Pentraxin 3: A Novel Biomarker for Predicting Progression from Prostatic Inflammation to Prostate Cancer

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Abstract

Pentraxin-3 (PTX3) is a member of the pentraxin family of innate immune regulators, which includes C-reactive protein (CRP). PTX3 has been implicated in angiogenesis, proliferation, and immune escape in cancer. In the present study, we evaluated PTX3 tissue expression and serum concentration as a biomarker to discriminate prostatic inflammation and benign prostatic hyperplasia (BPH) from prostate cancer, and to determine whether PTX3 status may predict progression from BPH to prostate cancer. We analyzed 40 patients with biopsy-proven BPH who underwent a second prostate biopsy 12 to 36 months later when they were diagnosed with prostate cancer or inflammation/BPH (n = 20 patients each group). Furthermore, we evaluated PTX3 serum concentrations in an independent set of patients with biopsy-proven inflammation/BPH (n = 61) and prostate cancer (n = 56). We found reduced PTX3 tissue expression in patients with prostatic inflammation/BPH compared with patients who developed prostate cancer. In the latter group, there was an increase in PTX3 tissue expression between the first and second prostate biopsy. PTX3 serum levels were also higher in patients with prostate cancer than in patients with inflammation/BPH. In contrast, there was no difference in serum PSA or CRP levels in these two groups. ROC curve analysis confirmed the reliability of PTX3 serum levels in predicting prostate cancer development, identifying a cutoff value of 3.25 ng/mL with a sensitivity and a specificity of 89.3% and 88.5%, respectively. In summary, our results encourage further evaluation of PTX3 as a tissue biopsy and blood-borne biomarker to discriminate BPH from prostate cancer. Cancer Res; 74(16); 4230–8. ©2014 AACR.

Introduction

Prostate cancer is the most common solid-organ malignancy and the second leading cause of cancer-related death in males (1). In the vast majority of cases, prostate cancer is diagnosed by a prostate biopsy (PBx) performed because of an elevated PSA level and/or an abnormal digital rectal examination (DRE). In current clinical practice, however, the diagnostic yield of a first extended PBx is in the range of 40% (2) even selecting patients by “improved” nomograms (3). Patients with an initial negative PBx have an approximately 20% incidence of prostate cancer at a subsequent PBx (4).

Although studies on saturation biopsy (5) provide evidence that the first PBx misses some of these cancers, studies on prostate cancer chemoprevention (6, 7) demonstrate that other cancers may develop in the time span to second PBx due to progressive changes in the prostate microenvironment.

Chronic inflammation has been reported to cause as much as 20% of human cancers and to be implicated in prostate carcinogenesis by several mechanisms (8). Basically, chronic inflammation creates a milieu rich in proinflammatory cytokines and growth factors that may lead to an uncontrolled proliferative response and genetic mutations of rapidly dividing cells (9, 10). An increased number of CD4+CD25+ and CD8+Foxp3+ regulatory T cells have been observed in prostate glands and in the peripheral blood of patients with prostate cancer, suggesting an important role for active immune suppression of antitumor immunity (11–13).

Pentraxins, a superfamily of evolutionary conserved proteins, are essential components of the humoral arm of the innate immune system and play a pivotal role in vascular biology (14, 15). Pentraxin-3 (PTX3), the prototype of long pentraxins, differs from short pentraxins for gene organization, cellular source, and ligand-binding capacities (15, 16). Like short pentraxins, PTX3 facilitates dysregulation of mitogenic signaling pathways, sustains cellular proliferation, angiogenesis, insensitivity to apoptosis, cancer cell invasion and migration, and tumor escape from immunosurveillance (17, 18). Unlike short pentraxins, such as C-reactive protein (CRP),
PTX3 is not produced by hepatocytes but synthesized by a variety of cell types at the site of inflammation (14, 19), whereby it seems to regulate complement activation (14, 17, 18). Recent findings suggest an insidious relationship between complement and cancer in terms of cellular proliferation and regeneration as well as angiogenesis (18, 20).

Considering that chronic inflammation is found in as much as 80% of PBxs (7) and the potential role of PTX3 in inflammatory-related carcinogenesis (21), the aim of present study was to determine whether PTX3 prostate tissue expression and serum levels could predict progression from chronic prostate inflammation to prostate cancer.

Patients and Methods

Study population

Our Institutional Review Board-approved prospective database on PBx was used to identify patients who were diagnosed with prostate cancer by a second PBx performed at least one year after an initial negative PBx (group A, n = 20). A matched dataset of patients with a second negative PBx performed at least one year after the first negative PBx (group B, n = 20) was created using a propensity score multidimensional matching technique based on the following clinical variables: age, serum PSA value, prostate volume, DRE findings, and time to second PBx. As previously described, all patients received, in the first and second PBx setting, a 18-core biopsy scheme following some form of local anesthesia (22, 23).

Histologic analysis of prostatic tissue

Two senior uropathologists performed the analysis using contemporary diagnostic criteria for prostatic intraepithelial neoplasia, atypical small acinar proliferation, and prostate cancer. Moreover, biopsy samples of both benign prostatic tissue and prostate cancer were graded on a 4-point scale for inflammation (0, no inflammatory cells; 1, scattered inflammatory cell infiltrate; 2, nonconfluent lymphoid nodules; and 3, large inflammatory areas with confluence of infiltrate) and aggressiveness (0, no contact between inflammatory cells and glandular epithelium; 1, contact between inflammatory cell infiltrate and glandular epithelium; 2, clear but limited, less than 25% of the examined material, glandular epithelium disruption; and 3, glandular epithelium disruption more than 25% of the examined material), as described by Irani and colleagues (24).

Tissue immunofluorescence and confocal laser scanning microscopy

PTX3 protein expression was evaluated on paraffin-embedded tissue sections using a rat monoclonal anti-PTX3 antibody (clone MNB4, Abcam). Specific fluorescence was identified by confocal microscopy using the Leica TCS SP5 (Leica) equipped with argon-krypton (488 nm), green-neon (543 nm), and helium-neon (633 nm) lasers. To stain the nuclei, samples were incubated with TO-PRO (Invitrogen, Molecular Probe). The slides were then mounted in Gel Mount (Biomed Corp.) and sealed. To perform fluorescence quantification, for every sample up to 10 optical slices of 0.6 μm in depth each were acquired with ×40 oil immersion differential interference contrast microscopy (DIC) objective by an observer blinded to the origin of the slides, recorded at the same photo multiplier tube, pinhole aperture, and laser voltage setting, and analyzed with Leica Application Suite Advanced Fluorescence software (LAS AF, Leica). In detail, each image was represented with 1,028 × 1,028 pixels measuring 387.5 × 387.5 μm² each, and recorded

<table>
<thead>
<tr>
<th>Table 1. Clinical and histologic characteristics of the PBx study population</th>
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<tr>
<td><strong>Baseline clinical and histologic variables</strong></td>
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<tr>
<td>Age, y</td>
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<tr>
<td>68.3 ± 9.4</td>
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<tr>
<td>Suspicious DRE ( %)</td>
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<td>Prostate volume (mL)</td>
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<td>Total PSA levels (ng/mL)</td>
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<td>PSA density (ng/mL * mL)</td>
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<td>International Prostate Symptom Score (IPSS)</td>
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<td>Flow max (mL/s)</td>
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<td>Post-void residual (mL)</td>
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<td>Histologic grading of prostatic inflammation (G ≥ 2)</td>
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<td>Aggressiveness of prostatic inflammation (A ≥ 2)</td>
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<tr>
<td><strong>Clinical and histologic variables at second biopsy</strong></td>
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<tr>
<td>Prostate volume (mL)</td>
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<td>Histologic grading of prostatic inflammation (G ≥ 2)</td>
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<td>Aggressiveness of prostatic inflammation (A ≥ 2)</td>
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<td>Biopsy Gleason score</td>
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<tr>
<td>≤ 6</td>
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<td>7</td>
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<td>≥ 8</td>
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with a line mode to reduce background noise (average on two scanning images). Image acquisition was performed selecting specific domains of the emission spectrum, that is, Alexa 488 was excited at 488 nm with a Argon/Krypton laser and its fluorescence emission was collected between 500 nm and 542 nm. Each 24-bit TIFF image was analyzed by LAS AF software to visualize every z-series of images in x-y side view. The mean fluorescence intensity (MFI), representing the intensity of each pixel in the selected channel/number of pixels included in the region of interest, was quantified by a specific tool of the LAS AF software.

To determine whether PTX3 expression was caused by infiltrating cells and not only by prostate cells, we performed a double staining with a mouse monoclonal anti-PSA antibody (clone ER-PR8, Dako), a rabbit polyclonal anti-Myeloperoxidase (MPO), a mouse monoclonal anti-CD20 (clone L26), and a rabbit monoclonal anti-CD3 (clone 2GV6; all from Ventana Medical Systems). The immune complexes were detected using the Alexa Fluor 488 goat anti-rat and 546 goat anti-mouse IgG (H+L) and goat anti-rabbit IgG antibodies (Molecular Probes), whereas nuclei were stained with TO-PRO-3 (Molecular Probes).

**PTX3 ELISA**

PTX3 serum levels were tested on serum samples drawn at the time of PBx in two independent groups of patients with biopsy-proven prostatic inflammation (n = 61) and prostate cancer (n = 56). Serum samples, frozen at −80°C, were assayed in duplicate using a commercially available ELISA Kit, according to the manufacturer’s instructions (R&D).

Figure 1. PTX3 protein expression in prostatic tissue (A–E). Indirect immunofluorescence for PTX3 showed limited staining in both first (A) and second (B) PBx samples of patients who did not develop prostate cancer (group B); conversely, PTX3 staining was moderate in the first (C) and diffuse in the second (D) PBx samples of patients who developed prostate cancer (group A). Quantification of specific PTX3 fluorescence demonstrated low PTX3 tissue levels in group B patients at both first and second PBx, as opposed to a higher PTX3 tissue levels at first PBx that further increased at second PBx in group A patients (E). Nuclei are highlighted with TO-PRO in blue. Results are representative of 10 patients. *, P = 0.02; **, P = 0.002; ***, P = 0.03.
Statistical analysis

Statistical analyses were performed using SPSS software (SPSS 17.0 Inc.). Normal distribution of the data was evaluated by Skewness and Kurtosis test. Continuous variables were compared by paired or unpaired Student *t* test, as appropriate. Correlation between two variables was ascertained by Pearson correlation test. Frequencies were compared by the $\chi^2$ test. To test the independent effects of different clinically relevant variables on histologic diagnosis of prostate cancer, a binary logistic regression analysis was used and serum levels of PTX3, PSA, and age were tested as covariates.

To validate the association of a variable with histologic diagnosis, an ROC curve analysis was performed. An operational cutoff level was defined to differentiate the risk of prostate cancer between the two groups. A two-sided $P < 0.05$ was considered statistically significant. Results were expressed in the text as mean ± SD unless otherwise stated.

Figure 2. Colocalization of PTX3 and PSA. A–H, double-label immunofluorescence showing expression of PTX3 (green; A and E) and PSA (red; B and F) in the prostate tissue of group A (A–D) and group B (E–H). TO-PRO-3 counterstains nuclei (blue; C and G). Merged images (yellow) demonstrate coexpression of PTX3 and PSA by resident prostate cells both in group A (D) and in group B (H), although PTX3 expression is limited in group B, as expected. Results are representative of 10 patients.
Figure 3. Colocalization of PTX3 with MPO, CD20, and CD3. A–H, double-label immunofluorescence showing expression of PTX3 (green; A and E) and MPO (red; B and F) in the prostate tissue of group A (A–D) and group B (E–H). TO-PRO-3 counterstains nuclei (blue; C and G). Merged images (yellow; D and H) demonstrate that numerous infiltrating neutrophils (MPO-positive cells, white arrows) express PTX3, but prostatic resident cells are the major source of this long pentraxin. I–P, double-label immunofluorescence showing expression of PTX3 (green; I and M) and CD20 (red; J and N) in the prostate tissue of group A (I–L) and group B (M–P). TO-PRO-3 counterstains nuclei (blue; K and O). (Continued on the following page.)
Results

Clinical and histologic features

The main clinical features of the two sets of patients as well as the pathologic features of cancers diagnosed at second PBx are summarized in Table 1. There was no difference in the clinical and pathologic characteristics of the two groups at the first PBx (Table 1). Tumor grading at the second biopsy in group A is reported in Table 1.

PTX-3 protein prostatic tissue expression

In group B, PTX3 protein expression was extremely low in both first and second PBx samples (Fig. 1A and B); conversely, group A showed a marked PTX3 protein expression already in the first PBx samples (Fig. 1C), which further increased in the second PBx (Fig. 1D). Specifically, patients in group B had a statistically significantly lower PTX3 expression than those in group A (Fig. 1E) at both first (MFI: 0.8 ± 0.3 vs. 2.2 ± 0.8; P = 0.02) and second PBx (MFI: 1.1 ± 0.9 vs. 3.5 ± 0.3; P = 0.002), with no significant changes between first and second PBx (MFI: 0.8 ± 0.3 vs. 1.1 ± 0.9; P = 0.6). Conversely, patients in group A showed a significant increase in PTX3 expression (Fig. 1E) from first to second PBx (MFI: 2.2 ± 0.8 vs. 3.5 ± 0.3; P = 0.03).

Interestingly, double immunofluorescence confocal microscopy revealed that PTX3 extensively colocalized with PSA in both group A (Fig. 2A–D) and group B biopsies (Fig. 2E–H). PTX3 also colocalized with MPO+ infiltrating cells, although they did not represent a major source of PTX3 within the prostate tissue (Fig. 3A–H). On the other hand, CD4+ or CD20+ infiltrating cells did not significantly express the long pentraxin (Fig. 3I–X).

PTX3 serum levels

Two independent groups of patients with biopsy-proven prostatic inflammation (n = 61) and prostate cancer (n = 56) were recruited and PTX3 serum levels were assessed. The sample size of these independent groups was assessed on the basis of the results of the PTX3 tissue expression to reach a power of 80%. No differences in main clinical features were observed between the two groups of patients (Table 2). Gleason grading of prostate cancer is reported in Table 2. At the time of PBx, patients with prostate cancer had statistically significantly greater PTX3 serum levels (6.1 ± 4.9 vs. 2.4 ± 1.1 ng/mL; P = 0.001) than those with prostatic inflammation (Fig 4A). Note-worthy, there was no difference in PSA serum levels (8.3 ± 4 vs. 7.5 ± 3.7, ng/mL; P = 0.7) between the two groups of patients (Fig 4B).

To rule out the potential influence of systemic inflammation on PTX3 serum levels, we also tested CRP serum levels and found no difference between patients with prostatic inflammation and prostate cancer (2.4 ± 2.8 vs. 2.8 ± 2.2 mg/dL; P = 0.8), with normal values in all patients (Fig 4C). Lack of correlation between CRP and PTX3 serum levels was further confirmed by regression analysis (Spearman R² = 0.002; P = 0.9).

To estimate the relative risk for prostate cancer at time of PBx, a binary logistic regression analysis was performed using histologic diagnosis of prostate cancer as dependent variable, and serum levels of PTX3 and PSA, prostate volume, and patient age at time of PBx as covariates. The only covariates significantly related to the risk of prostate cancer were the PTX3 serum levels and patient age [RR = 3.836; confidence interval (CI), 2.335–6.302; P < 0.001 and RR = 1.092; CI 1.007–

Table 2. Clinical and histologic characteristics of the independent set of patients with PTX3 serum evaluation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BPH (n = 61)</th>
<th>Prostate cancer (n = 56)</th>
<th>P</th>
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<tr>
<td>Age, y</td>
<td>61.5 ± 10.4</td>
<td>65.6 ± 6.6</td>
<td>0.2</td>
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<td>Suspicious DRE (%)</td>
<td>29.5</td>
<td>37.5</td>
<td>0.4</td>
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<td>Prostate volume (mL)</td>
<td>59.3 ± 20.9</td>
<td>55.9 ± 13.8</td>
<td>0.7</td>
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<tr>
<td>Total PSA levels (ng/mL)</td>
<td>7.5 ± 3.7</td>
<td>8.3 ± 4.4</td>
<td>0.7</td>
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<tr>
<td>PSA density (ng/mL)</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>0.5</td>
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<tr>
<td>International Prostate Symptom Score (I-PSS)</td>
<td>10.8 ± 8.1</td>
<td>13.1 ± 9.6</td>
<td>0.6</td>
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<td>Flow max (mL/s)</td>
<td>15.0 ± 9.3</td>
<td>11.7 ± 7.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Post-void residual (mL)</td>
<td>42.3 ± 21.5</td>
<td>33.0 ± 18.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Histologic grading of prostatic inflammation (G≥2)</td>
<td>15/61 (24.5%)</td>
<td>18/56 (34.1%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Aggressiveness of prostatic inflammation (A≥2)</td>
<td>9/61 (14.7%)</td>
<td>11/56 (19.6%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Biopsy Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>35 (62.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13 (23.2%)</td>
<td></td>
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<tr>
<td>≥8</td>
<td>8 (14.3%)</td>
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</table>

(Continued.) Merged images (yellow; D and H) show very rare infiltrating B cells (CD20-positive cells, white arrow) expressing PTX3. Q-X, double-label immunofluorescence showing expression of PTX3 (green; Q and U) and CD3 (red; R and V) in the prostate tissue of group A (Q–T) and group B (U–X). TO-PRO-3 counterstains nuclei (blue; S and W). In merged images (yellow; T and X), some infiltrating T cells (CD3-positive cells, white arrows) express PTX3. Results are representative of 10 patients for experimental groups.
Serum PTX3 (mg/mL)

A

PTX3

P < 0.001

B

PSA

P = ns

C

CRP

P = ns

BPH

Cancer

Figure 4. PTX3 (A), PSA (B), and CRP (C) serum levels. PTX serum levels were significantly higher in patients with prostate cancer (n = 56) in comparison with patients with BPH (n = 61; 6.1 ± 4.9 vs. 2.4 ± 1.1 pg/mL; t test for unpaired data; P < 0.001; A). PSA and CRP serum levels were assessed in the same cohort of patients, but no differences were found in relation to histologic diagnosis (8.3 ± 4.4 vs. 7.5 ± 3.7 ng/mL for PSA and 2.8 ± 2.2 vs. 2.4 ± 2.2 mg/dL for CRP in patients with prostatic cancer and BPH, respectively; P = nonsignificant [ns]; B and C, respectively). Data are expressed as median and 25th and 75th percentiles in boxes and 5th and 95th percentiles as whiskers.

1.183; P < 0.05, respectively), whereas PSA and prostate volume did not show any significant association (RR = 1.024; CI, 0.872–1.202; P = 0.773 and RR = 0.983; CI, 0.953–1.014; P = 0.289), respectively.

ROC analysis

A ROC curve analysis was carried out to further validate the association of PTX3 serum levels with the histologic diagnosis of prostate cancer and to define an operational cutoff value. The analysis showed that PTX3 serum levels were significantly associated with prostate cancer at PBx (AUC = 0.922, P = 0.0001), and defined a cutoff value of 3.25 ng/mL with a 88.5% specificity and a 89.3% sensitivity, a positive predictive value of 87.1% and a negative predictive value of 97.2% (Fig. 5A). Moreover, serum PTX3 curve performed much better than the serum PSA curve (AUC = 0.5569, P = 0.3; Fig. 5B) and CRP curve (AUC 0.336, P = 0.5; Fig. 5C).

Discussion

The present study demonstrates that PTX3 serum levels can reliably identify discriminate benign prostatic hyperplasia (BPH) from prostate cancer. In addition, PTX3 tissue expression may also identify those patients with chronic inflammation at PBx who are at risk to develop prostate cancer.

The potential role of PTX3 as a tissue and serum marker of carcinogenesis was recently postulated for human soft tissue liposarcoma (25) and lung carcinoma (20). Serum PTX3 elevation in patients with cancer are not well understood, but its known overexpression by endothelial cells and macrophages in response to inflammatory signals as well as its role in clearance of cells undergoing apoptosis (14) both suggest that PTX3 may act as a biomarker for neoplasia, through its elevation in the inflammatory microenvironment of the tumor and in the clearance of apoptotic cells (14).

In this context, the close relationship between complement cascade and PTX3 may represent an interesting pathogenic hypothesis. Indeed, there is a growing body of evidence linking a deregulation in complement cascade activation and carcinogenesis. Prostate cancer cells are well known to express different regulatory proteins of the complement system and this event may represent a mechanism of tumor escape from complement-mediated immune surveillance (14). PTX3 interaction with C1q result in complement activation on apoptotic cells, whereas in the fluid phase induces inhibition of the complement cascade (17). Because in our case, PTX3 is released within the prostate tissue, as demonstrated by its increased circulating levels, it is conceivable that its overexpression may represent a further mechanism of prostate cancer escape from complement-mediated immunosurveillance. It has been shown that PTX3 may also directly restrict the cross-presentation of tumor-derived antigens, thus limiting the immune response toward cancer cells (26).

The potential role of PTX3 in prostate carcinogenesis has been pointed out by Ravenna and colleagues (27), who demonstrated an increased expression of this long pentraxin and of several other mediators of innate immunity in the neoplastic but not in the normal prostate tissue of patients with prostate cancer. Unfortunately, their study had no control group of patients without prostate cancer and, most important, did not focus on patients who received a repeat PBx; thus, they could not provide any information on the role of PTX3 in disease progression. Conversely, our study demonstrated that PTX3 protein was significantly overexpressed in the prostate tissue of patients who developed prostate cancer, as compared with those who did not, at both first and repeat PBx; thus representing a reliable immunohistochemical marker to predict disease progression. Moreover, our study demonstrated that PTX3 serum levels were significantly higher in patients with prostate...
cancer as compared with those with chronic prostatic inflammation, thus suggesting that PTX3 could become a reliable blood test for prostate cancer diagnosis. Lack of correlation between PTX3 and CRP serum levels allowed ruling out the possibility of PTX3 increase being due to systemic inflammation, thus providing further support to the potential role of this marker in prostate cancer development/progression.

Potential study limitations include its retrospective nature and the relatively small number of patients. Conversely, evaluation of inflammation at first and second PBx, demonstration of PTX3/PSA colocalization, and correlation of PTX3 tissue expression with its serum levels certainly represent key strengths of our study.

In conclusion, the present study demonstrates for the first time that PTX3 prostatic tissue expression and PTX3 serum levels can reliably predict prostate cancer development. These findings shed light on a potential molecular mechanism linking prostatic inflammation and carcinogenesis and provide solid grounds for further testing the predictive value of this biomarker as well as its potential to represent a target for prostate cancer prevention.

Disclosure of Potential Conflicts of Interest
Nopotential conflicts of interest were disclosed.

References


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