Effects of vaginal prolapse surgery and ageing on vaginal vascularization
Weber, M.A.

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Focal depth measurements of the vaginal wall: a new method to non-invasively quantify vaginal wall thickness in the diagnosis and treatment of vaginal atrophy

Weber MA, Diedrich C, Ince C, Roovers JP

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ABSTRACT

Objective. The aim of the study was to evaluate if vaginal focal depth measurement could be a non-invasive method to quantify vaginal wall thickness.

Methods. Postmenopausal women undergoing topical estrogen therapy because of vaginal atrophy (VA) were recruited. VA was diagnosed based on the presence of symptoms and vaginal pH at least 5.5. The control group consisted of women above 40 years without VA. Focal depth measurements were performed before and after treatment using the Cytocam-Incident Dark Field device assessing the distance between the subepithelial microcirculation and the epithelial surface. Measurements were performed before and after treatment in the intervention group and at two different time points in the control group. Vaginal pH was measured. Symptoms were evaluated using the most bothersome symptom approach.

Results. Eight women with VA and nine controls were included. Pretreatment median focal depth was not significantly different between both groups. Pretreatment focal depth more than doubled after a median of 7 weeks of topical estrogen treatment (80 μm [interquartile range (IQR) 80-120 μm] to 220 μm [148-248 μm], \( p=0.02 \)), whereas the measurements in the control group did not change. Pre-treatment vaginal pH differed between both groups (5.5 vs 5.1, respectively, \( p<0.01 \)). Vaginal pH did not change after treatment.

Conclusions. Using in vivo microscopy we introduced a new non-invasive measure of vaginal wall thickness. A significant increase in vaginal focal depth was observed in participants with VA treated with topical estrogens. This innovative measurement of vaginal wall thickness could become the preferred objective measure to evaluate treatment effect. Moreover, it has great potential for other applications in the field of urogynaecology.
INTRODUCTION
Vaginal atrophy (VA) is a common condition in postmenopausal women, it is estimated that up to 40% of postmenopausal women experience symptoms of VA (1-3). With a further increase of life expectancy in many countries, women can experience a postmenopausal state of up to one third of their lives, with marked impact on everyday activities, sexual functioning, and body image (2, 4). Up till now, diagnosis and evaluation of treatment of VA is mainly based on symptoms presented by the patient or the clinical (subjective) judgement of the practitioner (5). VA is associated with symptoms like vaginal dryness, irritation and signs like a thin and pale aspect of the vaginal epithelium at physical examination. Objective measures include calculation of the vaginal maturation index or vaginal maturation value describing the proportion of parabasal, intermediate and superficial cells appearing on a vaginal smear (5). This objective measurement is, however, difficult (expensive and time consuming) to incorporate in daily clinical practice. An objective measure that is easy to perform in clinical practice is the assessment of the vaginal pH. The disadvantage of this measurement is the variability of the vaginal pH due to several patient characteristics, including smoking (6) and the presence of bacterial vaginosis, blood, semen or vaginal medications (7). Moreover, a large variation exists in the type of pH strip used and the location measured (5). With these issues in the objective assessment of VA, it is questionable not only how well we identify women with VA, but also how reliable we can assess the effects of interventions intended to treat VA.

VA is associated with thinning of the vaginal epithelial layer due to the declining levels of circulating oestrogen associated with VA (8, 9). Therefore, measurement of the vaginal wall thickness could form an objective measure in the diagnosis and evaluation of VA and its treatment. Treatment of VA often consists of topical oestrogen therapy. Previous studies have shown an increase in vaginal wall thickness after topical oestrogen treatment (10) with the use of histological assessment of biopsy specimens. This is however, an invasive method of assessing vaginal wall thickness and is therefore not applicable in a daily clinical setting.

We previously performed several investigations using handheld in vivo microscopy to investigate the vaginal microcirculation (11, 12). The microcirculation consists of all vessels with a diameter smaller than 100 micrometer (μm) (i.e., capillaries, arterioles, and
Chapter 6

venules) and is the site from which immune support, water, waste products, and gases (such as oxygen and carbon dioxide) between blood and tissue cells are exchanged (13). Vaginal microcirculation could therefore be a measure for vaginal oxygenation and for the function of the surrounding pelvic organs. We showed that the vaginal microcirculation can be reliably quantified using in vivo microscopy (11).

Recently a new generation hand held microscope called Cytocam-Incident Dark Field (IDF) was introduced with several new features enhancing the optical resolution. The enhanced resolution allows 30% more microvessels to be observed than was possible with previous generation devices (14, 15). In addition to this, the device is equipped with a focusing mechanism which allows the focal depth (i.e., the distance between the tissue surface and the epithelial microcirculation) to be quantified (14, 16).

We hypothesize that the depth of focus of the vaginal wall is much smaller in women with VA compared with women without VA and that this focal depth would be responsive to treatment with topical oestrogen known to correct vaginal atrophy. To this end we used the Cytocam-IDF device to quantitatively measure microcirculatory focal depth of the vagina. We aim to evaluate if the assessment of vaginal focal depth could be used as a non-invasive measurement to quantify vaginal wall thickness. One of the clinical applications of such measurement could be to monitor the effects of topical oestrogens in the treatment of VA and to assess the persistence of response to therapy and avoid unnecessary additional hormone therapy. Therefore, measurements were performed in women with and without VA and before and after treatment with topical oestrogen.

METHODS
Because this is a first explorative study to evaluate vaginal focal depth, we performed a pilot study in the outpatient clinic of the Department of Obstetrics and Gynaecology, Academic Medical Center, University of Amsterdam. The Medical Ethics Committee of the Academic Medical Center reviewed and approved this study. Women enrolled in this study received a full explanation of the study guidelines and procedures and written informed consent was obtained from each participant.
Setting and participants
Postmenopausal women that underwent treatment with topical oestrogens because of symptoms and signs associated with VA were recruited. A medical history was obtained and women with cardiovascular (e.g., angina pectoris, uncontrolled hypertension), inflammatory (e.g., rheumatoid arthritis, eczema), other systemic illness (e.g., inadequately controlled (non-) insulin dependent diabetes mellitus) or those taking medications (e.g., anticoagulants, anti-inflammatory, or immunosuppressive agents) that could influence the microcirculation were excluded from participation. In addition, women were excluded from participation if they had a previous history of vaginal surgery (e.g., anterior or posterior colporrhaphy).

The study group consisted of postmenopausal women with VA diagnosed based on the following three characteristics: 1] the presence of one or more of the following symptoms: vaginal dryness, vaginal itching or irritation or dyspareunia, rated with the use of the most bothersome symptom (MBS) approach (17, 18); 2] the presence of one or more of the following signs at physical examination: vaginal wall pallor and petechiae, friability of the vaginal wall (defined as any bleeding occurring during examination), conization (markedly decreased elasticity) or the absence of rugae (19); and 3] a vaginal pH at least 5.5, who received topical oestrogen as part of their standard treatment (Synapause-E3 ovules 0.5 mg daily for two weeks followed by two times per week for at least 4 weeks).

The control group consisted of women above 40 years of age attending to the outpatient department because of bothersome pelvic floor symptoms with no symptoms or signs of VA, no previous history of vaginal surgery and no use of topical estrogens.

Physical examination
All patients in the study group underwent a gynecological examination before and after treatment with topical estrogen. Women in the control group had a gynecological examination as part of their standard consult. Gynecological examination included evaluation of signs signifying VA including presence of vaginal wall pallor, petechiae, friability of the vaginal wall, conization (markedly decreased elasticity), and absence of
rugae (19). The vaginal pH was assessed in both groups using a pH strip with a pH range of 3.8-5.5 with intervals of 0.3 (Dosatext).

**Vaginal focal depth measurements**

All participants were accommodated in a gynaecological examination chair in a stable 45° semireclined position. In the VA group vaginal focal depth measurements were performed before and after topical oestrogen treatment on the anterior vaginal wall using Cytocam-IDF imaging (Braedius Scientific BV, Huizen, The Netherlands). The Cytocam, covered with a sterile disposable cap, was gently placed into contact with the anterior vaginal wall at exactly 3 cm above the hymen. To ensure measurements were performed exactly 3 cm above the hymen the disposable cap was marked at 3 cm from the tip (11, 12). The Cytocam is equipped with a precision-controlled focal depth measuring system (accuracy 4 µm) assessing the distance between the subepithelial microcirculation and the epithelial surface in micrometers. Previous studies identify the focal depth as being specific to each patient and as being constant in different sublingual areas (16). In the control group measurements were performed at two different time points. The investigator measuring vaginal focal depth was not blinded to whether the participant was in the VA group or in the control group. All measurements were performed by one investigator.

**Statistical analysis**

For differences in focal depth and vaginal pH between the two groups the (unpaired) Mann-Whitney U test was used. Differences between two time points within each patient were tested using the (paired) Wilcoxon signed rank test. A P of <0.05 was considered statistically significant. All analyses were performed using the statistical software SPSS (IBM SPSS Statistics version 20).

**RESULTS**

**Participants**

Eight postmenopausal women with VA were included (median age 65 years [interquartile range (IQR) 56 - 74 years]. Nine women were included in the control group (median 49
years [45 - 59 years], P<0.01). Parity of the women with VA (median 2.0 [range 0.5 – 2.8] was comparable to the women without VA (median 2.0 [range 0.0 – 2.5], P=0.68). The control group consisted of women whom presented with abnormal pap smears (n=2), urinary incontinence (n=4), recurrent urinary tract infections (n=1) and/or pelvic organ prolapse (n=4) without symptoms and signs of VA. In the VA group there were also four participants with concomitant POP. One participant in the VA group did not wish to complete the follow-up visit for personal reasons and two participants in the control group refused their second measurement at a different time point because they did not have a regular follow-up appointment at the clinic and an extra visit would take too much of their time.

*Vaginal focal depth measurements*

Median focal depth for achieving optimal image quality in the VA group before treatment was smaller although not statistically significantly different from the median focal depth in the control group (80 μm [IQR 80-120 μm] vs 160 μm [120-200 μm] respectively, P=0.07, Table 1). Pretreatment focal depth in the VA group more than doubled after a median of 7 weeks of topical oestrogen treatment (80 μm [80-120 μm] vs 220 μm [148-248 μm], P=0.02, Table 1, Figure 1). Focal depth also slightly increased between the first and second measurements in the control group, however, this difference was not statistically significant (P=0.07, Table 1). Median time between measurements in the control group was 11 weeks [6-12 weeks].
Chapter 6

Table 1. Comparison between women with vaginal atrophy and the control group and before and after topical estrogen treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Median (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA - Pre-treatment (n=8)</td>
<td>80 (80-120)</td>
<td>0.02</td>
</tr>
<tr>
<td>VA - Post-treatment (n=7)</td>
<td>220 (148-248)</td>
<td></td>
</tr>
<tr>
<td>Control - 1st measurement (n=9)</td>
<td>160 (120-200)</td>
<td>0.07</td>
</tr>
<tr>
<td>Control - 2nd measurement (n=7)</td>
<td>200 (180-200)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Figure 1. Focal depth measurements before and after treatment in the VA group
Table 1: Comparison between women with vaginal atrophy and the control group and before and after topical estrogen treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Median (IQR)</th>
<th>VA-Pre-treatment (n=8)</th>
<th>VA-Post-treatment (n=7)</th>
<th>Control-1st measurement (n=9)</th>
<th>Control-2nd measurement (n=7)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal depth [µm]</td>
<td>80 (80-120)</td>
<td>220 (148-248)</td>
<td>0.02</td>
<td>160 (120-200)</td>
<td>200 (180-200)</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Vaginal pH</td>
<td>5.5 (5.5-5.5)</td>
<td>5.0 (4.9-5.5)</td>
<td>0.07</td>
<td>5.1 (4.6-5.3)</td>
<td>5.2 (4.8-5.3)</td>
<td>0.32</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

VA: vaginal atrophy. IQR: interquartile range

* Wilcoxon signed rank test for comparison pre- and post-treatment;

* Wilcoxon signed rank test for comparison between Control-1st measurement and Control-2nd measurement;

* Mann-Whitney U test for comparison between VA-Pre-treatment and Control-1st measurement.
Chapter 6

Vaginal pH

Vaginal pH at baseline in the VA group differed significantly from the pH in the control group (5.5 vs 5.1 respectively, P<0.01, Table 1). Vaginal pH did not change significantly after treatment in the VA group (P=0.07) and between two time points in the control group (P=0.32, Table 1).

Changes in physical examination

In six of the seven women in the VA group that completed treatment and the follow-up visit the signs of VA at physical examination improved. Especially vaginal wall pallor, petechiae and friability of the vaginal wall were no longer present. In one participant vaginal wall pallor, petechiae, friability and decreased elasticity of the vaginal wall were still present and she experienced more vaginal irritation and itching. This patient had a history of recurrent urinary tract infections and during her follow-up period she was diagnosed with a urinary tract infection which required antibiotic treatment. She was treated with Synapause ovules for two months and reported to have been compliant.

MBS approach

All women in the VA group experienced symptoms of VA, five reported one of their symptoms as MBS at baseline and one participant did no longer report this symptom after treatment. One participant reported two symptoms as MBS at baseline; she was free of symptoms after treatment. For the other participants reporting of a MBS was comparable before and after treatment.

Adverse events

No adverse events were reported.

DISCUSSION

This study aimed to evaluate if the assessment of vaginal focal depth could generate a noninvasive measurement to objectively quantify vaginal wall thickness. The results of this study show that Cytocam-IDF imaging, a validated technique to assess vaginal microcirculation, was able to non-invasively measure the distance between the vaginal...
subepithelium and the superficial squamous epithelium. This distance increased significantly in women with VA after topical oestrogen treatment, indicating a more than doubled thickness of the vaginal wall squamous epithelium. The other objective measurement (vaginal pH), however, did not change significantly. Therefore, this new non-invasive measurement of vaginal wall thickness may be a better method to objectify the effect of treatment in women with VA.

Before further interpreting these results, some potential limitations need to be addressed. Despite our criterion to not include women below the age of 40 years in the control group, the women in the control group were still significantly younger than the women in the VA group. Although the age factor alone does not seem to cause marked changes in certain dimensions of the vagina like vaginal length (20) or smooth muscle content in the vaginal wall (21), the increased presence of other pelvic floor disorders like POP in older women could have an effect on vaginal wall thickness. The vaginal wall consists of four layers; the epithelial layer is a superficial nonkeratinized, stratified, squamous epithelium. The subepithelial layer or lamina propria is a connective tissue layer composed of elastin, collagens and fibroblasts. The lamina propria is perforated by small arterioles and venules (22). The muscularis is composed of smooth muscle cells surrounded by connective tissue and the adventitia is a loose connective tissue layer that separates the muscularis of the vagina and the paravaginal tissue. The lamina propria and muscularis are the two important layers providing tensile strength to the vaginal wall (23). In both our control group as well as the VA group, four participants had POP stage at least 2. In previous studies, it has been shown that in patients with POP the distance of the muscularis layer to the surface epithelium is increased compared to patients without POP (24, 25). In our study, no statistically significant differences in vaginal focal depth between the VA group and the control group were revealed at baseline. Our study group was too small to make a comparison in vaginal focal depth between patients with and without POP which was also not the focus of our study. Such comparison, however, could be the subject of a future study.

Another potential limitation is the lack of intra- and inter-observer variability analysis in this study and the comparison of our measurement to other vaginal wall depth measurements. With the Cytocam we measure the distance between the subepithelial
microcirculation and the epithelial surface for optimal focus in microvascular imaging. This implies we measure the depth at which the microcirculation is imaged most clearly. The microcirculation is situated at the level of the lamina propria layer of the vaginal wall. Therefore we do not measure the exact depth of the lamina propria and the layers below the lamina propria (i.e., muscularis and adventitia). With vaginal wall biopsies, a precise measurement of total vaginal wall thickness is, however, also difficult to obtain because cross-sections must be perfectly perpendicular to the tissue edge. Oblique sections would appear thicker than those perpendiculars (22). Studies that evaluated vaginal wall thickness using biopsies measured only the thickness of the muscularis (26) or the distance from the muscularis layer to the surface epithelium (24, 25). Others looked at the smooth muscle content of the vaginal muscularis (21, 27) or counted the number of layers of epithelial cells (28). Some authors report a vaginal epithelium thickness of 2.5 mm (29), while others report the vaginal wall thickness as 0.209 mm (30). Studies evaluating vaginal wall thickness have therefore always been difficult to compare.

Strength of our study is that we introduce a non-invasive evaluation of the vaginal (epithelial) wall thickness. Another relevant strength is that we performed measurements in a control group which shows that there is a very limited variation in focal depth measurements within this group. This means there is not only a limited variation in vaginal wall thickness within control patients without VA, but also the measurement itself shows little variation. A third strength is that we combined subjective and objective measures in our evaluation of VA.

This is the first explorative study to evaluate if focal depth could be measured vaginally. We selected a study population with VA and compared measurements to a control group without VA because, considering the available literature, in this specific study population differences in vaginal wall thickness can be expected, especially after topical oestrogen therapy (9, 10). And indeed, a significant difference in vaginal focal depth was observed after topical oestrogens were used. After this first explorative study showing that focal depth measurements are possible to perform vaginally and even showing significant changes after topical oestrogen treatment, it is important to evaluate this measurement further. A well designed study to investigate the intra- and inter-observer variability of these measurements will be of added value. Moreover, evaluating
Focal depth of the vaginal wall

if a reference range for ‘normal’ focal depth exists is worth exploring, for example by performing measurements in a much larger group of women of different age groups. Furthermore, the possible applicability of vaginal focal depth measurements in other research areas is intriguing, as mentioned before it would be interesting to explore vaginal focal depth in POP, but other vulvar conditions (e.g., lichen sclerosis) or measurements of vaginal focal depth before and after vaginal surgery also form great potential for future research.

CONCLUSION

In conclusion, with this study we achieved two important contributions to the field of urogynaecology; first, we introduced a new non-invasive measure of vaginal wall thickness using in vivo microscopy; second, we reported a significant increase in vaginal focal depth in patients with VA treated with topical estrogens. Therefore, this innovative non-invasive measurement of vaginal wall thickness has the potential to become the preferred objective criterion in the diagnosis of VA and evaluate treatment effect. Moreover, such objective measurement of vaginal wall thickness has a huge potential for other applications in the field of urogynaecology.
Chapter 6

References


132
References


