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Mucosal Integrity and Inflammatory Markers in the Female Lower Genital Tract as Potential Screening Tools for Vaginal Microbicides

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Study Design—A randomized, blinded crossover trial of 18 subjects comparing effects of Gynol II (putative inflammatory gel), HEC (putative inert gel) and no gel exposure on endometrial and vaginal epithelial integrity and endometrial and vaginal inflammatory markers (IL-1β, IL-6, IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α, IL-1RA, IL-10, SLPI).

Results—Gynol II was associated with more vaginal lesions. No endometrial disruptions were observed across conditions. In the vagina, RANTES (p=0.055) and IL-6 (p=0.04) were higher after HEC exposure than at baseline. In the endometrium, IL-1β (p=0.003) and IL-8 (p=0.025) were lower after Gynol II cycles than after no gel.

Conclusions—Gynol II and HEC may modulate inflammatory markers in the vagina and endometrium. How these changes relate to infection susceptibility warrants further study.

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Mucosal integrity and inflammatory markers in the female lower genital tract as potential screening tools for vaginal microbicides

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Keywords

Nonoxynol-9; inflammation; cytokines; chemokines; vagina; endometrium

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1. Introduction

Potential vaginal contraceptives or microbicides need to demonstrate safety *in vivo* prior to widespread use. Currently, methodology to measure in vivo inflammatory responses to vaginal products is primarily limited to colposcopy to assess vaginal epithelial integrity [1–3]. However, gross visual inspection may not detect subtle inflammatory changes induced by vaginal products [3,4].

Multiple cytokines and chemokines are present on mucosal surfaces of the lower female genital tract and are potential in vivo biomarkers of inflammation at these sites. Evaluation of vaginal cytokines through vaginal lavage has been incorporated into some assessments of candidate microbicides [4–6]. However, these initial studies need to be replicated to prove their validity. Furthermore, the impact of microbicides on the upper genital tract, namely, the endometrium, is largely unknown.

The overall goal of this study was to assess whether vaginal exposure to a putative inflammatory gel (3% nonoxynol-9 vaginal gel [Gynol II]) and a putative inert gel (hydroxyethyl cellulose gel [HEC]) can elicit significant changes in inflammatory response or disruptions in epithelial integrity in the vagina and endometrium. Nonoxynol-9 was selected as the positive control because it is known to induce vaginal inflammation [1,2,4] and has been clinically linked to increasing HIV transmission [7]. HEC was selected as the negative control because of its common use as a placebo. If consistent patterns of cytokines, chemokines or epithelial disruptions were observed using these well-characterized gels, these inflammatory biomarkers and their patterns of change could serve as in vivo biomarkers of contraceptive or microbicide safety. We hypothesized that use of Gynol II would result in greater pro-inflammatory responses and epithelial disruption in both the vagina and endometrium than use of HEC. To test this hypothesis, we compared the effect of the gels on 4 outcomes: 1) vaginal epithelial integrity; 2) inflammatory cytokines and chemokines in the vagina; 3) endometrial integrity; 4) inflammatory cytokines and chemokines in the endometrium. Furthermore, we assessed how cytokines and chemokines differed between the vagina and endometrium.

2. Methods

A randomized, assessor-blinded, optional-crossover trial was undertaken to assess the effect of Gynol II (Personal Products, Rariton, NJ) and/or HEC (Biosyn, Inc., Huntingdon Valley, PA) on the vagina and endometrium. Eligible subjects were between ages 18 to 45 years, had regular menstrual cycles between 25–35 days, were at low risk for sexually transmitted infections, did not use intravaginal or intrauterine contraceptives, had a normal Papanicolaou test within 6 months and abstained from vaginal intercourse during the study from the day before first day of product use until 5 days following vaginal and endometrial assessment, rendering the first sampling time point at least 4 days from any semen exposure. Subjects underwent a wet prep to screen for yeast, bacterial vaginosis using Amsel's criteria, and trichimonas. They were also screened for herpes simplex virus (HSV0, gonorrhea and chlamydia using PCR testing, and HIV using serum testing. Potential subjects were excluded if she had a sexually transmitted infection, yeast infection, bacterial vaginosis, cervicitis, endometritis, current pregnancy, fewer than 10 weeks since the end of the last pregnancy, current breastfeeding, recent gynecologic surgery, colposcopy or endometrial biopsy, or allergy to Gynol II or HEC. Women on hormone-based contraceptive methods (oral, transdermal, injectable or implanted) were not excluded.

Subjects underwent at least 1 (and up to 3) sets of colposcopy, vaginal lavage (VL), endometrial lavage (EL), and endometrial biopsy in the follicular phase of separate

menstrual cycles, between cycle days 6 and 12. Subjects were provided with kits containing assigned gels; study coordinators conducted teaching to demonstrate how to fill applicators and insert the product. During cycles when subjects were exposed to gel products, the exposure began in the early follicular phase between cycle days 3 and 9. A maximum of 1 intervening menstrual cycle between any 2 assessment cycles was allowed. The first evaluation was performed without any gel exposure. The second evaluation was after 3 days' use of one product. Assignment of Gynol II versus HEC at this evaluation was randomized using a computer-generated scheme blocked to assure near-equal numbers of cycles assigned to each gel. For individuals who underwent a third evaluation, this was performed after 3 days' use of the second product. Because each subject could be exposed to two gels and sampling was designed to occur during the same part of the menstrual cycle, this study required assessments over multiple menstrual cycles. The subject, study team and clinician performing the evaluations were blinded to which gel was administered in a given cycle.

Participants self-inserted a single applicator of study gel once a morning for 3 consecutive days during the follicular phase after cessation of menses. Each Gynol II applicator contained 150 mg of nonoxynol-9 (N-9). On the third day, the participant returned for colposcopy, VL, EL and endometrial biopsy, in that order. Evaluations were timed to occur 1–4 h after the third gel administration. For each colposcopy, lesions and the following findings were documented: degree of epithelial disruption, location, color, blood vessels, and size [8].

VL was performed by introducing 5 mL of normal saline into the posterior vaginal fornix. Saline was recovered and transferred to a tube with phosphate buffered saline (PBS). EL was performed by injecting 2 mL of normal saline into the uterine cavity via a sterile hysterosalpingogram catheter (Lyco, San Antonio, TX) and recovered by suction. Flushing was performed with fresh saline up to 5 times to obtain a minimum of 4 mL of EL fluid [9]. VL and EL specimens were centrifuged at 200×g for 10 min, and supernatants were frozen at −70°C.

Eleven inflammatory markers were selected based on presence in the vaginal epithelium or endometrium, and/or change after exposure to N-9 [4,6,10–13]. These chemokines and cytokines were assayed in duplicate in all VL and EL specimens (DuoSet ELISA Development kits, R&D Systems, Minneapolis, MN) according to manufacturer guidelines. Levels of 8 pro-inflammatory markers IL-1 β, IL-6, IL-8, MCP-1, MIP-1 α, MIP-1 β, RANTES, and TNF-α and 3 anti-inflammatory markers IL-1RA, IL-10 and SLPI were determined. The limits of detectability were protein levels at which no optical displacement (OD) values were recorded. The precise limit of detectability for each cytokine is not known but is lower than the lowest point on the standard curve. For each cytokine, the ranges of the standard curve were as follows: IL-1β (3–300 pg/mL), IL-6 (10–200 pg/mL), IL-8 (30–3000 pg/mL), MCP-1 (15–1000 pg/mL), MIP-1α (10–200 pg/mL), MIP-1β (15–200 pg/mL), RANTES (15–1000 pg/mL), TNF-α (10–1000 pg/mL), IL-1RA (30–3000 pg/mL), IL-10 (20–2000 pg/mL) and SLPI (60–6000 pg/mL). Samples with OD levels below the standard curve were given 1/2 value of the lowest point on the standard curve [14]. Samples were not diluted. Cytokines for which some samples had values above the linear range of the standard curve are presented, but formal statistical comparisons were not performed. Means of the duplicates were used for analysis.

Immediately following collection of endometrial lavage specimens, an endometrial biopsy was performed with a sterile 3 mL endometrial suction curette. Tissue from endometrial biopsies were fixed in formalin, dehydrated in ethanol, paraffin embedded and sectioned. Hematoxylin and eosin staining was performed using the Surgipath Hematoxylin Gill III

Formula (Surgipath, Inc, Richmond, IL, USA) according to manufacturer instructions. Specimens were examined by a single pathologist blinded to treatment allocation. The following findings were documented: discontinuity of uterine epithelium, hemorrhage, necrosis of the endometrial stroma or epithelium, polymorphonuclear leukocytes (PMNs) and other inflammatory cells and luminal debris in glands.

Categorical variables were summarized by frequencies and proportions. Continuous variables were summarized by mean, median, and range. Paired t-test (for normally distributed data), Wilcoxon signed-rank test (for non-normally distributed data), Test for Symmetry and McNemar's test were used to compare paired variables. Independent data were compared using Student's t-test (for normally distributed data), Wilcoxon rank-sum test (for non-normally distributed data) and Fisher's exact test, as appropriate. P-values ≤0.05 were considered significant.

The study sample size was determined a priori to detect a significant difference in endometrial epithelial changes, specifically the rate of inflammation. Inflammation was defined by the presence of moderate to many polymorphonuclear leukocytes (PMNs) and other inflammatory cells on endometrial biopsy specimens. The sample size calculation was performed using the equation for a dichotomous outcome assuming a 1% rate of inflammation among HEC cycles and a 50% rate of inflammation among N-9 cycles, 1:1 randomization of Gynol II to HEC, 80% power and a two-tailed p-value of 0.05. The calculated sample size was 14 cycles per treatment arm. The study was reviewed and approved by the institutional review board of the University of Pennsylvania.

3. Results

Of 27 screened women, 18 subjects were enrolled at a median age of 31. years (range 22–45). Fourteen subjects were Caucasian, 4 were African American, and 1 was Asian American. One subject was Hispanic in addition to being African American. At study conclusion, these 18 subjects completed 18 baseline, 14 Gynol II and 14 HEC cycles. Twelve subjects completed baseline followed by both HEC and Gynol II cycles. Two subjects completed baseline and HEC cycles only, and 2 subjects completed baseline and Gynol II cycles only. Finally, 2 subjects completed only the baseline cycle. Two subjects were on hormonal contraceptives (both combined oral contraceptive pills); both completed baseline, Gynol II and HEC cycles. Race and ethnicity were not associated with the likelihood of completing all three exposure cycles. Overall, participants reported minimal complaints with respect to both gels and study procedures.

3.1. Vaginal epithelial integrity

Vaginal epithelial integrity was assessed by colposcopy. At baseline, 10 subjects had a total of 15 lesions. During Gynol II cycles, 10 subjects had a total of 18 lesions, and in HEC cycles, 4 subjects had a total of 5 lesions. There appeared to be more lesions after Gynol II exposure, but the number of subjects with lesions and the number of lesions per subject were not statistically different from baseline (p=0.27) or HEC (p=0.09) (Test for Symmetry). The degree of epithelial disruption, diagnosis, location, color, blood vessels, and size of lesions did not differ significantly by treatment (Table 1).

3.2. Biomarkers of inflammation: vaginal specimens

VL fluid was assayed for 8 pro-inflammatory and 3 anti-inflammatory markers. In Table 2 and Fig. 1, all data are presented. Of these, IL-1β, IL-6, IL-8, RANTES, IL-1RA and SLPI levels had measurements above the lowest point on the standard curve. IL-1RA and SLPI had several measurements above the assay range; formal statistical comparisons of these two

cytokines were not performed. Most MCP-1, MIP-1a, MIP-1b, TNF-a, and IL-10 levels were not within the linear aspects of the standard curve with this assay method. Compared by Wilcoxon signed-rank tests, Cytokine/total protein ratios did not differ between Gynol II and baseline. In comparing HEC to baseline cycles, RANTES (p=0.055) and IL-6 (p=0.04) levels were significantly higher after HEC exposure.

3.3. Endometrial integrity

In endometrial biopsy specimens across all three conditions, there were no findings of uterine epithelial discontinuity, hemorrhage, necrosis, or luminal debris (Table 3). There were few PMNs visualized in most specimens, and this observation did not differ by treatment (Test for Symmetry). Qualitatively, PMNs in the Gynol II and HEC cycles tended to be peri-glandular compared to baseline cycles.

3.4. Biomarkers of inflammation: endometrial specimens

Inflammatory markers in EL fluid are summarized in Table 4 and Fig. 2, where all data are presented. Of these, IL-1β, IL-6, IL-8, RANTES and IL-1RA levels had measurements above the lowest point on the standard curve. IL-6, RANTES and IL-1RA had several measurements above the assay range; formal statistical comparisons of these three cytokines were not performed. Most MCP-1, MIP-1a, MIP-1b, TNF-a, IL-10 and SLPI levels were not consistently within the linear range of the standard curves with this assay method. Exposure to Gynol II was associated with lower IL-1β (p=0.003) and lower IL-8 (p=0.025). In the endometrium, measured cytokine/total protein ratios did not differ between HEC and baseline cycles.

3.5. Endometrial versus vaginal inflammatory markers

Finally, to assess for potential differences in immune response in the vagina versus endometrium, we compared baseline IL-1β and IL-8 between the vagina and endometrium (Tables 2, 4). In the endometrium, IL-1β levels were lower (p=0.02) than in the vagina.

4. Discussion

The overarching goal of this exploratory study was to examine *in vivo* the effects of two well-characterized, vaginally-applied gels on the vagina and endometrium. In hopes of characterizing an in vivo model for testing future vaginal microbicides and contraceptives, we compared gross breaches in vaginal epithelium, endometrial histopathology and vaginal and endometrial levels of inflammatory cytokines and chemokines after no treatment and exposure to Gynol II and HEC gels. As expected, Gynol II appeared to induce more vaginal epithelial lesions than control conditions. However, the experimental conditions did not result in any gross histologic changes or specific patterns of inflammatory cells in the endometrium. Of 11 inflammatory markers that were measured, multiples chemokines and cytokines were not consistently detectable with this assay method. Of measurable markers HEC exposure was associated with a rise in two pro-inflammatory molecules RANTES and IL-6 in the vagina. In the endometrium, Gynol II exposures reduced levels of IL-1β and IL-8

The objective of testing inflammatory biomarkers in the lower genital tract after exposure to N-9 was to confirm prior studies and delineate specific patterns of inflammatory response to this product in the vagina and endometrium. In the vagina, none of the pro-inflammatory measures that were hypothesized to increase after N-9 exposure exhibited a significant rise above baseline. These study findings are not completely consistent with prior works [4,15,16] and may have resulted from the lack of a standard approach in sampling timing, lavage volume, duration of N-9 exposure, study size and technical differences in assays. While sampling in this study may have occurred earlier than prior studies and missed a later

increase in inflammatory response, subjects had already been exposed to two consecutive daily doses of the study gel prior to the last dose and sampling. We are unable to rule out the possibility that earlier sample resulted in interference of assays by the vehicle gels. Prior studies have reported decrease of IL-1RA and no increase of pro-inflammatory mediators in vaginal washings after one dose of Gynol II [4]. These findings are corroborated by the present study. Delayed proinflammatory responses have been reported after multiple doses of Gynol, which could not be confirmed in the present study. Technical differences such as different ELISA kits and laboratories could also contributed to disparate findings between the two studies. Because a recent study evaluating IL-1β and IL-6 cytokine measurements found significant differences among ELISAs and laboratories [17], there may be a need for conversion coefficients among different immunoassays to facilitate comparing results among studies. Finally, while participants were asked to abstain from vaginal intercourse for at least 4 days before each endometrial and vaginal sampling, we did not assess for the presence of semen in the vaginal fluid, which may impact cytokine levels.

Assessing in vivo endometrial cytokines was novel. First, we noted that <u>at least one</u> baseline cytokine profile differed significantly between the vagina and the endometrium, as IL-1β levels was higher in the vagina. This analysis using cytokine/total protein ratios suggests that the cytokine profile differs by anatomic site in the lower genital tract. Unfortunately, we did not obtain cellular pellets of VL and EL samples to further account for differences in sampling volume. We found the reduction of two pro-inflammatory cytokines IL-1β and IL-8 in endometrial lavage samples after N-9 exposure in the setting of no epithelial disruption on histology. This observation may reflect more subtle toxicity that was not observed by gross histology. Alternatively, although the number of washings required to obtain 2 mL of endometrial lavage fluid was 2 for the majority of cycles, it is possible that variable numbers of washings may "dilute" cytokines. Changes in cytokine/chemokine profile in the human endometrium after N-9 exposure have also been documented by two ex vivo studies. The first of these used ex vivo human endometrial explants and also demonstrated falls in pro-inflammatory measures IL-1β, TNF-α, and IL-8 after N-9 exposure [13]. However, these observations may reflect cellular necrosis and a lack of inflammatory cells to produce cytokines after exposure to N-9. Similarly, an in vitro dual-chamber model using a human endometrial cancer cell line (HEC-1A) demonstrated that high doses of N-9 was associated with decreases in IL-8, MCP-1 and SLPI, but lower doses induced IL-8 [18]. Lower doses of N-9 also reduced secretion of the pro-inflammatory mediator MCP-1 and anti-inflammatory mediator SLPI. Although these data from Gali et al. support dose-dependent effects of N-9, the effect of lower doses on the immunomodulatory markers in their model is not entirely consistent. These early data support future studies assessing the in vivo response to vaginal products in both the vagina and endometrium, but careful consideration of study design to yield consistent, reproducible data is warranted.

The placebo exposure HEC was associated with significant increases in two proinflammatory chemokines (RANTES and IL-6) in the vagina. In the vagina, prior murine and ex vivo human vaginal epithelium studies have not identified cytokine changes after HEC exposure [16,19]. There have not been other previous in vivo human studies of the effect of HEC on these biomarkers. Our results need to be interpreted with caution. However, if replicated, this study suggests that HEC is not an "inert" gel and may not be the optimal "placebo" control in future studies.

Finally, endometrial histology by light microscopy was not a sensitive test for detecting differences in the endometrium after exposure to the test conditions. Histology was evaluated because prior work documented rapid movement of N-9 into the upper female reproductive tract after vaginal insertion [20]. Further, mouse and human explant models show histological changes with N-9 exposure [13,21]. In this study, light microscopy

detected no significant histologic changes or patterns of inflammatory cells in the endometrium. Potential future directions include examining these samples by electron microscopy.

In conclusion, this trial demonstrated that cytokines and chemokines are altered by exposure to Gynol II and HEC, but the observed patterns of change are not entirely consistent with prior reports. Overall, in vivo studies of these molecular markers in the lower genital tract have been few with small sample sizes. Given limited evidence, more human data will be needed to determine whether biomarkers of inflammatory response are good surrogates of microbicide and contraceptive safety. Specifically, the methodology for obtaining vaginal specimens ought to be more uniform and future work may benefit from universal controls. Finally, future work with larger sample sizes is needed to determine how these changes relate to infection susceptibility and their utility in clinical studies.

Acknowledgments

References

1. Roddy RE, Cordero M, Cordero C, Fortney JA. A dosing study of nonoxynol-9 and genital irritation. Int J STD AIDS. 1993; 4:165–170. [PubMed: 8391856]

2. Stafford MK, Ward H, Flanagan A, et al. Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. J Acquir Immune Defic Syndr Hum Retrovirol. 1998; 17:327–331. [PubMed: 9525433]

3. Harwood B, Meyn LA, Ballagh SA, Raymond EG, Archer DF, Creinin MD. Cervicovaginal colposcopic lesions associated with 5 nonoxynol-9 vaginal spermicide formulations. Am J Obstet Gynecol. 2008; 198:32 e1–32 e7. [PubMed: 18166301]

4. Fichorova RN, Tucker LD, Anderson DJ. The molecular basis of nonoxynol-9-induced vaginal inflammation and its possible relevance to human immunodeficiency virus type 1 transmission. J Infect Dis. 2001; 184:418–428. [PubMed: 11471099]

5. Anderson DJ, Williams DL, Ballagh SA, et al. Safety analysis of the diaphragm in combination with lubricant or acidifying microbicide gels: effects on markers of inflammation and innate immunity in cervicovaginal fluid. Am J Reprod Immunol. 2009; 61:121–129. [PubMed: 19143675]

6. Keller MJ, Guzman E, Hazrati E, et al. PRO 2000 elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity. AIDS. 2007; 21:467–476. [PubMed: 17301565]

7. Van Damme L, Ramjee G, Alary M, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. Lancet. 2002; 360:971–977. [PubMed: 12383665]

8. World Health Organization. Update 2004. Geneva: WHO; 2004. Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products.

9. Li TC, MacKenna A, Roberts R. The techniques and complications of out-patient uterine washing in the assessment of endometrial function. Hum Reprod. 1993; 8:343–346. [PubMed: 8473445]

10. Fichorova RN, Bajpai M, Chandra N, et al. Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicidal contraceptives. Biol Reprod. 2004; 71:761–769. [PubMed: 15128598]

11. Iqbal SM, Kaul R. Mucosal innate immunity as a determinant of HIV susceptibility. Am J Reprod Immunol. 2008; 59:44–54. [PubMed: 18154595]

12. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev. 2005; 206:306–335. [PubMed: 16048557]

13. Jain JK, Li A, Minoo P, Nucatola DL, Felix JC. The effect of nonoxynol-9 on human endometrium. Contraception. 2005; 71:137–142. [PubMed: 15707564]

14. Lubin JH, Colt JS, Camann D, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004; 112:1691–1696. [PubMed: 15579415]

15. Galen BT, Martin AP, Hazrati E, et al. A comprehensive murine model to evaluate topical vaginal microbicides: mucosal inflammation and susceptibility to genital herpes as surrogate markers of safety. J Infect Dis. 2007; 195:1332–1339. [PubMed: 17397004]

16. Trifonova RT, Pasicznyk JM, Fichorova RN. Biocompatibility of solid-dosage forms of anti-human immunodeficiency virus type 1 microbicides with the human cervicovaginal mucosa modeled ex vivo. Antimicrob Agents Chemother. 2006; 50:4005–4010. [PubMed: 17030562]

17. Fichorova RN, Richardson-Harman N, Alfano M, et al. Biological and technical variables affecting immunoassay recovery of cytokines from human serum and simulated vaginal fluid: a multicenter study. Anal Chem. 2008; 80:4741–4751. [PubMed: 18484740]

18. Gali Y, Arien KK, Praet M, et al. Development of an in vitro dual-chamber model of the female genital tract as a screening tool for epithelial toxicity. J Virol Methods. 165:186–197. [PubMed: 20138087]

19. Cone RA, Hoen T, Wong X, Abusuwwa R, Anderson DJ, Moench TR. Vaginal microbicides: detecting toxicities in vivo that paradoxically increase pathogen transmission. BMC Infect Dis. 2006; 6:90. [PubMed: 16740164]

20. Barnhart KT, Stolpen A, Pretorius ES, Malamud D. Distribution of a spermicide containing Nonoxynol-9 in the vaginal canal and the upper female reproductive tract. Hum Reprod. 2001; 16:1151–1154. [PubMed: 11387285]

21. Dayal MB, Wheeler J, Williams CJ, Barnhart KT. Disruption of the upper female reproductive tract epithelium by nonoxynol-9. Contraception. 2003; 68:273–279. [PubMed: 14572891]
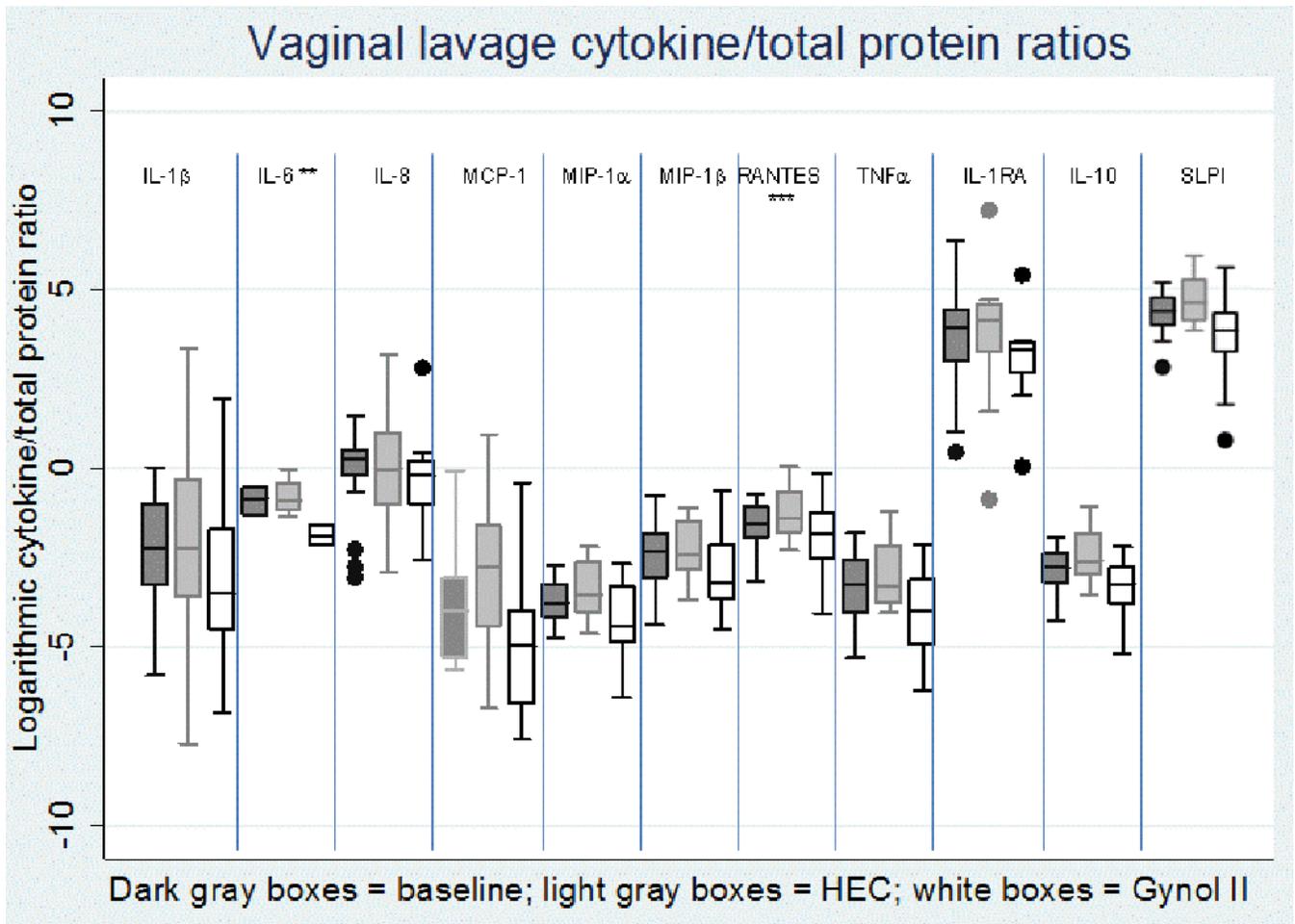
Figure 1*. Comparisons ** of vaginal cytokine/total protein ratios by exposure
* Box-Plots: Boxes depict the median (middle horizontal line), 25th percentile (lower horizontal border) and 75th percentile (higher horizontal border). Whiskers (distal ends of vertical lines) depict 5th and 95th percentiles.
** Wilcoxon signed-ranktests. For MCP-1, MIP-1α, MIP-1β TNFα, IL-10, measurements below lowest point on standard curve; no formal statistical comparisons were performed. For IL-1RA and SLPI, proportion of measurements above the highest point on the standard curve; no formal statistical comparison were performed.
*** RANTES (p = 0.055) and IL-6 (0.04) levels were significantly higher in HEC cycles than at Baseline
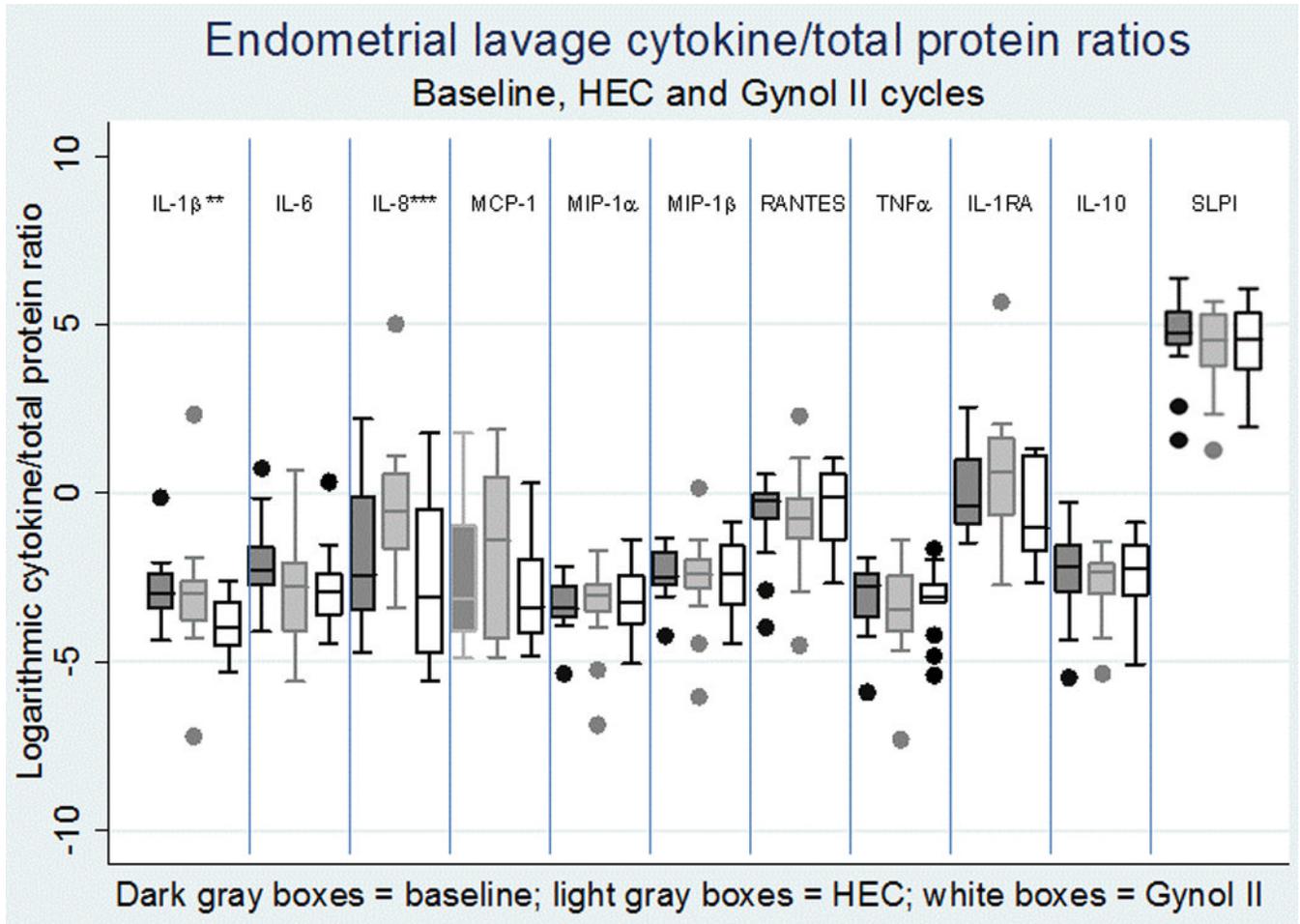
Figure 2*. Comparisons** of endometrial cytokine/total protein ratios by exposure

* Box-Plots: Boxes depict the median (middle horizontal line), 25th percentile (lower horizontal border) and 75th percentile (higher horizontal border). Whiskers (distal ends of vertical lines) depict 5th and 95th percentiles.

** Wilcoxon signed-ranktests. For MCP-1, MIP-1α, MIP-1β TNF α, IL-10, measurements below lowest point on standard curve; no formal statistical comparisons were performed. For IL-6, RANTES and IL-1RA, a proportion of measurements were above the highest point on the standard curve; no formal statistical comparison were performed.

*** IL-1β (p=0.003) and IL-8 (p=0.025) were significantly lower with Gynol II exposure than at Baseline

Table 1

Characteristics of colposcopic lesions[*], [**] among treatment cycles

	Baseline lesions (n=15)	HEC lesions (n=5)	Gynol II lesions (n=18)
Epithelial disruption			
Intact	12 (80%)	3 (60%)	15 (83%)
Superficial disruption	3 (20%)	2 (40%)	3 (17%)
Deep disruption	0	0	0
Diagnosis			
Erythema	7 (46%)	2 (40%)	10 (55%)
Petechiae	5 (33%)	1 (20%)	3 (17%)
Abrasion	1 (7%)	1 (20%)	1 (6%)
Other	1 (7%)	1 (20%)	2 (11%)
Location			
External genitalia	7 (46%)	2 (40%)	7 (39%)
Cervix	7 (46%)	2 (40%)	11 (61%)
Vagina	1 (7%)	1 (20%)	0
Color			
Normal	0	0	0
Slightly red	6 (39%)	2 (40%)	10 (56%)
Red	8 (54%)	3 (60%)	6 (33%)
White	1 (7%)	0	2 (11%)
Blood vessels			
Intact	8 (54%)	2 (40%)	12 (67%)
Disrupted	7 (46%)	3 (60%)	6 (33%)
Size			
<5 mm	7 (46%)	3 (60%)	5 (28%)
5–10 mm	4 (27%)	2 (40%)	5 (28%)
>10 mm	4 (27%)	0	8 (44%)

[*] 18 Baseline, 14 HEC and 14 Gynol II colposcopies were performed.

[**] McNemar's tests used to compare lesions by exposure group

Table 2

Vaginal lavage: comparisons[*] of cytokine/total protein ratios among treatment cycles

Cytokine	Baseline (n=18) median (range)	HEC (n=14) median (range)	Gynol II (n=14) median (range)
IL-1β	0.1 (0.003–1.0)	0.11 (0.0004–28.7)	0.03 (0.0009–6.98)
IL-6[**]	0.14 (0.06–0.59)	0.30 (0.06–0.96)	0.08 (0.01–0.25)
IL-8	1.27 (0.13–4.3)	0.97 (0.16–22.5)	0.81 (0.09–16.6)
MCP-1	0.018 (0.004–0.92)	0.065 (0.001–2.53)	0.007 (0.0005–0.66)
MIP-1α	0.023 (0.008–0.067)	0.029 (0.01–0.11)	0.012 (0.002–0.069)
MIP-1β	0.098 (0.012–0.46)	0.09 (0.025–0.33)	0.041 (0.01–0.53)
RANTES[**]	0.21 (0.04–0.49)	0.24 (0.10–1.03)	0.16 (0.02–0.87)
TNF-α	0.04 (0.005–0.17)	0.02 (0.002–0.12)	0.04 (0.02–0.3)
IL-1RA	52.0 (1.56–584)	61.3 (0.42–1365.5)	27.5 (1.04–221.3)
IL-10	0.06 (0.01–0.14)	0.074 (0.03–0.34)	0.04 (0.005–0.11)
SLPI	76.8 (15.3–176.9)	100.4 (44.3–379.6)	44.7 (1.84–274.7)

[*] Wilcoxon signed-rank tests. For MCP-1, MIP-1α, MIP-1β, TNFα, IL-10, measurements below lowest point on standard curve; no formal statistical comparisons were performed. For IL-1RA and SLPI, a proportion of measurements above the highest point on the standard curve; no formal statistical comparisons were performed.

[**] RANTES (p=0.055) and IL-6 (0.04) levels were significantly higher in HEC cycles than at Baseline

Table 3

Characteristics of endometrial biopsy specimens among treatment cycles[*]

	Baseline biopsies (n=18)	Gynol II biopsies (n=14)	HEC biopsies (n=14)
Discontinuity of uterine epithelium	0	0	0
Hemorrhage	0	0	0
Regeneration of uterine epithelium			
Proliferative	16 (89%)	12 (86%)	12 (86%)
Secretory	1 (5%)	0	1 (7%)
Interval	1 (5%)	1 (7%)	0
Not sufficient	0	1 (7%)	1 (7%)
Necrosis of endometrial stroma or epithelium	0	0	0
Polymorphonuclear leukocytes (PMNs) and other inflammatory cells			
Absent	5 (28%)	3 (21%)	1 (7%)
Few	10 (56%)	8 (57%)	12 (86%)
Moderate	3 (16%)	3 (21%)	1 (7%)
Many	0	0	0
Luminal debris in glands	0	0	0

[*] McNemar's test and Test of Symmetry used to compare lesions by exposure group

Table 4

Endometrial lavage: comparisons[*] of cytokine/total protein ratios among treatment cycles

Cytokine	Baseline (n=18) median (range)	HEC (n=14) median (range)	Gynol II (n=14) median (range)
IL-1β	0.05 (0.01–0.85)	0.05 (0.0007–10.3)	0.02 (0.005–0.08)
IL-6	0.10 (0.017–2.02)	0.06 (0.004–1.96)	0.053 (0.011–1.39)
IL-8	0.22 (0.02–8.9)	(0.53 (0.11–147.6)	0.09 (0.02–6.0)
MCP-1	0.044 (0.007–5.93)	0.25 (0.008–6.44)	0.036 (0.008–1.32)
MIP-1α	0.032 (0.005–0.11)	0.048 (0.001–0.18)	0.039 (0.006–0.25)
MIP-1β	0.083 (0.014–0.26)	0.089 (0.002–1.14)	0.09 (0.012–0.41)
RANTES	0.79 (0.055–1.74)	0.54 (0.011–9.76)	0.88 (0.069–2.83)
TNF-α	0.064 (0.003–0.15)	0.032 (0.0007–0.25)	0.05 (0.005–0.19)
IL-1RA	0.69 (0.23–12.8)	1.9 (0.065–286.7)	0.35 (0.069–3.74)
IL-10	0.11 (0.004–0.74)	0.098(0.005–0.23)	0.11 (0.006–0.42)
SLPI	117.5 (4.61–598.2)	94.3 (3.5–298.9)	97.8 (6.95–439.3)

[*] Wilcoxon signed-rank tests. For MCP-1, MIP-1α, MIP-1β, TNF α, IL-10 and SLPI, measurements below lowest point on standard curve; no formal statistical comparisons were performed. For IL-6, RANTES and IL-1RA, a proportion of measurements were above the highest point on the standard curve; no formal statistical comparisons were performed.

[**] IL-1β (p=0.003) and IL-8 (p=0.025) were significantly lower with Gynol II exposure than at Baseline