In a beautifully descriptive paper, authors from Rio de Janeiro and San Francisco report a quantitative and qualitative histological analysis of spongiosal tissue in patients with bulbar urethral strictures. They found that stricture formation was characterised by major alterations in extracellular matrix features.

A morphometric analysis of bulbar urethral strictures

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OBJECTIVE

To report a quantitative and qualitative histological analysis of spongiosum tissue in patients with bulbar urethral strictures.

MATERIALS AND METHODS

Urethral specimens from 15 patients who had end-to-end anastomotic urethroplasty were evaluated; the control group comprised five bulbar urethras from cadavers. The collagen content, elastic fibres, smooth muscle and vessels were analysed using stereological methods.

RESULTS

There was complete loss of the relationship between smooth muscle, extracellular matrix and sinusoids in the peri-luminal area (PLA), with collagen replacement. The extension of the fibrotic area was greater in those with a traumatic than in those with an atraumatic stricture. The content of smooth muscle and collagen in the peripheral spongiosum (PS) area was similar for the stricture and control groups, and results were comparable for traumatic and atraumatic groups and those with suprapubic cystostomy diversion or not before surgery. There was a remarkably lower vascular density in the traumatic than in the atraumatic group. There was an increase in type III collagen in the PLA and in type I collagen in the PS; collagen type III in the PLA was greater in the group with no suprapubic cystostomy diversion before surgery. There were fewer elastic fibres in both stricture areas (PLA and PS) than in the control group.

CONCLUSIONS

Urethral stricture formation is characterized by marked changes in extracellular matrix features, with consequent changes in organ function.

KEYWORDS

urethral stricture, morphometry, collagen, extracellular matrix, vessels

INTRODUCTION

Male urethral stricture is a fibrotic process resulting in uncompliant scarred tissue, decreasing the calibre of the urethral lumen. These changes have a variable impact on the patient's micturition. The bulbar segment is the most common site of urethral stricture, in up to a half of cases [1]. In an evaluation of 175 patients, Fenton *et al.* [1] showed that most urethral strictures are idiopathic (34%) or iatrogenic (32%), being less frequently inflammatory (20%) or traumatic (14%).

The normal male urethra is lined by a layer of pseudo-stratified columnar epithelium that lies on the basement membrane. Beneath the basement membrane there is a connective tissue layer containing vascular sinusoids of the corpus spongiosum and smooth muscle fibres [2]. The connective tissue includes mainly fibroblasts and an extracellular matrix (ECM), containing collagen, proteoglycans, elastic fibres and glycoproteins.

The histological, immunohistochemical and ultrastructural analyses of normal and strictured urethra show that the most dramatic changes appear within the connective tissue. Although the cause of stricture formation is thought to be a consequence of urothelial damage and extension into the supporting spongiosum tissue, the exact mechanism remains unknown. The wound-healing process in other tissues, e.g. skin, is characterized by an increase in the amount of collagen, which can lead to hypertrophied scar tissues [3]. However, to our knowledge, it is unknown if molecular mechanisms of urethral healing are completely comparable to those of the skin.

During the last decades there have been remarkable changes in the treatment of urethral stricture. Anastomotic urethroplasty (AU), with complete excision of scar tissue, is associated with better long-term results [4]. When AU is impossible, tissue transference techniques, e.g. penile fascio-cutaneous flaps or grafts (buccal mucosa or genital skin) are available [5]. Less aggressive procedures, e.g. direct visual internal urethrotomy (DVIU) or urethral dilatation can be used, but the results are inconsistent [6]. Despite these surgical options, the spongiosal urethral tissue is fundamental to all reconstructive techniques. The corpus spongiosum must provide an adequate blood supply to the anastomotic area, transfer tissues or to the epithelialization process.

The aim of the present study was to quantitatively and qualitatively analyse the spongiosum tissue in patients with bulbar urethral strictures, using histological methods to evaluate the distribution of collagen, elastic fibres and smooth muscle fibres.

MATERIALS AND METHODS

The local human research ethics committee approved the study, and all patients provided written informed consent. Urethral specimens from 15 patients (mean age 38 years, range 19–68) who had had end-to-end AU were evaluated. The control group comprised five bulbar urethras obtained from autopsies of individuals (mean age 41 years, range 28–52) who had died from causes unrelated to the urogenital system. The time elapsed between death and fixation of the excised controls was <6 h.

From the original urethral specimens only the central portion was selected. In all cases the stricture length was <2 cm (mean 1.3, range 0.5–2). The cause of the stricture was traumatic (TS, external trauma or urological instrumentation) in eight cases and atraumatic in seven (ATS).

The samples of urethral tissue were immersed in fixative solution containing 4% formalin in 0.1 M sodium phosphate buffer (pH 7.4) for 24 h at 4 °C. The specimens were then processed by routine histological methods. Transverse sections 5-μm thick were obtained, including the urethral epithelium and all spongiosum tissue. The material was stained using Hart's technique for elastic system fibres, with Gomori trichrome for collagen and smooth muscle fibres, and with Sirius red for collagen. The spongiosum tissue was analysed in two different regions, the peri-luminal area (PLA) and peripheral spongiosum (PS). Because the PLA and PS areas have similar components in the control group we did not differentiate these areas in the controls when comparing them with the PLA and PS areas in the stricture group.

From each urethral fragment, five different sections were selected. Five random fields were then evaluated from each section, resulting in the analysis of 25 test areas from each urethral fragment. For the stereological method, the analysed fields were then digitized to a final magnification of ×200 using a video camera coupled to a light microscope. The selected histological areas were then quantified by applying a test-grid system on the digitized fields on the screen of a colour monitor [7]. From stereological principles in isotropic tissue, the area distribution of a given structure, as determined on a two-dimensional section of the structure, is proportional to the volume distribution of the structure. The volume density of histological components was calculated as $Vv = Pp/Pt$, where Vv is the volume density, p is the tissue component under consideration, Pp is the number of test points associated with p, and Pt is the number of points in the test system. The stereological methods were described in detail elsewhere [7].

The results were compared between the TS and ATS groups. The effect of a suprapubic cystostomy at the time of surgery was evaluated in a group diverted ≥30 days before surgery (DSC) and in an undiverted (NSC) group.

The data were analysed using the unpaired Mann–Whitney *U*-test to determine whether the Vv differences were statistically significant, with $P \leq 0.05$ considered to indicate statistical significance.

RESULTS

There were more urethral epithelial layers in the stricture site and this was associated with an increase in the corneal layer. There was complete loss of the relationship between smooth muscle, ECM and sinusoids in the PLA, which was composed basically of collagen. The extension of the fibrotic area was variable, but was higher in the TS than the ATS group, Figs 1–3.

Table 1 shows the collagen and smooth muscle Vv in the PS area of the stricture and control groups. The content of smooth muscle and collagen was similar in the groups and results were comparable between the TS and ATS and DSC and NSC groups (Table 1).

Vascular density (VD, the Vv associated with blood vessels) was assessed in the TS and ATS groups; there was a markedly lower mean (SD) VD in the TS, at 13.1 (1.8)%, than in the ATS group, at 19.6 (2.7)% ($P < 0.001$; Fig. 4).

Qualitative analysis of collagen type I and III showed an increase in type III in the PLA and an increase in type I in the PS; collagen type III in the PLA was greater in the NSC group (Fig. 5).

Table 1 also shows the elastic fibre Vv in the PLA, PS and control groups; there were fewer elastic fibres in both stricture areas (PLA and PS) than in the control group (Fig. 6) . The distribution of elastic fibres in the different groups is also shown in Table 1; there were significantly fewer elastic system fibres in TS than ATS, but no difference in the DSC and NSC groups.

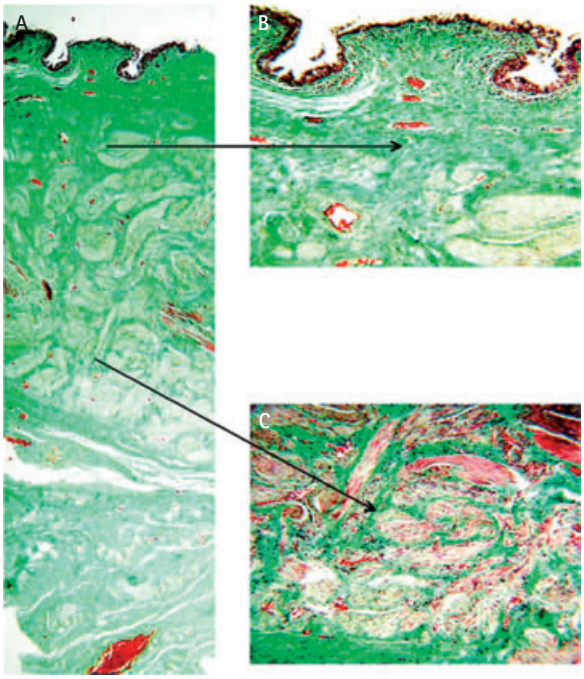


FIG. 1.
A normal bulbar urethra: (A) General view (×10); higher magnifications (×100) of (B) the PLA, and (C) the PS area. Gomori's trichrome stain.

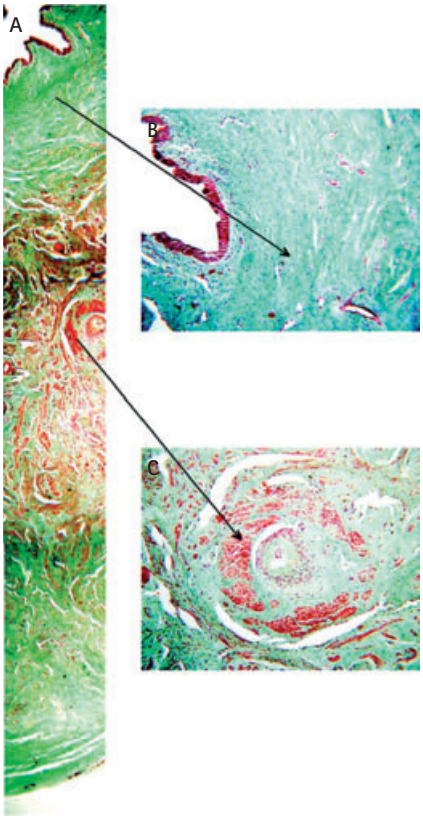


FIG. 2. A bulbar urethral stricture (ATS): (A) General view (× 10); higher magnification (× 100) of the spongiofibrosis in (B) the PLA and (C) the PS. Gomori's trichrome stain.

DISCUSSION

Despite the development of urethral surgical techniques during the last decades, studies on the arrangement of tissues in urethral stricture and the effect on surgical results are scarce. Scott and Foote [8] reported, in an animal model, that after trauma the urethral surface initially ulcerated and was covered by a stratified columnar epithelium. The stricture tissue is rich in myofibroblasts and giant multinuclear cells, and it was suggested that the first group of cells is related to stricture formation and the second to collagen production. The increase in collagen is associated with a loss of vascular characteristics of the normal urethra.

The collagen and elastic fibres are fibrotic components of the ECM and related to pathological changes in different tissues. The increase in collagen content represents a tissue response and the result is fibrosis [9]. Singh and Blandy [2] showed that the total collagen amount increases in strictured urethra, resulting in a dense fibrotic tissue associated with a decrease in smooth muscle. Baskin *et al.* [10], in a biochemical study, proposed that the total collagen amount in the stricture does not change, but rather its subtype ratio changes in favour of collagen type III. The changes in the ratio of collagen I/III were associated with a decrease in urethral compliance. However, the present samples were limited because they represented only a small portion of tissue from the whole resected urethra in the stricture and control groups. When we analysed the urethral stricture using transverse sections, the structural changes were not homogeneous. The PLA represents spongiosum tissue completely destroyed and replaced by connective tissue. This fibrotic area extends to the periphery to variable degrees, and in general is more extensive in TS, where there is a direct injury. The extension of spongiofibrosis can be evaluated using urethral ultrasonography [11].

There was an increase of 32% in the mean collagen concentration of the strictured bulbar urethra, associated with changes in the glycosaminoglycan concentration [9]. The predominant glycosaminoglycan in normal bulbar urethra is hyaluronic acid, while in urethral strictures there was an increase in hyaluronic acid and dermatan sulphate.

The intense fibrosis in the PLA was associated with a healthy PS, with a ratio between collagen and smooth muscle similar to that in the control group, with preservation of vascular structure. It is this area (the PS) that is used as a vascular support for grafts in the ventral onlay urethroplasty with spongioplasty [5]. When bulbar onlay urethroplasties were compared by graft position, ventral (supported by the PS) and dorsal (supported by the corpus cavernosum) the success rates were no different [5]. Despite the normal histological aspect, molecular changes can precede the disarrangement of the spongiosum tissue. In a previous study [12], urethral stricture was related to a decrease in immunoreactivity to neural nitric oxide synthase, despite the presence of a small amount of spongiofibrosis. It was confirmed that when neural nitric oxide synthase-carrying cavernosal nerves are damaged, a fibrotic process starts in the corpora cavernosa that is characterized by smooth muscle degeneration [13], and the authors postulated that the same process occurs in the pathogenesis of spongiofibrosis.

The qualitative evaluation of collagen types showed a greater collagen III/I ratio in the PLA than in the PS. This is probably associated with an increase in the collagen turnover

in this area, especially in NSC patients, where persistent obstructive flow together with urethral distension could be factors perpetuating the formation of fibrosis. Suprapubic diversion can modify the structure of the fibrotic urethral tissue, and therefore can affect the results of urethroplasty.

Urethral dilatation and DVIU are the most popular treatments for urethral stricture, with a simple technique and low morbidity. Despite this, two studies showed inadequate rates of long-term success [7]. Pansadoro and Emiliozzi [6] reported initial recurrence rates of 58% after DVIU and secondary procedures, with failures in almost all cases. The results were better for ATS (48%) than TS (16%). We found a significantly lower VD in TS than ATS, and this difference in stricture visualization could explain the clinical results.

During DVIU, fibrotic tissue of low vascularity is incised; the importance of healthy tissue in the epithelialization process was shown in experimental studies using models of hypospadias. Such an incision, through a normal urethral plate, was associated with epithelialization with no collagen deposition or scar formation [14]. Therefore, urethroplasty could be the first choice in cases of TS, rather then DVIU.

Bastos *et al.* [15] reported that the concentration of elastic fibres in the spongy urethra increased significantly with age, and this high concentration partly explained its high extensibility. This feature was markedly changed in urethral stricture, especially TS, where there was a dense and hypovascular scar, when compared with urethral strictures of other causes.

Changes in the penile elastic system were described in fibrosis of the tunica albuginea that characterises Peyronie's disease [16,17]. The spongiosum tissue has structural similarity with the erectile tissue of corpus cavernosum, and the relation between changes in elastic fibres and the quality of erectile tissue was reported [18]. Progressive fibrosis associated with denervation and ischaemia of the corpora cavernosa in patients after radical prostatectomy is associated with a decrease in elastic system fibres and smooth muscle [19].

In conclusion, urethral stricture formation is characterized by marked changes in ECM

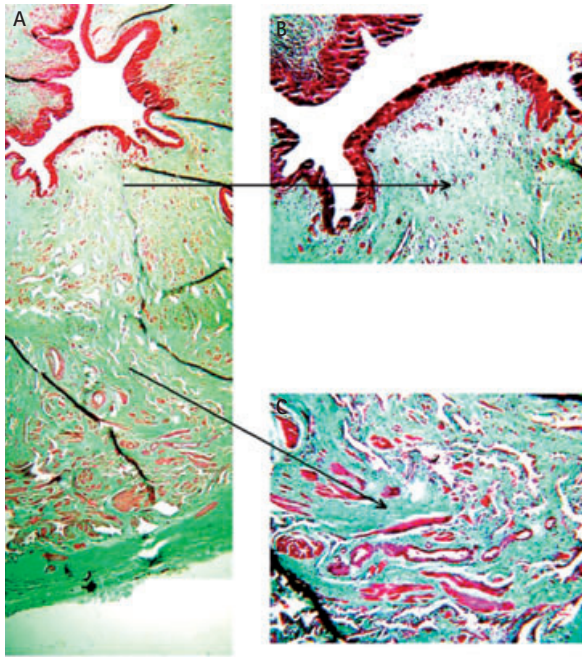


FIG. 3.
A bulbar urethral stricture (TS): (A) General view (×10); higher magnification (×100) of the spongiofibrosis in (B) the PLA and (C) the PS. The spongiofibrosis was greater in the TS than the ATS (Fig. 2). Gomori's trichrome stain.

FIG. 4. VD in the PLA of a bulbar urethral stricture: (A) in the ATS and (B) the TS. V, vessels. The VD was lower in TS than ATS. Gomori's trichrome stain, ×100.

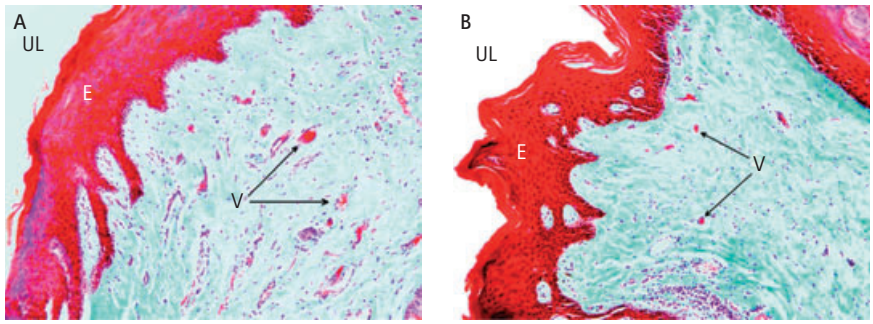


TABLE 1 The mean (SD) Vv (%) of smooth muscle cells, collagen and elastic fibres in the PLA and/or the PS of the stricture and control groups, and the subgroups (TS, ATS, DSC, NSC)

	PS			Elastic fibres		
Group	n	Smooth muscle	Collagen	n	PLA	PS
Stricture	15	47.8 (5.7)	42.3 (7.2)	13	52.0 (5.5)*	51.4 (5.2)*
Control	5	51.0 (7.1)	41.4 (7.0)	5	34.8 (18.7)	37.8 (12.8)
TS	8	48.4 (5.3)	44.4 (7.0)	7	16.7 (10.4)*	30.7 (10.4)*
NTS	7	47.0 (6.5)	40.1 (7.3)	6	45.7 (6.7)	46.3 (10.1)
DSC	6	47.5 (6.0)	44.8 (6.5)	5	26.4 (19.6)	38.7 (15.4)
NSC	6	48.2 (6.8)	38.6 (7.2)	8	32.4 (16.6)	42.4 (15.3)

$P < 0.05$ between groups.

FIG. 5. The PLA of a bulbar urethral stricture: (A) in the DSC group (×100) and (B) the NSC group (×200). The type III collagen (green) is greater in the NSC group (Sirius red under polarization, ×100).

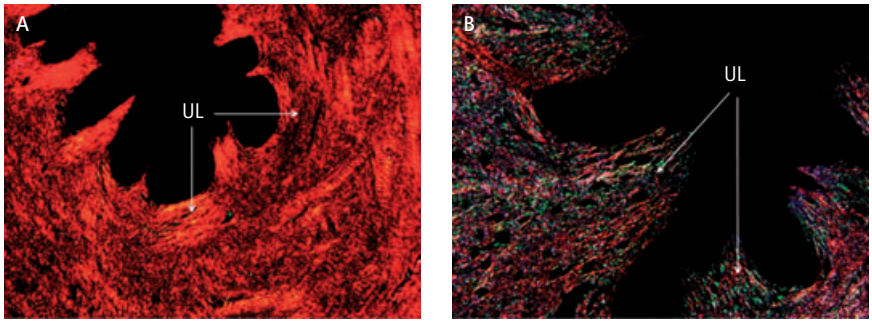
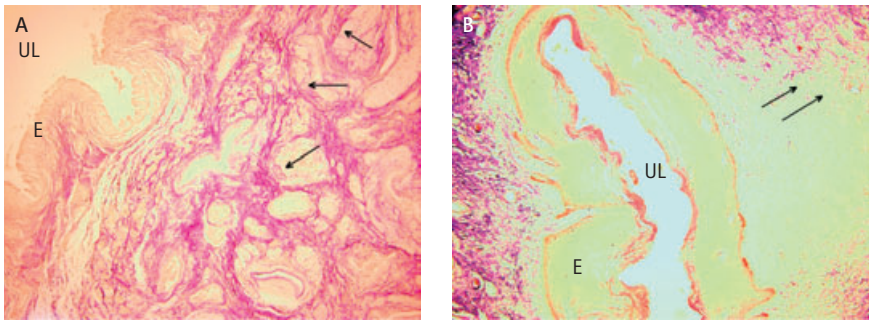


FIG. 6. A bulbar urethral section: (A) Normal urethra; (B) urethral stricture. Note that elastic fibres (arrows) are fewer in the urethral stricture (B) than in controls (A). Hart's stain, ×100.



components. To better understand the development of urethral stricture it is necessary to consider the changes in the PLA and PS separately. Suprapubic diversion can modify the structure of the fibrotic urethral tissue. The different causes of urethral strictures must be considered when choosing the surgical technique, because they affect the spongiosum tissue differently.

CONFLICT OF INTEREST

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Abbreviations: **ECM**, extracellular matrix; **AU**, anastomotic urethroplasty; **DVIU**, direct visual internal urethrotomy; **(A)TS**, (a)traumatic stricture; **PLA**, peri-luminal area; **PS**, peripheral spongiosum; **Vv**, volume density; **D(N)SC**, (not) diverted by suprapubic cystostomy; **VD**, vascular density.