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ORIGINAL ARTICLE

# Changes in elastin density in different locations of the vaginal wall in women with pelvic organ prolapse

**Laurent de Landsheere · Silvia Blacher · Carine Munaut · Betty Nusgens · Chrystèle Rubod · Agnès Noel · Jean-Michel Foidart · Michel Cosson · Michelle Nisolle**

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**Abstract**
*Introduction and hypothesis* The purpose of this study was to analyze the histomorphometric properties of the vaginal wall in women with pelvic organ prolapse (POP).
*Methods* In 15 women undergoing surgery for POP, full-thickness biopsies were collected at two different sites of location from the anterior and/or posterior vaginal wall. Properties of the precervical area (POP-Q point C/D) were compared with the most distal portion of the vaginal wall (POP-Q point Ba/Bp) using histological staining and immunohistochemistry. The densities of total collagen fibers, elastic fibers, smooth muscle cells, and blood vessels were determined by combining high-resolution virtual imaging and computer-assisted digital image analysis.
*Results* The mean elastin density was significantly decreased in the lamina propria and muscularis layer of the vaginal wall from the most distal portion of the prolapsed vaginal wall compared with the precervical area. This difference was statistically significant in the lamina propria for both anterior (8.4±1.2 and 12.1±2.0, $p$=0.048) and posterior (6.8±0.5 and 10.1±1.4, $p$=0.040) locations, and in the muscularis for the anterior (5.2±0.4 and 8.4±1.2, $p$=0.009) vaginal wall. There were no statistically significant differences in the mean densities of collagen fibers, smooth muscle cells or blood vessels between the two locations.
*Conclusions* In this study, we observed changes in elastin density in two different locations of the vaginal wall from women with POP. The histomorphometric properties of the vaginal wall can be variable from one place to another in the same patient. This result supports the existence of most vulnerable locations within the vaginal wall and the potential benefit of site-specific prolapse surgery.

**Keywords** Vagina · Pelvic organ prolapse · Collagen · Elastin · Connective tissue

**Abbreviations**

| | |
|---|---|
| POP | Pelvic organ prolapse |
| ECM | Extracellular matrix |
| αSMA | Alpha smooth actin |
| RGB | Red green blue |

## Introduction

The pathophysiology of pelvic organ prolapse is multifactorial. Multiparity, trauma, obesity, and aging are associated with alterations in the pelvic tissues that lead to the development of pelvic organ prolapse (POP) [1]. In addition to these environmental factors, genetic predisposition is an important contributing factor to the pathogenesis of POP [2].

Pelvic organ support is the result of a complex balance and interactions between the connective tissue of the pelvic support structure [3]. The loss of pelvic support holding the

L. de Landsheere (✉) · J.-M. Foidart · M. Nisolle
Department of Obstetrics and Gynecology, University of Liège, CHR La Citadelle, Boulevard du 12e de ligne, no. 1, 4000 Liège, Belgium
e-mail: ldelandsheere@gmail.com

L. de Landsheere · S. Blacher · C. Munaut · A. Noel · J.-M. Foidart · M. Nisolle
Laboratory of Tumor and Development Biology, CHU, GIGA-Cancer, University of Liège, Avenue de l'Hôpital, 3—Pathology Tower (B23), Sart-Tilman, Belgium

B. Nusgens
Laboratory of Connective Tissues Biology, GIGA-Cancer, University of Liège, Avenue de l'Hôpital, 3—Pathology Tower (B23), Sart-Tilman, Belgium

C. Rubod · M. Cosson
Department of Gynecologic Surgery, Hôpital Jeanne de Flandre, Centre Hospitalier Régional Universitaire de Lille, Avenue Oscar Lambret, no. 2, 59037 Lille Cedex, France

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bladder in place may cause cystocele [4]. Two theories have been proposed to explain the etiology of anterior vaginal wall prolapse. The lateral wall defect theory suggests that cystocele results from the detachment of the lateral vaginal support to the arcus tendineus fasciae pelvis (ATFP) [5]. The central defect theory is based on the site-specific weakness of the pubocervical fascia [6]. Improving knowledge about changes in the composition of the vaginal wall may help to better understand the anatomical changes that lead to POP.

Histologically, the vaginal wall is composed of four layers. The epithelial layer is a superficial nonkeratinized stratified squamous epithelium. The subepithelial layer, or lamina propria, is a dense connective tissue layer primarily composed of fibrillar collagens and elastin populated by fibroblasts. The muscularis is composed of inner circular and outer longitudinal smooth muscle cells surrounded by connective tissue. The adventitia is a loose connective tissue layer that separates the vaginal wall muscularis and the paravaginal tissue [7].

The connective tissue of the vagina is composed in varying proportions of cellular elements surrounded by an extracellular matrix (ECM). Collagen and elastin are fundamental fibrillar components of the ECM that play an important role in the mechanical strength of the vaginal tissue [7]. Collagens I, III, and V are the primary collagen subtypes present in the vagina. Elastic fibers are key architectural elements of connective tissues. These fibers provide extensibility and recoil ability to elastic tissues [8]. This property of resilience is important for the maintenance of the structural integrity of the vagina when subjected to mechanical strain.

Boreham et al., by specifically evaluating the histomorphometric features of the vaginal wall in women with POP, suggested that the characteristics of the vaginal wall might be significantly altered in women with POP [9, 10]. However, assessing the changes in the vaginal tissue that occur in women with POP is challenging for many reasons. The methodology varied greatly from one study to another. The biopsy sites and sample sizes can be variable, and specimens are not often well defined histologically, making it impossible to determine which layer of the vaginal wall is being analyzed [11].

In this work, a novel histomorphometric analysis method was used to characterize the histomorphometric properties in two different locations of the vaginal wall from women with POP. This methodology combines high-resolution virtual imaging of full-thickness biopsies and computer-assisted image analysis [12, 13].

## Materials and methods

### Patients

From February to May 2012, a total of 15 women underwent vaginal surgical procedures for pelvic organ prolapse at the Department of Gynecological Surgery of the University Hospital Jeanne de Flandre, Lille, France. Institutional Review Board approval was obtained prior to the start of the study (CPP 09/62) and all patients signed an informed consent form prior to surgery. Demographic characteristics (age, parity, BMI, menopausal status, POP-Q stage, and type of surgery) were collected prospectively and stored in a dedicated database. Study exclusion criteria were previous pelvic surgery, connective tissue disease, and history of endometriosis or pelvic cancer. All patients underwent an assessment of their POP stage according to the International Pelvic Organ Prolapse Quantification system [14].

### Tissue collection

Full-thickness vaginal tissue samples were taken from each woman at two different sites of the vaginal wall. Biopsies were collected only in the compartment involved during the POP repair. The first sample was obtained with Metzenbaum scissors, after carrying out deep infiltration with diluted lidocaine solution followed by a sagittal midline incision, in the most distal portion of the anterior (POP-Q point Ba) and/or posterior (POP-Q point Bp) vaginal wall. The second sample was collected from the precervical area of the anterior (POP-Q point C) and/or posterior (POP-Q point D) vaginal wall. Each specimen measured $\geq 10 \times 5$ mm. We performed standard light microscopic techniques to confirm that all the layers were present in the collected tissues.

### Histological material

Human biopsy specimens were fixed in buffered 4 % formalin, embedded in paraffin, and serially sectioned at a thickness of 5 μm. After sectioning, the samples were stained with routine dyes (hematoxylin-eosin) for conventional histopathological evaluation or with specific stains, such as Masson's trichrome or orcein, to evaluate collagen and elastic fibers respectively [15].

### Alpha SMA immunohistochemistry

To unmask antigens in paraffin-embedded sections, the slides were autoclaved for 11 min at 126 °C and 1.4 bar in citrate buffer (S 2032; Dako, Glostrup, Denmark). To analyze alpha smooth muscle actin, a mouse monoclonal antibody was used (αSMA, clone 1A4, M 0851; Dako). The slides were washed in Tris-HCl, pH 7.6. Endogenous peroxidases were blocked with 3 % H2O2 for 20 min at room temperature (RT). The slides were incubated with normal goat serum (NGS; Hormonology Laboratory, Marloie, Belgium) before being incubated with the second primary Ab, αSMA (1/400 in NGS/Tris 10 %), for 90 min at 37 °C. A goat anti-mouse Ab conjugated to biotin (E 0433; Dako) diluted 1/400 in Tris

buffer for 30 min at RT was used as the secondary Ab, followed by incubation for 30 min at RT with streptavidin-alkaline phosphatase (P 0397; Dako) diluted 1/500. DAB + (Liquid DAB + substrate chromogen system, K3468; Dako) was applied for 15 min at RT in the dark as a chromogen, and the sections were rinsed in $H_2O$. The slides were finally counterstained with hematoxylin and mounted with Eukitt medium for microscope observation. Negative controls were performed both by omission of the primary antibody and by replacement of this primary antibody by an appropriate isotype control (mouse IgG2, kappa; Dako).

### Image acquisition

Virtual images were acquired with the fully automated digital microscopy system dotSlide (BX51TF; Olympus, Aartselaar, Belgium) coupled with a Peltier-cooled high-resolution digital color camera (1,376 x 1,032 pixels; XC10, Olympus). Images of the whole tissue sections were digitized at high magnification (×100), producing virtual images in which the pixel size was 0.65 μm.

### Image processing and measurements

Image processing consisted of the transformation of an original color image into a binary image in which all pixels belonging to the objects of interest, i.e., smooth muscle cells, elastic fibers, and blood vessels in the present case, took a value of 1 and those of the background a value of 0. Images were registered in full red/green/blue (RGB) color.

Smooth muscle cells in the muscularis layer were identified by immunohistochemical staining with αSMA antibodies and could be distinguished by a brownish color. Because structure detection was primarily based on color segmentation, weakly stained regions could have remained unrecognized. To increase the contrast between the muscle and the surrounding tissue, the excess red component (two times the red value minus the blue value minus the green value) was calculated. After splitting the resulting color image into its RGB components, muscular structures were extracted from the enhanced red component using automatic entropy thresholding [16]. Finally, an erosion filter was used to eliminate small artifacts, and the resulting image was used as a marker for a geodesic reconstruction of the final binary image [17]. It is worth noting that the vaginal muscularis consisted of smooth muscle bundles and vascular complexes, both of which were composed of smooth muscle cells detected by αSMA antibodies. Therefore, the staining of these two structures was similar.

Orcein appears to be an appropriate specific stain, providing optimal contrast between elastic fibers and the tissue background. After splitting the original color image into its RGB components, elastic fibers could be directly extracted from the red component using automatic entropy thresholding [16]. In only a few cases did the operator have to increase the contrast.

Blood vessel lumens were identified as the lightest structures in the tissue (the value of the threshold depended on the average value of the background). To avoid the selection of other structures (for example, holes in the tissue), only lumens neighboring previously detected vessel walls were considered. Finally, the resulting binary images were systematically compared with the corresponding originals, and lumen vessels not detected automatically were added manually by a single physician (L.d.L.).

Masson's trichrome staining identified vaginal wall collagen fibers, which appeared green. This stained area represents the non-elastic fraction of the ECM and does not allow the determination of the density of each subtype of collagen fiber. The green component of the RGB color image was first extracted, and a median filter was applied to eliminate noise [17]. Collagen fibers were then extracted using automatic entropy thresholding [16]. The resulting binary images were systematically compared with the corresponding originals, and in cases in which the detection of collagen fibers was not accurate, the threshold was fixed manually.

To perform local measurements, the boundary between the two layers of interest, i.e., the lamina propria and the muscularis layer, was drawn manually on each histological section image by the same physician (L.d.L.).

The following values were determined:

1. Proportions of the area occupied by the lamina propria and the muscularis in relation to the total histological section
2. Muscle density in the muscularis: area occupied by smooth muscle cells in the muscularis layer divided by the area of the muscularis layer.
3. Vessel density in the muscularis: area occupied by blood vessels in the muscularis layer divided by the area of the muscularis region.
4. Vessel density in the lamina propria: area occupied by blood vessels divided by the area of the lamina propria.
5. Elastin density in the muscularis: area occupied by elastic fibers divided by the area of the muscularis.
6. Elastin density in the lamina propria: area occupied by elastic fibers divided by the area of the lamina propria.
7. Total collagen density in the muscularis: area occupied by the total collagen content divided by the area of the muscularis.
8. Total collagen density in the lamina propria: area occupied by the total collagen content divided by the area of the lamina propria.

Image processing and measurements were performed using the image analysis toolbox of MATLAB (7.9; Mathworks). Statistical analysis was performed with the statistics toolbox

of the Matlab (9.2) (Mathworks). Previously described densities were first calculated for each patient at each considered location. Mean densities were determined by each location. The results were expressed as the means ± standard error of the mean (SEM). The Mann–Whitney test was used to compare the mean parameters values for the two groups. The results were considered significant at $p<0.05$.

## Results

The demographic and surgical characteristics of the women included in the study are presented in Table 1. Most of the patients were postmenopausal (86.7 %), and none of the patients had received any hormonal treatment in the 3 months prior to surgery.

Among the 15 patients, 6 patients (40 %) underwent isolated anterior repair, 5 patients (33.3 %) underwent isolated posterior repair, and 4 patients (26.7 %) underwent combined anterior and posterior repair. Tissue samples were only taken from the compartment involved in the POP repair at two different sites from the anterior vaginal wall in 10 cases and from the posterior vaginal wall in 9 cases.

In order to prove the homogeneity of the standardized tissue collection from the two predefined locations, we evaluated the mean area of the two layers of interest, the muscularis and lamina propria, in all biopsies ($n=38$; Fig. 1). No significant difference was observed in terms of sample size, either in the anterior ($p=0.30$) or the posterior region ($p=0.80$).

Smooth muscle cells, blood vessels, elastic and collagen fibers were first detected in the muscularis and lamina propria in histological sections in the anterior and/or the posterior regions from each patient (Fig. 2, 3). The corresponding densities in each layer were calculated, and the mean values for each location, middle third or precervical portion of the anterior ($n=10$) and the posterior vaginal wall ($n=9$) are reported in Table 2.

The total collagen content of the two tissue locations was not statistically significantly different. The mean smooth muscle and blood vessel density were not significantly different for the middle third portion of the vaginal wall and the precervical region (Table 2).

A lower mean elastin density was observed in the lamina propria and in the muscularis layer of the most distal portion of the vaginal wall. This difference was statistically significant in the lamina propria for both anterior ($p=0.048$) and posterior ($p=0.040$) locations and in the muscularis for the anterior ($p=0.009$) vaginal wall .

More detailed analysis was performed to compare the elastin densities in the two regions for each patient individually. We found that for all the studied cases, the elastin density was systematically lower in the middle third portion of the vaginal wall (Fig. 4).

**Table 1** Demographic and surgical characteristics

| Characteristic | $n=15$ |
|---|---|
| Age, years (mean ± SD) | 62.2±10.8 |
| BMI, kg/m$^2$ (mean ± SD) | 29.3±2.5 |
| Vaginal parity, median (range) | 3 (1–8) |
| Menopause, *n* (%) | 13 (86.7) |
| POP stage, median (range) | 3 (2–3) |
| Type of surgery, *n* | |
| Anterior colporrhaphy | 2 |
| Anterior Prolift® | 4 |
| Posterior colporrhaphy | 1 |
| Posterior Prolift® | 4 |
| Anterior and posterior Prolift® | 4 |

*SD* standard deviation, *BMI* body mass index, *POP* pelvic organ prolapse

## Discussion

The main finding of this study is the significant decrease in the elastin density in the lamina propria and muscularis layer in the most distal portion of the vaginal wall of women suffering from POP. This difference was statistically significant for the muscularis of the anterior vaginal wall and for the lamina propria of both the anterior and posterior vaginal wall. Elastic fibers are the key architectural elements of connective tissues that are subjected to mechanical strain, providing extensibility and recoil ability to elastic tissues. The decrease in elastin content may cause changes in the properties of the vaginal wall, making it more rigid and less resistant to mechanical forces.

Elastin density was lower in the anterior vaginal wall, but the degree of prolapse is often greater in the anterior vaginal wall than in the posterior vaginal wall. To assess the correlation with the severity of POP, we calculated the elastin density based on the POP-Q stage. We did not find any relationship between these variables, but the number of patients in the present study is most likely too low to be able to draw reliable conclusions.

The finding of this study allows the variability of vaginal composition to be highlighted in different locations of the vaginal wall from the same women presenting with POP. Tissue quality is worse at the most distal portion of the vaginal wall compared with regions where stress is lower. This observation confirms that the repair of this damage by the technique of site-specific repair must be a good option in the surgical management of genital prolapse, at a time when the use of synthetic mesh is controversial.

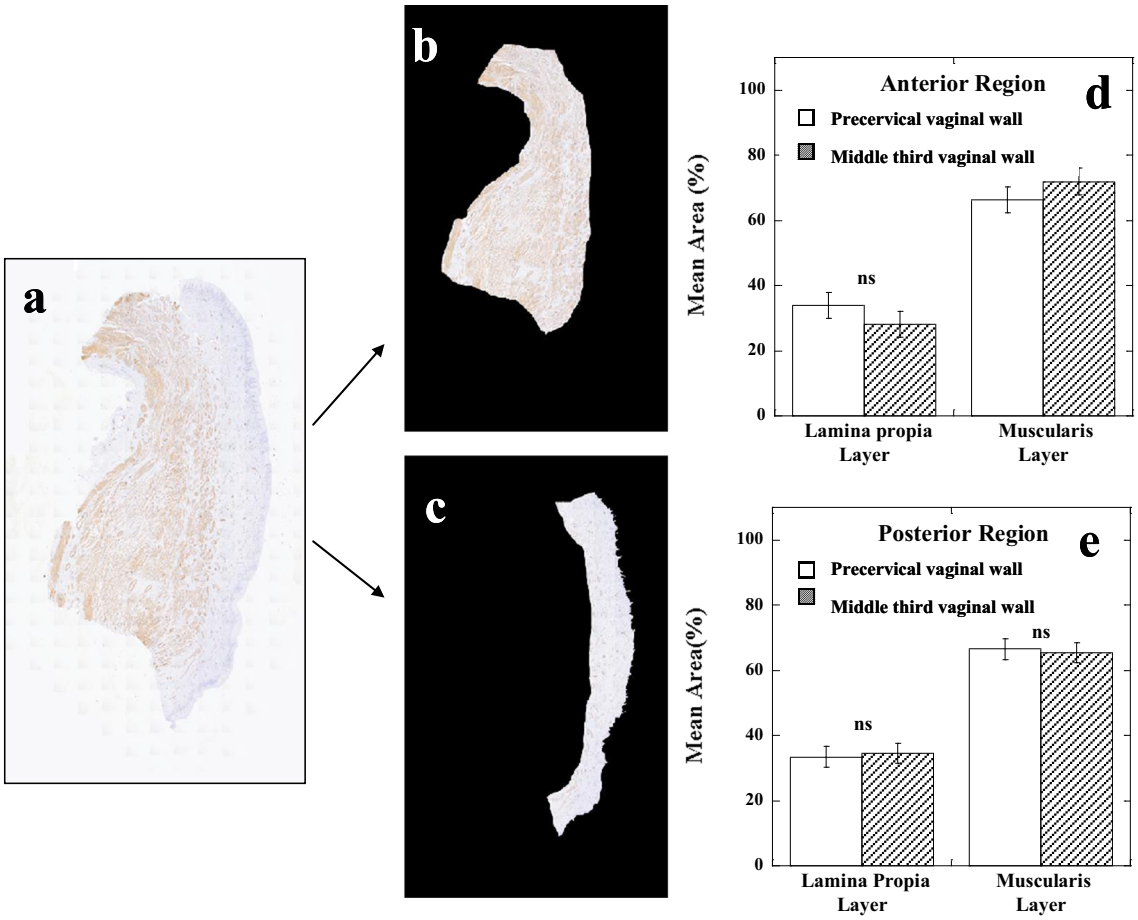


**Fig. 1** **a** αSMA-stained histological section, **b** muscularis layer, **c** lamina propria. Mean area of lamina propria and muscularis for precervical and middle third portion of **d** the anterior ($n$=10 and **e** the posterior vaginal wall ($n$=9). The mean area is expressed as a percentage of the total histological section

The mean strength of this study is the comparison of two different biopsy sites from the same patient, thus avoiding interindividual variability and allowing the collection of samples of similar quality. In previous studies, the major limitation was matching the prolapsed and control groups in terms of age, menopausal status, hormone therapy, and sample location because the characteristics of patients treated for POP are often different from those of control patients for several reasons [9, 18–20]. First, patients undergoing surgery for POP are frequently postmenopausal and older than patients undergoing hysterectomy for benign reasons. Second, it is difficult to obtain full-thickness and large-sized biopsies from the middle third portion of the vaginal wall from patients without POP. Samples taken from the control group are most often collected from the vaginal apex during hysterectomy for benign disease, limiting the sample size [20]. However, we cannot determine whether these changes are related to other factors that could potentially influence the histological properties of the vaginal tissue such as age, menopausal status, previous surgery for POP, etc. A larger study is required to assess the relative contribution of these factors in the vaginal tissue composition.

The major limitation of this study is that the two biopsies were obtained from patients presenting with genital prolapse without any comparison with control patients without POP. Therefore, the absence of a control group prevents us from determining whether these changes are a cause or an effect of POP. The main reasons for the lack of control group are ethical, as collecting large tissue samples at the middle third vaginal wall of patients operated for reasons other than POP can be considered to be too invasive. In our experience, biopsies obtained at this level in patients without POP do not have the same quality as those of patients with POP and cannot be compared without introducing bias. Using a similar methodology of sample collection, Kannan et al. found very subtle changes between prolapsed and non-prolapsed tissues [21]. In a previous report, Karam et al. found a significant difference in elastin expression in the vaginal wall of patients with cystocele compared with control patients of similar age (10.6 vs 14.4, $p$=0.049) [22]. In the control group, vaginal

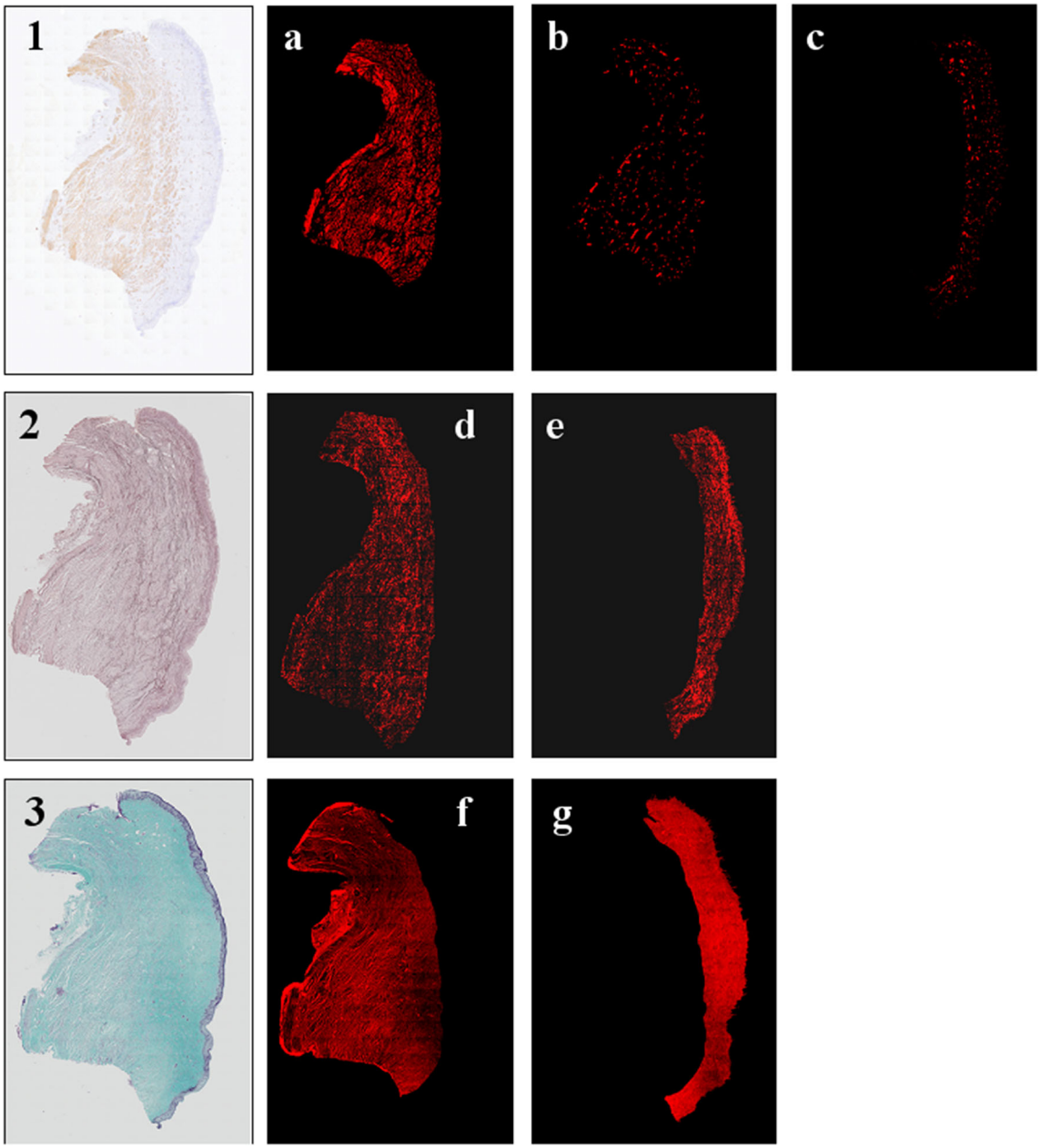


**Fig. 2** *1* αSMA-stained histological section of the precervical vaginal tissue in the anterior region. Binary images corresponding to **a** smooth muscle cells and **b** blood vessels in the muscularis layer and blood vessels in **c** the lamina propria. *2* Orcein-stained histological section of the same patient. **d** Binary images corresponding to elastic fibers in the muscularis layer and in **e** the lamina propria. *3* Masson's trichrome-stained histological section of the same patient. Binary images corresponding to collagen fibers in **f** the muscularis layer and in **g** the lamina propria

tissue was obtained from the same location in patients with no prolapse undergoing radical cystectomy for bladder urothelial carcinoma. It is interesting to note that the mean elastin expression in the muscularis layer of the upper lateral anterior vaginal wall in the prolapse group (10.6±5.0) was comparable to our results (8.4±1.2). However, for both studies, the biopsy sites and/or analytical methods were different, and it is therefore difficult to make comparisons with our findings.

In the study outlined herein, we used high-resolution and computerized image analysis to provide an objective assessment of the different component densities of the vaginal wall without using subjective visual scales for density. In addition, the whole histological section was analyzed, rather than randomly selected fields, allowing the more accurate analysis of each component from the full-thickness vaginal tissue. However, another limitation of this study is the small number of patients and the lack of power statistical calculation, although the number of subjects is comparable to that used in other studies in the same field. These small sample populations highlight the difficulty of obtaining good-quality

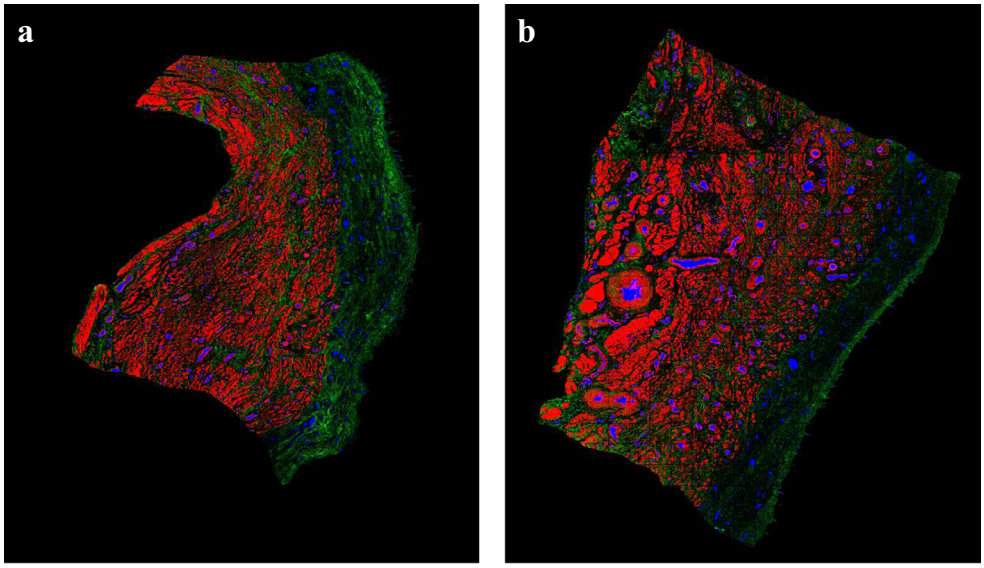


**Fig. 3** Composed images of **a** the precervical area and **b** the middle third portion of the anterior vaginal wall from the same patient. Smooth muscle cells are represented in *red*, elastic fibers in *green*, and blood vessels in *blue*

samples from two different sites from the same patient. However, it is important to note that the decrease in elastin density was found in all cases, as shown in Fig. 4. This consistency further supports our observations, despite the small number of tissue samples.

Unlike previous studies, ours did not find any significant differences in the smooth muscle cell density between the two locations [9, 18, 19, 23, 24]. However, the muscularis layer architecture was globally altered, and the smooth muscle bundles appeared disorganized. It is unclear whether these changes are related to the pathogenesis of genital prolapse or simply to the aging of the tissues.

We did not observe any significant differences in the distribution or density of blood vessels between the two locations of the vaginal wall. Boreham et al. observed large dilated veins more frequently in the lamina propria of women with POP and suggested that these dilated vessels are related to an increase in stasis and gravity, but we could not confirm this hypothesis [9]. We observed very subtle changes in the smooth muscle cell and blood vessel densities, suggesting that the fibrillar components might play important roles in the integrity of the pelvic connective tissue and that the alteration of these components is most likely involved in the development of POP.

**Table 2** Density measurement of collagen, elastin, blood vessels, and smooth muscle cells in the lamina propria, and the muscularis layer of the precervical and middle third anterior ($n$=10) and posterior ($n$=9) vaginal wall

| | Lamina propria | | | Muscularis layer | | |
|---|---|---|---|---|---|---|
| | Middle third area | Precervical area | *P* value | Middle third area | Precervical area | *P* value |
| Mean collagen density | | | | | | |
| Anterior vaginal wall | 88.6±1.2 | 84.3±1.9 | 0.18 | 75.3±1.6 | 74.2±1.7 | 0.79 |
| Posterior vaginal wall | 87.8±1.5 | 90.9±0.6 | 0.11 | 75.1±1.8 | 73.4±1.7 | 0.60 |
| Mean elastin density | | | | | | |
| Anterior vaginal wall | 8.4±1.2 | 12.1±2.0 | 0.048* | 5.2±0.4 | 8.4±1.2 | 0.009* |
| Posterior vaginal wall | 6.8±0.5 | 10.1±1.4 | 0.040 * | 7.3±0.6 | 9.0±1.1 | 0.44 |
| Mean blood vessels density | | | | | | |
| Anterior vaginal wall | 2.7±0.2 | 2.9±0.2 | 0.35 | 3.4±0.4 | 3.3±0.2 | 0.85 |
| Posterior vaginal wall | 2.1±0.3 | 2.0±0.3 | 0.39 | 4.7±0.3 | 4.4±0.8 | 0.30 |
| Mean smooth muscle cells density | | | | | | |
| Anterior vaginal wall | – | – | – | 14.1±1.0 | 14.7±1.8 | 0.74 |
| Posterior vaginal wall | – | – | – | 12.1±1.7 | 12.7±1.9 | 0.54 |

Data are presented in percent (mean ± SD)

*Statistically significant

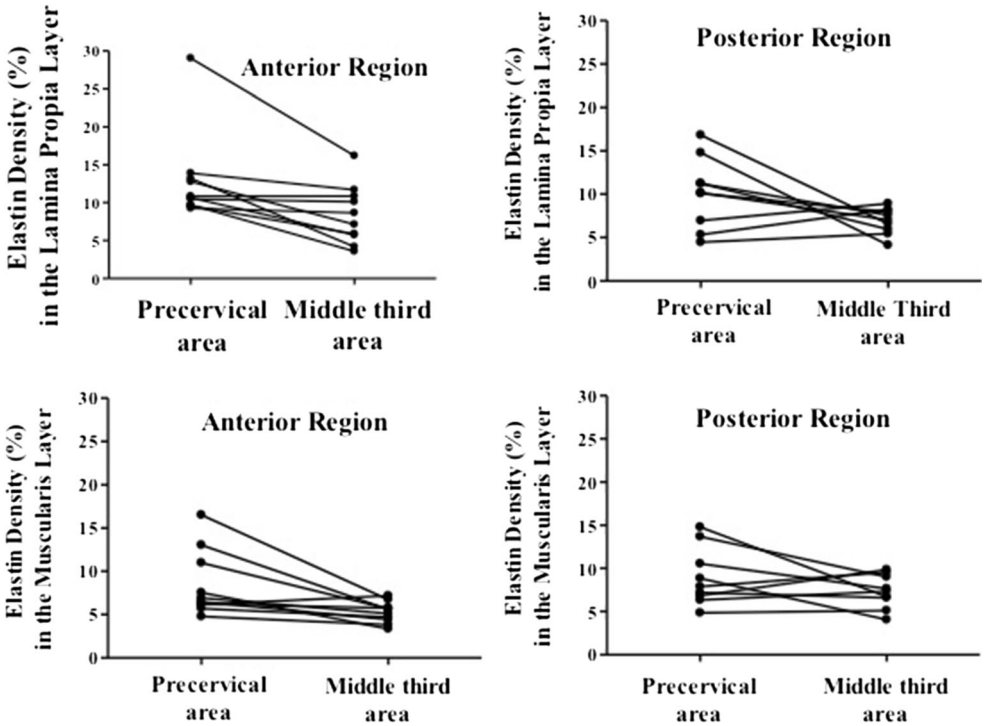


**Fig. 4** Elastin density measurement from each patient in the lamina propria and the muscularis layers form the precervical and middle third portion of the anterior ($n$=10) and posterior vaginal wall ($n$=9). Elastin densities are presented as percentages

There was no significant difference in the total amount of collagen between the two tissue locations. The collagen content measured in the present study corresponds more precisely to the area occupied by the non-elastic fraction of the ECM and was not assessed by specific immunohistochemical staining of collagens I, III, and V. Indeed, in our experience, the immunohistochemical study of collagen subtypes is nonspecific. Therefore, one shortcoming of this study is that it did not specifically assess changes in densities of the different collagen subtypes, including collagen III, which is often involved in the pathogenesis of prolapse [25]. We did not observe significant changes in the total amount of collagen or in the vaginal thickness between the two locations, but, as previously mentioned, the sample size of this study is limited.

In this study, a new method for quantifying the components of vaginal tissue in women with POP was applied. The whole-slide image acquisition method enabled the inspection of entire histological sections. This whole-slide imaging overcomes the limitations of the commonly used "hot spot" approach, which is reported to be observer-dependent and thus lacks reproducibility [12, 13, 26, 27]. The development of image processing algorithms, adapted to the specific immunohistochemical staining of the tissue, allows the automated detection and quantification of the elastin, collagen, smooth muscle cell, and vessel densities. The precise quantification of these parameters can be used to improve our knowledge of the vaginal wall's properties and integrate these elements with other methods of analysis, such as molecular biology or biomechanical assessments.

In conclusion, genital prolapse is a complex disease whose pathophysiology is poorly understood. This study suggests that elastin might play a role in this pathological condition because its density was significantly lower in the most distal portion of the vaginal wall. These findings illustrate the existence of more vulnerable locations within the vaginal tissue and support the central defect theory. However, this study is not conclusive of a causal effect of low elastin in POP and also highlights the difficulty of studying histological changes in vaginal tissue. Further studies are needed to determine the mechanisms that cause these changes and lead to POP.

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**Conflicts of interest** none

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