, particularly age, have been observed previously and are likely rooted in historical misperceptions that NET-EN is more appropriate for younger women desiring future fertility,\textsuperscript{101} suggesting socio-demographic differences
between users persist despite contemporary messaging to discourage age-based prescribing.\textsuperscript{102,103}

Our results stratified by geographic metropolitan area showed a lack of consistency between two regions of South Africa. Findings in the Durban region, the dominant area in the accumulation of study person-time overall, were consistent with overall findings. While power in the Johannesburg/Klerksdorp sub-group analysis was limited compared to power in the Durban region analysis, the inconstancy in the direction of the adjusted hazard ratio raises suspicion that our overall findings may be related to factors other than injectable type.

2.5.6 Public health implications

HIV-1 infection does not exist in isolation from other significant health risks for women in sub-Saharan Africa, including maternal mortality. In South Africa, modern contraception has averted an estimated 58\% of potential maternal deaths.\textsuperscript{12} Benefits of contraception include reduced newborn deaths, improved child health/education, and increased household income, among others.\textsuperscript{104} Thus, concerns about possible increased HIV-1 risk with DMPA use must be weighed against beneficial effects of effective contraception on a broad range of reproductive outcomes. Despite findings that may implicate DMPA in HIV-1 acquisition, observational findings remains mixed, and withdrawing any common effective contraceptive could increase maternal mortality rates.\textsuperscript{74} Given the majority of observational data suggesting no increased HIV-1 risk specific to NET-EN, and the fact that this equally effective alternative exists for those preferring injectable contraceptives, policy makers, clinician-scientists and community stakeholders should review emerging data to determine if women, in consultation with
providers, should consider switching from DMPA to NET-EN in high HIV-1 incidence settings where NET-EN is available.
2.6 FIGURE AND TABLES

Figure 2.1: Aim 1 cohort profile

5,029 total VOICE participants

4,077 randomized at South African sites

952 enrolled in countries not using NET-EN (Uganda and Zimbabwe)

22 HIV-seropositive at baseline

761 never used injectable contraception

33 missing injectable type

13 never returned after study enrollment

106 initiated injectable contraception after HIV outcome or censoring
  11 after HIV seroconversion
  84 after pregnancy censoring
  11 after administrative censoring

3,141 injectable contraceptive users included in analysis

1,788 DMPA users

1,097 NET-EN users

256 users of both methods
Table 2.1: Aim 1 baseline characteristics of women by first injectable type on study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole cohort (n=3,141)</th>
<th>DMPA first (n=1,927)</th>
<th>NET-EN first (n=1,214)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (IQR)</td>
<td>23 (21-27)</td>
<td>24 (21-27)</td>
<td>23 (20-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married/cohabitating</td>
<td>603 (19.2%)</td>
<td>408 (21.2%)</td>
<td>195 (16.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parous</td>
<td>2,632 (83.8%)</td>
<td>1,786 (92.7%)</td>
<td>846 (69.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any secondary education</td>
<td>3,017 (96.2%)</td>
<td>1,839 (95.6%)</td>
<td>1,178 (97.1%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Formal employment</td>
<td>299 (9.5%)</td>
<td>178 (9.3%)</td>
<td>121 (10.0%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Home owned by self/family</td>
<td>2,585 (82.4%)</td>
<td>1,585 (82.3%)</td>
<td>1,000 (82.4%)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Sexual behaviors**

| >1 sexual partner                          | 118 (3.8%)             | 53 (2.8%)            | 65 (5.5%)              | <0.001   |
| Median sex acts past week (IQR)            | 2 (1-3)                | 2 (1-3)              | 2 (0-3)                | 0.004    |
| Condom at last sex                         | 2,132 (74.6%)          | 1,299 (74.3%)        | 833 (75.1%)            | 0.66     |
| Anal sex past 3 months                     | 628 (20.3%)            | 364 (19.2%)          | 264 (22.2%)            | 0.04     |
| Sex for money past year                    | 155 (5.0%)             | 93 (4.9%)            | 62 (5.2%)              | 0.67     |

**Primary partner**

| Any secondary education                    | 2,891 (92.7%)          | 1,764 (92.0%)        | 1,127 (93.8%)          | 0.19     |
| Has other partners                         |                        |                      |                        |          |
| Yes                                        | 285 (9.4%)             | 163 (8.6%)           | 122 (10.6%)            | 0.03     |
| Don’t know                                 | 1,952 (64.3%)          | 1,247 (66.0%)        | 705 (61.4%)            |          |
| Circumcised                                |                         |                      |                        |          |
| Yes                                        | 1,001 (32.1%)          | 556 (29.0%)          | 445 (37.0%)            | <0.001   |
| Don’t know                                 | 392 (12.6%)            | 238 (12.4%)          | 154 (12.8%)            |          |
| HIV-infected                               |                         |                      |                        |          |
| Yes                                        | 104 (3.4%)             | 61 (3.2%)            | 43 (3.7%)              | 0.001    |
| Don’t know                                 | 893 (29.4%)            | 602 (31.9%)          | 291 (25.3%)            |          |

**Genital tract infections**

| Bacterial vaginosis‡                       | 1,246 (39.7%)          | 763 (39.6%)          | 483 (39.9%)            | 0.91     |
| Trichomoniasia                             | 174 (5.5%)             | 114 (5.9%)           | 60 (4.9%)              | 0.25     |
| Chlamydia                                  | 469 (14.9%)            | 289 (15.0%)          | 180 (14.8%)            | 0.90     |
| Gonorrhea                                  | 110 (3.5%)             | 67 (3.5%)            | 43 (3.5%)              | 0.92     |
| Herpes simplex virus type 2                | 1459 (46.6%)           | 986 (51.2%)          | 473 (39.3%)            | <0.001   |

Abbreviations: DMPA, depot medroxyprogesterone acetate; IQR, interquartile range; NET-EN, norethisterone enanthate.

Continuous variables are median (IQR). Categorical variables are number (%). *Comparison between participants using DMPA and NET-EN as their first injectable method on study, calculated with Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables.

‡ Nugent score ≥7 for Gram-stained vaginal smear.
Table 2.2: Aim 1 incidence and relative hazard of HIV-1 infection, by injection type: primary comparison and pre-specified sub-group analyses

<table>
<thead>
<tr>
<th></th>
<th>HIV-1 sero-conversions/py</th>
<th>Incidence/100 py (95% CI)</th>
<th>Unadjusted Cox proportional hazards regression</th>
<th>Adjusted Cox proportional hazards regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Primary comparison in whole cohort (n=3,141)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>55/989.8</td>
<td>5.56 (4.27-7.24)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>152/1,744.2</td>
<td>8.71 (7.43-10.22)</td>
<td>1.58 (1.16-2.15)</td>
<td>1.46 (1.10-1.95)*</td>
</tr>
<tr>
<td>Total</td>
<td>207/2,733.9</td>
<td>7.57 (6.61-8.68)</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>Women who reported no condom use for vaginal sex at baseline (n=304)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>1/108.1</td>
<td>0.92 (0.13-5.52)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>7/160.2</td>
<td>4.37 (2.08-6.57)</td>
<td>5.09 (0.63-41.5)</td>
<td>6.16 (3.29-11.52)**</td>
</tr>
<tr>
<td>Total</td>
<td>8/268.3</td>
<td>2.98 (1.49-5.96)</td>
<td>0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women under 25 years old at baseline (n=1,890)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>41/636.5</td>
<td>6.44 (4.74-8.75)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>104/964.6</td>
<td>10.78 (8.90-13.07)</td>
<td>1.69 (1.17-2.42)</td>
<td>1.44 (1.04-2.00)*</td>
</tr>
<tr>
<td>Total</td>
<td>145/1,601.1</td>
<td>9.06 (7.70-10.66)</td>
<td>0.005</td>
<td>0.03</td>
</tr>
<tr>
<td>Women who were HSV-2 seronegative at baseline (n=1,671)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>34/588.6</td>
<td>5.78 (4.13-8.08)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>55/856.1</td>
<td>6.42 (4.93-8.37)</td>
<td>1.11 (0.72-1.70)</td>
<td>1.12 (0.81-1.56)***</td>
</tr>
<tr>
<td>Total</td>
<td>89/1,444.8</td>
<td>6.16 (5.00-7.58)</td>
<td>0.63</td>
<td>0.50</td>
</tr>
<tr>
<td>Women who were HSV-2 at seropositive baseline (n=1,459)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>21/396.0</td>
<td>5.30 (3.46-8.13)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>97/887.0</td>
<td>10.94 (8.96-13.34)</td>
<td>2.09 (1.30-3.35)</td>
<td>2.10 (1.17-3.74)***</td>
</tr>
<tr>
<td>Total</td>
<td>118/1,283.0</td>
<td>9.20 (7.68-11.02)</td>
<td>0.002</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: py, person-years; CI, confidence interval; NET-EN, norethisterone enanthate; DMPA, depot medroxyprogesterone acetate, REF, reference.

*Adjusted for baseline age, marriage/cohabitation, education, and HSV-2 status, and time-varying oral contraceptive pill use, primary partner has other partners, and condom use at last sex, with stratification by study site.  **Adjusted for all of the above except condom use.  ***Adjusted for all of the above except prevalent HSV-2. Baseline HSV-2 status available for 99.6% (n=3130) of whole cohort.
Table 2.3: Aim 1 incidence and relative hazard of HIV-1 infection, by injection type: non-pre-specified sub-group analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence/100 py (95% CI)</th>
<th>Unadjusted Cox proportional hazards regression</th>
<th>Adjusted Cox proportional hazards regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>p-value</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>Whole cohort plus person-time following pregnancy outcomes (n=3,209)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>5.59 (4.30-7.26)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>8.82 (7.54-10.32)</td>
<td>1.59 (1.17-2.16)</td>
<td>1.48 (1.08-2.02)*</td>
</tr>
<tr>
<td>Total</td>
<td>7.65 (6.69-8.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women with trichomoniasis, chlamydia or gonorrhea infection at baseline (n=674)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>8.74 (5.51-13.88)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>15.51 (11.99-20.06)</td>
<td>1.78 (1.05-3.02)</td>
<td>1.70 (1.07-2.70)*</td>
</tr>
<tr>
<td>Total</td>
<td>13.11 (10.47-16.41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women who used both DMPA and NET-EN at different times during follow-up (n=256)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>2.43 (0.78-7.54)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>13.32 (8.28-21.42)</td>
<td>5.99 (2.36-15.18)</td>
<td>6.57 (3.25-13.28)*</td>
</tr>
<tr>
<td>Total</td>
<td>7.97 (5.14-12.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durban region (n=2,419)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>6.56 (4.88-8.82)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>9.41 (7.99-11.08)</td>
<td>1.44 (1.03-2.02)</td>
<td>1.52 (1.11-2.09)*</td>
</tr>
<tr>
<td>Total</td>
<td>8.54 (7.40-9.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johannesburg/Klerksdorp region (n=722)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>3.44 (1.90-6.21)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>DMPA</td>
<td>3.75 (1.88-7.51)</td>
<td>1.09 (0.44-2.70)</td>
<td>0.98 (0.44-2.20)*</td>
</tr>
<tr>
<td>Total</td>
<td>3.57 (2.27-5.59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: py, person-years; CI, confidence interval; NET-EN, norethisterone enanthate; DMPA, depot medroxyprogesterone acetate.

*Adjusted for baseline age, marriage/cohabitation, education, and HSV-2 status, and time-varying oral contraceptive pill use, primary partner has other partners, and condom use at last sex, with stratification by study site.
Chapter 3: MANUSCRIPT 2
Gonorrheal and chlamydial infection in users of injectable progestin contraception in South Africa

3.1 ABSTRACT

Background
Observational findings conflict regarding the potential impact of injectable progestin contraception on acquisition of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infection. Previously, we reported that risk of HIV-1 acquisition may differ between DMPA and NET-EN users, and that age differences persist in the prescription of these contraceptives. In the current analysis, we compared prevalent and incident CT and NG between DMPA and NET-EN users among reproductive age women in South Africa.

Methods
We used prospective data from 2,986 South African women who used injectable contraception while participating in the VOICE study (MTN-003). Participants enrolled between 2009 and 2011 and were evaluated annually and as clinically indicated for CT and NG infection using a DNA amplification assay performed on urine. We used an Andersen-Gill proportional hazards model to compare risk for CT and NG infection between DMPA and NET-EN users. We conducted additional analyses among women reporting no condom use at baseline, those under 25 years old, and those who did not switch injectable contraceptive types during follow-up.
Results

Among 2,986 users of injectable contraception, 1,711 (57.3%) used DMPA, 1,028 used NET-EN (34.4%), and 247 (8.3%) used both methods at different times during study follow-up. Overall baseline prevalence was 14.9% for CT and 3.6% for NG. Users of DMPA and NET-EN did not differ in prevalence of either infection at baseline (CT: 15.1 versus 14.7%, p=0.77; NG: 3.5 versus 3.6%, p=0.93). Over 2,630 person-years (py) of follow-up, 400 CT infections and 93 NG infections were detected (CT incidence: 15.2/100 py; NG incidence 3.5/100 py). Users of DMPA and NET-EN did not differ significantly in their risk for incident CT (HR 0.92, 95% CI 0.75 to 1.13) or NG (HR 0.85 (0.56 to 1.29). When adjusted for baseline (time-fixed) age, education, and marriage/cohabitation, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use, no difference between DMPA and NET-EN users was detected for either incident CT (adjusted HR [aHR] 1.05, 95% CI 0.85 to 1.29) or NG (aHR 1.00, 95% CI 0.65 to 1.54).

Discussion

In a prospective cohort of women participating in HIV prevention research, DMPA and NET-EN users did not differ in either prevalent or incident CT or NG. Differential acquisition of HIV-1 previously observed in this cohort does not appear to be explained by differential acquisition of CT or NG. Women considering injectable contraception can be counseled that risk of CT and NG does not appear to be impacted by injectable progestin type.
3.2 INTRODUCTION

3.2.1 Injectable hormonal contraception and acquisition of gonorrheal and chlamydial infection

Uptake of contraception, particularly injectable progestin contraception, has increased substantially over the past twenty years, particularly in Africa. As unintended pregnancy contributes to maternal mortality, family planning is a vital strategy for protecting women’s health. However, some observational findings have suggested that hormonal contraception (HC), particularly depot medroxyprogesterone acetate (DMPA), may increase women’s risk of acquiring sexually transmitted HIV. Study results also conflict regarding the impact of DMPA on two types of sexually transmitted cervical infection: Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infection, both of which are common among young women in South Africa. This lack of clear evidence is particularly relevant in areas such as South Africa, where the HIV and sexually transmitted infection (STI) burden are high among young women and injectable hormonal contraceptive use is common.

3.2.2 Types of injectable progestin contraceptives in South Africa

In South Africa, the two injectable hormonal contraceptive methods commonly used are the progestogen derivative, DMPA, and the first generation synthetic progestin, norethisterone enanthate, or NET-EN. Both of these highly effective methods are available in public access clinics and have the same medical eligibility criteria.
However, they differ in their duration of contraceptive effectiveness and consequently their administration schedule (three-monthly for DMPA versus two-monthly for NET-EN). In our secondary analysis of data from injectable contraception users in the VOICE trial, we found that users of DMPA tended to be slightly older than users of NET-EN, a finding consistent with historic trends in South Africa. To date, no studies that disaggregated injectable progestin type have implicated NET-EN in acquisition of chlamydial or gonorrheal infection.

Among South African women who used injectable progestin contraception in the VOICE study, we found that users of DMPA had approximately 50% increased risk of HIV-1 acquisition, compared to users of NET-EN. In part, a comparison between DMPA and NET-EN users in VOICE was undertaken with the hypothesis that restriction to a single contraceptive delivery system (injection) might reduce the behavioral confounding potentially associated with a comparison of injectable contraceptive use to no use of HC. However, one potential explanation for the difference we observed in HIV-1 acquisition might be unreported or otherwise unaccounted for differences in behavioral risk for STI between DMPA and NET-EN users. In such a case, it would be reasonable to also expect higher rates of CT and NG acquisition for users of DMPA compared to NET-EN.

In the cohort of injectable contraception users in VOICE, HIV-1 infection was strongly associated with having had a chlamydial, gonorrheal or trichomonas infection at screening in the study (HR 2.15, 95% CI 1.66 to 2.79, p<0.001), which may have been reflective of sexual risk behavior (e.g., lack of consistent condom use). However, strong evidence supports the role of both ulcerative and non-ulcerative STI in promoting HIV-1 transmission by increasing HIV-1 infectiousness and susceptibility through various
biological mechanisms. Increased risk for STI has also been proposed as one potential mechanism of mediation by which HC could increase women’s risk of HIV-1 infection. In this analysis, we compared prevalent and incident CT and NG between users of DMPA and NET-EN enrolled at South African sites in the VOICE study.

3.3 METHODS

3.3.1 Study population

We included prospective data from 2,986 women enrolled at 11 sites in South Africa (Durban [8], Johannesburg [2] and Klerksdorp [1]) between 2009 and 2011 in the VOICE trial, a Phase 2B, randomized, multi-site trial of the safety and effectiveness of three formulations of tenofovir-based HIV-1 chemoprophylaxis in healthy, sexually active women (Appendix I). Healthy, non-pregnant women using an effective method of contraception (defined by the protocol as a hormonal method, intrauterine device, or sterilization) were eligible for participation in VOICE (Appendix II). Few women elected to use intrauterine devices or were sterilized. Consequently, most women were using a form of hormonal contraception at the time of enrollment. Other details of the VOICE trial have been described elsewhere. None of the three active intervention study arms were associated with a decreased risk of HIV-1 acquisition compared to placebo, likely due to poor adherence to study product regimens. We excluded 33 participants missing data on injectable contraceptive type and 22 identified by plasma HIV-1 ribonucleic acid (RNA) polymerase chain reaction (PCR) as HIV-infected at enrollment (Figure 3.1). As switching between injectable types was rare, we conducted comparisons
between DMPA and NET-EN users based on the first injectable exposure type recorded on study (Table 3.1).

3.3.2 Characterization of exposure

Contraception was provided on-site at all sites, and methods obtained off-site were transcribed from participants’ family planning cards. Exposure lengths per injection (17 weeks (DMPA) and ten weeks (NET-EN) were based a priori on WHO guidelines for duration of contraceptive coverage. Exposure was further categorized to distinguish periods where combined oral contraceptive pill (COC) and injectable exposure overlapped, for example, to treat breakthrough vaginal bleeding. We then estimated distinct segments of exposure representing the days that each woman used the contraceptive method. Participant-years of use for each method thus include all segments of use.

3.3.3 Chlamydial and gonorrheal infection

Women were screened for CT and NG using the Becton Dickinson ProbeTec ET® (Franklin Lakes, NJ) strand displacement amplification (SDA) conducted on urine at study screening, annually, and at end of study product use, with additional testing performed as clinically indicated (see Appendix III). Results counseling and treatment were provided on-site. Single-dose, directly observed therapy for both infections was strongly encouraged across sites but not required. While national guidelines promote syndromic management for genital tract infections, all research sites used protocol-specific guidance for laboratory assay-based screening and treatment of infections. Consistent with local standards of care, tests of cure for CT and NG and expedited partner therapy were not routinely carried out.
3.3.4 Statistical methods

We compared baseline demographic, sexual behavioral, and primary partner characteristics between participants using DMPA and NET-EN as their first injectable method on study, using the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. Prevalence of CT and NG (at study screening) and herpes simplex virus type 2 (HSV-2) (at enrollment) was calculated and compared between DMPA and NET-EN users using Pearson’s chi-squared test. We calculated overall CT and NG incidence rates per 100 person-years of follow-up with 95% confidence intervals. We also calculated separate CT and NG incidence rates similarly for person-time specific to DMPA and NET-EN exposure.

Recent partner change was measured as a binary variable. Frequency of vaginal sex was measured as the number of vaginal sex acts in the past week. Primary partner’s additional partners were reported categorically as “yes”, “no”, or “don’t know”. Condom use was a binary variable indicating condom use at last vaginal intercourse.

We used an Andersen-Gill proportional hazards model, which allows for multiple failure events per participant, to investigate the potential association between time-varying injectable contraceptive type and acquisition of CT and NG during follow-up. We confirmed that data met the proportional hazards assumption. Follow-up time was initiated from the enrollment visit, with first documented injectable use during study participation used as time origin for the Anderson-Gill model. Observations were censored at estimated date of HIV-1 infection, detection of pregnancy, or last SDA performed during follow-up. Date of HIV-1 infection was estimated using midpoint between the last negative and first confirmed positive test (see Appendix III). Censoring
at HIV-1 infection and pregnancy was undertaken due to the likelihood of differential screening and treatment for chlamydial and gonorrheal infection following these diagnoses. We recalculated the hazard ratio comparing DMPA to NET-EN use for both infections, adjusting for potential confounding variables (time-fixed age, education, and marriage/cohabitation, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use).

We did not estimate the pooled impact of injectable progestin contraceptive use compared to non-use on CT or NG acquisition for several reasons. First, there was not an adequately large group of women who did not use HC in VOICE. Moreover, recently published guidance on the design and analysis of observational studies such as this one recommends more precise characterization of HC exposure, in part by disaggregating HC type in analyses. As confidence intervals for incidence rates of CT overlapped substantially across sites, we did not calculate a site-stratified hazard ratio in the unadjusted or adjusted Andersen-Gill model for CT. A similar approach was undertaken for NG due to overlapping confidence intervals for NG incidence rates.

Because routine screening for CT and NG occurred on an annual basis following enrollment, we conducted a sensitivity analysis restricting participants to those women who did not switch their injectable contraceptive type during follow-up (n=2,739). We also conducted analyses among women reporting no condom use at baseline, those under 25 years old, and those who did not switch injectable contraceptive types during follow-up.

The comparison of DMPA to NET-EN to examine risk for incident CT and NG was not pre-specified in the VOICE protocol, but was designed prior to knowledge of final
VOICE results. We hypothesized that there would be no difference in incident CT or NG infection between DMPA and NET-EN users. Statistical tests used a two-sided alpha of 0.05. Analyses were conducted using Stata version 13.1 (StataCorp, Inc, College Station, TX).

3.3.5 Human subjects and regulatory review
All participants provided written informed consent. The VOICE trial underwent all required institutional review board (IRB) and country-specific regulatory reviews, and is registered with Clinicaltrials.gov (NCT00705679). The Johns Hopkins Bloomberg School of Public Health IRB provided additional approval for this secondary analysis.

3.4 RESULTS
3.4.1 Characteristics of cohort participants
Among 4,077 women enrolled at South African sites, 3,316 (81.3%) used injectable progestin contraception during follow-up. Of injectable progestin contraception users, 2,986 (90.0%) were included in the analysis (Figure 3.1). Among injectable contraceptive users, 1,711 (57.3%) used DMPA, 1,028 used NET-EN (34.4%), and 247 (8.3%) used both methods at different times during study follow-up (Figure 3.1). Median length of follow-up was 13.5 months (IQR, 10.1 to 16.5 months), which did not differ significantly by injectable type used (p=0.68). Table 3.1 includes demographic and behavioral characteristics of participants at baseline. We observed several significant differences between users of DMPA and NET-EN. Overall, users of DMPA were older (median age 24 versus 23 years, p<0.001), more likely to be married (21.3 versus 16.2%, p=0.001),
and more likely to have had at least one child (92.6 versus 70.5%, p<0.001), compared to users of NET-EN. Compared to NET-EN users, DMPA users were slightly less likely to have completed any secondary education (95.5 versus 97.0%, p=0.04), and less likely to report at baseline having more than one sexual partner in the prior three months (2.5 versus 5.3%, p<0.001).

3.4.2 Prevalent and incident chlamydial infection
The overall prevalence of CT among South African users of injectable progestin contraception at baseline was 14.9% (Table 3.1). Users of DMPA and NET-EN did not differ in likelihood of diagnosis at baseline (p=0.77). Over 2,630 py of follow-up, 400 chlamydial infections occurred for an overall incidence of 15.2/100 py among users of injectable contraception (Table 3.2). Incidence of chlamydial infection did not differ significantly by injectable progestin type (incidence, DMPA 14.9/100 py, 95% CI 13.1 to 16.8/100 py) versus NET-EN 15.8/100 py, 95% CI 13.5 to 18.5/100 py) (HR 0.92, 95% CI 0.75 to 1.13, p=0.45). Adjustment for potential confounding variables (time-fixed age, education, and marriage/cohabitation, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use) did not qualitatively change this estimate (adjusted HR [aHR] 1.05, 95% CI 0.85 to 1.29, p=0.65).

3.4.3 Prevalent and incident gonorrheal infection
The overall prevalence of NG among South African users of injectable progestin contraception at baseline was 3.6% (Table 3.1). Users of DMPA and NET-EN did not differ in likelihood of diagnosis at baseline (p=0.93). Among injectable progestin contraceptive users overall, 93 gonorrheal infections occurred over 2,630 person-years of
follow-up for an overall incidence of 3.5/100 person-years (Table 3.2). Gonorrhea incidence did not differ significantly by injectable progestin type (DMPA versus NET-EN: 3.4/100 py, 95% CI 2.6 to 4.4/100 py, versus 3.8/100 py, 95% CI 2.7 to 5.3/100 py) (HR 0.85, 95% CI 0.56 to 1.29, p=0.44). Adjustment for potential confounding variables (time-fixed age, education, and marriage/cohabitation at baseline, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use) also did not qualitatively change the estimate for difference in hazard of NG infection (aHR 1.00, 95% CI 0.65 to 1.54, p=1.00).

3.4.4 Sensitivity analyses
Among women who never switched to the other injectable contraceptive method (n=2,739), we found no difference in CT acquisition (HR 0.87, 95% CI 0.70 to 1.08; aHR 1.01, 95% CI 0.81 to 1.26) or NG acquisition (HR 0.75, 95% CI 0.49 to 1.14, p=0.18; aHR 0.90, 95% CI 0.57 to 1.40, p=0.63) between DMPA and NET-EN users. Among women who disclosed at baseline that they did not use condoms, we also found no significant difference in CT acquisition between DMPA and NET-EN users (HR 1.25, 95% CI 0.63 to 2.47; aHR 1.53, 95% CI 0.74 to 3.18). We did not generate estimates similarly for NG acquisition, due to a small number of NG endpoints in this sub-group (n=7). Restricting the analyses to women less than 25 years old also did not qualitatively change our findings (CT: HR 1.11, 95% CI 0.88 to 1.40, aHR 1.13, 95% CI 0.89 to 1.43; NG: HR 1.02, 95% CI 0.63 to 1.66; aHR 1.13, 95% CI 0.69 to 1.86).
3.5 DISCUSSION

3.5.1 Overview

Several studies have investigated whether use of HC may increase women’s risk of contracting CT or NG; however, results have been mixed. Our findings, which showed no significant difference in CT or NG acquisition for DMPA compared to NET-EN users, were consistent with our pre-specified hypothesis. However, these results were unexpected, given the difference we observed in HIV-1 acquisition between DMPA and NET-EN users in VOICE. The non-significant higher risk of chlamydial and gonorrheal infection among the generally younger NET-EN users is consistent with the known association of younger age for both chlamydial and gonorrheal infections.

3.5.2 DMPA and NET-EN users differ in acquisition of HIV-1 but not chlamydia or gonorrhea

Our findings for chlamydial and gonorrheal infection do not demonstrate a clear explanation for the difference observed in HIV-1 acquisition between DMPA and NET-EN users in VOICE. Had we seen higher rates of incident chlamydia and/or gonorrhea in DMPA compared to NET-EN users, this might have suggested our HIV-1 findings were related either to differences in behavioral risk for STI, or a biologic mediating effect of these infections between injectable progestin contraception exposure and HIV-1 acquisition. However, we did not observe such a trend in this sub-cohort. Our results do not rule out the possibility that the difference in HIV-1 acquisition between DMPA and NET-EN users in this cohort is related to unmeasured confounding. Chlamydial and gonorrheal exposure likely overlaps with HIV-1 exposure in many, but not necessarily all, sexual networks. Thus, it is possible that DMPA and NET-EN users had similar
exposure risk for CT and NG infection, and differential exposure risk for HIV-1 infection. Our exclusion of those who initiated injectable progestin contraception following their last SDA means that this cohort differed slightly in composition from the cohort previously analyzed for difference in HIV-1 acquisition. However, this difference was modest (n=155) and increased risk for HIV-1 acquisition persisted in the Aim 2 cohort for DMPA compared to NET-EN users.

3.5.3 Strengths

Our analysis included 400 incident chlamydial and 93 incident gonorrheal infections that occurred over 2,630 person-years of follow-up, constituting one of the largest datasets for analyses of hormonal contraceptive use and STI acquisition in a single study. The more common usage of DMPA compared to NET-EN in our cohort is consistent with national trends in South Africa. Characterization of exposure for the model was strengthened by frequent measurement and on-site provision of contraception throughout study participation. While incidence of CT and NG was unfortunately high in our cohort, we were not faced with the problem of limited power that may have affected some previous analyses of HC use and cervical STI acquisition.

3.5.4 Limitations

The VOICE trial was not designed to investigate the potential association between contraceptive type and STI acquisition. Thus, our design, like all observational designs, may be subject to bias, and results must be interpreted cautiously. Screening occurred for CT and NG infection annually, with additional testing as clinically indicated. More frequent assessment of our outcomes may have been more informative. As asymptomatic and sub-clinical infections are common for both STIs, it is possible that the exposure
contemporaneous with diagnosis was not the contraceptive exposure at the time of CT or NG acquisition. We did not collect data on completion of partner treatment, so we do not know if this factor differed between users of DMPA and NET-EN. As single-dose, directly observed therapy was encouraged but not required, it is possible that some cases of CT and NG were not adequately treated between tests. However, we saw no data to suggest that any under-treatment, if it occurred, differed by type of injectable exposure. If women were evaluated and treated off-site for CT and/or GC without the knowledge of site staff, this may have impacted our results. Lastly, cohort participants were drawn from South African women taking part in a clinical trial of HIV prevention and receiving regular clinical screening and treatment for reproductive health outcomes. Therefore, our results may have limited generalizability.

3.5.5 Public health implications and directions for future research

Overall, these data suggest that users of different types of hormonal contraception may have a similar risk of incident CT and NG infection, after adjusting for potential confounders. Thus, our results also suggest that women considering injectable contraception can be counseled that risk of CT and NG does not appear to be impacted by injectable progestin type. Based on potential increased HIV-1 risk associated with DMPA use in some observational studies, some have called for the removal of DMPA as a contraceptive option for women. However, limiting access to effective contraception has the potential to increase dramatically a number of serious adverse health outcomes, including rates of unwanted pregnancy, abortion, maternal morbidity, mortality and preterm birth. Recommendations for use of HC must consider its impact in a broad public health context. Our findings support WHO Expert Working Group
recommends that there should be no restriction of use for any HC for any reproductive-age women, based on their risk of acquiring an STI.\textsuperscript{115}
3.6 FIGURE AND TABLES

Figure 3.1: Aim 2 cohort profile

5,029 participants in VOICE

- 952 enrolled at sites not using NET-EN (Uganda and Zimbabwe)

4,077 at South African sites

- 22 HIV-infected at enrollment
- 761 never used injectable contraception
- 33 missing contraceptive type
- 170 never had follow-up SDA
- 105 initiated injectable use after censoring
  - 11 after HIV seroconversion
  - 85 after pregnancy outcome
  - 9 after last SDA during follow-up

2,986 included in cohort

- 1,711 DMPA users
- 1,028 NET-EN users
- 247 users of both methods
Table 3.1 Aim 2 baseline characteristics of women by first injectable type on study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>DMPA first</th>
<th>NET-EN first</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 2986</td>
<td>n = 1,843</td>
<td>n = 1,143</td>
<td></td>
</tr>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>23 (21-27)</td>
<td>24 (21-27)</td>
<td>23 (20-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married/cohabitating</td>
<td>577 (19.3)</td>
<td>392 (21.3)</td>
<td>185 (16.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Parous</td>
<td>2,513 (84.2)</td>
<td>1,707 (92.6)</td>
<td>806 (70.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,865 (96.1)</td>
<td>1,757 (95.5)</td>
<td>1,108 (97.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Formal employment</td>
<td>286 (9.6)</td>
<td>170 (9.2)</td>
<td>116 (10.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Home owned by self or family</td>
<td>2,463 (82.5)</td>
<td>1,520 (82.5)</td>
<td>943 (82.6)</td>
<td>0.97</td>
</tr>
<tr>
<td>Sexual behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has &gt;1 sexual partner</td>
<td>104 (3.5)</td>
<td>45 (2.5)</td>
<td>59 (5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median vaginal sex acts past week (IQR)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (0-3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Condom at last sex</td>
<td>2,359 (87.1)</td>
<td>1,458 (86.9)</td>
<td>901 (87.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Any anal sex past 3 months</td>
<td>609 (20.7)</td>
<td>354 (19.5)</td>
<td>255 (22.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex for money past year</td>
<td>146 (4.9)</td>
<td>89 (4.9)</td>
<td>57 (5.1)</td>
<td>0.81</td>
</tr>
<tr>
<td>Primary partner</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,750 (92.7)</td>
<td>1,687 (92.0)</td>
<td>1,063 (93.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Has other partners</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>273 (9.4)</td>
<td>157 (8.7)</td>
<td>116 (10.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1,865 (64.5)</td>
<td>1,193 (66.1)</td>
<td>672 (61.9)</td>
<td></td>
</tr>
<tr>
<td>Genital tract infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydia</td>
<td>446 (14.9)</td>
<td>278 (15.1)</td>
<td>168 (14.7)</td>
<td>0.77</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>106 (3.6)</td>
<td>65 (3.5)</td>
<td>41 (3.6)</td>
<td>0.93</td>
</tr>
<tr>
<td>Herpes simplex virus type 2</td>
<td>1,388 (46.6)</td>
<td>938 (50.9)</td>
<td>450 (39.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: DMPA, depot medroxyprogesterone acetate; NET-EN, norethisterone enanthate; IQR, interquartile range. Characteristics are n (%), unless otherwise indicated. *Fisher exact test for categorical variables, and Wilcoxon rank-sum test for comparison of medians.
Table 3.2 Acquisition of chlamydial and gonorrheal infection among users of injectable progestin contraception

<table>
<thead>
<tr>
<th>Cases/py</th>
<th>Incidence/100 py (95% CI)</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted Andersen-Gill model</td>
<td>Adjusted Andersen-Gill model*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chlamydial infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>150/949.0</td>
<td>15.8 (13.5-18.5)</td>
<td>REF</td>
<td>0.92 (0.75-1.13)</td>
<td>0.45</td>
</tr>
<tr>
<td>DMPA</td>
<td>250/1,680.8</td>
<td>14.9 (13.1-16.8)</td>
<td>0.85 (0.56-1.29)</td>
<td>0.44</td>
<td>1.00 (0.65-1.54)</td>
</tr>
<tr>
<td>Total</td>
<td>400/2,629.8</td>
<td>15.2 (13.8-16.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gonorrheal infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>36/949.0</td>
<td>3.8 (2.7-5.3)</td>
<td>REF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPA</td>
<td>57/1680.8</td>
<td>3.4 (2.6-4.4)</td>
<td>0.85 (0.56-1.29)</td>
<td>0.44</td>
<td>1.00 (0.65-1.54)</td>
</tr>
<tr>
<td>Total</td>
<td>93/2629.8</td>
<td>3.5 (2.9-4.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: py, person-years; CI, confidence interval; NET-EN, norethisterone enanthate; DMPA, depot medroxyprogesterone acetate.

*Adjusted for baseline age, marriage/cohabitation, and any secondary education, and time-varying oral contraceptive use, recent change in primary partner, partner has other partner(s), frequency of intercourse, and condom use at last vaginal sex.
Chapter 4: MANUSCRIPT 3
 Injectable progestin contraception and vaginal infection among South African women

4.1 ABSTRACT

Background

Observational data suggest that abnormal shifts in the vaginal microenvironment increase a woman’s risk of acquiring sexually transmitted HIV-1 infection. Bacterial vaginosis (BV) and trichomoniasis are associated with changes in the vaginal microenvironment that provide a biologically plausible explanation for increased risk of HIV-1 infection among women with these vaginal infections. We assessed whether users of two common injectable progestin contraceptives (depot medroxyprogesterone acetate [DMPA] and norethisterone enanthate [NET-EN]) differed in their risk for these two common types of vaginal infection.

Methods

We used prospective data from injectable contraceptive users enrolled at South African sites in the VOICE trial, a multi-center randomized trial of topical and oral HIV-1 chemoprophylaxis conducted among women of reproductive age. Incidence rates for 100 person-years for BV and trichomonas infection were estimated separately by type of injectable progestin injection used. Separate Andersen-Gill proportional hazards regression analyses allowing for repeat infections (multiple failure time events) were used to compare risk for BV and trichomoniasis between DMPA and NET-EN users. We also calculated estimates for both infections with adjustment for potential confounders.
(baseline age and HSV-2 status, and time-varying recent partner change, combined oral contraceptive pill use, recent washing with water inside the vagina, frequency of sex, real-time diagnosis of BV during follow-up (as proxy for metronidazole treatment), and condom use).

**Results**

Among 3,029 users of injectable contraception who had follow-up testing for BV, 1,719 (56.8%) used DMPA, 1,058 (34.9%) used NET-EN, and 252 (8.3%) used both types of injectable progestin contraception at different times during study participation. Similar distribution for injectable types was noted among 2,957 injectable progestin contraceptive users who had follow-up testing for trichomoniasis. Users of DMPA and NET-EN did not differ in prevalence of either infection at baseline (BV: 39.5 versus 39.6%, p=0.96; trichomoniasis: 5.8 versus 5.0%, p=0.36). Overall, users of DMPA were slightly older and less likely to report multiple partners, compared to NET-EN users. Users of NET-EN were less likely to be seropositive for herpes simplex virus type 2. However, DMPA and NET-EN users were similar in terms of reported unprotected sex. We did not observe any difference in prevalent or incident trichomoniasis between DMPA and NET-EN users (HR 0.92, 95% CI (0.70 to 1.20, p=0.54; aHR 1.06, 95% CI 0.78 to 1.45, p=0.69). Contrary to our pre-specified hypothesis, we observed that among those negative for BV at baseline, incident BV was reduced among DMPA users, compared to NET-EN users after adjusting for potential confounders (aHR 0.86, 95% CI 0.75 to 0.98, p=0.02).
Discussion

Progestin type does not appear to impact risk for trichomoniasis among injectable progestin contraceptive users. However, our analysis, which is the first to compare BV risk directly between DMPA and NET-EN users, suggests that DMPA use may be protective for BV, compared to NET-EN use. These results are consistent with earlier findings, which suggested that DMPA users in the VOICE study did not have a higher risk of genital tract infection (chlamydia and gonorrhea), compared to NET-EN users, despite having a higher risk for incident HIV-1 infection during study follow-up.
Injectable progestin contraception and vaginal infection among South African women

4.2 BACKGROUND

4.2.1 Injectable progestin contraception and risk for HIV-1 acquisition in women

Effective contraception is an important strategy for reducing maternal mortality and protecting the health of women and their families. In Eastern and sub-Saharan Africa, where high maternal mortality ratios persist, use of injectable progestin contraception is an important family planning strategy, accounting for over 40 percent of contraceptive usage. However, some observational analyses have found a link between the use of injectable progestin contraception, particularly depot medroxyprogesterone acetate (DMPA), and HIV-1 acquisition in women.

Recent observational findings suggest that different progestins may confer different susceptibility to HIV-1 acquisition in women. In a cohort of injectable contraceptive users participating in an HIV prevention study in South Africa, users of DMPA had approximately 50% increased risk of HIV-1 acquisition compared to users of another common but different progestin contraceptive, norethisterone enanthate (NET-EN). This difference in hazard persisted in multivariable Cox models and did not appear to be explained by differential risk of chlamydial or gonorrheal infection.

4.2.2 Bacterial vaginosis, trichomoniasis, and risk for HIV-1 acquisition in women

Bacterial vaginosis (BV) is a common vaginal infection characterized by a reduced concentration of normally dominant hydrogen-peroxide producing lactobacilli, and
overgrowth of other bacteria, usually gram variable coccobacilli and anaerobic gram negative rods. Typically, bacteria detected in BV include *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and species of *Prevotella*, *Porphyromonas*, *Bacteroides*, *Peptostreptococcus*, and *Mobiluncus*. Infection may be asymptomatic, but typical symptoms may include an increased and thin malodorous vaginal discharge. Elevated vaginal pH (>4.5) and the presence of clue cells (epithelial cells with borders completely obscured by bacteria) on microscopy are also typical findings. In observational studies, women already infected with herpes simplex type 2 virus (HSV-2) were more likely to acquire BV, compared to women without HSV-2. Although condom use has been associated with decreased risk of acquiring BV, BV has been termed a “sexually associated” rather than sexually transmitted infection (STI). Observational findings have suggested that BV is more commonly diagnosed in sexually experienced compared to sexually inexperienced women.

Trichomoniasis is a common curable STI caused by the organism, *Trichomonas vaginalis*. Like BV, trichomoniasis may be asymptomatic, but when symptomatic, trichomoniasis is also characterized by excessive vaginal discharge. High prevalence is common among women with risk factors for STI (e.g., multiple partners, exchanging sex for money). While trichomoniasis is often detected in women with BV, recent observational findings also suggest that BV predisposes women to trichomoniasis.

Risk for HIV-1 infection has been linked to presence of BV (as well as intermediate vaginal flora by Nugent score) and trichomonas infection. Both vaginal infections have been associated with younger age and recent changes in sexual partnership. While pathologic processes differ, with trichomonas clearly having an infectious agent (the *T. 
vaginalis organism), both vaginal infections disrupt the normal vaginal microenvironment, and potentially decrease genital mucosal defenses against other sexually transmitted pathogens, e.g., HIV-1.

4.2.3 Injectable progestin contraception type and acquisition of vaginitis

The possibility that different types of injectable progestin contraception might have different impacts on BV and trichomoniasis remains relatively unexplored. Few analyses investigating vaginitis prevalence and acquisition have reported results for DMPA and NET-EN users in the same study, and data specific to NET-EN are particularly lacking. Pettifor and colleagues estimated the impact of DMPA and NET-EN use compared to use of no hormonal contraception on acquisition of BV and trichomoniasis infection. In that analysis, DMPA was marginally associated with decreased risk of trichomoniasis, and both DMPA and NET-EN were associated with decreased risk of BV acquisition (DMPA versus no HC: aIRR 0.77, 95% CI 0.56 to 1.06; NET-EN versus no HC: aIRR 0.91, 95% CI 0.70 to 1.18). In a different cohort of South African family planning clinic patients, Kleinschmidt et al found that DMPA and NET-EN users had similar prevalence of BV and trichomoniasis at baseline. In the present analysis, we compared DMPA and NET-EN users in terms of their risk for incident BV and trichomoniasis.

4.3 METHODS

4.3.1 Study participants

The VOICE study was a Phase 2B study of the safety and effectiveness of oral and topical formulations of tenofovir for prevention of sexually transmitted HIV-1 in women.
Participants were required to be 18 through 45 years old, HIV-uninfected, non-pregnant, and sexually active, defined as having vaginal intercourse at least one in the prior three months (see Appendix II). Participants were also required to be using an effective method of contraception at entry, and intending to use an effective method for the next 24 months. Effective methods included hormonal methods, intrauterine contraceptive device, and sterilization of the participant or her partner(s). Women with recent pregnancy (less than 42 days ago) and reproductive tract infection (other than asymptomatic BV) were excluded. However, women with genital tract infections could be enrolled after any clinical signs and symptoms resolved and treatment was completed. Additional details regarding the design and results of the VOICE study have been previously described.\textsuperscript{76,77}

For this secondary analysis, we included injectable progestin contraception users enrolled at South African sites in the VOICE study (Figures 1 and 2). We restricted analysis to women enrolled at sites in South Africa (Durban, Klerksdorp, and Johannesburg), where use of both DMPA and NET-EN is common.\textsuperscript{51}

4.3.2 Study procedures

4.3.2.1 Contraception

All study sites provided a range of contraceptive options on-site. Exposure was estimated using injection dates, which were recorded in charts for on-site injections and transcribed from family planning cards for off-site injections. Exposure lengths per injection (17 weeks (DMPA) and ten weeks (NET-EN) were based a priori on WHO guidelines for duration of contraceptive coverage.\textsuperscript{96} Exposure was further categorized to distinguish
periods where combined oral contraceptive pill (COC) and injectable exposure overlapped, for example, to treat breakthrough vaginal bleeding.

4.3.2.2 Bacterial vaginosis

All participants had Gram-stained vaginal smears performed at study screening visits. Symptomatic participants were screened with the BVBLUE® rapid test. Symptomatic BV was exclusionary in the VOICE study. However, participants could still be enrolled if they completed BV treatment during the screening period, tested negative for BV, and were otherwise eligible for participation. Treatment was according to current WHO guidance, using single-dose observed therapy whenever possible. Thereafter, participants were screened for BV as clinically indicated. Study clinicians were instructed to test for BV if participants reported excessive or malodorous vaginal discharge, vaginal erythema, vaginal edema, and/or pruritus of the external genitalia.

4.3.2.3 Trichomonas infection

All participants were tested at study screening for trichomonas infection. Although trichomoniasis was exclusionary in the VOICE study, participants could still be enrolled if they completed treatment for trichomonas infection detected during the screening period, had a negative test of cure, and were otherwise eligible for enrollment.

Participants were treated according to current WHO guidance, with single-dose observed regimens whenever possible. Thereafter, participants were tested for trichomoniasis annually and as clinically indicated. Clinical indications included excessive, frothy,
diffuse, yellow-green discharge, vaginal erythema, vaginal edema, pruritus of the external genitalia, dysuria and dyspareunia.

4.3.3 Laboratory methods

4.3.3.1 Bacterial vaginosis

Gram-stained vaginal smears, the gold standard for diagnosis of BV, were conducted for research purposes. The OSOM BVBLUE® rapid test (Sekisui, Lexington, MA), which screens for presence of vaginal fluid sialidase, was used for clinical management during study conduct. However, clinicians also treated women whose clinical presentation and wet prep findings (performed as clinically indicated to investigate symptoms of candidiasis) met Amsel criteria for diagnosis of BV.¹²⁰ Gram-stained vaginal smears were performed at study screening, annually, and at study exit. Nugent scores of these smears were used to define BV endpoints in this analysis.³³ Gram-stained vaginal smears were prepared by collecting fluid from the lateral vaginal wall via swab and rolling the swab across the slide. Specimens were air-dried on slides (not heat-fixed) and shipped to a central laboratory for batched (not real-time) Nugent scoring.

4.3.3.2 Trichomonas vaginalis infection

Routine screening and clinically indicated testing for trichomoniasis were performed using the OSOM Rapid Trichomomas® test (Sekisui, Lexington, MA). Specimens were collected from the lateral vaginal wall or posterior fornix using a Dacron® swab. Testing was performed during participant visits, when possible, but storage at room temperature for 24 hours or refrigeration for 36 hours was permitted before testing. Due to low
sensitivity, wet prep data was not used to define trichomoniasis outcomes in this analysis.

4.3.4 Statistical analysis

We compared baseline demographic, sexual behavioral, and primary partner characteristics between participants using DMPA and NET-EN as their first injectable method on study, using the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. Prevalence of BV, trichomoniasis, CT and NG (at study screening) and herpes simplex virus type 2 (HSV-2) (at enrollment) was calculated and compared between DMPA and NET-EN users using Pearson’s chi-squared test. Date of HIV-1 infection was estimated using midpoint between last negative and first confirmed positive test. We excluded 33 participants missing data on injectable contraceptive type and 22 identified by plasma HIV-1 ribonucleic acid (RNA) polymerase chain reaction (PCR) as HIV-infected at enrollment (Figure 4.1). As confidence intervals for incidence rates of BV overlapped substantially across sites, we did not calculate a site-stratified hazard ratio in the unadjusted or adjusted Andersen-Gill model for BV. A similar approach was undertaken for trichomoniasis due to overlapping confidence intervals for incidence rates of that infection, as well. We confirmed these data met the proportional hazards assumption.

4.3.4.1 Bacterial vaginosis

We calculated overall BV incidence rates per 100 person-years of follow-up with 95% confidence intervals among the subset of women who were negative for BV by Nugent score at baseline (n=1,829). We also calculated separate BV incidence rates similarly for person-time specific to DMPA and NET-EN exposure. The failure time variable for the analysis of BV acquisition was defined as a Nugent score greater than or equal to seven.33
As Gram-stained vaginal smears were read centrally in batches, results were not available in real time. Treatment was initiated only for those participants who were positive for BV via rapid testing or Amsel criteria.\textsuperscript{120}

To compare BV incidence between DMPA and NET-EN users, accounting for recurrent infections, Andersen-Gill proportional hazards regression was used.\textsuperscript{112} Origin for the analysis was first use of injectable progestin contraception on record during study participation. Observations were censored at HIV-1 seroconversion, pregnancy, or last Gram stain performed during study participation. Censoring for HIV-1 seroconversion and pregnancy was undertaken due to the likelihood of changes to BV screening and treatment strategies for participants, based on these conditions.\textsuperscript{126} Based on clinical relevance, we adjusted for baseline age and HSV-2 status, and time-varying recent partner change, same-day washing with water inside the vagina, frequency of sex, real-time diagnosis of BV during follow-up (as a proxy for metronidazole treatment), and condom use. Statistical tests used a two-sided alpha of 0.05. Analyses were conducted using Stata version 13.1 (StataCorp, Inc, College Station, TX).

4.3.4.2 Trichomonas infection

We calculated overall trichomoniasis incidence rates per 100 person-years of follow-up with 95% confidence intervals. We also calculated separate trichomoniasis incidence rates similarly for person-time specific to DMPA and NET-EN exposure. The failure time variable for the trichomoniasis analysis was a positive result for the OSOM Rapid Trichomonas® test during study follow-up. Observations were censored at HIV-1 seroconversion, pregnancy, or last OSOM Rapid Trichomonas® test during study follow-
up. Censoring for HIV-1 seroconversion and pregnancy was undertaken due to the likelihood of changes to trichomoniasis screening and treatment strategies for participants, based on these conditions. Based on clinical relevance, we adjusted for baseline age, and bacterial vaginosis status, and time-varying recent partner change, COC use, real-time diagnosis of BV (as proxy for treatment with metronidazole), frequency of vaginal sex, time since washing inside the vagina, and condom use at last vaginal sex. Treatment for BV is relevant for an analysis of incident trichomoniasis, due to the action of the recommended medication (metronidazole) against both BV and *T. vaginalis*.

4.3.4.3 Measurement of covariates

Baseline bacterial vaginosis status, baseline trichomoniasis status, and herpes simplex virus type 2 serostatus were binary variables. Time-varying COC use, recent partner change (since last study visit), real-time diagnosis of BV and condom use at last vaginal sex were binary variables. Frequency of vaginal sex referred to number of vaginal sex acts in the past week. Washing inside the vagina was a categorical variable that characterized time since washing with water inside the vagina (today, past three days, past 4 days, past eight days, or never).

4.3.4.4 Human subjects and regulatory review

All participants provided written informed consent. The VOICE trial underwent all required institutional review board (IRB) and country-specific regulatory reviews, and is registered with Clinicaltrials.gov (NCT00705679). The Johns Hopkins Bloomberg School of Public Health IRB provided additional approval for this secondary analysis.
4.4 RESULTS

4.4.1 Study participants

*Bacterial vaginosis analysis.* Among 3,029 injectable contraception users in this analysis, DMPA users comprised 56.8% (n=1,719), NET-EN users comprised 34.9% (n=1,058), and those who switched injectable types during follow-up comprised 8.3% (n=252). As few women switched injectable type during follow-up, comparisons below refer to first method used during study follow-up. Median age for DMPA users was slightly higher compared to NET-EN users (24 versus 23 years old, \(p<0.001\)). Overall marriage and cohabitation were uncommon (n=590, 19.5%), but more common among DMPA compared to NET-EN users (21.5 versus 16.4%, \(p=0.001\)). Users of DMPA were more likely to be parous (93.9 versus 70.1%, \(p<0.001\)), but less likely to have completed at least some secondary education, compared to NET-EN users (95.6 versus 97.0%, \(p=0.05\)). Users of NET-EN were more likely to report having multiple partners, compared to DMPA users (5.5 versus 2.7%, \(p<0.001\)). While median number of sex acts was similar for DMPA and NET-EN users, interquartile range for DMPA users was shifted slightly higher compared to NET-EN users (median 2, IQR (1-3) versus median 2, IQR (0-3), \(p=0.01\)). Seropositivity for HSV-2 at baseline was more common in DMPA users compared to NET-EN users (50.9 versus 39.8%, \(p<0.001\)).

*Trichomoniasis analysis.* This secondary analysis included 2,957 injectable contraception users at South African sites in the VOICE study (Table 4.2). As few women switched injectable type during follow-up, comparisons below refer to first method used during study follow-up. Overall, DMPA use (n=1,804, 61.0%) was more common than NET-EN use (n=1,153, 39.0%) as a first injectable method used during study follow-up. The
median age of DMPA users was slightly older than that of NET-EN users (24 versus 23 years old, p<0.001). Users of DMPA were significantly more likely to be married or cohabitating (21.7 versus 16.1%, p<0.001) and parous (92.9 versus 70.2%, p<0.001), but slightly less likely to report completing some secondary education (95.4 versus 97.3%, p=0.01). Compared to NET-EN users, DMPA users were less likely to report multiple partners (2.9 versus 5.2%, p=0.001). While median number of sex acts in the past week was similar for DMPA and NET-EN users, the interquartile range trended slightly lower for NET-EN users, compared to DMPA users (0-3 versus 1-3, p=0.001). However, DMPA users were more likely to be seropositive for HSV-2 at baseline (51.3 versus 39.2%, p<0.001).

4.4.2 Bacterial vaginosis

No difference in prevalence of BV at baseline was observed between DMPA and NET-EN users (39.5 versus 39.6%, p=0.96) (Table 4.1). Overall, 1,829 women included in this analysis were negative for BV at enrollment by Nugent score. Of these, we observed 388 cases over 630.3 person-years of follow-up among NET-EN users (61.6/100 person-years, 95% CI 55.7 to 68.0/100 person-years), and 604 cases over 1,110.1 person-years (54.4/100 person-years, 95% CI 50.2 to 58.9/100 person-years) of follow-up among DMPA users. The overall hazard of BV was lower for DMPA compared to NET-EN users in the unadjusted model (HR 0.86, 95% CI 0.76 to 0.98, p=0.02). Adjusting for baseline age and HSV-2 status, and time-varying oral contraceptive use, frequency of vaginal intercourse, condom use at last vaginal sex, same-day washing inside vagina, real-time diagnosis of BV, and recent change in primary partner, risk for BV was still lower among DMPA compared to NET-EN users (aHR 0.86, 95% CI 0.75 to 0.98,
Further adjustment for number of sexual partners did not change the direction or magnitude of the estimate.

4.4.3 Trichomoniasis

Users of DMPA and NET-EN did not differ in prevalent trichomoniasis at baseline (5.8 versus 5.0%, p=0.36) (Table 4.2). During 2,716.6 person years of follow-up, 227 positive rapid tests for trichomonas infection occurred (8.4/100 person-years, 95% CI 7.3 to 9.5/100 person-years) (Table 4.3). Of these outcomes, 85 were among NET-EN users (8.7/100 person-years, 95% CI 7.1 to 10.8/100 person-years) and 142 were among DMPA users (8.2/100 person-years, 95% CI 6.9 to 9.6/100 person-years). No significant difference was observed in incident trichomoniasis between DMPA and NET-EN users (HR 0.92, 95% CI 0.70 to 1.20, p=0.54) (Table 4.4). This similar risk for trichomoniasis for DMPA and NET-EN users persisted in multivariable analysis adjusted for baseline age, HSV-2 serostatus, and bacterial vaginosis status, and time-varying recent partner change, COC use, real-time diagnosis of BV, frequency of vaginal sex, same-day washing inside the vagina, and condom use at last vaginal sex (aHR 1.07, 95% CI 0.79 to 1.45, p=0.67) (Table 4.4). Additional adjustment for marriage, cohabitation and education did not change the direction or magnitude of the estimate.

4.5 DISCUSSION

4.5.1 Summary

In this prospective cohort of injectable progestin contraceptive users in South Africa, we found a similar prevalence of bacterial vaginosis and trichomonas infection for DMPA
and NET-EN users at baseline. To our knowledge, this is the first analysis to compare DMPA and NET-EN users directly in terms of their risk for prevalent and incident BV and trichomoniasis. Contrary to our pre-specified hypothesis for this analysis, we observed a significantly decreased risk for BV among users of DMPA compared to NET-EN, in both unadjusted and adjusted models. However, DMPA and NET-EN users did not differ significantly in incident trichomoniasis in our unadjusted or adjusted analysis. Our findings in multivariable analysis confirmed the results of other studies showing an increased risk for incident BV among women seropositive for HSV-2.\textsuperscript{122,123}

As contraception was not a randomized exposure in this study population, selection bias is a possible explanation for the differential acquisition of BV between DMPA and NET-EN users. However, several biological explanations are also plausible that would decrease risk for alterations in flora for DMPA compared to NET-EN users. First, amenorrhea is more frequently associated with DMPA compared to NET-EN use.\textsuperscript{92} Thus, differential acquisition of BV between DMPA and NET-EN users might be explained by decreased incidence of vaginal bleeding, which appears to trigger shifts and increased diversity in vaginal flora.\textsuperscript{128} Lactoferrin is considered an important source of iron for BV-associated bacteria such as \textit{Gardnerella vaginalis}.\textsuperscript{43} We did not measure vaginal lactoferrin in this study; however, lower lactoferrin levels in amenorrheic DMPA users could contribute to a decreased risk for BV. While BV risk was decreased in those who had washed inside the vagina on the day of the study visit, this may be more likely due to interference with collection of adequate specimens for Gram-stained vaginal smears, as opposed to clinical protection against BV.
The lack of difference in trichomoniasis acquisition between DMPA and NET-EN users suggests that sexual risk behaviors for trichomoniasis (e.g., unprotected vaginal intercourse) may be similar between users of these two different injectable contraceptive methods. Thus, these results may add credence to the possibility that our findings for HIV-1 acquisition in a cohort of injectable contraception users in the VOICE study are not necessarily due to unaccounted for differences in sexual risk behavior. Had we observed DMPA users to have a clearly higher risk for BV and/or trichomoniasis, this would suggest a behavioral explanation or biologic mediating role for vaginitis in differential HIV-1 acquisition between DMPA and NET-EN users.

4.5.2 Strengths

This analysis was strengthened by the frequent measurement of contraceptive exposure. As contraception was provided on-site or transcribed from family planning clinic cards, the likelihood of exposure misclassification is less than if participant recall had been used to define exposures. Extensive monitoring of data collection and management in this large multi-center trial also strengthened our analysis. Lastly, we used Nugent scoring of Gram-stained vaginal smears, the gold standard for BV diagnosis, to define BV outcomes.

4.5.3 Limitations

It is important to note that women in the VOICE trial were not randomized to contraceptive methods. All findings from secondary analyses have an inherent risk of bias and thus must be interpreted with care. Our findings do not rule out the possibility of selection bias as an explanation for similarities or differences in incident vaginitis.
between DMPA and NET-EN users. In our analysis, the study population consisted of healthy women willing to participate in clinical research, and thus participants are not representative of the general population of women in South Africa. Study endpoints were likely measured with a high degree of accuracy, but not necessarily precision in time. More frequent testing for vaginitis could have been more informative. We did not collect information on female sexual partnerships, which are known to impact risk for BV. We focused on incident BV infection, rather than recurrent infection among those who had asymptomatic BV at baseline. Thus, these results cannot be extended to those women with recurrent BV. Social desirability bias may have limited reporting of sensitive behaviors that have a relationship to BV and trichomoniasis risk, namely new sexual partnerships, frequency of vaginal sex and condom use. However, in multivariable analysis, reported condom use at last sex was protective against incident trichomoniasis, suggesting some degree of accuracy in reporting.

4.5.4 Public health implications

These results suggest that DMPA and NET-EN use do not confer different risk for trichomoniasis infection, but that DMPA use may be more protective against bacterial vaginosis. If the relationship we observed between progestin type and acquisition of BV is causal, these results have implications other than the potential effect on HIV-1 acquisition in women. Bacterial vaginosis is known to impact many other important women’s health outcomes, including peri-partum infection, spontaneous abortion, preterm birth, low birth weight in infants, and risk for STI.38-41 However, given the limited data specific to NET-EN use, further research is necessary to inform any clinical guidance around injectable contraceptive type and risk for different forms of vaginitis.
4.6 FIGURE AND TABLES

Figure 4.1: Aim 3a Cohort Profile

5,029 participants in VOICE

952 enrolled at sites not using NET-EN (Uganda and Zimbabwe)

4,077 randomized at South African sites

22 HIV-seropositive at enrollment

761 never used injectable contraception

33 missing progestin type

129 missing follow-up Gram stain

103 began injectable method after censoring
   11 after HIV diagnosis
   85 after pregnancy
   7 after last Gram stain

3,029 DMPA and NET-EN users

1,719 DMPA users

1,058 NET-EN users

252 users of both methods
5,029 participants in VOICE

- 952 enrolled at sites not using NET-EN (Uganda and Zimbabwe)

4,077 randomized at South African sites

- 22 HIV-seropositive at enrollment
- 761 never used injectable contraception
- 33 missing progestin type
- 201 never had TV test during follow-up

2,957 DMPA and NET-EN users

- 1,674 DMPA users
- 1,038 NET-EN users
- 245 users of both methods

- 103 began injectable method after censoring
  - 11 after HIV diagnosis
  - 85 after pregnancy
  - 7 after last TV test
## Table 4.1 Aim 3a baseline characteristics of women by first injectable contraceptive type used on study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n=3,029</th>
<th>DMPA first n=1,855</th>
<th>NET-EN first n=1,174</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>23 (21-27)</td>
<td>24 (21-27)</td>
<td>23 (20-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married/cohabitating</td>
<td>590 (19.5)</td>
<td>398 (21.5)</td>
<td>192 (16.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Parous</td>
<td>2,546 (84.1)</td>
<td>1,723 (93.9)</td>
<td>823 (70.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,908 (96.1)</td>
<td>1,770 (95.6)</td>
<td>1,138 (97.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Formal employment</td>
<td>288 (9.5)</td>
<td>171 (9.2)</td>
<td>117 (10.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>Home owned by self or family</td>
<td>2,495 (82.4)</td>
<td>1,524 (82.2)</td>
<td>971 (82.8)</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Sexual behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has &gt;1 sexual partner</td>
<td>113 (3.8)</td>
<td>50 (2.7)</td>
<td>63 (5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median vaginal sex acts past week (IQR)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (0-3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Condom at last sex</td>
<td>2,050 (74.3)</td>
<td>1,247 (74.0)</td>
<td>803 (74.8)</td>
<td>0.66</td>
</tr>
<tr>
<td>Any anal sex past 3 months</td>
<td>607 (20.4)</td>
<td>353 (19.3)</td>
<td>254 (22.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex for money past year</td>
<td>148 (4.9)</td>
<td>89 (4.8)</td>
<td>59 (5.1)</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Primary partner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,791 (92.8)</td>
<td>1,701 (92.2)</td>
<td>1,090 (93.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Has other partners</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>278 (9.5)</td>
<td>158 (8.7)</td>
<td>120 (10.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1,879 (65.8)</td>
<td>1,197 (65.8)</td>
<td>682 (61.3)</td>
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</tr>
<tr>
<td><strong>Genital tract infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>1,196 (39.5)</td>
<td>732 (39.5)</td>
<td>464 (39.6)</td>
<td>0.96</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>163 (5.4)</td>
<td>107 (5.8)</td>
<td>56 (4.8)</td>
<td>0.24</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>452 (14.9)</td>
<td>282 (15.2)</td>
<td>170 (14.5)</td>
<td>0.59</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>109 (3.6)</td>
<td>66 (3.6)</td>
<td>43 (3.7)</td>
<td>0.88</td>
</tr>
<tr>
<td>Herpes simplex virus type 2</td>
<td>1,406 (46.6)</td>
<td>943 (50.9)</td>
<td>463 (39.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: DMPA, depot medroxyprogesterone acetate; NET-EN, norethisterone enanthate; IQR, interquartile range. Characteristics are n (%), unless otherwise indicated. *Fisher exact test for categorical variables, and Wilcoxon rank-sum test for comparison of medians.
### Table 4.2 Aim 3b baseline characteristics of women by first injectable contraceptive type used on study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n=2,957</th>
<th>DMPA first n=1,804</th>
<th>NET-EN first n=1,153</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>23 (21-27)</td>
<td>24 (21-27)</td>
<td>23 (20-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married/cohabitating</td>
<td>577 (19.5)</td>
<td>391 (21.7)</td>
<td>186 (16.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parous</td>
<td>2,485 (84.0)</td>
<td>1,676 (92.9)</td>
<td>809 (70.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,839 (96.1)</td>
<td>1,718 (95.4)</td>
<td>1,121 (97.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Formal employment</td>
<td>282 (9.5)</td>
<td>167 (9.3)</td>
<td>115 (10.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>Home ownership</td>
<td>2,435 (82.4)</td>
<td>1,483 (82.3)</td>
<td>952 (82.6)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Sexual behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has &gt;1 sexual partner</td>
<td>110 (3.8)</td>
<td>51 (2.9)</td>
<td>59 (5.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Median vaginal sex acts past week (IQR)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (0-3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Condom at last sex</td>
<td>2,013 (74.6)</td>
<td>1,222 (74.4)</td>
<td>791 (74.9)</td>
<td>0.76</td>
</tr>
<tr>
<td>Any anal sex past 3 months</td>
<td>592 (20.3)</td>
<td>348 (19.6)</td>
<td>244 (21.5)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex for money past year</td>
<td>147 (5.0)</td>
<td>90 (5.0)</td>
<td>57 (5.0)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Primary partner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any secondary education</td>
<td>2,726 (92.9)</td>
<td>1,653 (92.1)</td>
<td>1,073 (94.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Has other partners</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>271 (9.5)</td>
<td>156 (8.8)</td>
<td>115 (10.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1,839 (64.3)</td>
<td>1,167 (66.0)</td>
<td>672 (61.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Genital tract infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>1,167 (39.5)</td>
<td>715 (39.7)</td>
<td>452 (39.3)</td>
<td>0.83</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>163 (5.5)</td>
<td>105 (5.8)</td>
<td>58 (5.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>448 (15.2)</td>
<td>277 (15.4)</td>
<td>171 (14.8)</td>
<td>0.70</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>105 (3.6)</td>
<td>63 (3.5)</td>
<td>42 (3.6)</td>
<td>0.83</td>
</tr>
<tr>
<td>Herpes simplex virus type 2</td>
<td>1,374 (46.6)</td>
<td>924 (51.3)</td>
<td>450 (39.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: DMPA, depot medroxyprogesterone acetate; NET-EN, norethisterone enanthate; IQR, interquartile range. Characteristics are n (%), unless otherwise indicated. *Fisher exact test for categorical variables, and Wilcoxon rank-sum test for comparison of medians.
Table 4.3 Incidence of bacterial vaginosis and trichomoniasis among users of injectable progestin contraception

<table>
<thead>
<tr>
<th></th>
<th>Cases/py</th>
<th>Incidence/100 py (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial vaginosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>388/630.3</td>
<td>61.6 (55.7-68.0)</td>
</tr>
<tr>
<td>DMPA</td>
<td>604/1,110.1</td>
<td>54.4 (50.2-58.9)</td>
</tr>
<tr>
<td>Total</td>
<td>992/1740.5</td>
<td>57.0 (53.6-60.7)</td>
</tr>
<tr>
<td><strong>Trichomoniasis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET-EN</td>
<td>85/974.3</td>
<td>8.7 (7.1-10.8)</td>
</tr>
<tr>
<td>DMPA</td>
<td>142/1742.3</td>
<td>8.2 (6.9-9.6)</td>
</tr>
<tr>
<td>Total</td>
<td>227/2716.6</td>
<td>8.4 (7.3-9.5)</td>
</tr>
</tbody>
</table>

Abbreviations: py, person-years; NET-EN, norethisterone enanthate; DMPA, depot medroxyprogesterone enanthate. Note: bacterial vaginosis incidence results are for those women who were negative for bacterial vaginosis at baseline in the trial, according to Nugent score results.
### Table 4.4: Unadjusted and adjusted hazard ratios for bacterial vaginosis and trichomoniasis acquisition

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Bacterial vaginosis</th>
<th>Trichomoniasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted Andersen-Gill Model</td>
<td>Adjusted Andersen-Gill Model</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Baseline (at Enrollment unless otherwise indicated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>0.97 (0.95-0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BV (by Nugent score)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Herpes simplex virus type 2</td>
<td>1.18 (1.06-1.33)</td>
<td>0.004</td>
</tr>
<tr>
<td>Time-varying</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPA (versus NET-EN)</td>
<td>0.86 (0.76-0.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>1.26 (1.05-1.51)</td>
<td>0.01</td>
</tr>
<tr>
<td>Recent partner change</td>
<td>1.31 (1.10-1.57)</td>
<td>0.003</td>
</tr>
<tr>
<td>Vaginal sex acts past week</td>
<td>1.02 (0.99-1.05)</td>
<td>0.16</td>
</tr>
<tr>
<td>Condom at last sex</td>
<td>0.96 (0.83-1.11)</td>
<td>0.58</td>
</tr>
<tr>
<td>Washed inside vagina today</td>
<td>0.61 (0.54-0.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Real-time diagnosis of BV</td>
<td>14.71 (9.19-23.56)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval; BV, bacterial vaginosis; NA, not applicable; DMPA, depot medroxyprogesterone acetate; NET-EN, norethisterone enanthate. Note: bacterial vaginosis incidence data are for those women who were negative for bacterial vaginosis at baseline in the trial (n=1829), according to Nugent score results.
Chapter 5: SUMMARY AND CONCLUSIONS
5.1 OVERVIEW OF RATIONALE

The inter-relationship of factors influencing women’s reproductive health is undoubtedly complex. We undertook these analyses in an effort to shed light on the relationship between two critical factors in women’s health: effective family planning and risk for HIV-1 infection. Both injectable progestins and genital tract pathogens have effects on vaginal mucosa, which could result in adverse reproductive health outcomes. Better understanding the relationships among these cofactors for women of reproductive age is important.

As the leading cause of death for women in South Africa is HIV/AIDS, new strategies are urgently needed to reduce new HIV-1 infections among women. However, to be protective of women’s health overall, HIV/AIDS prevention strategies must avoid deleterious effects on other important causes of morbidity and mortality for women.

While some observational data has accumulated suggesting increased risk of HIV-1 and genital tract infection for women using DMPA, findings are mixed. New strategies to address the outstanding questions around injectable hormonal contraception use HIV-1 infection should be undertaken where possible. Here, we undertook the novel strategy of a direct comparison between two injectable methods of contraception to investigate potential associations with HIV-1 and genital tract infection.

5.2 OVERVIEW OF METHODS

Aim 1. To compare the potential impact of DMPA to NET-EN injection use on incident HIV-1 among South African women. We hypothesized that there would be no
difference in risk of incident HIV-1 between the two injectable contraceptive methods.

For Aim 1, we compared baseline characteristics of women using DMPA and NET-EN via the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. A Cox proportional hazards regression analysis was then used to compare the hazard of HIV-1 infection between DMPA and NET-EN users. Several sensitivity analyses were carried out in sub-groups to test the robustness of our results. These sub-groups included women under 25 years old, women who reported not using condoms (at baseline), women who tested HSV-2-seronegative at baseline, women who tested HSV-2-seropositive at baseline, women diagnosed with curable sexually transmitted infection (STI) at baseline, and women who used both DMPA and NET-EN at different times during study follow-up.

**Aim 2. To compare the potential impact of DMPA to NET-EN injection use on gonorrhea and chlamydia acquisition among women in South Africa.** Sexually transmitted infections, which have been associated with incident HIV-1, are common in South Africa, and impact of HC on incidence has not been clearly characterized. We hypothesized that there would be no difference in risk of chlamydial or gonorrheal infection between the two injectable contraceptive methods. In Aim 2, we conducted an analysis of injectable contraception users who had completed at least one strand displacement amplification for chlamydia and gonorrhea during follow-up. We compared baseline characteristics of women using DMPA and NET-EN via the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. We then used Anderson-Gill proportional hazards regression to compare the risk for chlamydial and gonorrheal infection between DMPA and NET-EN users. We tested the
robustness of our results in three cohort sub-groups: women who did not switch injectable contraceptive type during study follow-up, women under 25 years old, and women who reported not using condoms at baseline.

**Aim 3. To compare the potential impact of DMPA to NET-EN injection on acquisition of vaginal infection among women in South Africa.** We hypothesized that there would be no difference in acquisition of bacterial vaginosis or trichomonas infection between the two injectable contraceptive methods. In Aim 3, we conducted an analysis of incident vaginal infections among injectable contraception users at South African sites. We compared baseline characteristics of women using DMPA and NET-EN via the Wilcoxon rank-sum test for continuous variables and Pearson’s chi-squared test for categorical variables. This included a comparison of DMPA and NET-EN users for prevalence of bacterial vaginosis and trichomoniasis at screening. We then used an Andersen-Gill proportional hazards model to compare bacterial vaginosis acquisition between DMPA and NET-EN users, among women who were negative for BV at baseline. Anderson-Gill proportional hazards regression was also used to compare DMPA and NET-EN users for risk of incident trichomoniasis.

**5.3 SUMMARY OF RESULTS**

5.3.1 Aim 1: HIV-1 infection

Among 3,141 South African injectable contraception users, 1,788 (56.9%) solely used DMPA, 1,097 (34.9%) solely used NET-EN, and 256 (8.2%) used both injectable types at different times during follow-up. As most did not switch injectable type, we compared
baseline characteristics of women whose first injectable was DMPA (n=1,927, 61.4%) versus NET-EN (n=1,214, 38.7%) (Table 2.1). Overall, most women were young and unmarried. Women whose first injectable was DMPA were slightly older, more likely married or cohabitating, more likely parous, and less likely to report multiple partners or a circumcised partner. At baseline, reported condom use at last sex was similar for both injectable contraceptive groups. Median number of sex acts for past week was comparable, but with the distribution weighted toward fewer acts among NET-EN users. Recent anal sex was more commonly reported by those whose first injectable was NET-EN. While prevalence of curable genitourinary infection was comparable between groups, baseline seropositivity for HSV-2 was higher for those whose first method was DMPA.

Overall median follow-up time for women was 13.8 months (IQR 11.5-17.0), and was similar between groups (p=0.27). Women whose first injectable was DMPA more frequently continued participation until scheduled termination (92.8% versus 89.4%, p=0.001). Users of DMPA were less likely to use COCs (alone or for management of breakthrough bleeding) during follow-up, compared to NET-EN users (14.0 versus 27.5%, p<0.001). While pregnancy during follow-up was less common among those who initiated injectable use with DMPA compared to NET-EN (1.7 versus 3.3% ever pregnant, p=0.003), overall pregnancy incidence (0.5% for both methods) did not differ during presumed DMPA and NET-EN use, based on modeled lengths of exposure (p=0.76). Pregnancy failure rates were consistent with published failure rates for injectable progestin contraceptive use, suggesting that our defined exposures were not inconsistent with those relevant for contraceptive effect.
Overall HIV-1 incidence was 7.57 per 100 py (95% CI 6.61 to 8.68; 207 new HIV-1 infections over 2733.9 py of follow-up) (Table 2.2). One hundred fifty-two HIV-1 infections occurred during 1744.2 py of DMPA use (8.71/100 py, 95% CI 7.43 to 10.22) and 55 during 989.8 py of NET-EN use (5.56/100 py, 95% CI 4.27 to 7.24). The overall hazard of HIV-1 infection was higher among DMPA compared to NET-EN users (HR 1.58, 95% CI 1.16 to 2.15, p=0.004). This association persisted in the multivariable model adjusted for baseline and time-varying factors, with stratification by study site (aHR 1.46, 95% CI 1.10 to 1.95, p=0.01).

Among women HSV-2-seronegative at baseline, no significant difference in risk was observed between DMPA and NET-EN users (Table 2.2). However, among women HSV-2-seropositive at baseline, we observed a two-fold higher risk of incident HIV-1 infection in DMPA compared to NET-EN users (aHR 2.10 versus 1.12, pinteraction= 0.07). Repeating the primary comparison in the whole cohort, but including additional person-time after pregnancy outcomes, did not substantially alter results. Results were also not substantially changed by restricting analysis to those diagnosed with trichomonas, chlamydia or gonorrhea infection at baseline. When we restricted analysis to those who used both DMPA and NET-EN at different times during follow-up, DMPA was strongly associated with a higher risk of HIV-1 acquisition, compared to NET-EN use in the analysis adjusted for potential confounders (aHR 6.57, 95% CI 3.25 to 13.28, p<0.001).

5.3.2 Aim 2: Chlamydial and gonorrheal infection

Among 4,077 women enrolled at South African sites, 3,316 (81.3%) used injectable progestin contraception during follow-up. Among injectable progestin contraception users, 2,986 (90.0%) were included in the analysis (Figure 3.1). Median length of follow-
up was 13.5 months (IQR, 10.1 to 16.5 months), which did not differ significantly by injectable type used (p=0.68). Table 3.1 includes demographic and behavioral characteristics of participants at baseline. We observed several significant differences between users of DMPA and NET-EN. Overall, users of DMPA were older (median age 24 versus 23 years, p<0.001), more likely to be married (21.3 versus 16.2%, p=0.001), and more likely to have had at least one child (92.6 versus 70.5%, p<0.001), compared to users of NET-EN. Compared to NET-EN users, DMPA users were slightly less likely to have completed any secondary education (95.5 versus 97.0%, p=0.04), and less likely to report at baseline having more than one sexual partner in the prior three months (2.5 versus 5.3%, p<0.001).

5.3.2.1 Aim 2a: Chlamydial infection

The overall prevalence of CT among South African users of injectable progestin contraception at baseline was 14.9% (Table 3.1). Users of DMPA and NET-EN did not differ in likelihood of diagnosis at baseline (p=0.77). Over 2,630 py of follow-up, 400 chlamydial infections occurred for an overall incidence of 15.2/100 py among users of injectable contraception (Table 3.2). Incidence of chlamydial infection did not differ significantly by injectable progestin type (DMPA 14.9/100 py, 95% CI 13.1 to 16.8/100 py versus NET-EN 15.8/100 py, 95% CI 13.5 to 18.5/100 py) (HR 0.92, 95% CI 0.75 to 1.13, p=0.45). Adjustment for potential confounding variables (time-fixed age, education, and marriage/cohabitation, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use) did not
qualitatively change this estimate (adjusted HR [aHR] 1.05, 95% CI 0.85 to 1.29, p=0.70).

5.3.2.2 Aim 2b: Gonorrheal infection

The overall prevalence of NG among South African users of injectable progestin contraception at baseline was 3.6% (Table 3.1). Users of DMPA and NET-EN did not differ in likelihood of diagnosis at baseline (p=0.93). Among injectable progestin contraceptive users overall, 93 gonorrheal infections occurred over 2,630 person-years of follow-up for an overall incidence of 3.5/100 person-years (Table 3.2). Gonorrhea incidence did not differ significantly by injectable progestin type (DMPA versus NET-EN: 3.4/100 py, 95% CI 2.6 to 4.4/100 py versus 3.8/100 py, 95% CI 2.7 to 5.3/100 py; HR 0.85, 95% CI 0.56 to 1.29, p=0.44). Adjustment for potential confounding variables (time-fixed age, education, and marriage/cohabitation, and time-varying oral contraceptive use, recent partner change, partner’s other partnerships, frequency of sex, and condom use) also did not qualitatively change the estimate for difference in hazard of NG infection (aHR 1.00, 95% CI 0.65 to 1.54, p=1.00).

5.3.2.3 Aim 2: Sensitivity analyses

Among women who never switched to the other injectable contraceptive method, we found no difference in CT acquisition between DMPA and NET-EN users (HR 0.87, 95% CI 0.70 to 1.08; aHR 1.01, 95% CI 0.81 to 1.26). Among women who disclosed at baseline that they did not use condoms, we found no difference in CT acquisition between DMPA and NET-EN users (HR 1.25, 95% CI 0.63 to 2.47; aHR 1.53, 95% CI 0.74 to 3.18). We did not generate estimates similarly for NG acquisition, due to a small number of NG endpoints in this sub-group (n=7). Restricting the analyses to women less
than 25 years old also did not qualitatively change our findings (CT: HR 1.11, 95% CI 0.88 to 1.40, aHR 1.13, 95% CI 0.89 to 1.43; NG: HR 1.02, 95% CI 0.63 to 1.66; aHR 1.13, 95% CI 0.69 to 1.86).

5.3.3 Aim 3a: Bacterial vaginosis

Among 3,029 injectable contraception users in this analysis, exclusive DMPA use was more common than exclusive NET-EN use (56.8% versus 34.9%); 8.3% of participants used both types of injectable method (at different times) during study follow-up. As switching injectable type was uncommon, we compared injectable users by first method used during study follow-up. Median age for DMPA users was slightly higher compared to NET-EN users (24 versus 23 years old, p<0.001). Few women were married or cohabitating (n=590, 19.5%), but marriage/cohabitation was more common for DMPA compared to NET-EN users (21.5 versus 16.4%, p=0.001). Users of DMPA were more likely to be parous (93.9 versus 70.1%, p<0.001), but less likely to have completed some secondary education compared to NET-EN users (95.6 versus 97.0%, p=0.05). Users of NET-EN were more likely to report having multiple partners, compared to DMPA users (5.5 versus 2.7%, p<0.001). While median number of sex acts was similar for DMPA and NET-EN users, interquartile range for DMPA users was shifted slightly higher compared to NET-EN users (median 2, IQR (1-3) versus median 2, IQR (0-3), p=0.01).

No difference in prevalence of BV at baseline was observed between DMPA and NET-EN users (39.5 versus 39.6%, p=0.96) (Table 4.1). Overall, there were 1,829 women included in this analysis who were negative for BV at baseline by Nugent score. Of these, we observed 388 cases over 630.3 person-years of follow-up among NET-EN users (61.6/100 person-years, 95% CI 55.7 to 68.0/100 person-years), and 604 cases over
1,110.1 person-years (54.4/100 person-years, 95% CI 50.2 to 58.9/100 person-years) of follow-up among DMPA users. The overall hazard of BV was lower for DMPA compared to NET-EN users in the unadjusted model (HR 0.86, 95% CI 0.76 to 0.98, p=0.02). Adjusting for baseline age and HSV-2 status, and time-varying oral contraceptive use, frequency of intercourse, condom use at last vaginal sex, same-day washing inside the vagina, real-time diagnosis of BV, and recent change in primary partner, risk for BV was still lower among DMPA compared to NET-EN users (aHR 0.86, 95% CI 0.75 to 0.98, p=0.02).

5.3.3.1 Aim 3b: Trichomoniasis

This secondary analysis included 2,957 injectable contraception users at South African sites in the VOICE study (Table 4.2). Overall, exclusive DMPA users (n=1,674, 56.6%) were more common than exclusive NET-EN users (n=1,038, 35.1%); 245 women (8.3%) used both methods at different times during study follow-up. The median age of DMPA users was slightly older than that of NET-EN users (24 versus 23 years old, p<0.001). Users of DMPA were significantly more likely to be married or cohabitating (21.7 versus 16.1%, p<0.001) and parous (92.9 versus 70.2%, p<0.001), but slightly less likely to report completing some secondary education (95.4 versus 97.3%, p=0.01). Compared to NET-EN users, DMPA users were less likely to report multiple partners (2.9 versus 5.2%, p=0.001). While median number of sex acts in the past week was similar for DMPA and NET-EN users, the interquartile range trended slightly lower for NET-EN users, compared to DMPA users (0-3 versus 1-3, p=0.001). However, DMPA users were more likely to be seropositive for HSV-2 at baseline (51.3 versus 39.2%, p<0.001).
Users of DMPA and NET-EN did not differ for risk of prevalent trichomoniasis at baseline (5.8 versus 5.0%, p=0.36) (Table 4.2). During over 2,716.6 person years of follow-up, 227 positive rapid tests for trichomonas infection occurred (8.4/100 person-years, 95% CI 7.3 to 9.5/100 person-years). Of these outcomes, 85 were among NET-EN users (8.7/100 person-years, 95% CI 7.1 to 10.8/100 person-years) and 142 were among DMPA users (8.2/100 person-years, 95% CI 6.9 to 9.6/100 person-years). In a multivariable analysis that adjusted for multiple potential demographic and behavioral confounders, no significant difference was observed in incident trichomoniasis between DMPA and NET-EN users (aHR 1.07, 95% CI 0.79 to 1.45, p=0.67).

5.4 STRENGTHS OF THE STUDY

5.4.1 Characteristics of the VOICE trial

Analyzing HC and HIV-1 acquisition was a planned objective in VOICE. Thus, we prospectively implemented a robust data collection and site monitoring strategy for this cohort, one of the largest to date with regard to person-time and HIV-1 infections. The study population included participants from multiple sites in a diverse geographic area in South Africa. The VOICE design also permitted simultaneous investigation of genital tract infection, allowing us to investigate potential differences between exposure groups for genital tract infection outcomes. The study sponsor provided for an independent data monitoring committee, the US NIH Vaccine and Prevention Data and Safety Monitoring Board, which monitored interim findings and conduct throughout the study’s execution.
5.4.2 Potential reduced confounding by contraceptive delivery system

By using a direct comparison between two different progestin types of injectable contraception, we may have reduced potential confounding, compared to analyses that have employed a no HC comparison group. Women not using HC may have different coital frequency and patterns of condom use compared to those using HC. Moreover, those not using HC may include women seeking pregnancy. Pregnancy intention may be associated with different coital frequency and potentially different partner choices.

5.4.3 Characterization of contraceptive exposure

The frequency with which we updated contraceptive exposure status, sexual behavior data, and pregnancy and HIV-1 status data is also high compared to many other studies investigating the HC-HIV association. Contraception was offered on-site to all participants, and injection types and dates were directly observed or determined from family planning cards, rather than obtained via self-report. This study design included frequent (monthly) pregnancy testing. Such frequent measurement of documented contraceptive use allowed for more precise characterization of exposures relative to HIV-1 outcome, reducing potential exposure misclassification.

5.4.4 Number and characterization of HIV-1 endpoints

The unfortunately high HIV-1 incidence we observed in this cohort contributed to adequate power to compare the potential impact of DMPA and NET-EN use on HIV-1 infection. Testing for HIV-1 infection was conducted monthly, which is among the highest frequency used in similar observational analyses to date. An independent endpoint adjudication committee reviewed all cases where a participant’s HIV-1 status
was not unequivocal. Lastly, we used the midpoint between the last negative test and the first algorithm-confirmed positive test as the estimated date of HIV-1 infection.

5.4.5 Methodologic strengths

Our analysis aimed to address concerns related to the behavioral confounding encountered in previous studies (e.g., differential condom use and coital frequency) by directly comparing two types of the same delivery method: DMPA to NET-EN injection use, as opposed to a comparison of very different delivery methods (e.g., hormonal injection versus no method). The VOICE study provided a large longitudinal dataset with a high number of injectable contraception users and HIV-1 outcomes to address this question. Design of the case report forms allowed for disaggregation of contraceptive exposure types, a distinction not possible in some previous analyses of injectable HC and HIV-1 acquisition. Use of time-varying exposure also strengthens our results. Our data did meet the proportional hazards assumption, making Cox and Andersen-Gill proportional hazards regression analyses appropriate methodologic choices for these data.

By using HIV RNA PCR testing to analyze stored specimens from baseline, we were able to omit 22 participants from the analyses who were actually HIV-infected at the time of enrollment. Lastly, method failure rates for DMPA and NET-EN (0.5% for both) using our assumptions for hormonal contraceptive exposure were consistent with pregnancy failure rates in the published literature.129
5.5 LIMITATIONS OF THE STUDY

5.5.1 Potential sources of bias

As all analyses in this dissertation were observational, our primary concern is selection bias, which is inherent to nearly all secondary data analyses. Measurement error in our primary exposure variable (DMPA versus NET-EN use) is also a potential concern. Pharmacokinetic profiles may differ between injectable progestins and among women, and physiologic effects may outlast detectable drug.\textsuperscript{100} Thus, our exposure definitions may not demarcate the most relevant exposure periods, raising the possibility of information bias in our exposure data. As many of our important covariates (e.g., condom use, frequency of sex) were necessarily self-reported, information bias in the form of social desirability bias is also potentially of concern.

Screening for CT, NG, and trichomonas infection was undertaken annually, with additional testing as clinically indicated. More frequent assessment of our outcomes may have provided more informative results in Aims 2a and 2b. As asymptomatic and sub-clinical infections are common for STIs, it is possible that the exposure contemporaneous with diagnosis was not the contraceptive exposure at the time of STI acquisition. We did not collect data on completion of partner treatment, so we do not know if this factor differed between users of DMPA and NET-EN. As single-dose, directly observed therapy was encouraged but not required, it is possible that some cases of CT and NG were not adequately treated between tests. However, we saw no data to suggest that any under-treatment, if it occurred, differed by type of injectable exposure.

In this cohort, which did not determine partners’ HIV-1 serostatus, true HIV-1 exposure levels are unknown and may have differed between injectable groups; this is also the case
for CT, NG and trichomoniasis. While we hypothesized that DMPA and NET-EN users were more similar to each other regarding HIV-1 risk, than to women not using HC, we observed differences between women in the two contraceptive groups. First, DMPA users were more likely to continue follow-up in VOICE until scheduled termination, which may have impacted estimates for comparative risk of HIV-1 acquisition. Some factors, such as partner’s circumcision status, uncertainty about a partner’s other partners or HIV-1 status, and participant’s HSV-2 serostatus might suggest higher HIV-1 risk for DMPA compared to NET-EN users. However, other differences between groups, such as age, extra-primary partnerships, recent anal sex, and knowing a primary partner had other partners or HIV-1, potentially indicate higher HIV-1 risk in NET-EN users, although we did not observe this in our results. Thus, demographic and behavioral differences between DMPA and NET-EN users do not clearly indicate a consistent direction for bias.

Of note, some differences between DMPA and NET-EN users, particularly age, have been observed previously and are likely rooted in historical misperceptions that NET-EN is more appropriate for younger women desiring future fertility. Our results suggest that socio-demographic differences between users persist despite contemporary messaging to discourage age-based prescribing. Given that we saw differences in baseline demographic and behavior characteristics between DMPA and NET-EN users, it is likely that provider bias was present, in the form of selective prescriptive patterns.
5.6 DIRECTIONS FOR FUTURE RESEARCH

5.6.1 Better quality for future observational analyses of the potential HC-HIV association

In January 2013, a meeting was convened by the United States Agency for International Development (USAID) and FHI 360 to discuss and compile recommendations for the collection and analysis of observational data on the potential relationship of HC and HIV acquisition. This meeting, entitled, “Best practices in analytic approaches to assess the effect of hormonal contraception on HIV acquisition with observational data,” was held in Seattle, WA. Among others, recommendations included the disaggregation of contraceptive type, treating contraceptive exposure as time-varying, and assessment of contraceptive exposure using the shortest possible intervals. Recommendations also addressed methodologic strategies to reduce measurement error in self-reported sexual behavioral data, a significant challenge in analyses of the HC-HIV association. Our analyses have incorporated many of these recommendations. Future analyses may also be strengthened by considering the inclusion of these and other analytic strategies recommended in the report.

5.6.2 Consideration of a randomized trial of contraceptive method and HIV-1 acquisition

A randomized trial to investigate the potential impact of contraceptive method on HIV-1 acquisition has been proposed. The proposed trial includes an open-label randomization of approximately 8,600 HIV-uninfected women desiring contraception. Study arms would include DMPA, contraceptive implant and the copper IUD. Participants would be followed for 15 months, and the trial in its entirety is expected to
last four years. The proposed trial has been the subject of some controversy for several reasons, including cost, diversion of trial site capacity, potential for migration from randomized method, and potential ethical issues, among others. As currently designed, this trial is not positioned to determine whether DMPA and NET-EN confer different risk of HIV-1 acquisition. However, a modification to allow randomization to DMPA versus NET-EN for those women desiring a switch from a non-injectable to an injectable method could potentially contribute some high-quality data to address this question. At this time, complete funding has not been secured for the trial’s conduct, and it is not clear whether or not the trial will move forward.

5.6.3 Growing in vitro data for differential impact of progestins on mucosal immunity
As previously noted, recent data suggest that different progestin hormones may impact different components of the immune system. Notably, findings from the Hel (University of Alabama at Birmingham) and Hapgood (University of Cape Town) laboratories suggest that medroxyprogesterone and norethisterone have different effects on T cell function. Research is currently underway to characterize immune cell populations and HIV-tropic receptor expression in the genital tract and blood of users of different methods of contraception (including DMPA and NET-EN) in Zimbabwe and Pittsburgh. This study complements other work in the US, which has focused on the impact of DMPA on HIV target cells, vaginal microflora and vaginal epithelial thickness. Measuring the levels of endogenous and exogenous hormones at different time points during injectable contraceptive exposure could also be an important adjunct strategy to understanding their differential impact on mucosal immunity.
5.6.4 Better estimation of HIV-1 exposure

With the possible exception of study within HIV-serodiscordant couples, HIV prevention research, including study of comparative risk of HIV-1 infection, is hampered by the inability to measure, or even reasonably estimate true HIV-1 exposure in study participants. When unaccounted for differential risk of HIV-1 infection is associated with exposure type in observational research on the HC-HIV association, confounding is present. The sensitive nature of individual behavioral risk for HIV-1 infection impedes reporting, as does women’s understandably imperfect knowledge of their partners’ HIV-1 serostatus. Measurement of genital tract HIV-1 DNA has been proposed as one strategy for better measurement of true HIV-1 exposure.64 Other possible approaches include the incorporation of sexual network data and expanded use of partner testing, when available, although both of these may be limited to small numbers.

5.7 PUBLIC HEALTH IMPLICATIONS

5.7.1 Tools for curbing new HIV-1 infections in women

Despite substantial progress in managing the HIV/AIDS crisis, the rate of new HIV-1 infections in South Africa remains unacceptably high. The burden of HIV-1 on young women is particularly concerning. Thus, strategies to combat the rate of new HIV-1 infections in women are urgently needed. Known effective methods for prevention of HIV-1 infection in women face considerable challenges for implementation. Condom use can be difficult or impossible to negotiate for many women, particularly women in long-term partnerships or marriages, as the suggestion may prompt accusations of infidelity, and even trigger domestic violence.135 Woman-initiated methods, such as microbicide
and PrEP use, which in theory circumvent the issue of negotiation with partners, rely upon product adherence, which has been shown to be challenging among women at high risk for HIV-1 acquisition.\textsuperscript{76,136} Once available, multi-purpose technologies designed to prevent both pregnancy and HIV-1 may prove to be one strategy by which women are motivated to adhere to HIV-1 prevention products, as fear of unplanned pregnancy may supersede that of HIV-1 acquisition among many young women.\textsuperscript{137}

However, all of the above strategies require a degree of behavior change that may prove elusive for some women and their partners. Where ethical and feasible, structural level interventions that obviate or reduce the need for behavior change may have a role in combating the HIV epidemic.\textsuperscript{138,139} One such structural intervention, already suggested by some advocates, is the omission of DMPA as a contraceptive choice in areas where women are at high risk for HIV-1 acquisition.\textsuperscript{113,114} However, several factors make this option unjustified, impractical, and ultimately risky for the health of women and their families.

First, many areas where DMPA use is common in Eastern and Southern Africa currently have few or no alternatives for modern family planning, particularly long-acting methods.\textsuperscript{19} Thus, if DMPA were to be removed as a contraceptive option, the rate of unplanned pregnancies and maternal deaths would likely increase.\textsuperscript{74} Use of DMPA also plays an important role in HIV prevention, as it is a common, safe and effective family planning method for HIV-infected women,\textsuperscript{140} thus contributing to decreased perinatal HIV transmission. In the absence of definitive evidence of harm, and especially without an equally acceptable, affordable and accessible alternative in all areas, it would be
unwise to omit DMPA as a contraceptive option, particularly where uptake is currently high.

Approximately 40 percent of women in Eastern and South Africa currently use injectable progestin contraception. The high prevalence of injectable progestin contraceptive usage and the known protective effect of modern family planning on many women’s health and social outcomes makes the potential link between DMPA and HIV-1 acquisition especially important for further study. Our results, which do not appear to be explained by differences in acquisition of genital tract infection, suggest that different injectable progestin contraceptives may confer different risk of HIV-1 acquisition.

5.7.2 Clinical and public health relevance of direct comparisons between effective methods of contraception

Ultimately, a direct comparison to another contraceptive cannot produce an estimate of the direct effect of DMPA usage on HIV-1 acquisition. However, direct comparisons such as ours may offer more clinically relevant information for the millions of women at risk for HIV-1 infection who want to use progestin-only injectable contraception. This is particularly true for those women unable to negotiate condom use and those in areas where non-hormonal alternatives are scarce. The more common comparison of DMPA to no HC is appropriate for a scientific goal of estimating the direct effect of hormonal contraception on HIV-1 acquisition. Future analyses in other large trials of biomedical HIV prevention, for example, MTN-020 (the ASPIRE study), may permit the comparison of individual types of injectable HC to non-HC use, due to an anticipated larger group of participants using the copper IUD. Ultimately, comparisons between effective methods
have greater clinical utility for women desiring to avoid pregnancy, and for their contraceptive providers.

Past research comparing hormonal contraceptive use to no use of hormonal contraception has not only been plagued with potential unmeasured confounding, but also runs the risk of setting up a false dichotomy when results are interpreted. This dichotomy implies that a reasonable alternative to DMPA use may be using no hormonal contraception. In settings with few alternatives to DMPA other than male condoms, avoiding hormonal contraception may essentially mean avoiding the only effective, female-initiated method of contraception locally available to avoid the serious health risks associated with pregnancy in low resource settings. Women seeking family planning services generally do not do so with the question, “Should I start a method of family planning?” Often, the question is, “Which method of family planning should I start?” or in settings with few contraceptive options, “Can I start a family planning method today?” Thus, in areas with high rates of maternal morbidity and mortality, a comparator group of no hormonal contraception lacks some public health relevance. Future analyses of the HC-HIV association should, where possible, consider including such direct comparisons between methods.

5.7.3 Implications for WHO guidance

Given the high HIV-1 incidence observed in this cohort of injectable contraception users in South Africa, our findings support WHO recommendations on consistent condom use by couples in which the woman is using injectable progestin contraception. However, condom use is frequently outside women’s control. This persistent inequity itself contributes to demand for injectable contraceptives, which can be used independently of
a sexual partner’s knowledge or consent. However, as a body issuing public health
guidance, WHO must continue to reinforce messages regarding known effective
strategies for HIV prevention. Ultimately, our results do not change the conflicting nature
of the observational data used by WHO to generate contraceptive use guidance for
women at risk of HIV-1 infection, or the need to use condoms when this can be
negotiated.

HIV-1 infection does not exist in isolation from other significant health risks for women
in sub-Saharan Africa, including maternal mortality. In South Africa, modern
contraception has averted an estimated 58% of potential maternal deaths.12 Benefits of
contraception include reduced newborn deaths, improved child health and education, and
increased household income, among others.104 Thus, concerns about possible increased
HIV-1 risk with DMPA use must be weighed against beneficial effects of effective
contraception on a broad range of outcomes, as well as region-specific risk for HIV-1
acquisition among women. Despite findings that may implicate DMPA in HIV-1
acquisition, the overall body of data remains mixed, and withdrawing any common
effective contraceptive could increase maternal mortality rates.74 The majority of
observational data suggests no increased HIV-1 risk specific to NET-EN, an equally
effective alternative for those preferring injectable contraceptives. Thus, policy makers,
clinician-scientists and community stakeholders should continue to review emerging
findings to determine if women, in consultation with providers, should consider
switching from DMPA to NET-EN in high HIV-1 incidence settings where NET-EN is
available.
Appendix I: VOICE trial design

VOICE participants
n = 5,000

Tablet
n = 3,000
- TDF tablet
  n = 1,000
- FTC/TDF tablet
  n = 1,000
- Placebo tablet
  n = 1,000

Gel
n = 2,000
- TFV gel
  n = 1,000
- Placebo gel
  n = 1,000
Appendix II: Eligibility criteria in the VOICE study

Inclusion Criteria

Women must meet all of the following criteria to be eligible for inclusion in the study:

1) Age 18 through 45 years (inclusive) at screening, verified per site SOPs; within this range sites may restrict the upper age limit per site SOPs, to target women at high risk of HIV infection

2) Able and willing to provide written informed consent to be screened for and to take part in the study.

3) Able and willing to provide adequate locator information, as defined in site SOPs

4) HIV-uninfected based on testing performed by study staff at screening and enrollment (per applicable algorithms)

5) Per participant report, sexually active, defined as having vaginal intercourse at least once in the 3 months prior to Screening Part 1

6) Per participant report, using an effective method of contraception at enrollment, and intending to use an effective method for the next 24 months; effective methods include hormonal methods; intrauterine contraceptive device (IUCD); and sterilization (of participant or her sexual partner or partners as applicable and with verification as defined in site SOPs)

7) At screening and enrollment, agrees not to participate in other research studies involving drugs, medical devices, or vaginal products for the next 24 months
Exclusion Criteria

Women who meet any of the following criteria are excluded from the study:

1) Participant reported any of the following:
   a) Known adverse reaction to any of the study products (ever)
   b) Known adverse reaction to latex (ever)
   c) Pathologic bone fracture not related to trauma (ever)
   d) Non-therapeutic injection drug use in the 12 months prior to Screening Part 1
   e) Post-exposure prophylaxis (PEP) for HIV exposure within 6 months prior to enrollment
   f) Last pregnancy outcome 42 days or less prior to enrollment
   g) Gynecologic or genital procedure (e.g., biopsy, tubal ligation, dilation and curettage, piercing) 42 days or less prior to enrollment
   h) Participation in any other research study involving drugs, medical devices, or vaginal products 30 days or less prior to enrollment
   i) Currently breastfeeding
   j) Currently using spermicide; interferon or interleukin therapy; medication(s) with significant nephrotoxic potential, including but not limited to amphotericin B, aminoglycosides, cidofovir, foscartern and systemic chemotherapy; medication(s) that may inhibit or compete for elimination via active renal tubular secretion (including but not limited to probenecid)

2) As determined by the IoR/designee, any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric,
endocrine, respiratory, immunologic disorder or infectious disease, including active tuberculosis

3) Has any of the following laboratory abnormalities:

a) AST or ALT greater than 1.5 x site laboratory ULN

b) Calculated creatinine clearance less than 60 mL/min by the Cockcroft-Gault formula where creatinine clearance (female) in mL/min = (140 - age in years) x (weight in kg) x 0.85/72 x (serum creatinine in mg/dL)

c) Serum creatinine greater than the site laboratory ULN for women

d) Hemoglobin less than 10.0g/dl 3

e) Platelet count less than 100,000/mm

f) Serum phosphate level below site laboratory LLN (lower limit of normal)

g) Positive for HBsAg test result

h) Grade 2 or higher Pap result (at sites with capacity, where standard of care)

i) Dipstick urinalysis results for protein
   i) Any result of 2+ or greater at a single visit
   ii) At least two results of 1+ or greater at separate visits

j) Dipstick urinalysis results for glucose
   i) Any single result of 2+ or greater at a single visit
   ii) At least two results of 1+ or greater at separate visits

Note: Otherwise eligible participants with an exclusionary test may be re-tested during the screening process.
Note: Dipstick retesting is allowable in cases where results are attributable to urinary tract infection or menses, according to the judgment of the IoR/designee. If a participant is re-tested and a non-exclusionary result is documented within 56 days of providing informed consent for screening, the participant may be enrolled.

Note: Women with a documented normal result within the 12 months prior to enrollment need not have Pap smear during the screening period. Women with abnormal Pap smears can be enrolled upon completion of the initial phase of evaluation if no current treatment is indicated (based on local standard of care for management of abnormal cervical cytology). Need for a repeat Pap within 6 months does not preclude enrollment prior to that result becoming available.

Note: Serum creatinine results below the site laboratory LLN will be repeated during the Screening period.

4) Is pregnant

Note: Self-reported pregnancy is adequate for exclusion from the study. A documented negative pregnancy test performed by study staff is required for inclusion.

5) Per participant report at Screening Part 1:

a) Intends to become pregnant in the next 24 months

b) Plans to relocate away from the study site in the next 24 months

c) Plans to travel away from the study site for more than 8 consecutive weeks during the next 24 months

6) Diagnosed with urinary tract infection (UTI)
Note: Otherwise eligible participants diagnosed with UTI during screening are offered treatment and may be enrolled after completing treatment and all symptoms have resolved. If treatment is completed and symptoms have resolved within 56 days of obtaining informed consent for screening, the participant may be enrolled.

7) Diagnosed with pelvic inflammatory disease, an STI or reproductive tract infection (RTI) requiring treatment per current WHO guidelines

Note: Otherwise eligible participants diagnosed during screening with pelvic inflammatory disease or STI/RTI requiring treatment per WHO guidelines — other than asymptomatic BV and asymptomatic candidiasis — are offered treatment and may be enrolled after completing treatment and all symptoms have resolved. If treatment is completed and symptoms have resolved within 56 days of obtaining informed consent for screening, the participant may be enrolled. Genital warts requiring treatment also must be treated prior to enrollment. Genital warts requiring therapy are defined as those that cause undue burden of discomfort to the participant, including bulky size, unacceptable appearance, or physical discomfort.

8) Has a clinically apparent Grade 2 or higher pelvic exam finding (observed by study staff)

Note: Cervical bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the IoR/designee is considered expected non-menstrual bleeding and is not exclusionary.

Note: Otherwise eligible participants with exclusionary pelvic exam findings may be enrolled/randomized after the findings have improved to a non-exclusionary severity
grading or resolved. If improvement to a non-exclusionary grade or resolution is
documented within 56 days of providing informed consent for screening, the
participant may be enrolled.

9) Has any other condition that, in the opinion of the IoR/designee, would preclude
informed consent, make study participation unsafe, complicate interpretation of study
outcome data, or otherwise interfere with achieving the study objectives
## Appendix III: Schedule of evaluations in VOICE

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<th>SCR 2</th>
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<th>MLY</th>
<th>QRT</th>
<th>SEM</th>
<th>ANN</th>
<th>PUEV</th>
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* = required, ▲ = as indicated, • = at sites with capacity where local standard of care, ▲ = first monthly visit, + = UA, pelvic exam components, and other relevant assessments may be done on day of ENR to confirm eligibility. **Note:** Informed consent for specimen storage and possible future research testing may be deferred (no later than Month 3 follow-up visit) in accordance with site SOPs. ENR includes procedures conducted as part of final screening procedures and confirmation of eligibility. Monthly visit procedures also occur at quarterly, semiannual, and annual visits; likewise, quarterly visit procedures occur at semiannual and annual visits, and semiannual visit procedures occur at annual visits. If Sample 2 is drawn (per Appendix III), blood is also collected for the following analyses: Plasma archive, CD4+ T-cell count, HIV-1 RNA PCR. For hepatitis B susceptible participants randomized to oral study product who do not receive hepatitis B vaccination, HBsAg additionally is checked annually and 6 months after PUEV; serum chemistries are checked 6 months after PUEV.
Appendix IV: HIV testing algorithm

START
Sample 1 rapid test

Requires additional testing.

Sample 1 WB

Sample 1 HIV viral load

Consult the MTN Network Lab and continue with algorithm

Consult the MTN Network Laboratory for further testing and follow up

Sample 2 WB

STOP. HIV infection confirmed Report to participant as HIV-infected

STOP. Report to participant as HIV-uninfected

+ or ind

- or ind
References


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large individual participant data meta-analysis. 20th International AIDS Conference. Melbourne; 2014.


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105. Alkema L, Kantorova V, Menozzi C, Biddlecom A. National, regional, and global rates and trends in contraceptive prevalence and unmet need for family planning between


141. MTN. Comparison of Injectable Contraceptives DMPA and NET-EN Suggests Some Women Using DMPA Were at Higher Risk of Acquiring HIV. First Comparison Between Injectable Contraceptives DMPA and NET-EN Suggests Some Women Using DMPA Were at Higher Risk of Acquiring HIV. Pittsburgh, PA: MTN; 2014.
Curriculum Vitae
Lisa M. Noguchi, CNM, MSN

PERSONAL DATA:

SCHOOL ADDRESS: Johns Hopkins Bloomberg School of Public Health
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PLACE OF BIRTH: Pittsburgh, PA

CITIZENSHIP: United States

EDUCATION AND TRAINING:

2011-2014 Johns Hopkins Bloomberg School of Public Health
Baltimore, MD
PhD, Infectious Disease Epidemiology

1996-1997 University of Pennsylvania, School of Nursing
Philadelphia, PA
Master of Science in Nursing
1996-1997  University of Pennsylvania, School of Nursing  
Philadelphia, PA  
Certificate, Division of Nurse-Midwifery  

1993-1995  University of Pennsylvania, School of Nursing  
Philadelphia, PA  
Bachelor of Science, *cum laude*, Nursing  

1989-1993  University of Pennsylvania, College of Arts and Sciences  
Philadelphia, PA  
Bachelor of Arts, *magna cum laude*, Biomedical Anthropology, Asian Studies  

1992  Kansai University  
Osaka, Japan  
Certificate, Academic Honors, Asian Studies  

2003  University of California School of Medicine, San Francisco, CA  
Certificate, UCSF Summer Training in Clinical Research Program  
Department of Epidemiology and Biostatistics
APPOINTMENTS AND POSITIONS:

2014-present  Maternal and Child Survival Program/Jhpiego

Washington, DC

Maternal Health Team

2014-present  Senior Technical Advisor

2013-present  Johns Hopkins Bloomberg School of Public Health

Baltimore, MD

T-32 Training Program in Sexually Transmitted Infections

2013-present  Pre-doctoral Trainee

2006-present  University of Pittsburgh Medical Center

Pittsburgh, PA

Microbicide Trials Network

2011-present  Scientific Director, Pregnancy Research

2008-present  MTN Executive Committee


2008-2009  Director of Operations

2006-2008  Protocol Development Manager
2004-2006  University of Pittsburgh Medical Center  

Pittsburgh, PA  
Reproductive Infectious Disease and Immunology  
Department of OB/GYN/RS  
2004-2006  Research Clinician/CNM Member of Medical Staff  

1999-2004  St. Luke’s Women’s Center  
San Francisco, CA  
2000-2004  Director, Nurse-Midwifery Service  
1999-2000  Staff Nurse-Midwife  

Hopedale, MA  
Staff Nurse-Midwife  

1998-1999  Brigham and Women’s Hospital  
Boston, MA  
Registered Nurse for Labor and Delivery, Gyn/Oncology, High-Risk Antepartum, Nursery, Postpartum
1996  Cantabridgia Healthcare Center
Cambridge, MA
Registered Nurse/Charge Nurse

1996  Greenery Healthcare Center
Beverly, MA
Registered Nurse

1994-1995  University of Pennsylvania School of Medicine
Philadelphia, PA
Research Coordinator, SHARE Study, Case-Control Study of Risk Factors for Epithelial Ovarian Cancer, Center for Clinical Epidemiology and Biostatistics, (R01CA61095, National Cancer Institute, PI: Ness)

1992-1994  Thomas Jefferson University Hospital
Philadelphia, PA
1993-1994  Research Assistant, Infant Feeding Decisions Study
1992  Research Assistant, Very Low Birthweight Study
ACADEMIC:

2014  Lecturer, Advanced Issues in HIV/AIDS
      32nd Annual Graduate Summer Institute of Epidemiology and Biostatistics
      Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2013  Visiting Scholar (January 2013)
      Department of Global Health
      University of Washington, Seattle, WA

2013  Teaching Assistant, Epidemiologic Inference in Outbreak Investigation
      Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2013  Teaching Assistant, Epidemiologic Methods 3
      Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2012  Lecturer, Advanced Issues on HIV/AIDS
      30th Annual Graduate Summer Institute of Epidemiology and Biostatistics
      Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2011  Teaching Assistant, Infectious Disease Epidemiology
      Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2005-2006 Lecturer/Preceptor
Waynesburg College, Graduate School of Nursing, Waynesburg, PA

2000-2004 Clinical Faculty, Department of OB/GYN/RS
UCSF School of Medicine, San Francisco, CA

2000-2004 Clinical Instructor, Nurse-Midwifery Division
UCSF School of Nursing, San Francisco, CA

1998 Lecturer/Clinical Instructor
Holy Family Hospital School of Nursing, Phalombe, Malawi

NON-ACADEMIC:
1999-2006 American College of Nurse-Midwives
2003-2004: Co-Chair, Region VI, Chapter 4, San Francisco
2002-2003: Chair, Region VI, Chapter 4, San Francisco
2000-2001: Secretary, Region VI, Chapter 4, San Francisco

2002-2003 California Nurse-Midwives Association, Board of Directors
2004-2005 Pennsylvania Association of Licensed Midwives, Executive Board

HOSPITAL PRIVILEGES:
2005-2008 Dept. of Obstetrics and Gynecology, MWH of UPMC
2002 Dept. of Obstetrics and Gynecology, California Pacific Medical Center
1998-1999 Dept. of Obstetrics and Gynecology, Milford-Whitinsville Regional Hospital

**CERTIFICATION AND TRAINING:**

**Specialty certification:**
American College of Nurse-Midwives Certification Council #08942 1998-present

**Additional training:**
CITI Human Subjects Research Ethics 2012
Current Challenges in HIV Disease, IAS-USA 2005
Research Ethics, Family Health International 2005
Limited Obstetric Ultrasound 2004
Good Clinical Practice 2004
Fetal Heart Rate Monitoring 2002
Advanced Cardiac Life Support 2002
UCSF Maternal Fetal Medicine Review Course 2001
First Assist, Cesarean Section 2000
Fetal Heart Rate Monitoring 1998

**MEMBERSHIPS IN PROFESSIONAL SOCIETIES:**
Infectious Diseases Society of Obstetrics and Gynecology 2014-present
Society for Epidemiologic Research 2013-2014
International AIDS Society 2012-2014
American Public Health Association 2002-2005
American Institute of Ultrasound in Medicine 2001-2002
California Nurse-Midwives Association 2000-2004
ACNM Service Directors Network 2000-2004
American College of Nurse-Midwives 1995-2006
American College of Obstetricians and Gynecologists 1999-2000

**HONORS AND AWARDS:**

2014 American Board of Obstetrics and Gynecology Education Foundation Scholar, Infectious Diseases Society of Obstetrics and Gynecology
2014 Johns Hopkins University CFAR Annual Meeting Award, Best Poster (Prevention)
2014 Young Investigator Award, Conference on Retroviruses & Opportunistic Infections
2013 The Ruth Freeman Memorial Fund ($10,000 for PhD research)
2013 The Charlotte Ferencz Scholarship in the Department of Epidemiology
2012 U.S. Public Health Service Training Grant
2012 Jean Coombs Award, Johns Hopkins Bloomberg School of Public Health
2011 Mary B. Meyer Scholar in Maternal Child Health
2011 Department Scholarship, Johns Hopkins Bloomberg School of Public Health
2010 MTN Achievement Award (First Topical Microbicide Study in Pregnancy)
1995  Oxford Endowment, University of Pennsylvania
1995  Federal Nurse Traineeship
1995  *Cum laude*, University of Pennsylvania
1995  Sigma Theta Tau, International Honor Society of Nursing
1993  *Magna cum laude*, Distinction in Major, University of Pennsylvania
1992  Academic Honors with Scholarship, Kansai University

**PUBLICATIONS:**

**Journal articles:**


**Invited publications**


Abstracts


Bunge K, Macio I, Meyn L, **Noguchi LM**, Campbell T, Hillier S. The safety, persistence, and acceptability of an antiretroviral microbicide candidate UC-781. [oral presentation at 2007 Annual Scientific Meeting of the Infectious Diseases Society for Obstetrics and Gynecology].


**Noguchi LM**. Collaborative Initiative between Researchers and Community Representatives to Facilitate Community Understanding of Interim Analyses in an HIV Prevention Trial. [oral presentation at Unite for Sight Global Health, Yale University, 2009].


Other publications


ONGOING RESEARCH SUPPORT:

T32 AI 050056 Sherman/Jennings 07/31/13-06/30/15

Johns Hopkins Training Program in Sexually Transmitted Infections

The Johns Hopkins Training Program in Sexually Transmitted Infections is a multidisciplinary grant to fund the training of pre-doctoral students interested in sexually transmitted disease research. Role: Pre-doctoral Trainee (PhD Candidate).

UM1 AI 068633 Hillier/McGowan 06/01/06-present

Microbicide Trials Network

The Microbicide Trials Network (MTN) is one of six HIV/AIDS international clinical trials networks funded by US NIH/NIAID, with co-funding from the National Institute of Mental Health and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The MTN aims to evaluate the safety, acceptability, and effectiveness of candidate topical and oral HIV prevention products among various populations at risk in both US and non-US settings. Role: Scientific Director, Pregnancy Research.

PROFESSIONAL ACTIVITIES:

Selected seminars and presentations

2014 Injectable contraception and HIV/STI acquisition in the VOICE study, MTN Regional Meeting, Cape Town, South Africa.
2014 Phase 1 study of tenofovir vaginal gel in breastfeeding mother-infant pairs, MTN Annual Meeting, Bethesda, MD, USA.

2013 Physical exam of pregnant women, MTN Regional Meeting, Cape Town, South Africa.

2013 Planned analyses in the VOICE study, Best practices in analytic approaches to assess the effect of hormonal contraception on HIV acquisition with observational data. Bill and Melinda Gates Foundation, Seattle, WA USA.

2012 HIV Prevention Research Panel Discussion. Be the Generation Bridge – Partners’ Meeting, Washington, DC USA.

2012 Post-Trial Access Studies, MTN Regional Meeting, Cape Town, South Africa.

2011 Understanding Interim Analysis – Community Working Group Capacity-building:
VOICE Trial DSMB Outcome. MTN Regional Meeting, Cape Town, South Africa.


2010 MTN Community Working Group – Preparing for HIV Prevention Trial Results, MTN Annual Meeting, Arlington, VA, USA.

2010 MTN Community Working Group– Informed Consent in VOICE, MTN Regional Meeting, Cape Town, South Africa.

2008 MTN Community Working Group Consultation – Stopping Rules in VOICE, Johannesburg, South Africa.

2007 MTN Community Working Group Consultation – VOICE Protocol, Durban, South Africa.

2006 Grand Rounds, Binh Duong Hospital, Binh Duong, Vietnam. Evidence-Based PMTCT of HIV, Evidence-Based Management of Chorioamnionitis and Endometritis, Evidence-Based Management of Pelvic Inflammatory Disease


2002 Labor Assistant Training Program, St. Luke’s Hospital, San Francisco, CA

2000 IUD Insertion and Removal, St. Luke’s Women’s Center, San Francisco, CA

1998 Holy Family Hospital, School of Nursing and Midwifery, Phalombe, Malawi.

Care of the Infertility Patient
Induction/Augmentation of Labor
Birth-Related Injury
Neonatal Infection
Vesicovaginal and Rectovaginal Fistulae

JOURNAL ACTIVITIES:

2014-present Reviewer, *Journal of Acquired Immune Deficiency Syndromes*

2014-present Reviewer, *Sexually Transmitted Diseases*
2003-2005  Guest Co-Editor, *Journal of Midwifery and Women’s Health*
   Issue 50-4, July/August 2005

2004-2007  Reviewer, *Journal of Midwifery and Women’s Health*

**Volunteer/Service/Other:**

2006  Infection Control Consultant (WHO Standards)
   Project Vietnam, Binh Duong Hospital, Binh Duong, Vietnam
   American Academy of Pediatrics, California Chapter 4

2003-2004  Research Intern, University of California, San Francisco
   Women’s Global Health Imperative, Center for AIDS Prevention Studies

**Clinical/Research**

MTN Community Resource Working Group  2013-present

Trans-NIH Plan for HIV-Related Research: Women and Girls Planning Group  2011-present


MTN Manual of Operational Procedures Task Force  2006-2010

Sutter Regional Nurse-Midwives Network  2001-2004

Sutter SLH Committee on Interdisciplinary Practice  2000-2004

Sutter SLH Perinatal Education Task Force  2000-2002

**Community**

Nen Daiko, Japanese Percussion, Training and Performance  2010-present

Pittsburgh Refugee Center  2005


Admissions Interviewer, University of Pennsylvania  1998