Short- and long-term influence of the levonorgestrel-releasing intrauterine system (Mirena®) on vaginal microbiota and Candida

Gilbert Gerard Ghislain Donders,1,2,3, G. Bellen,1 Kateryna Ruban1 and Ben Van Bulck4

Abstract

Background. Recurrent vulvovaginal infections are a frequent complaint in young women in need of contraception. However, the influence of the contraceptive method on the course of the disease is not well known.

Aim. To investigate the influence of the levonorgestrel-releasing intrauterine-system (LNG-IUS) on the vaginal microflora.

Methods. Short-term (3 months) and long-term (1 to 5 years) changes of vaginal microbiota were compared with pre-insertion values in 252 women presenting for LNG-IUS insertion. Detailed microscopy on vaginal fluid was used to define lactobacillary grades (LBGs), bacterial vaginosis (BV), aerobic vaginitis (AV) and the presence of Candida. Cultures for enteric aerobic bacteria and Candida were used to back up the microscopy findings. Fisher’s test was used to compare vaginal microbiome changes pre- and post-insertion.

Results. Compared to the pre-insertion period, we found a temporary worsening in LBGs and increased rates of BV and AV after 3 months of LNG-IUS. After 1 and 5 years, however, these changes were reversed, with a complete restoration to pre-insertion levels. Candida increased significantly after long-term carriage of LNG-IUS compared to the period before insertion [OR 2.0 (CL95 1.1–3.5), P=0.017].

Conclusions. Short-term use of LNG-IUS temporarily decreases lactobacillary dominance, and increases LBG, AV and BV, but after 1 to 5 years these characteristics return to pre-insertion levels, reducing the risk of complications to baseline levels. Candida colonization, on the other hand, is twice as high after 1 to 5 years of LNG-IUS use, making it less indicated for long-term use in patients with or at risk for recurrent vulvovaginal candidosis.

INTRODUCTION

Disturbed vaginal microflora, with decreased numbers of lactobacilli, leading to bacterial vaginosis (BV) and/or aerobic vaginitis (AV), is a risk factor for the acquisition of sexually transmitted infections (STIs) [1, 2] and for preterm birth [3, 4] and can have a major impact on the development of human papilloma virus (HPV)-induced cervical lesions into cancer [5, 6]. Antibiotic intake, smoking, vaginal douching, genetic influences, sexual intercourse and hormonal factors play an important role in the maintenance of a healthy microflora [7–11]. The reduced amount of vaginal bleeding due to the use of progestins may influence vaginal pH and affect the likelihood of anaerobic overgrowth or Candida infections [12], but this influence on variations in vaginal microflora patterns is far from having been elucidated. The levonorgestrel-releasing intrauterine system (LNG-IUS, Mirena) combines the presence of an intrauterine foreign body with the slow release of 50 mcg levonorgestrel daily, accomplishing reliable long-term contraceptive activity (5 years), reduced menstrual bleeding and a good tolerability and continuation rate as compared to other contraceptive methods. In theory, the decrease of menstrual bleeding would make it a favourable device for women suffering from recurrent BV, while the continuous presence of progestin would be favourable for women suffering from recurrent episodes of symptomatic Candida. As the LVG-IUS is increasingly popular amongst fertile women who do not wish to be pregnant any time soon,
we urgently need to understand the short- and long-term influence of this device on these infections. Hence, the aim of this study was to assess the influence of LNG-IUS on vaginal infections and microflora constitution from 6 weeks to 5 years after insertion.

**METHODS**

In this prospective study we studied the short- and long-term effects of LNG-IUS (Mirena) insertion on the bacteriological and mycological aspects of the vaginal microflora. The study was approved by the central Ethical Committee of Leuven University Hospital [number S50518 (ML 4243)], and registered in the Belgian ministry of Health with the BUN number B32220072240. Subsequently, the study was approved by the ethical committees of the respective collaborating centres and was carried out in full accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Assessment of the vaginal flora was offered to 252 women presenting for insertion of the LNG-IUS, provided that they provided signed informed consent and filled out a questionnaire. Patients with vaginal bleeding were asked to return when no bleeding was present for vaginal sampling.

To assess the prevalence of BV, AV and *Candida* infections, vaginal smears were obtained with a cytobrush from the lateral upper vault through a non-lubricated speculum. pH was measured by pressing the cytobrush onto a Macherey-Nagel pH strip with a range 3.3–7.0 (Macherey-Nagel GmbH and Co, Duren, Germany) [13]. A cotton swab placed in Amies-modified Stuart medium was sent for culture to detect overgrowth of Gram-positive or Gram-negative aerobic bacteria and *Candida* sp. Microscopic findings were categorized according to the Femicare scoring system [14]. In brief, one droplet of saline was added to an object glass slide and microscopy was performed at 400× magnification with a phase-contrast microscope (Leica MD1000, Wetzlar, Germany). Lactobacillary grades (LBG) were classified as LBG I when *Lactobacillus* morphotypes were predominant, LBG IIA as a normal lactobacillary pattern but with a significant quantity of non-*Lactobacillus* morphotypes, LBG IIb when non-lactobacillary flora exceeded the quantity of *Lactobacillus* and finally LBG III when total replacement of the lactobacillary flora by other morphotypes was observed [15]. Diagnosis of BV was performed according to the extension of the typical anaerobe granular microflora on the smear. If the typical granular flora of BV-like bacteria were observed all over the smear, or if more than 20% of epithelial cells were covered with bacteria (clue cells), a ‘full BV’ diagnosis was given. When fewer than 20% of clue cells were observed or there were areas with streaks of BV-like bacteria mixed with other...
morphotypes, a ‘partial BV’ diagnosis was given [14]. AV flora was defined as the presence of small bacilli and/or cocci in pairs or chains, and scored according to severity, taking into account the lactobacillary grade, pattern of the background, number of leucocytes, presence of toxic leucocytes and presence of parabasal cells [16]. The presence of \textit{Candida} was diagnosed when unequivocal pseudo-hyphae and/or blastospores were detected on the smears and/or microbiology cultures were positive for \textit{Candida} sp.

The 88 patients with Mirena \textit{in situ} for 5 years were compared to the group without Mirena before insertion, and at intervals of 6 weeks and 12 months after insertion, allowing us to acquire an idea of both short- (3 months) and long-term changes (1 to 5 years) to the vaginal flora. Data were collected and stored in Excel 2007. For continuous variables with normal distribution, Student’s \( t \)-test was used, otherwise a Welch correction was performed. Numeric values were compared by the \( \chi^2 \) test or Fisher’s exact test in cases of low numbers.

**RESULTS**

The demographics of the 252 subjects are presented in Table 1. There was an expected trend towards older age in women carrying the Mirena for a longer period of time. We detected no differences in body mass index, percentage of nulliparous women and the level of schooling between women in the different groups, indicating that patients lost during follow-up had similar characteristics to those women who stayed in the follow-up. The mean number of bleeding days, as well as amount of bleeding, reduced significantly over time, with an initial flare up involving longer duration of bleeding and more low-grade bleeding at 3 months after
insertion, and there were almost no patients with heavy or extended bleeding after 1 to 5 years of use ($P<0.001$, Fig. 1).

In general, the markers of bacterial infection showed a worsening of bacterial equilibrium (increased BV and AV scores and lactobacillary grades) and the presence of *Escherichia coli* and other Gram-negative enteric rods at 3 months post-insertion, the period of the heaviest and longest bleeding (Table 2). After 1 year there was a normalization of all these markers towards pre-insertion levels. After 5 years of LNG-IUS use, the prevalence of both AV and BV, as markers of bacterial infection, stabilized or improved slightly (Fig. 2a).

*Candida* was cultured more often after long-term use (1 to 5 years) compared to before that period (OR 2.0, CL 95 1.1–3.5, $P=0.017$) (Table 2). Furthermore, if the *Candida* cultures were found in combination with microscopy, a similar association with long-term use was found (OR 1.9, CL 95 1.1–3.1, $P=0.035$). The likelihood of finding *Candida* was particularly great after 1 year of LNG-IUS use (OR 2.4, CL 95 1.1–4.9, $P=0.037$) and it was still high after 5 years (Fig. 2b).

**DISCUSSION**

In an earlier study using conserved conventional Pap smears as a method to diagnose microflora alterations, we were unable to detect an increased risk for single abnormal flora types, such as BV, *Candida* or *AV* at 1 year after insertion as compared to baseline [17]. However, in that study there was not only a significant increase for all abnormalities combined, but also a small, non-significant trend towards higher *Candida* prevalence after 1 year of LNG-IUS use. We concluded that a prospective cohort study with better diagnostic techniques and longer follow-up should be performed. The data from this study are presented here.

In this study we were able to observe more detailed characteristics of the vaginal microbiome prospectively in a cohort of LNG-IUS users, starting from a baseline situation before insertion, and then following up up 3, 12 and 60 months post-insertion. Microscopy data were backed up by culture data, in particular to confirm the presence or absence of *Candida* in case microscopy was not possible, difficult to interpret (e.g. due to menstrual bleeding) or false negative. The bacterial abnormalities studied were bacterial vaginosis, aerobic vaginosis and the presence of abnormal vaginal flora (disturbed lactobacillary grades, i.e. LBG IIb and III). It is known that the latter three conditions play a crucial role in the risk of acquiring several sexually transmitted diseases, including herpes simplex virus (HSV) and HIV, and in the enhanced development rate of cervical cancer in HPV-infected patients [1–6]. Hence it is important to know whether the use of LNG-IUS is safe in poor-resource areas, especially central Africa, where the risk of having abnormal vaginal microflora and the prevalence of STIs are both high, and at the same time the need for reliable long-term contraception is imperative.

Our cohort study clearly demonstrated that short-term use of the LNG-IUS leads to increased alert signs of a deteriorating vaginal microflora. However, this is also the period where prolonged mild-to-moderate bleeding is highly prevalent, indicating that the microflora have less chance to fully recover. It is generally accepted that symptomatic BV occurs more often

<table>
<thead>
<tr>
<th>Timing to LNG-IUS insertion</th>
<th>Before insertion</th>
<th>3 months after insertion</th>
<th>1 year after insertion</th>
<th>5 years after insertion</th>
<th>Statistical difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy findings</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LBG IIb-III</td>
<td>n=141</td>
<td>n=121</td>
<td>n=62</td>
<td>n=68</td>
<td>$P=0.024^*$</td>
</tr>
<tr>
<td><em>Candida</em></td>
<td>55 (39, 0 %)</td>
<td>65 (53, 7 %)*</td>
<td>24 (38, 7 %)</td>
<td>23 (33, 8 %)</td>
<td></td>
</tr>
<tr>
<td>Aerobic vaginitis &gt;5</td>
<td>9 (6, 4 %)</td>
<td>11 (9, 1 %)</td>
<td>2 (3, 2 %)</td>
<td>4 (5, 9 %)</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>11 (7, 8 %)</td>
<td>17 (14, 0 %)</td>
<td>3 (4, 8 %)</td>
<td>5 (7, 4 %)</td>
<td></td>
</tr>
<tr>
<td>Culture results</td>
<td>n=155</td>
<td>n=120</td>
<td>n=53</td>
<td>n=88</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>71 (45, 8 %)</td>
<td>42 (35,0 %)</td>
<td>25 (47, 1 %)</td>
<td>35 (39, 7 %)</td>
<td></td>
</tr>
<tr>
<td><em>Candida</em></td>
<td>20 (12, 9 %)</td>
<td>11 (9, 2 %)</td>
<td>10 (18, 8 %)†</td>
<td>20 (18, 2 %)†</td>
<td>$P=0.017†$</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td>51 (32, 9 %)</td>
<td>54 (45, %)</td>
<td>17 (32, 0 %)</td>
<td>35 (39, 7 %)</td>
<td></td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>26 (16, 7 %)</td>
<td>39 (32, 5 %)*</td>
<td>7 (13, 2 %)</td>
<td>10 (11, 4 %)</td>
<td>$P=0.015^*$</td>
</tr>
<tr>
<td><em>Candida</em> microscopy or culture positive</td>
<td>n=164</td>
<td>n=126</td>
<td>n=56</td>
<td>n=87</td>
<td>$P=0.035†$</td>
</tr>
<tr>
<td></td>
<td>22 (13.4 %)</td>
<td>18 (14.3 %)</td>
<td>15 (26.8 %)‡</td>
<td>13 (20.7 %)‡</td>
<td>$P=0.02‡$</td>
</tr>
</tbody>
</table>

*p*-value of the difference of this group versus the others.

†*p*-value of group after 1 year versus the others.

‡*p*-value of group after 1 year and after 5 years combined versus groups before 1 year combined.
after menses and is increased in women with menorrhagia and frequent bleeding. Furthermore, research focusing on full-genome analysis of the vaginal microbiome revealed evidence that menses can cause temporary bacterial disturbances in some women [18], and a twofold decrease of the most stable marker of healthy microflora, *Lactobacillus crispatus*, was found in a vaginal sample of 11 women tested by whole-genome analysis before and 3 months after LNG-IUS insertion [19]. It is known that BV and AVF are linked to increased growth of *Prevotella sp.* and other anaerobes during menses and correlate with the amount and duration of blood loss, although cause and effect are difficult to elucidate [13].

On the other hand, as could be expected, with reduced blood loss over time, long-term LNG-IUS users showed a reconstitution of the flora to the baseline levels in our study, so we are confident to conclude that Mirena does not expose women to an increased risk of entrapping genital infections as a result of the presence of BV, AV or other types of adverse microflora. Hence, in low-resource countries, LNG-IUS can be considered as a proper alternative to injectable depot progestins and other contraceptive methods that are thought to increase abnormal microflora and consequent risk of HIV and STI acquisition [20, 21]. For women living in these settings, our findings could even have more profound implications.

The prevalence of *Candida* followed a reciprocal path to the bacterial abnormalities. *Candida* was present at lower levels during the first months post-insertion, during the period that bleeding was more abundant. In general, menses decrease symptoms in women with *Candida* infections, while estrogens enhance the disease severity [22]. In this

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**Fig. 2.** Temporal presentation of selected patterns of (a) bacteria- and (b) *Candida*-related vaginal microflora abnormalities before the insertion of Mirena and at 3, 12 and 60 months post-insertion.
immediate post-insertion period, in parallel with the drop in Candida presence, the lactobacillary flora were also reduced, while after 3 months the lactobacillary flora improved in parallel with the increase in Candida colonization. The preferential co-habitation of Candida with lactobacilli is known [23], while Candida is rare in low-estrogen, lactobacilli-deprived, conditions such as those in the prepubertal and postmenopausal period [24].

Long-term use of LNG-IUS, then, obviously improves the lactobacillary flora, but at the expense of an increased risk of Candida colonization. However, a word of caution is due, as colonization does not necessarily indicate a higher prevalence of symptomatic candidosis. This study was not empowered to follow up on symptomatic infections. In line with our results, Lessard et al. reported a trend towards increased candidosis in long-term LNG-IUS users [24].

In conclusion, short-term use of LNG-IUS (Mirena) temporarily decreases lactobacillary dominance, increases bacterial abnormalities and decreases Candida detection during the first 3 months post-insertion, but after 1 to 5 years of use the vaginal bacterial characteristics return to pre-insertion levels. Therefore an increased risk of STI acquisition is unlikely to occur with this long-acting contraceptive system, making it a preferred method in countries where abnormal vaginal flora patterns and STIs are highly prevalent. On the other hand, vigilance is warranted as colonization does not necessarily indicate a higher prevalence of symptomatic candidosis. To confirm whether the increased rates of vaginal colonization with Candida is also linked to more frequent symptomatic recurrences requires further prospective clinical studies.

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Conflicts of interest
Professor Dr Gilbert Donders is a member of the Medical Advisory Board of Bayer NV. The other authors have no conflicts of interest.

Ethical statement
The study was approved by the central Ethical Committee of Leuven University Hospital [number S50518 (ML 4243)], and registered in the Belgian Ministry of Health with the BUN number B32220072240. Subsequently, the study was approved by the ethical committees of the respective collaborating centres and was carried out in full accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent before entering the study.

References