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Significant decrease of extracellular matrix in prostatic urethra of patients with benign prostatic hyperplasia

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Summary. Background: Benign prostatic hyperplasia (BPH) nodules increase urethral resistance, resulting in "pressure" of tissue expansion to the urethra and leads to an increase in outflow resistance, accompanied by characteristic lengthening of the prostatic urethra. The goal of this investigation was to analyze and quantify changes of the histological components in the prostatic urethra of patients with BPH and compare with a control group.

Methods: Prostatic urethra tissue samples were obtained from ten patients (age range 63 to 79 years, mean 66) with clinical symptoms of bladder outlet obstruction who had undergone open prostatectomy. The ten control group samples (urethral tissue samples from the transitional zone) were collected from prostates obtained during autopsy of accidental death adults of less than 25 years. The Volumetric density (Vv) of the histological components was determined with stereological methods from 25 random fields per sample using the point-count method with a M-42 grid test system. The quantitative data were analyzed using the Kolmogorov-Smirnov and Mann-Whitney U tests.

Results: The Vv (mean ± SD) in the control and BPH groups respectively were: 20.3±0.3 and 17.12±1.1 in the elastic fiber system ($p<0.007$); and 29.7±1.9 and 25.1±2.4 in the collagen compartment ($p<0.03$). Smooth muscle cell volume was increased in BPH cases, 49.9±0.4 and 52.3±2.3 (not statistically significant).

Conclusion: BPH nodules caused a significant decrease of elastic system fibers and collagen in prostatic urethra.

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Introduction

A healthy first segment of urethra, which traverses the prostate, is an essential ingredient of urinary continence (Tanagho and McAnich, 2000). For a long time, its structure was ignored in morphological studies. McNeal (1972) was the first to point out the morphologic synthesis of the prostatic urethra and pathophysiological conditions. Therefore, the transitional zone (TZ) is particularly relevant when considering benign prostatic hyperplasia (BPH) and tumors. Thus, as a rule, this area (TZ) is considered to be the main glandular region which enlarges in BPH, in turn, the TZ it is contiguous with prostatic urethra (McNeal, 1984, 1990, 1997).

This urological disorder is most common in men over 50 years of age (Berry et al., 1984; McNeal, 1997; Wei et al., 2005), is associated with complex, not well understood interactions between acinar epithelial cells (Babinski et al., 2003) and their supportive stroma (McNeal, 1997; Chagas et al., 2002; Babinski et al., 2007). Several autocrine and paracrine stimuli are involved in these interactions, which ultimately modulate cell proliferation and expression of stromal extracellular matrix (ECM) molecules (Doll et al., 2001). Epithelial-stromal interactions and stromal ECM (Babinski et al., 2003, 2007) components also play key roles in normal prostate physiology and in tumour growth (nodules) (Cunha, 1994; Nagle, 2004).

The majority of prostatic urethral injuries have a compressive origin from the BPH nodules (McNeal

1978, 1990; Roehrborn and McConnell, 2002; Chagas et al., 2002). BPH nodular growth occurs in the TZ in >70% of the cases and compresses the prostatic urethra, resulting in bladder outlet obstruction (BOO) (McNeal 1978, 1990; Roehrborn and McConnell, 2002). Morphological and quantitative features of BPH nodules are known (McNeal 1997; Shapiro et al., 1992; Chagas et al., 2002; Babinski et al., 2003; Costa et al., 2004). It is well known that BOO in BPH results from mechanical obstruction by the enlarged prostate and the tone of the prostate smooth muscles in the prostatic tissue (Caine, 1986; Lepor et al., 1993; Ichiyanagi et al., 1999; Tanagho and McAnich, 2000).

Presumably, BPH nodules increase urethral resistance, resulting in "pressure" of tissue expansion to the urethra and leads to an increase in outflow resistance, accompanied by characteristic lengthening of the prostatic urethra (McNeal 1987, 1990; Roehrborn and McConnell, 2002). As a consequence of these changes, lower urinary tract symptoms (LUTS) may occur (Ichiyanagi et al., 1999; Mirone et al., 2004).

The prostatic urethra is lined by transitional epithelium (Tanagho and McAnich, 2000). Regional differences may exist and vary from squamous, stratified or pseudostratified columnar epithelium (Orlandini and Orlandini, 1989). These variations may extend into the prostatic ducts (Roehrborn and McConnell, 2002). It is primarily composed of a mucosal layer covered by urothelium. Beneath the basal membrane a layer of connective tissue (submucosa) (Tanagho and McAnich, 2000) contains vascular sinusoids, cells (mainly fibroblasts), an extracellular matrix of collagen, proteoglycans, elastic fibers, glycoproteins (Da-Silva et al., 2002a,b; Bastos et al., 2004), and an inner longitudinal and an outer circular layer of smooth muscle (Dorschner et al., 2001; Roehrborn and McConnell, 2002, Stolzenburg et al., 2002; Yucel and Baskin., 2004). More recent anatomical studies consider it as a completely independent structure (Dorschner et al., 2001; Stolzenburg et al., 2002; Yucel and Baskin, 2004).

The fibroelastic tissue, collagen and elastic fibers, forms an insoluble ECM and is found in different organs, for which they provide elasticity and some biomechanical resistance (Kielty et al., 2002). Thus, elastic fibers and collagen are critical matrix components, mainly in organs that change shape under physiological conditions, e.g., the male urethra. These elements in the spongy urethra have been studied previously (Sing and Blandy, 1976; Baskin et al., 1993; Hsu et al., 1994; Da-Silva et al., 2002a; Bastos et al., 2004). In particular studies (Sing and Blandy, 1976; Baskin et al., 1993; Hsu et al., 1994), significant collagen content was observed through the interaction of elastic fibers with collagen and it was suggested that elastic fibres are important for urethral compliance. The ECM of the prostatic urethra has received less attention than smooth muscle disposition and innervation (Dorschner et al., 2001; Stolzenburg et al., 2002; Yucel and Baskin, 2004; Karam et al., 2005). So, the structure of this region has still not been properly studied in the normal prostatic urethra, or compared with hyperplastic samples. A better knowledge of the histological components in the normal and the hyperplasic prostatic urethra is necessary.

Thus, we studied the volumetric density (Vv) of the fibroelastic elements (collagen and elastic fibers) and smooth muscle in the prostatic urethral samples from patients with BPH mass and control (normal) groups, to define the quantities of the ECM components.

Materials and methods

Ethics procedures

This study complies with the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh, 2000). Our Internal Review Board approved study guidelines. Also, the protocol received approval by the Ethics Committee on Human Research of the State University of Rio de Janeiro. All patients provided written informed consent for the use of prostatic tissue material for research prior to inclusion in the study.

Groups and samples

BPH tissue samples of the TZ with prostatic urethra tissue were obtained from ten patients without symptoms of BOO who did not undergo any treatment for symptomatic BPH. Each patient had undergone open prostatectomy (retropubic or transvesicular). All patients studied had prostates larger than 40g (mean 60), a urodynamic flow rate of 15 ml/s - voiding pressure above the maximum flow rate of 40 cm H_2O, Schaffer nomogram with average degree of obstruction of 4.5 and an International Prostate Symptom Score (IPSS) of 18. Patient age range was 63 to 79 years [mean 66] and all patients had shown histological diagnosis of BPH with no foci of prostatic carcinoma.

The control samples were obtained from autopsies of young males ranging in age from 18 to 30 years (mean 24), who died of causes unrelated to the urogenital system. After removing the entire prostate cadaver, the fragments (1x1x0.3 cm) were obtained through longitudinal incisions, parallel to the urethra in the transition zone (TZ) and median incision in the periurethral zone of the prostatic urethra (ZPU). The methods described in detail previously were respected (McNeal, 1997; Chagas et al., 2002). The time elapsed between death and fixation of the excised controls was less than six hours.

Histological procedures and morphometrical method

The samples of prostatic urethra tissue were immersed in Bouin's solution for 24 hours and then embedded in paraffin. All samples were initially stained with hematoxylin-eosin and examined by the same

pathologist to detect any foci of carcinoma and to exclude samples with artifacts. A pathologist from the University Hospital, who was not involved in the research protocol, analyzed the prostate samples obtained in the surgical unit. Then, a pathologist from the Urogenital Research Unit confirmed the diagnosis.

From each prostate, five different samples of the prostatic urethra were taken from the transitional zone. From each sample, five different sections were selected. Five random fields were evaluated from each section, resulting in the analysis of 25 test areas from each prostatic urethra, totaling 250 fields that were analyzed for elastic fibers, collagen and smooth muscle in each group. For the stereologic analysis, the 5 μm sections were stained with Weigert's Fuscin-Resorcin with previous oxidation to detect the elastic system fibers (stained violet) and Masson's thricrome to detect collagen (blue stained) and smooth muscle (red stained).

The analyzed fields were then digitized to a final magnification of x400 using a video camera coupled to a light microscope (BH-2 Olympus). The selected histological areas were then quantified by applying a test-grid system (M42) on the digitized fields on the screen of a color monitor (Sony). From stereologic 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 the histological components was calculated as Vv = Pp/Pt, where Vv was the volume density, p was the tissue component under consideration, Pp was the number of test points associated with p, and Pt was the number of points in the test system. The stereologic methods have been described in detail previously (Weibel et al., 1966; Gundersen et al., 1988).

Statistical analysis

The data were analyzed using the Kolmogorov-Smirnov test to verify normal distribution (Gaussian) and variance of data, as well as the unpaired t test to demonstrate whether the Vv differences were statistically significant. $P \leq 0.05$ was considered statistically significant.

Results

The reported findings are exclusively from the transitional zone of the assessed human prostatic urethras. The prostatic urethra of the control group was primarily composed of a mucosa with transitional epithelium (Fig. 1). Beneath the mucosa, the submucosa was observed containing collagen interspersed with longitudinal bundles of smooth muscle (Fig. 2) and

Table 1. Results of elastic system fibers, connective tissue and smooth muscle cell quantitative evaluation (volumetric density) in prostatic urethra transitional zone.

Element	Controls	BPH	n	P Value
Elastic Fiber	20.3± 0.3	17.1±1.1	10	0.007†
Connective tissue	29.7±1.9	25.1±2.4	10	0.03†
Smooth Muscle Cells	49.9±0.4	52.3±2.3	10	0.09*

Data presented as the mean percentage ± SD. *: Not statistically significant, unpaired t test. †: Statistically significant, unpaired t test.

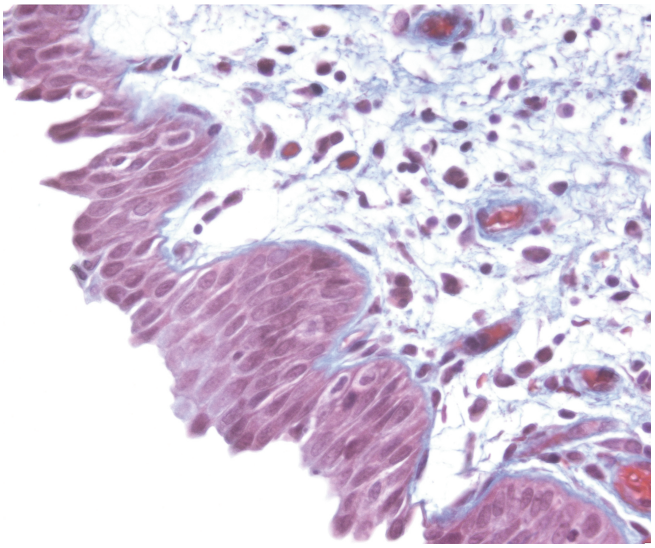

Fig. 1. Photomicrograph of a transition zone prostatic urethra section from control group illustrating mucosal layer with transitional epithelium preserved. Masson's Trichrome staining. x 40

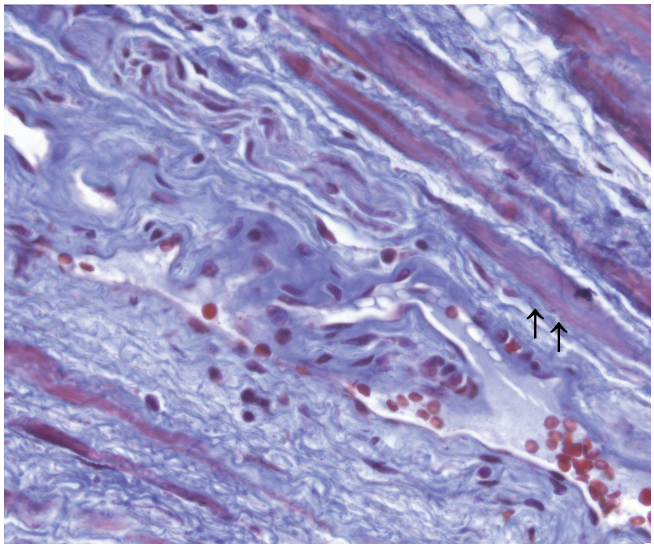

Fig. 2. Photomicrograph of a control group showing the connective tissue and parallel smooth muscle cells (arrows) in submucosa layer. Masson's Trichrome staining. x 40

elastic fibers (Fig. 3). These two parallel layers constitute the major component of the urethral wall.

In the BPH group, histological disorders were observed in the urethral mucosa with the epithelium damaged (Fig. 4). Statistically, significant decreases in Vv of collagen (18.3%) (Fig. 5) and elastic fibers (18.7%) (Fig. 6) were observed, while the Vv of smooth muscle was increased but not statistically significant (4.6%). The relative values (% mean±SD) of the quantitative evaluation in both groups are shown in Table 1.

Discussion

Area density has been used by many studies, attempting to quantify the ECM of urogenital structures

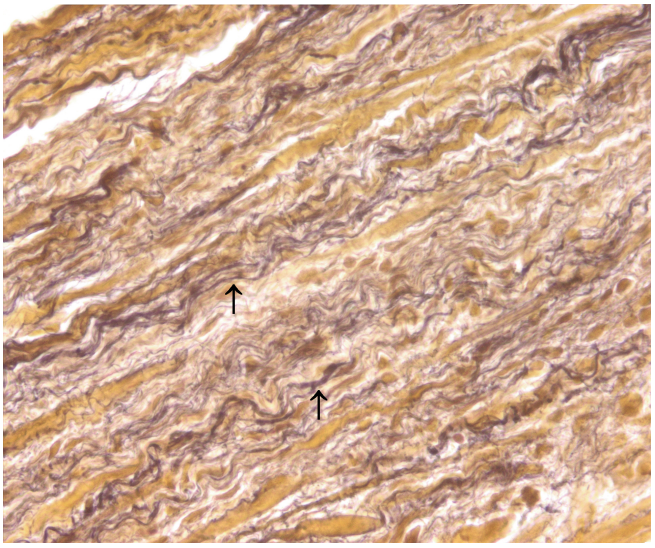

Fig. 3. Photomicrograph of a control group showing the elastic system with longitudinal and parallel arrangement fibers (arrow) in submucosa layer. Note their variable thickness. Weigert's Resorcin-Fuchsin staining. x 40

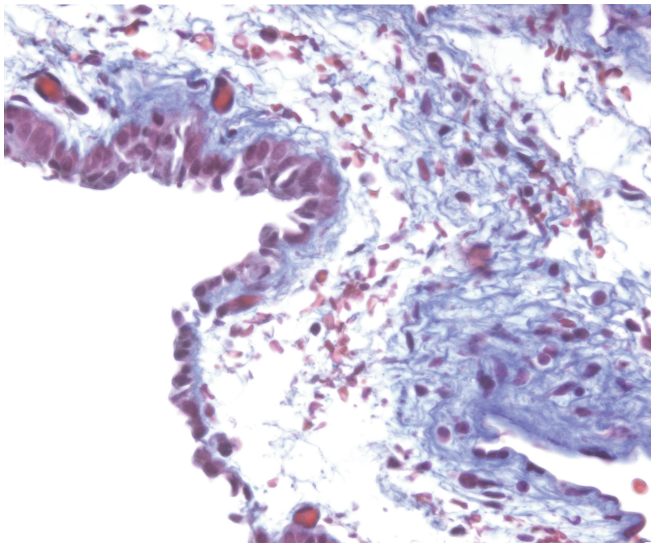

Fig. 4. Photomicrograph of prostatic urethra from patient in BPH group showing damage to epithelium and mucosa layer. Masson's Trichrome staining. x 40

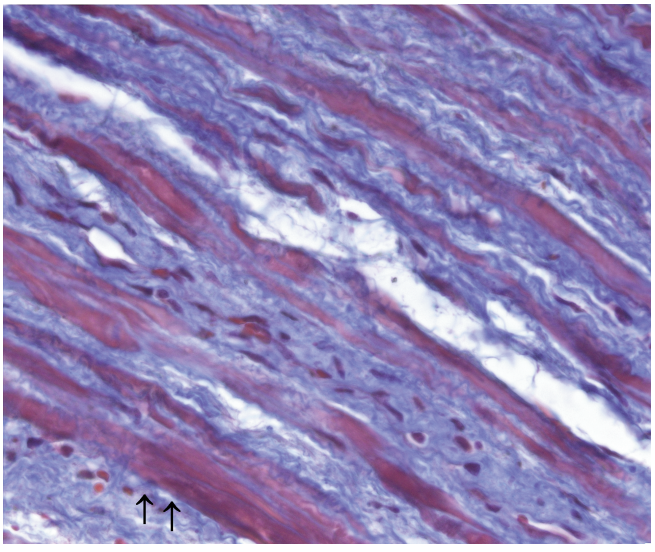

Fig. 5. Photomicrograph of prostatic urethra from patient in BPH group showing a decrease in connective tissue and non-significant increase in smooth muscle cells. Masson's Trichrome staining. x 40

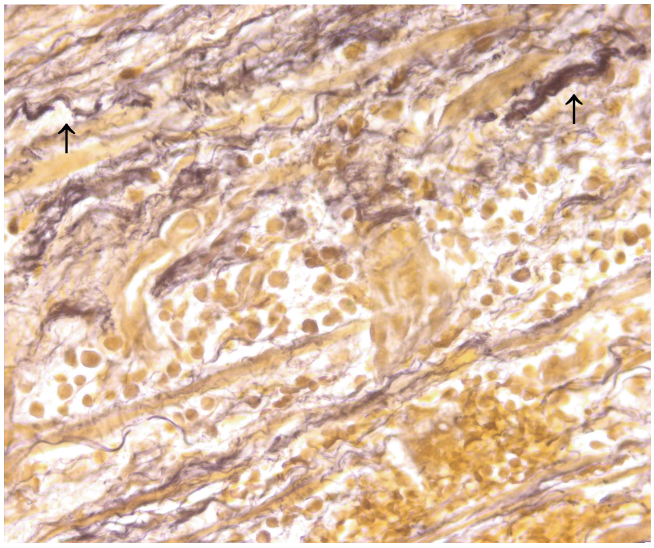

Fig. 6. Photomicrograph of prostatic urethra from patient in BPH group Demonstrating a loss in quantity of elastic system fibers. Note, in some areas the fibers are disorganized. Weigert's Resorcin-Fuchsin staining. x 40

by using computer-aided image analysis software (Rotten et al., 1988; Shapiro et al., 1992; Schuster and Schuster, 1999; Morrison et al., 2000). These programs use the color property of the elements (pixels) of an image to determine a threshold level for inclusion. However, this method is limited to quantifying only thin and line-shaped structures (Battlehner et al., 2003).

Stereological methods have been used in quantification studies (Bartsch et al., 1979; Chagas et al., 2002; Babinski et al., 2003) specifically to determine the number or proportion of fibrous components of the ECM (Chagas et al., 2002; Babinski et al., 2003). The point counting method has proven to be very efficient. It avoids the bias that frequently occurs with computerized image analyses, which may overestimate or underestimate the analyzed structures (Rotten et al., 1988; Schuster and Schuster, 1999; Morrison et al., 2000).

There are several conflicting opinions concerning the relationship between the size of the hyperplastic prostate and the grade of urethral compression (Shapiro et al., 1992; Kaplan et al., 1995; Ishigooka et al., 1996; Kojima et al., 1997; Terris et al., 1998; Ichiyanagi et al., 1999). The relationship between the IPSS and the prostate total volume suggests that the bigger prostate impinges more making the symptoms more severe (Kojima et al., 1997). On the other hand, the lack of relationship between the total prostatic volume and BOO suggests that the symptoms and urodynamic outcomes of the infra-vesical obstruction from HPB is more associated with the enlargement of the prostatic transitional zone than to the total prostatic volume (Kaplan et al.,1995; Ishigooka et al., 1996; Kojima et al., 1997; Terris et al.,1998; Ichiyanagi et al., 1999). The relationship between the histological changes in the prostatic urethra and the beginning of the obstructive symptoms is not completely recognized.

The ECM is very important in the development of tissues and organs, rebuilding and repairing damage, and maintaining tissue normality (Hay, 1991; Streuli, 1999; Eckes et al., 2000; Kielty et al., 2002). Therefore, the location and arrangement of the ECM can be associated with the functional features of each kind of tissue, pointing out their particular biomechanical proprieties (Hay, 1991; Streuli, 1999; Eckes et al., 2000; Kielty et al., 2002). Changes in the elements of the ECM of an organ also change these proprieties (Peters et al., 1997; Da-Silva et al., 2002a).

The observed human male urethras presented a uniform gross anatomical appearance, while changes in the ECM could be noted in its various segments (membranous, spongy and glandular), justifying the functional (Hsu et al., 1994; Da-Silva et al., 2002a) and pathological differences (Singh and Brandy, 1976; Baskin et al., 1993; Da-Silva et al., 2002b). A significant decrease of fibrous elements of the ECM (collagen and elastic fibers) was found. The external pressure from the hyperplastic nodules of the transitional zone probably creates a urethral rebuilding. This process imposes a large urethral distension, together with a characteristic increase in length (longitudinal and anteroposterior axis) of the proximal prostatic urethra, damaging the mucosa and submucosa (McNeal, 1997). McNeal (1997) reported that this change could take place due to the urethral position between the two transitional zone masses. This could compress the urethra and create an enlargement in the urethral mucosa, which increases as much as the hyperplastic nodules expand. Thus, the fibrous elements of the ECM could have an important role in preservation of the normal structure of the prostatic urethra. However, this connection has not been related previously.

The decrease in the elastic and collagen fibers affects the prostatic urethra by diminishing its elasticity and resistance. Therefore, should be considered during the urological procedures, such as urethrocystoscopy. The rebuilding changes of the ECM (elastic and collagen fibers) in the prostatic urethra are quite different from the spongy urethra, where the fibrous elements of the ECM increase (Da-Silva et al., 2002a). In this research, the remodeling of ECM from prostatic urethra is also different from previous studies, which examined the prostatic parenchyma (Chagas et al., 2002; Babinski et al., 2003; Costa et al., 2004).

There were no significant changes in the volumetric density of the smooth muscle in the submucosa on the urethral samples. Hence, the most important modifications in the prostatic urethra in patients with BOO for BPH are in the ECM. These changes are associated with great enlargement of urethra and the increase the external pressure from the prostatic hyperplastic nodules of the transitional zone (McNeal, 1972).

Yucel and Baskin (2004), in anatomical studies, reported that the vesical musculature of the trigonum extends caudally over the anterior half of the prostatic urethra, to the level of the *verumontanum*, as continuous muscular strata. Inui and colleagues (Inui et al., 1999) suggest that the HPB/BOO process could cause a compensatory hypertrophy of the detrusor muscle, which could be elongated onto the prostatic urethra (Yucel and Baskin, 2004). However, no significant change was found in the Vv of the smooth muscle, which suggests that the continuous muscular stratum from the vesical trigonum to the verumontanum are not altered, as reported in the vesical segment (Gosling, 1997; Kojima et al., 1997; Inui et al., 1999).

The results of this study offer new information about urethral morphology, mainly with reference to damage of the prostatic urethra by the hyperplastic nodules of the TZ previously published (McNeal, 1972, 1997). These results may add data about the urodynamic outcome concerning BOO (Ishigooka et al., 1996; Kojima et al., 1997; Gosling, 1997; Terris et al., 1998; Ichiyanagy et al., 1999; Inui et al., 1999). Briefly, the decrease in the ECM (elastic and collagen fibers) could be associated with an increase at the resistance and a decrease at the complacence of the urethra.

Conclusion

BPH nodules caused a significant decrease of elastic system fibers and collagen in prostatic urethra.

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Conflict of interest. None declared.

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