Detecting Novel Urine Biomarkers for the Early Diagnosis of Prostate Cancer: Platelet Derived Growth Factor-BB as a Possible New Target

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Abstract

Introduction: Although the prostate specific antigen revolutionized the diagnosis of prostate cancer (PCa), it has its limitations. We prospectively examined the potential use of the platelet-derived growth factor-BB (PDGF-BB) as a urine biomarker for the early diagnosis of PCa.

Materials and Methods: The urine samples of 118 patients were collected after a prostatic massage and all the patients subsequently underwent ultrasound-guided transrectal biopsy. PDGF-BB was detected in the urine by enzyme-linked immunosorbent assay. Results: Patients with PCa had greater levels of prostate specific antigen and PDGF-BB. Receiver operating characteristic curve analysis showed that the optimal cut-off of PDGF-BB for the prediction of PCa was 1,504.9 with a sensitivity of 60% and a specificity of 51.3%. Conclusion: PDGF-BB showed a significant predictive ability for PCa. Detection of PDGF-BB in urine with Elisa was easy and improved our diagnostic accuracy in the diagnosis of PCa.
Materials and Methods

This was a prospective study of the First University Urology Clinic, Laiko Hospital, the University of Athens, Greece, in conjunction with the Department of Pharmacology of the University of Athens. The study was approved by the scientific board and Ethics Committee of Laiko Hospital (approval No. 461/22–7–09) and the samples were collected between July 2009 and July 2013. Written informed consent was obtained from every patient after a detailed explanation of the purpose of the study.

Patients

The urine of 118 consecutive high risk for PCa patients were collected after a vigorous prostatic massage from an experienced urologist and immediately stored to -80°C. All the patients made up the study population since according to statistical analysis this number of patients was adequate for the preliminary results and conclusions of the possible efficacy of PDGF-BB. The patients were candidates for a TRUS-b due to a positive DRE, and either due to an elevated PSA > 4 ng/ml or abnormal PSA kinetics (PSA velocity > 0.75 ng/ml/year). Exclusion criteria were an already diagnosed PCa, previous operation for benign prostatic hyperplasia (BPH), 5α-reductase inhibitor medications (finasteride and dutasteride), patients who had already submitted to a TRUS-b, and patients with PSA > 25 ng/ml. All the patients after a thorough medical history and the prostatic massage were submitted from different urologists in order to eliminate any influence from the results of the DRE during the sampling of the prostate (blind method) to a 10-core per prostatic lobe TRUS-b. Histology was performed by an experienced pathologist in a completely “blind” method. The patients with a negative for PCa histology were the control group, meaning that the control group was not a selection of patients but a blind group that occurred in the same way as the PCa patients.

Enzyme-Linked Immunosorbent Assay (ELISA)

The urinary concentration of PDGF-BB was measured using ELISA kits according to the manufacturers’ instructions (Elabscience Biotechnology Co., Ltd.).

PDGF-BB levels were determined using the Sandwich-ELISA method. Plates had been pre-coated with an antibody specific to PDGF-BB. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for PDGF-BB and avidin horseradish peroxidase (HRP) conjugate were successively added to each plate well and incubated. The optical density (OD) was spectrophotometrically measured at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of PDGF-BB. Then, the concentration of PDGF-BB, in the samples was determined by comparing the OD of the samples in the linear portion of the corresponding standard curve of each protein (fig. 1). All samples were run in duplicate and the values were averaged. The urinary concentrations of PDGF-BB were measured using an ELISA kit according to the manufacturer’s instructions (Elabscience Biotechnology Co., Ltd., Catalogue Number E-EL-H1577). The standard PDGF-BB and all the reagents are included in the ELISA kit, which are produced by using raw material from a world-renowned manufacturer and professional manufacturing technology of ELISA kits. The determination of urine concentrations of PDGF-BB took place using the ELISA method.

Statistical Analysis

Quantitative variables are presented as mean and standard deviation (SD) and/or median (interquartile range = IQR). Qualitative variables are presented as absolute and relative frequencies. For the comparisons of proportions, chi-square and Fisher’s exact tests were used. Student’s t-tests were computed for the comparison of mean values when the distribution was normal and the Mann-Whitney test for the comparison of median values when the distribution was not normal. PDGF-BB and PDGF-BB/Cr were tested for their ability to predict prostate cancer, using receiver operating characteristic (ROC) curves. The overall performance of the ROC analysis was quantified by computing the area under the curve (AUC). An area of 1 indicated perfect performance, while 0.5 indicated a performance that was not different from chance. Using ROC analysis the optimal cut-offs along with the sensitivity and specificity were also determined. Logistic regression analysis in a stepwise method was used in order to find independent factors associated with prostate cancer. Adjusted odds ratios (OR) with 95% confidence intervals (95%CI) were computed from the results of the logistic regression analyses. Statistical significance was set at 0.05 and analyses were conducted using STATA statistical software (version 11.0).

Fig. 1. Representative standard curve of PDGF-BB concentrations between 31.25 and 2,000 pg/ml. The y axis represents mean corrected absorbance (optical density) measured at 450 nm for the stated PDGF-BB concentrations on the x axis. The values were used to calculate PDGF-BB urine levels in prostate cancer samples.

Initially, the OD for all samples was measured using an ELISAReader machine. Therefore, the calculation of all concentrations was determined by using the standard curve and therefore all the data were compatible with quality assurance/quality control.

Since the concentration of any analysis in urine is influenced by the hydration status of each individual, the urinary levels of PDGF-BB were normalized by the urinary level of creatinine (PDGF-BB/Cr) and were expressed as ng/mg. Creatinine normalization is a standard procedure in the clinical laboratory for assessing the levels of biomarkers in urine.
Table 1. Sample demographics and clinical characteristics

| Variable                                      | Value                  |
|----------------------------------------------|------------------------|
| Age, years (mean ± SD)                       | 65.5 ± 8.6             |
| BMI, kg/m² (mean ± SD)                       | 27.6 ± 3.5             |
| Family status, n (%)                         |                        |
| Single                                       | 8 (6.8%)               |
| Married                                      | 109 (92.4%)            |
| Divorced                                     | 1 (0.8%)               |
| Educational level, n (%)                     |                        |
| 6 years                                      | 27 (22.9%)             |
| 9 years                                      | 17 (14.4%)             |
| 12 years                                     | 36 (30.5%)             |
| > 12 years                                   | 38 (32.2%)             |
| Smoking, n (%)                               |                        |
| No                                           | 41 (34.7%)             |
| Ex-smoker                                    | 44 (37.3%)             |
| Yes                                          | 33 (28%)               |
| Family history of cancer, n (%)              |                        |
| No                                           | 57 (48.3%)             |
| Yes                                          | 61 (51.7%)             |
| Family history of PCa, n (%)                 |                        |
| No                                           | 95 (80.5%)             |
| Yes                                          | 23 (19.5%)             |
| PSA, ng/ml (mean ± SD)                       | 9.1 ± 5.4              |
| < 4                                          | 6 (5.1%)               |
| 4–10                                        | 83 (70.3%)             |
| > 10                                         | 29 (24.6%)             |
| Positive DRE (mean ± SD)                     | 54 ± 46.2              |
| PDGF-BB, pg/ml, median (IQR)                 | 1,560.4 (988.5–1,829.7)|
| Cr, pg/ml, median (IQR)                      | 109 (71–163)           |
| PDGF-BB/Cr, *100, median (IQR)               | 1,440.3 (721.4–2,839.4)|

Results

The sample consisted of 118 men with a mean age of 65.5 years (SD = 8.6 years). Sample demographics and clinical characteristics are shown in table 1. Most of the patients were married (92.4%) and 28% were currently smokers. A family history of cancer and family history of prostate cancer were present in 51.7% and 19.5% of the sample, respectively. The mean PSA level was 9.1 (SD = 5.4) and 54 patients (46.2%) had a positive DRE. Forty patients had histopathologically verified prostate cancer, 26 showed prostatic intraepithelial neoplasia, and the histological examination did not reveal carcinoma in 52 patients.

Table 2 shows the univariate association of study variables with PCa. Patients with PCa were older, had more frequent positive DREs and had greater levels of PSA, PDGF-BB, and PDGF-BB/Cr.

ROC analysis (table 3) showed a significant predictive ability for PCa from PDGF-BB (fig. 2) and PDGF-BB/Cr (fig. 3). The AUCs were 0.62 (95%CI: 0.51–0.72) and 0.64 (95%CI: 0.53–0.74) for PDGF-BB and PDGF-BB/Cr, respectively. ROC curve analysis (fig. 2) showed that the optimal cut-off of PDGF-BB for the prediction of PCa was 1504.9 with a sensitivity of 60% and a specificity of 51.3%. Furthermore, ROC analysis (fig. 3) showed a cut-off of 1217.7 for PDGF-BB/Cr with a sensitivity of 67.5% and a specificity of 52.6%.

Separate analysis in patients with PSA levels 4–10 ng/ml, showed that PDGF-BB had a discriminative ability with AUC of 0.65 (table 3). The optimal cut-off was
Table 2. Association of study variables with prostate cancer

| Variable                                      | Negative                  | PCa                      | p    |
|-----------------------------------------------|---------------------------|--------------------------|------|
| Age, years (mean ± SD)                        | 63.3 ± 7.9                | 69.8 ± 8.4               | < 0.001† |
| BMI, kg/m² (mean ± SD)                        | 27.2 ± 3.4                | 28.3 ± 3.6               | 0.114‡      |
| Married, n (%)                                |                           |                          | 1.000‡      |
| No                                            | 6 (66.7)                  | 3 (33.3)                 |      |
| Yes                                           | 72 (66.1)                 | 37 (33.9)                |      |
| Educational level, years, n (%)               |                           |                          | 0.436†      |
| 6–9                                           | 26 (59.1)                 | 18 (40.9)                |      |
| 9–12                                          | 26 (72.2)                 | 10 (27.8)                |      |
| > 12                                          | 26 (68.4)                 | 12 (31.6)                |      |
| Smoking, n (%)                                |                           |                          | 0.255†      |
| No                                            | 29 (70.7)                 | 12 (29.3)                |      |
| Ex-smoker                                     | 25 (56.8)                 | 19 (43.2)                |      |
| Yes                                           | 24 (72.7)                 | 9 (27.3)                 |      |
| Family history of PCa, n (%)                  |                           |                          | 0.514†      |
| No                                            | 36 (63.2)                 | 21 (36.8)                |      |
| Yes                                           | 42 (68.9)                 | 19 (31.1)                |      |
| Family history of PCa, n (%)                  |                           |                          | 0.696†      |
| No                                            | 62 (65.3)                 | 33 (34.7)                |      |
| Yes                                           | 16 (69.6)                 | 7 (30.4)                 |      |
| PSA, ng/ml, median (IQR)                      | 6.9 (5.5–8.8)             | 9.2 (6.2–13.9)           | 0.010†      |
| PSA, ng/ml, n (%)                             |                           |                          | 0.014†      |
| < 4                                           | 4 (66.7)                  | 2 (33.3)                 |      |
| 4–10                                          | 61 (73.5)                 | 22 (26.5)                |      |
| > 10                                          | 13 (44.8)                 | 16 (55.2)                |      |
| DRE, n (%)                                    |                           |                          | 0.002†      |
| Negative                                      | 50 (79.4)                 | 13 (20.6)                |      |
| Positive                                      | 28 (51.9)                 | 26 (48.1)                |      |
| PDGF-BB, pg/ml, median (IQR)                  | 1504.7 (824–1746.7)       | 1657.1 (1258–2594.4)     | 0.040†      |
| PDGF-BB/Cr, *100, median (IQR)                | 1022.2 (647.5–1888.8)     | 1531 (639.2–3495.8)      | 0.016†      |
| 1 = Chi-square test; † = Fisher’s exact test; ‡ = Student’s t test; * = Mann-Whitney test.

Table 3. ROC analysis results for the prediction of prostate cancer

| Variable                                      | AUC  | 95%CI   | p    |
|-----------------------------------------------|------|---------|------|
| All                                           | 0.62 | 0.51–0.72 | 0.040 |
| PDGF-BB, pg/ml                                | 0.64 | 0.53–0.74 | 0.016 |
| PSA 4–10 ng/ml                                | 0.65 | 0.51–0.78 | 0.044 |
| PDGF-BB/Cr, *100                              | 0.63 | 0.49–0.78 | 0.065 |

1534.5 for PDGF-BB with a sensitivity of 63.6% and a specificity of 55.7%. The discriminative ability of PDGF-BB/Cr in patients with PSA levels 4–10 ng/ml did not reach statistical significance (p = 0.065).

When multiple logistic regression in a stepwise method was conducted (table 4), it was found that PDGF-BB was independently associated with PCa. Specifically, for a 100 unit increase in PDGF-BB the likelihood for PCa increased about 4%. Other variables that were found to be independently associated with PCa were increased age, increased PSA, and positive DRE.

Table 4. Odds ratios and 95%CI derived from stepwise multiple logistic regression analysis with the dependent variable the presence of PCa

| Variable                                      | OR (95%CI) | p    |
|-----------------------------------------------|------------|------|
| Age, years                                    | 1.10 (1.03–1.16) | 0.002 |
| PSA, ng/ml                                    | 1.09 (1.01–1.18) | 0.039 |
| DRE                                           | 1.0        |      |
| Negative                                      | 2.46 (1.01–5.98) | 0.048 |
| Positive                                      | 1.04 (1.01–1.08) | 0.019 |

† = Indicates reference category.
Discussion

The most important trials concerning PCa screening and disease management of the prostate, lung, colon, and ovary, the European Randomized Study of Prostate Cancer, the Prostate Cancer Prevention Trial and Prostate Cancer Intervention Versus Observation Trial reached confusing and contradictory conclusions concerning PSA screening [3, 6–8]. The prostate, lung, colon, and ovary failed to show benefits from screening in PCa mortality, the European Randomized Study of Prostate Cancer concluded a 20% reduction in PCa death but also a high incidence of over-diagnosis, the Prostate Cancer Prevention Trial suggested that there is not a definite PSA threshold with high sensitivity and specificity and finally the Prostate Cancer Intervention Versus Observation Trial concluded that there is no benefit of treating localized PCa compared with only observation [3, 6–8]. The conclusion is that PSA although the best biomarker to our disposal for the diagnosis of PCa is still far from the ideal biomarker both due to the fact that there is no accurate threshold that can be used with relative security for the diagnosis of PCa and that PSA also detects low-risk localized PCa that would never really be a threat to the survival of the patient, and thus submitting the patient to aggressive treatments (radical prostatectomy and radiation therapy) with severe inherent procedural complications such as incontinence and erectile dysfunction. Our goal is to improve the diagnostic accuracy of PSA especially in detecting high risk aggressive PCa and PCA3 was one of the first examples of urine biomarkers that evolved in PSA and helped in the discrimination of patients that needed a repeat biopsy after previous negative biopsies but a persistent elevated PSA, sparing an unnecessary invasive TRUS-b [9]. PDGF-BB has been connected to PCa in various studies [10, 11]. PCa cells produce PDGF-BB, which enables PCa cells to escape immunity surveillance [10]. The PDGF-BB receptor has been suggested to be over-expressed and activated during PCa progression [11]. Although PDGF-BB seems to participate in the pathophysiology of PCa and is over-expressed during the tumorigenic process, this is the first time its potential use has been examined as a urine biomarker. Even though urine is not an easy biological fluid to analyze due to its complex nature, it can be easily obtained with a non-invasive procedure and its anatomical relation to the prostate and the direct release of prostate cells and biochemical products in urine especially after a vigorous DRE makes urine ideal for biomarker testing [5]. PDGF-BB was easily detected and analyzed in urine without any technical difficulty concerning both the qualitative and the quantitative detection (fig. 1). Even though urinary levels of PDGF-BB were normalized according to creatinine, statistical analysis was performed both for PDGF-BB and PDGF-BB/Cr. Prostate cancer patients had greater levels of PDGF-BB and PDGF-BB/Cr (table 2). The ROC curve analysis (fig. 2) showed that the optimal cut-off of PDGF-BB for the prediction of PCa was 1504.9 with a sensitivity of 60% and a specificity of 51.3%, while the optimal cut-off (fig. 3) for PDGF-BB/Cr was 1217.7 with a sensitivity of 67.5% while maintaining a specificity of 52.6%. A separate analysis in order to evaluate the discriminative ability of PDGF-BB, was performed in the subpopulation of patients with a PSA 4–10ng/ml, which according to the previous used threshold for performing a TRUS-b (PSA > 4 ng/ml) was considered the grey zone of PSA. Although the levels of PSA were increased, the high levels were not necessarily attributed to PCa, but also to inflammation and BPH and these patients usually submitted to consecutive negative TRUS-b (table 3) [12]. In the subpopulation of patients with PSA 4–10 ng/ml, PDGF-BB with an optimal cut-off of 1534.5 had a discriminative ability with AUC of 0.65 (table 3), with a sensitivity of 63.6% while maintaining a specificity of 55.7%. This is one of the most important results since an ideal biomarker must have the ability to diagnose the requested disease when it is localized (increased in relation to a predefined threshold, but low levels of PSA) and of course to discriminate the specific disease (PCa) from other conditions that could affect the biochemistry and confuse the diagnostic accuracy [13]. The subpopulation of patients with PSA 4–10 ng/ml is the group of patients that although being the best biomarker at our disposal, increased (PSA > 4 ng/ml) patients may suffer from prostatitis or BPH and the patients are submitted to numerous invasive negative TRUS-b with possible complications in order to reach a diagnosis explaining the rise of PCa and mainly in order to exclude PCa [2, 3]. An ideal biomarker could discriminate these conditions sparing the need of invasive and painful procedures such as TRUS-b [1, 13]. A contradictory result was that this discriminative ability in patients with PSA levels 4–10 ng/ml even though showing a clear trend, did not reach statistical significance (p = 0.065) when the levels of PDGF-BB were normalized according to creatinine levels. Nevertheless, the subpopulation of PSA 4–10ng/ml is not the only problem of PSA as a biomarker, since there are patients (15% of PCa patients) with PSA < 4ng/ml that are diagnosed with PCa on the Gleason score of 7 or higher and even patients with PSA < 1ng/ml have a
9% risk of being diagnosed with PCa [12]. An important parameter of a reliable biomarker is that its levels have a continuous relation with the under examination disease and higher levels indicate the presence of the disease and is specific for PCa. The ideal is that higher levels indicate high-risk disease [12, 14]. In our case a significant result was that PDGF-BB was independently associated with PCa and for a 100 unit increase in PDGF-BB increased the likelihood for PCa by about 4% when a multiple logistic regression in a stepwise method was conducted (table 4). Creatinine normalization is a standard procedure in the clinical laboratory for assessing the levels of biomarkers in urine.

A recent review on creatinine normalization of albumin levels in the context of chronic kidney disease supports our choice of the normalization method [15]. Normalization to creatinine can provide results with more accuracy because the urine sample can be influenced by hematuria, a condition prevalent in patients with bladder cancer and other genitourinary disorders [15].

Our limitations are possibly the small number of patients (n = 118) and the fact that we did not performed a correlation of the PDGF-BB levels with the Gleason score of the PCa patients. Of course this is an ongoing study and more results with a larger population and Gleason score correlation is planned.

Our strengths are that this is a prospective well-designed study that both patients with a negative histology and PCa were included and that in every step there was a blind method of acquiring and analyzing the data. Also PDGF-BB showed a clear signal in a difficult biological fluid such as urine without compromising the accurate detection of its levels.

**Conclusion**

PSA is still the best biomarker at our disposal for the diagnosis and follow-up of PCa but it is far from being characterized as the ideal biomarker. The inability to define an accurate threshold that definitely discriminates PCa patients from healthy individuals and of course the fact that PSA cannot diagnose high-risk PCa patients constitutes the imperative need of novel biomarkers. PDGF-BB showed significant predictive ability for PCa both in the overall population and the population with PSA 4–10 ng/ml where there are diagnostic dilemmas of patients with persistent increased levels of PSA but negative TRUS-b occurs. Of course, more studies are needed in order to establish PDGF-BB as a urine biomarker that could potentially help improve the diagnostic ability of PSA.

**References**

1. Truong M, Yang B, Jarrard DF: Toward the detection of prostate cancer in urine: a critical analysis. J Urol 2013;189:422–429.
2. Dijkstra S, Mulders PF, Schalken JA: Clinical use of novel urine and blood based prostate cancer biomarkers: A review. Clin Biochem 2014;47:889–896.
3. Cary KC, Cooperberg MR: Biomarkers in prostate cancer surveillance and screening: past, present, and future. Ther Adv Urol 2013;5:318–329.
4. Pin E, Fredolini C, Petricoin EF 3rd: The role of proteomics in prostate cancer research: biomarker discovery and validation. Clin Biochem 2013;46:524–538.
5. Hessels D, Schallken JA: Urinary biomarkers for prostate cancer: a review. Asian J Androl 2013;15:333–339.
6. Andriole GL, Crawford ED, Grubb RL 3rd, Buys SS, Chia D, Church TR, Fouad MN, Gelmann EP, Kvale PA, Reding DJ, Weissfeld JL, Yokochi LA, O’Brien B, Clapp JD, Rathmell JM, Riley TL, Hayes RB, Kramer BS, Izmirlian G, Miller AB, Pinsky PF, Prorok PC, Gohagan JK, Berg CD: Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 2009;360:1310–1319.
7. Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, Lilja H, Zappa M, Denis LJ, Recker F, Berenguer A, Määtänniemi L, Bangma CH, Aus G, Villers A, Rebillard X, van der Kwast T, Blijenberg BG, Moss SM, de Koning HJ, Auvinen A: Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360:1320–1328.
8. Thompson IM, Ankerst DP, Chi C, Lucia MS, Goodman PJ, Crowley JJ, Parnes HL, Colman CA Jr: Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA 2005;294:66–70.
Groskopf J, Aubin SM, Deras IL, Blase A, Bodrug S, Clark C, Brentano S, Mathis J, Pham J, Meyer T, Cass M, Hodge P, Macairan ML, Marks LS, Rittenhouse H: APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Clin Chem 2006;52:1089–1095.

Cheng J, Ye H, Liu Z, Xu C, Zhang Z, Liu Y, Sun Y: Platelet-derived growth factor-BB accelerates prostate cancer growth by promoting the proliferation of mesenchymal stem cells. J Cell Biochem 2013;114:1510–1518.

Najy AJ, Won JJ, Movilla LS, Kim HR: Differential tumorigenic potential and mastriptase activation between PDGF B versus PDGF D in prostate cancer. Mol Cancer Res 2012;10:1087–1097.

Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parmes HL, Minasian LM, Ford LG, Lippman SM, Crawford ED, Crowley JJ, Coltman CA Jr: Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. N Engl J Med 2004;350:2239–2246.

Kulasingam V, Diamandis EP: Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. Nat Clin Pract Oncol 2008;5:588–599.

Semjonow A, Brandt B, Oberpenning F, Roth S, Hertle L: Discordance of assay methods creates pitfalls for the interpretation of prostate-specific antigen values. Prostate Suppl 1996;7:3–16.

Levey AS, Becker C, Inker LA: Glomerular filtration rate and albuminuria for detection and staging of acute and chronic kidney disease in adults: a systematic review. JAMA 2015;313:837–846.