

Prostatic calculi detected in peripheral zone of the gland during a transrectal ultrasound biopsy can be significant predictors of prostate cancer

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Summary *Purpose: Prostatic calculi (PC) are usually associated with benign prostatic hyperplasia or chronic inflammation. However, in several studies prostatic inflammation and calcification have been implicated in the pathogenesis of prostate cancer (CaP). We evaluated the prevalence of PC during transrectal ultrasound (TRUS) and correlate the ultrasonographic patterns with histological findings.*

Methods: A prospective study of 664 patients undergoing TRUS and prostate biopsy was planned. A standardized reproducible technique was used with using a GE Logiq 7 machine equipped with a 5-9MHz multi-frequency convex probe "end-fire". We defined marked presence of PC as multiple hyperechoic foci with significant area (≥ 3 mm in the largest diameter). PC were classified according to zone distribution into the gland: transitional zone (TZ), central zone (CZ), and peripheral zone (PZ).

Results: No significant difference was noted between the patients with PC and without PC, when comparing age, preoperative PSA level, prostate volume, and biopsy number, except for DRE findings. 168 patients (25.3%) had marked presence of PC on TRUS: 50.6% in TZ, 20.2% in CZ, and 29.2% in PZ. 31 patients (63.3%) with presence of PC in PZ had CaP on biopsy. The correlation observed between CaP and the presence of PC in PZ was statistically significant ($p < 0.001$). However, among patients in the CaP group there was no statistical association between PC and moderate or high Gleason grade.

Conclusions: This study suggests that chronic prostatic inflammation and PC have a role in the biogenesis of cancer. CaP was more frequent in patients with PC in PZ of the gland, but was not associated with higher Gleason grade among these patients ($p < 0.001$).

KEY WORDS: Prostatic calculi; Prostate cancer; Inflammation; Ultrasound; Risk factors.

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INTRODUCTION

Prostatic calculi (PC) are usually ovoid bodies, with variable sizes and shapes, and they are found in alveoli of prostatic glands (1). PC are created as a result of deposition of calcareous calcium salts on corpora amylacea (2). Their incidence is believed to begin after puberty and increase

with age (3, 4). The study of prostate with transrectal ultrasound (TRUS) provides the evaluation of the number, location, and dimension of the PC through axial and sagittal views. PC are usually accepted associated with benign prostatic hyperplasia (BPH) or chronic prostatitis (5). However, in several studies prostatic inflammation and calcification have been implicated in pathogenesis of prostate cancer (CaP) (6-8). In this study we wished to evaluate the prevalence of PC during TRUS and correlate the ultrasonographic patterns with histological findings.

MATERIALS AND METHODS

A single center prospective randomized study of 664 consecutive patients referred for transrectal ultrasound-guided prostate biopsy (TRUSBx) to our Department was performed between November 2013 to April 2016.

All patients underwent an initial TRUSBx for abnormal digital rectal examination (DRE), high prostate-specific antigen (PSA) levels (≥ 4 ng/ml), or both. Patients with a history of biopsy, surgical treatment for prostatic disease or neoadjuvant therapy were excluded from our study. All patients enrolled in the study signed a consent form for the procedure. TRUSBx was performed with the patient in the left lateral decubitus using a General Electric Logiq 7 machine (GE Healthcare, Milwaukee, WI, USA) equipped with a 5-9MHz multi-frequency convex probe "end-fire". The patients were treated under local anaesthesia with Lidocaine Spray 10 gr/100 ml (ECOCAIN[®], Molteni Dental, FI, Italy) applied two minutes before the procedure (9). Each TRUS performed included an assessment of the prostatic diameter, the volume of the whole prostate, the transition zone, capsular, seminal vesicle characteristics, presence/absence of prostatic calcification, and a morphological description of potential pathological features. The prostate volume was invariably calculated using prostate ellipse formula ($0.52 \times \text{length} \times \text{width} \times \text{height}$).

We defined moderate/marked presence of PC as multiple (≥ 3 in number) hyperechoic foci with significant area (≥ 3 mm in the largest diameter) and coarse shadow detected in both dimensions (Figure 1). Mild calcifications were defined as 1 or multiple small foci without

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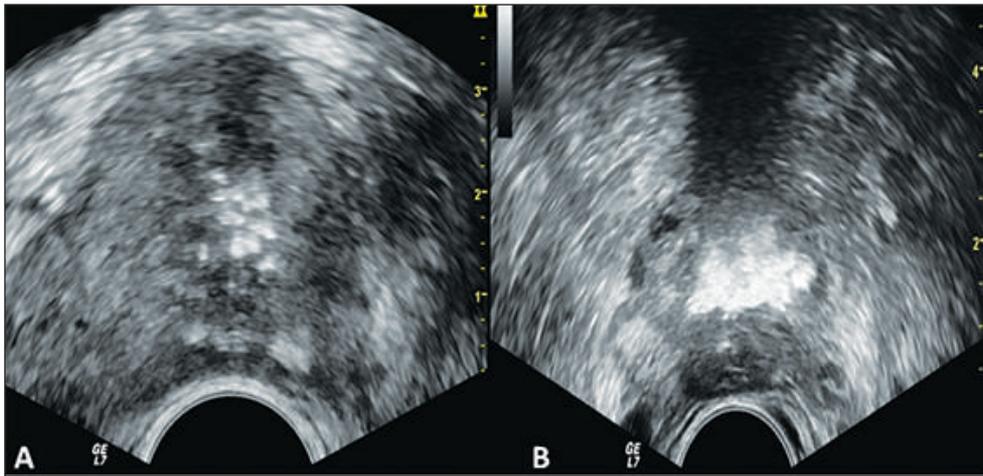


Figure 1. Transrectal ultrasound in axial section of the prostate gland: middle (A) and apex (B) zone show marked calcifications with multiple hyperechoic foci, and significant area (≥ 3 mm) with coarse shadow.

coarse shadow. PC were classified according to zone distribution into the gland [transitional zone (TZ), central zone (CZ), and peripheral zone (PZ)]. All measurements were analysed and recorded by an experienced urologist. After having images of the prostate, sampling was carried out with a 18-Gauge Tru-Cut (Bard Biopsy Systems, Tempe, AZ, USA) needle powered by an automatic spring-loaded biopsy disposable gun. A 14-core biopsy scheme was performed in each patient, as first intention, including 2 basal samples (lateral and medial), 2 parasagittal samples (lateral and medial), 2 apical samples (lateral and medial), and 1 transitional zone sample on each side. All biopsy cores were analysed internally by our Pathology Department specializes in genitourinary pathology. The Gleason grading was based on the recommendations of the 2005 international society of urological pathology consensus conference.

Statistical analysis

Comparisons between patients with PC and without PC were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Univariate logistic regression analysis was used to identify the individual clinical factors predictive of CaP presence. All statistical analyses were conducted on Microsoft Excel 2010 platform version 10.1. A $p < 0.05$ was considered to indicated statistical significance.

RESULTS

The mean \pm standard deviation age of enrolled patients was 61.4 ± 6.6 years, with a prostate volume (PV) of 46.2 ± 18.9 ml, initial PSA levels of 7.5 ± 5.3 ng/ml. The number of biopsy cores was 11.4 ± 4.6 . No significant difference was noted between the patients with PC and without PC, when comparing age, preoperative PSA level, prostate volume, and number of biopsy fragments, except for DRE findings. In patients with PC more frequently abnormal DRE findings were observed. CaP was detected in 213 patients (32.1%) and their Gleason scores were ≤ 6 (51.2%), 7 (32.4%), and ≥ 8 (16.4%) (Table 1). High-grade prostatic intraepithelial neoplasia (PIN) was shown in 81 patients (12.2%), and atypical

Table 1.

Demographic, clinic-pathologic features and outcomes of patients undergoing transrectal ultrasound biopsy.

	Patients with prostatic calculi (n: 168)	Patients without prostatic calculi (n: 496)	P
Age (year), mean \pm SD*	61.1 \pm 6.3	62.4 \pm 6.5	NS**
Prostate volume (ml), mean \pm SD*	45.4 \pm 17.7	47.2 \pm 18.3	NS**
PSA [†] level (ng/ml), mean \pm SD*	6.9 \pm 5.8	7.7 \pm 5.1	NS**
N° biopsy cores, mean \pm SD*	11.2 \pm 4.4	11.9 \pm 4.6	NS**
Abnormal DRE [‡] , n (%)	91 (54)	156 (31)	< 0.001
Prostate cancer, n (%)	45 (26.8)	168 (33.9)	NS**
Biopsy Gleason score, n (%)			
≤ 6	31 (68.9)	78 (46.4)	< 0.001
7	10 (22.2)	59 (35.1)	< 0.001
≥ 8	4 (8.9)	31 (18.5)	< 0.001

SD* = standard deviation; PSA[†] = prostate-specific antigen; NS** = not significant; DRE[‡] = digital rectal examination.

small acinar proliferations (ASAP) in 55 patients (8.3%). One hundred and sixty eight patients (25.3%) had marked presence of PC on TRUS: 85 patients (50.6%) in TZ, 34 patients (20.2%) in CZ, and 49 (29.2%) in PZ (Table 2). Prostatic calculi in TZ were frequently associated with histological findings of inflammation (55.3%; $p < 0.002$) and BPH (41.2%; $p < 0.001$). However, in 31 patients (63.3%) with presence of PC in PZ had CaP on biopsy ($p < 0.001$). The frequency of CaP increased as

Table 2.

Distribution of prostatic calculi and histological findings in patients undergoing transrectal ultrasound biopsy.

Histological findings, n (%)	Prostatic calculi distribution			P
	Transitional zone (n: 85)	Central zone (n: 34)	Peripheral zone (n: 49)	
Hyperplasia	35 (41.2)	10 (29.5)	10 (20.4)	< 0.001
Inflammation	47 (55.3)	13 (38.2)	8 (16.3)	< 0.002
Cancer	3 (3.5)	11 (32.3)	31 (63.3)	< 0.001

patient age increased. In addition, patients with CaP had higher PSA levels and smaller prostates than men without CaP ($p < 0.001$). The correlation observed between CaP and the presence of PC in PZ of the prostatic gland was statistically significant ($p < 0.001$). However, among patients in the CaP group there was no statistical association between PC and moderate or high Gleason grade.

DISCUSSION

In literature, no studies have systematically looked for PC in a general population. However, it is generally accepted that the incidence of PC increases with age (10). In one study of 612 TRUS, PC were seen in 47.2% of patients younger than 50 years old and in 86% of those older than 50 years. The authors showed that calcifications are associated with chronic inflammation (11). *Geramoutsos et al.* (12) screened 1374 men younger than 50 years old and found 101 cases of PC (7.4%). The patients with larger significant calcifications were much more likely to have chronic inflammation. A casual connection between chronic inflammation and carcinogenesis was supposed for the first time by *Virchow* in 1863 (13). Today it is accepted that almost 25% of all cancers are associated to chronic inflammatory diseases. Standard examples are gastric cancer occurring on a history of *Helicobacter pylori* infection or colon carcinoma after history of Crohn's disease (14). In the last years, the casual link between inflammation and PCa has been investigated (15). However, the cause of chronic prostatic inflammation as well as its presumed role in carcinogenesis remain unclear. Inflammation and PC are often histologically apparent in the examination of the CaP specimens from older men (4). Histologically, chronic inflammatory cell infiltrates are commonly observed in prostate specimens in the peripheral and transitional zone of the gland (7). Immune surveillance may clarify the association between the inflammation and CaP. The prostate gland is clearly an immunocompetent organ. Besides epithelial and stromal cells, the prostate also contains a small number of immunocompetent cells (lymphocytes, macrophages and granulocytes), which are collectively known as human prostate-associated lymphoid tissue (16). *Vignozzi and Maggi* demonstrated that prostate stromal cells can act as antigen-presenting cells, stimulating alloreactive CD4+ T cells to produce several inflammatory cytokines, chemokines and growth factors (including IL 8, IL 6 and bFGF) in response to a variety of inflammatory stimuli (17). The formative mechanism of PC is not well understood, but is usually observed more often in an inflammation of prostate gland than in the prostates of men with CaP. Prostatic stones are hypothesized to be calcifications of prostatic secretions, with a core of calcium apatite surrounded by concentric layers (18). Multiple different aetiological agents are thought to contribute to initiation of PC, including infections, bacterial biofilm production, dietary factors, physical trauma, hormonal changes, desquamation of prostatic epithelium with obstruction of intraprostatic ducts and urine reflux (3, 8, 19). *Griffiths et al.* (20) reported 63% correlation between PC and CaP on TRUS. Similar results were found by *Hwang et al.* (21). In their study the authors deduced that, although not sta-

tistically significant, PC were more common in men with CaP and were associated with higher Gleason grade among these patients. Contrary to the aforementioned researches *Woods et al.* (8) showed that PC were less commonly associated with CaP than with BPH.

Epidemiological, genetic, molecular and rodent model studies have suggested an association between PC and CaP. *Zhang et al.* (22) further examined the relation between inflammation, PC and PCa in surgical specimens. Inflammation was more common in radical prostatectomy specimens. The limitation of this tissue source relates to the fact that these prostates were removed from patients with an established diagnosis of PCa, and their aggressiveness warranted surgery. However, in our study, inflammation was presented histologically in association with PC in 46.4% of patients who were diagnosed PCa on prostate biopsy. This proportion of positive findings for CaP in the biopsy specimens is relatively high compared with those in previous studies. To our knowledge, this is the first prospective study based on a large number of patients to show a link between CaP and PC. However, it had several limitations. A first limitation, we had no data available regarding the ethnic background of the patients. This details could be of special interest, because in multi-ethnic populations, some subgroups might have more unfavourable CaP characteristics than others (23). However, although we did not expressly documented race, the majority of the patients of our study cohort were white and Italian population. Thus, the number of Asian and black patients was very small and surely did not exceed 1% of the entire cohort. Second in this study only a visual assessment of PC was performed. The description of PC, calcification size, hyperechoic prostatic images as signs of an inflammation is not usually accepted in the urological community (24). We did not quantify the extent of PC and distinguished only 2 distinct types of severity. To clarify the actual role of PC on CaP development more molecular research, cytokines and inflammatory markers are required, and would provide a more accurate measure of inflammation. Finally, long term follow-up of patients with PC might clarify the possible association between PC, inflammation and CaP. There is no evidence confirming a temporal relationship between tissue inflammation and prostatic carcinogenesis.

CONCLUSIONS

Although we don't completely understand the role of the PC in the development of BPH or CaP. There is emerging evidence that inflammation is crucial for the etiology of BPH, whether inflammation has a role in the pathogenesis of CaP remains unclear. Chronic prostatic inflammation may result from the immunologic response of different pathogenic causes that produce a tissue damage and subsequent vulnerability to developing cancer. However, further studies are necessary to more fully elucidate the relationship between inflammation, PC, and CaP.

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