Immunologic evaluation of the endometrium with a levonorgestrel intrauterine device in solid organ transplant women and healthy controls

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Abstract

Objective: The objective was to describe the endometrial milieu of stable transplant patients and healthy women before and after levonorgestrel intrauterine system (LNG-IUS) insertion.

Study design: Women between 18 and 45 years of age desiring LNG-IUS insertion were enrolled with a 2:1 ratio of healthy to stable solid organ transplant patients. The first visit entailed a blood draw, uterine lavage and endometrial biopsy followed by LNG-IUS insertion. Follow-up visit involved a repeat serum draw, uterine lavage and endometrial biopsy. Cytokine levels were measured in the uterine lavage and serum by quantifying inflammatory biomarkers. Immunohistochemistry staining was performed on the endometrial tissue to measure macrophage levels. Statistical analysis included a nonparametric analysis that compared medians of the marker levels before and after intrauterine device (IUD) insertion within the group and between the two groups.

Results: Sixteen participants completed the study: 5 solid organ transplant patients and 11 healthy patients. For the serum, there were no marked changes in the cytokines or soluble receptor levels in either group after IUD insertion. However, in the uterine lavage, there was an increase in cytokine levels post-IUD insertion for both healthy and transplant women. For the endometrial tissue, there was evidence of macrophage activity in both groups after device insertion.

Conclusions: This pilot study investigated the uterine environment of the transplant patient population. Findings have pointed to the strong local inflammatory response following LNG-IUS insertion for the transplant recipients. In addition, these preliminary findings will help power a larger study that can investigate the safety and effectiveness of the IUD in this patient population.

Implications: Findings from this pilot study suggest that the IUD is inducing a local inflammatory reaction in the uterus of the transplant patient as in the healthy control. A larger study can build on these preliminary results to pursue the efficacy and safety of IUD use among solid organ transplant patients.

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Keywords: Intrauterine device; Immunology; Transplant; Endometrium

1. Introduction

Limited data exist on the use of intrauterine contraception in women who require chronic immunosuppression, such as solid organ transplant (SOT) recipients. Pregnancy during an immunosuppressed state has the potential for serious morbidity, secondary to worsening of maternal health conditions, which directly affects fetal and maternal health [1]. Providing reliable, long-term contraception for these women is important for the morbidity associated with unplanned pregnancy. However, practitioners are reticent to provide intrauterine devices (IUDs) for SOT recipients in the absence of studies revealing not only the safety and effectiveness of IUDs but their mechanism of action and effects on immune/inflammatory biomarkers in this select group.

Investigations on the IUD’s mechanism of action have centered on the healthy, immunocompetent endometrium. These studies revolve around the local inflammatory reaction with the IUD. Macrophages, neutrophils and plasma cells...
have played the main roles in this inflammatory process [2–6]. In turn, the macrophages produce various cytokines that further trigger the inflammatory response. Therefore, macrophages and cytokines are our players of interest in this study.

Macrophages are present in the endometrium with an active IUD in place [4,7]. The levonorgestrel (LNG)-IUD remodels the endometrium with stromal cell decidualization and has been associated with the marked presence of neutrophils and macrophages. [8] An earlier study has also highlighted the endometrial changes with elevated leukocytes and tissue IL-8 levels 1 month after LNG-IUD insertion [7].

Another approach to studying the endometrial environment is investigating the infiltrates surrounding and attaching to the IUD. One study demonstrated attachment of leukocytes to the IUD, and these cells produced prostaglandins, contributing to the inflammatory response [9]. Although this study focused on the inert IUD, there have been subsequent studies following the same idea by performing a uterine lavage to examine cells on the device [10]. Given these findings, we quantified biomarker levels through uterine lavage and endometrial sampling.

There were several studies that informed the selection of cytokines in this study. One study reported the endometrial tissue and fluid in postmenopausal women with an IUD and found that there was an increase in IL-6 and TNF-α in the endometrium with the IUD in place [10]. Another noted IL-1α, IL-1β and IL-6 as products of the macrophages and T lymphocytes in their evaluation of endometrium [3]. We were also curious to investigate the macrophage subtypes 1 and 2 (M1 and M2) due to their distinct properties. M1s are proinflammatory cells that produce proinflammatory cytokines (IL-6, TNF-α, IL-23), whereas the M2s have anti-inflammatory properties, producing IL-10 [11].

Before investigating the safety and effectiveness of the IUD in the SOT group, there has to be an understanding of the IUD’s effects on the endometrium in this population. The main objective of this study was to investigate the uterine environment of both the transplant patient and healthy controls by examining the histology and inflammatory markers, focusing on the macrophage with its subtypes. Our hypotheses were the following: (a) Transplant patients would display a smaller difference in endometrial inflammatory markers after IUD insertion compared to the healthy controls, and (2) proinflammatory macrophages (type 1 macrophages) would predominate in the healthy women, while anti-inflammatory macrophages (type 2 macrophages) would predominate in the transplant patient.

2. Materials and methods

2.1. Study participants

We recruited women between the ages of 18 to 45 years interested in the LNG-IUD as a birth control method. Inclusion criteria for the healthy controls included no immunosuppressive drug use in the past 3 months and no contraindications to IUD insertion. Exclusion criteria were current pregnancy, current pelvic infection and known to be HIV positive. For the SOT patient, we applied similar inclusion/exclusion criteria in addition to the criterion of being stable on her immunosuppressant regimen. We aimed for a case: control ratio of 1:2. After establishing eligibility, we reviewed and signed appropriate consents with the participants. We obtained the Institutional Review Board approval prior to study initiation.

2.2. Study visits

The study consisted of two visits. Visit 1 for both the SOT and healthy patients started with a blood draw by using a BD Vacutainer Serum collector. To exclude those with any local infections, we performed gonorrhea and chlamydia (GC/CT) cultures on all patients prior to IUD insertion per clinic protocol. The SOT patients had a prescreening visit that entailed contraceptive counseling and STD screening including HIV and rapid plasma reagin testing, GC/CT cultures and wet mounts. Prior to IUD insertion, we performed a uterine lavage followed by an endometrial biopsy (EMB). For the uterine lavage, we inserted a saline sonohysterography catheter through the cervix. We then pushed 3 ml of sterile normal saline through the catheter and withdrew the wash contents using a syringe. We performed the lavage under ultrasound guidance to ensure proper placement of the catheter in the uterine cavity. We avoided placement in the cervical canal to minimize withdrawal of cervicovaginal secretions. In addition, the reason to perform the lavage prior to the EMB was to minimize blood contamination of the wash that an EMB could induce if performed first. For the EMB, we used a Pipelle to withdraw endometrial tissue with a maximum of three passes.

The study participant returned 4–6 weeks later for visit 2. The participants completed a five-question exit survey at this visit. We took a repeat blood draw. Then we performed a uterine lavage and endometrial biopsy while keeping the IUD in place.

2.3. Immunoassay of cytokines and soluble receptors in serum and lavage fluids

We transported serum and lavage samples to the lab within 2 h of collection. We centrifuged the fluid sample and then subaliquoted the supernatant and serum into microtubes.

The assays used in this study were two multiplexed immunometric assay panels (Luminex platform) for cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α and IFN-γ) and for soluble receptors (sIL-2R, sIL-6R, sTNFR2 and sCD14) (R&D Systems). The Luminex xMAP system uses spectrally addressed bead sets, each of which is conjugated with a different capture monoclonal antibody specific for a given target molecule. The antibody-conjugated beads react with the sample and a secondary detection antibody to form a
capture sandwich immunoassay. After completion of the assay, the Bio-Plex 200 Luminex array reader (BioRad) quantifies the amount of each analyte in the assay solution. The intraassay variations were <12%. The interassay coefficients of variance were the following: 19.95 (IL-6), 9.32 (IL-8), 27.28 (IL-10), 17.81 (TNF-α), 25.69 (IFN-γ), 25.26 (IL-1β), 6.86 (CD14), 4.88 (TNF), 3.73 (IL-6) and 7.96 (IL-2). Reporting of the cytokines was in pg/ml.

2.4. Immunohistochemistry of endometrial tissue

For the endometrial tissue samples, we prepared hematoxylin and eosin stains for pathology interpretation. We studied several markers for the immunohistochemical (IHC) staining. M1 macrophages phenotypically express CD16 and CD86, whereas M2s express arginase and CD206.[11,12] We used the lowest limit of detection (LLOD) as a measure of assay sensitivity. LLODs for these analytes ranged from 0.31 to 24.93. The analytes were well above the LLODs, so we have concluded that since the analytes were readily detectable, the assay was sufficiently sensitive.

2.5. Statistical analysis

We performed a nonparametric analysis since the data distribution did not follow the normal distribution and was nonsymmetric. As a result, we compared medians on the original scale. We computed the p values for comparing the markers levels before and after IUD insertion within a group using the nonparametric Wilcoxon signed rank test. We used the Wilcoxon rank sum method to compare the change between the two groups.

3. Results

3.1. Total participants

We enrolled a total of 17 participants in the study: 5 SOT patients and 12 healthy patients. However, 1 of the healthy participants was lost to follow-up, giving 11 healthy patients with complete data.

Table 1 provides the participants’ characteristics. The age range for the healthy and transplant patients were similar, with mean age of 27.6 years for the healthy and 29.4 years for the transplant patients. Majority of the candidates were nulliparous. In the healthy group, majority were white, while in the transplant group, majority were minorities.

The type of SOTs varied. One patient had bilateral renal transplants, two were lung transplant patients, one had a heart transplant, and one patient was both a heart and renal transplant recipient. Alongside this variation was the mix of medication regimens. All five patients were on tacrolimus, three were on prednisone, and two were on CellCept. Three were also on antibiotics.

3.2. Serum concentration of cytokines and soluble receptors

The concentrations of the serum cytokines and soluble receptors were variable. Given the nonsymmetric data set, we calculated the medians for each marker before and after IUD placement. The median change reflected the change in the inflammatory marker levels after IUD placement.

When inspecting the changes within the groups, the change in serum inflammatory markers went mostly downwards for the healthy group, while there was a small

<table>
<thead>
<tr>
<th>Markers</th>
<th>Healthy before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change [within-group p value] pg/ml (SE)</th>
<th>Transplant before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change [within-group p value] pg/ml (SE)</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>5.59 (1.06)</td>
<td>3.97 (1.49)</td>
<td>0.24 [97] (0.65)</td>
<td>5.96 (1.86)</td>
<td>9.57 (2.71)</td>
<td>1.78 [31] (1.36)</td>
<td>.28</td>
</tr>
<tr>
<td>IL-8</td>
<td>9.24 (9.15)</td>
<td>9.27 (3.45)</td>
<td>−0.72 [24] (6.21)</td>
<td>9.49 (2.69)</td>
<td>12.35 (7.90)</td>
<td>2.52 [31] (6.65)</td>
<td>.10</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.24 (0.54)</td>
<td>2.43 (0.42)</td>
<td>−0.10 [65] (0.43)</td>
<td>1.35 (1.41)</td>
<td>2.18 (1.22)</td>
<td>0 [63] (0.37)</td>
<td>.46</td>
</tr>
<tr>
<td>TNF-α</td>
<td>21.58 (2.24)</td>
<td>19.83 (2.55)</td>
<td>−1.33 [17] (1.59)</td>
<td>22.22 (6.70)</td>
<td>25.73 (7.14)</td>
<td>5.85 [06] (1.53)</td>
<td>.01</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.73 (0.81)</td>
<td>1.63 (0.59)</td>
<td>−0.25 [37] (0.63)</td>
<td>1.10 (0.93)</td>
<td>1.97 (1.34)</td>
<td>0.61 [13] (0.56)</td>
<td>.02</td>
</tr>
<tr>
<td>IL-1β</td>
<td>4.04 (0.64)</td>
<td>4.19 (0.67)</td>
<td>−0.03 [2] (0.24)</td>
<td>2.48 (1.83)</td>
<td>2.77 (2.09)</td>
<td>1.41 [31] (0.57)</td>
<td>.03</td>
</tr>
</tbody>
</table>

We used the lowest limit of detection (LLOD) as a measure of assay sensitivity. LLODs for these analytes ranged from 0.31 to 24.93. The analytes were well above the LLODs, so we have concluded that since the analytes were readily detectable, the assay was sufficiently sensitive.
increase in marker levels for the transplant group (all values >.5) (Tables 2 and 3). When comparing the marker changes between the two groups, there were three markers worth noting: TNF-α (p=.01), IFN-γ (p=.02) and IL-1β (p=.03) (Table 2). Fig. 1 provides a visual presentation of these findings.

3.3. Uterine lavage

We also measured intrauterine concentrations of the cytokines and soluble receptors. There was a marked increase in lavage fluid levels of IL-1β, IL-6, IL-8, IL-10 and TNF-α in the control group post-IUD insertion (Table 4). IFN-γ levels did not appear to increase. Similarly, the transplant patients showed increased post-IUD insertion levels of IL-1β, IL-6, IL-8 and TNF-α (p<.06-.13). The transplant group did not exhibit an increase in post-IUD insertion lavage levels of either IL-10 or IFN-γ. Additionally, the median levels of these inflammatory cytokines were generally lower in the transplant group than in the controls, although the fold increase was often comparable. For example, lavage median IL-6 levels post-IUD insertion were nearly twice as high in the control group as in the transplant group, but the fold increase in lavage IL-6 levels was 52 in the control group and >100 in the transplant group.

Lavage levels of the sIL2-R, TNF-R2, sCD14 and sIL-6R also increased post-IUD insertion both in transplant recipients and in controls (Table 5). Median lavage levels of these soluble receptors post-IUD insertion were generally similar to the levels seen in the transplant group when compared to the control group. In fact, the magnitude increases seen were greater in the transplant group than in the control group. For instance, the transplant group held a >40-fold increase in lavage median sCD14 levels, while the control group showed about a 3-fold increase in lavage sCD14.

3.4. Endometrial biopsy results (pathology and immunohistochemistry)

3.4.1. Pathology

A gynecologic pathologist reviewed the endometrial biopsy samples from the first visit to rule out abnormalities. None of the specimens showed hyperplasia or malignancy. Majority of the endometrium samples from healthy controls were in the proliferative phase. Three of the specimens had inactive endometrial glands with deciduaized stromal cells. Two specimens showed scant weakly secretory to inactive endometrium only. Majority of the transplant EMB specimens showed inactive endometrial glands with decidualized stromal cells or secretory phase endometrium.

3.5. IHC: M1 activity

M1 activity included markers that reflect a proinflammatory response. There was an increase in IHC staining for the following markers in both groups: CD 16 and IL-8 (p<.05 for healthy, p>.06-.13 for transplant) (Table 6). There was a decrease in IHC staining for both groups of CD 86, IFN-γ and TNF-α post-IUD insertion (Table 6). It is again noted that the decrease in IHC staining was blunted for the transplant group for the TNF-α and CD 86 when compared to the healthy group. For the IL-6 marker, the healthy group had a decrease in IHC staining post-IUD (p=.17), whereas the transplant group had a small increase in staining (p=1).

3.6. IHC: M2 activity

M2 activity included markers that reflect an anti-inflammatory state. An increase in IHC staining occurred post-IUD for the healthy group for the following M2 markers: arginase and CD 206 (Table 7). The trend was opposite for the transplant group where the IHC staining decreased post-IUD for these two markers (p values for all changes>.44–1).
IL-1α were any systemic inflammatory effects of the IUD. We included sCD14 since it is released by activated macrophages. Overall, it appeared that IUD insertion did not result in any marked changes in systemic inflammation, at least as detected by these biomarkers.

The uterine lavage results were interesting to note. The inflammatory markers markedly increased in both groups after IUD insertion, which was consistent with the notion that the IUD induces a strong local inflammatory effect. The only marker that had no median change in either group was IFN-γ, which is associated with T-cell activation but is not necessarily associated with inflammatory responses. We also noticed that the majority of the median changes in the lavage cytokine levels in the healthy group were larger compared to those in the transplant group. In particular, we observed an increase in the following markers for both groups: IL-6, IL-8, IL-10, TNF-α, IL-1β, sTNF-R2. This aligned well with the findings of Archer et al. of an increase of IL-6 and TNF-α after IUD placement in healthy, postmenopausal women [10].

Overall, there were marked increases in median lavage levels of most cytokines and soluble receptors in both the transplant and control groups. Therefore, transplant recipients clearly exhibited strong local inflammatory responses following insertion of the IUD.

We conducted immunohistochemistry staining of the endometrial biopsy specimens to discern the subtypes of macrophages with the IUD in place. The M2 markers (anti-inflammatory subtype) did not predominate in the transplant group as theorized. Arginase, CD 206 and IL-4 all (anti-inflammatory subtype) did not predominate in the transplant group as theorized. Arginase, CD 206 and IL-4 all

4. Discussion

The main objective of this pilot study was to describe the endometrial milieu of the SOT group. It also investigated the healthy endometrium by looking at an array of inflammatory markers rather than a single inflammatory marker. The other unique feature to this project was the combination of endometrial biopsy, serum and uterine washings for specimen collection. The results of these entities provided additional information that may inform future studies on the endometrial milieu of the SOT group. It also investigated the healthy endometrium by looking at an array of inflammatory markers.

The reason for testing the serum was to determine if there were any systemic inflammatory effects of the IUD. Essentially, there were no notable changes in these inflammatory markers pre- and post-IUD insertion in both groups. Most of the markers were proinflammatory markers. We also measured soluble receptors as they are found at higher levels and are more readily detectable than cytokines, which are often found at low levels in biological specimens. We included sCD14 since it is released by activated macrophages. Overall, it appeared that IUD insertion did not result in any marked changes in systemic inflammation, at least as detected by these biomarkers.

For IL-4, both groups showed a decrease in percentage staining after IUD placement. The decrease was more marked for the transplant group. The IL-10 staining was 100% before and after IUD placement for both the healthy and transplant endometrium.

3.7. Exit survey

In the exit survey, the common side effect was irregular bleeding. For the healthy patients, 8 of the 11 respondents noted an increase in their bleeding since the IUD placement. For the transplant patients, two of the five respondents noted an increase, and the other three noted a decrease in their bleeding since IUD placement.

The transplant patients also responded that since receiving their IUD, their medical condition either had remained stable or had improved. When asked about their satisfaction towards the IUD, all transplant patients replied as “satisfied” or “very satisfied.” For the healthy patients, 10 of the 11 respondents were satisfied or very satisfied with their IUD. The one person who had reported “neutral” requested for its removal at the end of her follow-up study.

Table 4
Lavage cytokine levels after IUD placement

<table>
<thead>
<tr>
<th>Markers</th>
<th>Healthy before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change pg/ml (SE) [p value]</th>
<th>Transplant before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change pg/ml (SE) [p value]</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>6.8 (5.3)</td>
<td>352.7 (236)</td>
<td>352.1 (235) [.002]</td>
<td>1.8 (4.6)</td>
<td>198 (43.3)</td>
<td>194 (4.07) [.06]</td>
<td>.28</td>
</tr>
<tr>
<td>IL-8</td>
<td>46.5 (55)</td>
<td>900.2 (1398)</td>
<td>6745 (1397) [.001]</td>
<td>40.5 (147.4)</td>
<td>2164.7 (608)</td>
<td>2112.0 (504.5) [.06]</td>
<td>.19</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.44 (0.30)</td>
<td>2.26 (0.89)</td>
<td>1.77 (0.95) [.02]</td>
<td>0.56 (0.14)</td>
<td>0.56 (0.37)</td>
<td>0.16 (0.38) [.31]</td>
<td>.23</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.41 (0.93)</td>
<td>16.41 (8.61)</td>
<td>15.14 (8.64) [.005]</td>
<td>0.41 (0.93)</td>
<td>8.14 (2.19)</td>
<td>5.02 (2.14) [.13]</td>
<td>.19</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.29 (0.38)</td>
<td>0.50 (0.12)</td>
<td>0 (0.36) [.71]</td>
<td>0.52 (0.13)</td>
<td>0.68 (0.25)</td>
<td>0.00 (0.26) [.50]</td>
<td>.33</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.55 (0.91)</td>
<td>180.6 (103.1)</td>
<td>180.3 (103.4) [.002]</td>
<td>0.56 (3.90)</td>
<td>40.9 (66.2)</td>
<td>40.3 (62.9) [.06]</td>
<td>.46</td>
</tr>
</tbody>
</table>

Table 5
Lavage soluble receptor levels after IUD placement

<table>
<thead>
<tr>
<th>Markers</th>
<th>Healthy before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change pg/ml (SE) [p value]</th>
<th>Transplant before IUD pg/ml (SE)</th>
<th>After IUD pg/ml (SE)</th>
<th>Median change pg/ml (SE) [p value]</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 14</td>
<td>25,590 (10,798)</td>
<td>76,147 (17,237)</td>
<td>42,655 (22,927) [.07]</td>
<td>1851 (31,341)</td>
<td>76,717 (24,531)</td>
<td>26,783 (19,455) [.13]</td>
<td>.95</td>
</tr>
<tr>
<td>sTNF-R</td>
<td>182 (62.9)</td>
<td>956 (208)</td>
<td>424 (209) [.003]</td>
<td>25.5 (84.5)</td>
<td>590 (166)</td>
<td>482 (121) [.06]</td>
<td>.53</td>
</tr>
<tr>
<td>sIL-6R</td>
<td>1083 (357)</td>
<td>1949.6 (445)</td>
<td>1035 (733) [.28]</td>
<td>161 (686)</td>
<td>1396 (285)</td>
<td>634 (463) [.31]</td>
<td>.87</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>18.8 (8.5)</td>
<td>65.5 (26)</td>
<td>42.7 (31.4) [.12]</td>
<td>0.9 (15)</td>
<td>104 (37.5)</td>
<td>76 (27.8) [.13]</td>
<td>.78</td>
</tr>
</tbody>
</table>
Using the Definiens’ Tissue Studio, we performed morphometric analysis to determine the percentage of positively stained cells. Briefly, using the predefined nuclear detection module or cell simulation module (for cytoplasmic staining) and classification tool, positive and negative cells within each tissue section were identified. Thresholds were set to classify hematoxylin staining for nuclei and DAB staining for positive nuclei or cytoplasm.

As for the M1 markers (proinflammatory), they did not predominate in the healthy endometrium. CD 16 and IL-8 were the only markers that increased in the healthy endometrium. Additional results from this study included the high satisfaction and acceptability of the IUD as their form of contraception for the SOT patients. The irregular bleeding and cramping were common effects after IUD placement in both groups. In their exit survey, the transplant patients also noted no complications in their disease since the IUD placement.

There were several limitations to this study. It was a small study where recruitment of the transplant patients was difficult, as recruitment was impaired by insurance and logistical barriers. Another limitation was the nonuniformity of immunosuppressant regimens given that the SOT participants had different types of transplants. The follow-up time was not long term as well. In addition, when using a fluid wash, studies have shown highly variable concentrations of cytokines. [15,16] We did not adjust or correct for total protein concentration. However, to minimize variability, we used a fixed volume of saline for the lavages and used the same collection protocol for all cases. These limitations will help inform the next steps in formulating a larger study investigating this same patient population.

In conclusion, our study has peered into the endometrium of the SOT patient. Although this pilot study could not discern significant differences between the healthy and transplant endometrium with an LNG-IUD in place, it at least allowed us to recognize certain patterns that align with prior studies. Serum results reflected that the LNG-IUD does not exhibit a systemic inflammatory effect, whereas the uterine lavage results correlated to the idea that the IUD is inducing a local inflammatory effect. In addition, for the lavage, the inflammatory marker increase may be blunted in the transplant group compared to the healthy controls, but there was still a marked increase in local inflammation seen in the transplant recipients. These preliminary findings will help power a larger study that can look into the safety and effectiveness of the IUD in this special patient population. This study has, at the very least, started us on the pathway of reassurance that the IUD is a feasible contraceptive option for the transplant patient.

### Acknowledgments

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The authors thank the following people who provided help in various aspects of this detailed project: Dr. Sharon Achilles, Dr. David Archer, Dr. Mitchell Creinin, Wen Ching Tran and Nessa Riazi. We also would like to acknowledge the invaluable help and collaboration with the transplant teams, including the coordinators: Stephanie Fraschilla, Nidhi Ralhan, Joselina Fuentes, Linda Rangel, Ilana Berg and Jonathan Smith.

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**Table 6**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Healthy before IUD % (SE)</th>
<th>After IUD % (SE)</th>
<th>Median change % (SE) [p value]</th>
<th>Transplant before IUD % (SE)</th>
<th>After IUD % (SE)</th>
<th>Median change % (SE) [p value]</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 16</td>
<td>21.7 (7.8)</td>
<td>51.2 (7.2)</td>
<td>28.5 (11.4) [0.014]</td>
<td>15 (16.6)</td>
<td>36.9 (10.4)</td>
<td>21.6 (6.6) [0.13]</td>
<td>.46</td>
</tr>
<tr>
<td>CD 86</td>
<td>3.2 (1.1)</td>
<td>0.9 (1.7)</td>
<td>−1.4 (2.0) [0.28]</td>
<td>3.3 (1.0)</td>
<td>3.2 (0.8)</td>
<td>−0.8 (0.8) [0.63]</td>
<td>.46</td>
</tr>
<tr>
<td>IL-6</td>
<td>14.2 (5.2)</td>
<td>7.4 (2.1)</td>
<td>−5.0 (4.4) [0.17]</td>
<td>21 (8.4)</td>
<td>21.9 (7.5)</td>
<td>0.9 (9.6) [1.0]</td>
<td>.87</td>
</tr>
<tr>
<td>IL-8</td>
<td>2.7 (5)</td>
<td>15.8 (9.4)</td>
<td>3.1 (10.2) [0.03]</td>
<td>2.0 (1.2)</td>
<td>25.4 (10.2)</td>
<td>24 (10.2) [0.06]</td>
<td>.40</td>
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<td>IFN-γ</td>
<td>76.2 (3.9)</td>
<td>57.8 (7.6)</td>
<td>−2.2 (8.6) [0.03]</td>
<td>84.6 (10.4)</td>
<td>58.5 (7.6)</td>
<td>−5.2 (8.6) [0.13]</td>
<td>.46</td>
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<tr>
<td>TNF-α</td>
<td>41.1 (8.6)</td>
<td>23.3 (7.9)</td>
<td>−25.6 (11.1) [0.21]</td>
<td>50.1 (17.6)</td>
<td>42.2 (16.6)</td>
<td>−3.7 (13.5) [0.44]</td>
<td>.53</td>
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</tbody>
</table>

**Table 7**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Healthy before IUD % (SE)</th>
<th>After IUD % (SE)</th>
<th>Median change % (SE) [p value]</th>
<th>Transplant before IUD % (SE)</th>
<th>After IUD % (SE)</th>
<th>Median change % (SE) [p value]</th>
<th>Between-group p value</th>
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</thead>
<tbody>
<tr>
<td>Arginase</td>
<td>10.2 (2.6)</td>
<td>12.9 (2.8)</td>
<td>3.0 (4.2) [0.83]</td>
<td>11.7 (6.1)</td>
<td>20.3 (6.3)</td>
<td>−3.4 (6.1) [0.81]</td>
<td>.69</td>
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<tr>
<td>CD 206</td>
<td>12.1 (6.8)</td>
<td>13.8 (5.3)</td>
<td>4.6 (9.9) [1.0]</td>
<td>16.8 (16.7)</td>
<td>13 (4.6)</td>
<td>−6.7 (17.6) [0.44]</td>
<td>.40</td>
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<tr>
<td>IL-4</td>
<td>64.8 (7.2)</td>
<td>53.3 (4.3)</td>
<td>−11.5 (8.1) [0.37]</td>
<td>71 (10)</td>
<td>50.1 (6.9)</td>
<td>−20.9 (10.3) [0.19]</td>
<td>.40</td>
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<td>IL-10 0*</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

* Analysis not conducted.
References


