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MOLECULAR MEDICINE | RESEARCH ARTICLE

The potential of PAI-1 expression in needle biopsies as a predictive marker for prostate cancer

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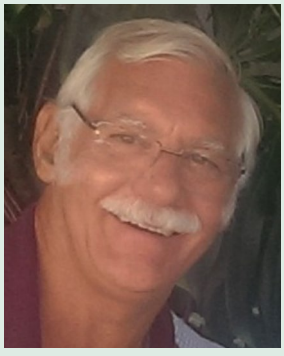
Abstract: The relative abundance of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) in transurethral resections of the prostate (TURP) has been shown to correlate with disease state. The objective of this study was to assay for uPA and PAI-1 in prostate needle biopsies, and to test their potential as predictive markers for prostate cancer (PCa). uPA and PAI-1 levels were determined for 111 patients (55 PCa; 56 benign prostatic hyperplasia (BPH)), using the FEMTELLE enzyme-linked immunosorbent (ELISA) assay. The PAI-1 concentrations for PCa and BPH patients differed significantly ($p = 0.0403$) and a level of ≥ 4.5 ng/mg protein in men 60 years and older appears to be predictive of PCa, with a sensitivity of 63%. uPA plays a minor role as a potential marker in biopsy tissue, a feature noted in our recent TURP tissue studies, and elsewhere. The potential utility of the uPA/PAI-1 ratio as a predictor of prostate disease, as previously suggested for TURP tissue, is not apparent in needle biopsy tissue. PAI-1 concentration in prostate biopsies could be a candidate marker for distinguishing between PCa and BPH in older patients.

Subjects: Bioscience; Health and Social Care; Medicine, Dentistry, Nursing & Allied Health

Keywords: prostate biopsies; uPA marker; PAI-1 marker; prostate cancer; benign prostatic hyperplasia



Antonio Serafin

ABOUT THE AUTHOR

Antonio Serafin has worked in the Division of Radiobiology at the University of Stellenbosch for 30 years, and is on the cusp of retirement. His interest in prostate cancer dates back to his PhD work which centered on the cell biological responses of novel prostate tumor cell lines to radiation and anticancer drugs.

The primary objective of the prostate biomarker project was to move from more invasive to less invasive medical procedures (from transurethral resection of the prostate tissue to prostate biopsy tissue), and to eventually test the research observations using venous blood samples. Antonio Serafin is currently a member of a small team investigating biomarkers in prostate cancer, antibody cocktails for targeted therapy, the cytotoxicity and radiomodifying effects of aqueous plant extracts, and high throughput biodosimetry tools.

PUBLIC INTEREST STATEMENT

The specificity of PSA is limited by a frequency of falsely elevated values in benign prostatic hyperplasia (BPH), and approximately two-thirds of PSA values greater than 4 ng/ml in men over 50, are due to BPH. This leads to anxiety and over-treatment.

Our project aimed at developing a bioassay which would characterize the presence and severity of prostate disease. Tissue of defined pathology was homogenized, and proteins extracted. We analyzed the extract using an enzyme-linked immunosorbent assay to determine the concentration of the proteins in question. We tested the correlation between disease stage, PSA value, and age, with the abundance of protein under investigation. No relationship existed between PAI-1 concentration and PSA level or age, but tumor stage correlated with PAI-1, albeit not strongly.

Our findings suggest that PAI-1, and not uPA, levels in prostate biopsies from elderly patients might potentially be useful as a predictive marker for prostate disease.

1. Introduction

Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) play an important role in tumor invasion and metastasis (Duffy, McGowan, Harbeck, Thomssen, & Schmitt, 2014; Schmitt et al., 2011; Shariat et al., 2007). Optimized cut-off concentrations of >3 ng/mg protein and >14 ng/mg protein for uPA and PAI-1, respectively, are associated with tumor aggressiveness and a poor prognosis in node-negative breast cancer (Annecke et al., 2008; Harbeck et al., 1999). Overexpression of PAI-1 is associated with a poor prognosis in cervix, lung, kidney, colon, ovarian, non-small cell lung, and oral cancers (Abe et al., 1999; Gershtein & Kushlinskii, 2001; Hundsdorfer et al., 2005; Kobayashi, Fujishiro, & Terao, 1994; Ohba et al., 2005; Pavey et al., 1996; Werle et al., 2004). The clinical value of uPA and PAI-1 determination is, at present, still limited to breast cancer. To date, no Level 1 evidence of clinical usefulness has been demonstrated for these two markers in other malignancies.

Despite its widespread use, the prostate-specific antigen (PSA) test has several major limitations. In addition to being found in patients with prostate cancer (PCa), a raised PSA level may be found in individuals with BPH, inflammation of the prostate, or an infection. There is little correlation between PSA levels and tumor aggressiveness. The value of PSA screening in decreasing the mortality rate has been questioned by PCa screening studies (Andriole et al., 2009; Schröder et al., 2009).

Of particular relevance, then, is the need to identify additional reliable markers for prostate malignancies. Such predictors should assist in reducing inadvertent incidences of under or overtreatment, in cases of false-negative and false-positives, respectively. Errors in identifying patients who could benefit from therapeutic interventions can impact negatively on treatment prognosis. In recent studies, we demonstrated that uPA/PAI-1 ratios derived from transurethral resection of the prostate (TURP) tissues from patients with PCa were significantly higher than those in their BPH counterparts (Akudugu, Serafin, & Böhm, 2015; Böhm et al., 2013). Although there is a strong correlation between prostate epithelial volumes derived from TURP and biopsy tissues (Fukatsu et al., 2003), the sole use of either tissue type for the prediction of PCa remains largely controversial (Epstein, 2000; Otto et al., 2014; Puppo, Introini, Calvi, & Naselli, 2006; Zigeuner et al., 2003). Overall, prediction strength is strongly influenced by tissue site and size. It is, therefore, conceivable that the previously demonstrated potential of the uPA/PAI-1 ratio in TURP tissue as a PCa marker (Akudugu et al., 2015; Böhm et al., 2013) may not emerge in needle biopsy tissue. Interrogation of the idea of using the uPA/PAI-1 ratio in TURP tissue as a PCa marker is thus warranted in biopsy tissue.

In the following, we present data on uPA and PAI-1 levels in prostate biopsy samples taken from 111 patients (56 BPH and 55 PCa). It is now shown that the potential utility of the uPA/PAI-1 ratio as a predictor of prostate disease, as previously suggested for TURP tissue (Akudugu et al., 2015; Böhm et al., 2013), is not apparent in core needle biopsy tissue. The data further demonstrate that a trend emerges in favor of PAI-1 acting possibly as a sole PCa biomarker, and suggest that a single biopsy might be adequate for assessing levels of the uPA and PAI-1 marker pair.

2. Materials and methods

2.1. Patients and samples

Core needle prostate biopsy tissue was obtained from 111 patients, after signed consent, according to ethical guidelines. Single biopsies weighing 5–9 mg were received from 35 patients, while 2–3 biopsies (10–57 mg) were obtained from the remaining 76 patients. The patients were recruited from the Gatesville Medical Centre in Athlone and the Tygerberg Academic Hospital (Cape Town, South Africa). Of these, 56 patients were diagnosed as BPH and 55 patients as PCa using a scoring system, based on PSA, digital rectal examination (DRE), Gleason score and histopathology, as described previously (Böhm et al., 2013). Initial screening of patients was by PSA and DRE. An abnormal PSA and/or DRE finding resulted in an 8-core transrectal prostate biopsy. A histology positive score was added to the PSA and DRE scores to obtain a 10-point final score. For example, patients with a negative DRE, a PSA value of 4 µg/L and one positive core (cT1) were given a rating of 1, indicating a low

probability of PCa and a high probability of BPH. A distinctly abnormal DRE with a PSA value of 100 µg/L, and one or more positive cores received a rating of 10, indicating a high probability of PCa and a low probability of BPH. Due to the scoring system not being validated, only patients with a score of 8 or higher, in the PCa category, were included. In order to reduce errors, a second urologist reviewed the clinical data and scores of patients. The objective of the scoring system was to obtain a high level of certainty of PCa identification with a low probability of missing same.

The mean ages of the BPH and PCa groups were 62 years (range: 41–78 years) and 65 years (range: 46–80 years), respectively. The corresponding PSA concentrations for the two groups of patients emerged as 11.6 ng/mL (range: 2.7–43.0 ng/mL) and 38.8 ng/mL (range: 0.67–246 ng/mL). The study was approved (reference N09/11/330) by the Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University (South Africa), and was conducted in accordance with the Helsinki Declaration of 1975.

2.2. Sample preparation and measurement of uPA and PAI-1 content

For protein extraction, the deep frozen prostate biopsy tissue samples were placed in cold extraction buffer, composed of 240 µL pH 8.5 Tris-buffered saline and 10 µl of 25% Triton X-100 (Sekisui Diagnostics Product R22), at 4°C for 24 h on a rotating roller, followed by centrifugation at 20,000 × *g* for 20 min at 4°C. Aliquots of the extract were then subjected to the Pierce Bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, USA). Briefly, the total protein concentration is manifest by a color change of the sample solution from green to purple in proportion to protein concentration, which is then measured using a colorimetric technique. Determination of the uPA and PAI-1 content in the biopsy tissue samples was by the FEMTELLE enzyme-linked immunosorbent (ELISA) assay (Sekisui Diagnostics, LLC, Lexington, MA, USA), as described elsewhere (Jänicke et al., 1994). Total protein was expressed as mg/mL, while the content of uPA and PAI-1 was expressed in ng/mg total protein. To test the ability of the pair of markers to predict disease state as proposed previously for TURP tissue (Akudugu et al., 2015; Böhm et al., 2013), the uPA/PAI-1 ratio was calculated for each patient sample, and data for the PCa and BPH groups were compared.

3. Statistical analysis

Statistical analyses were performed using the GraphPad Prism computer program (GraphPad Software, San Diego, CA, USA). To compare two data-sets, the unpaired *t* test was used. The *P* values and the coefficients of determination, R^2, were calculated from two-sided tests. A *P* value of < 0.05 indicates a statistically significant difference between the data-sets. The predictive power of the PAI-1 and uPA parameters was assessed using receiver operating characteristic (ROC) analysis. Briefly, a cut-off point was chosen within the range of each endpoint above which patients would have PCa. By moving the cut-off point across the data range, an array of true positive rates (sensitivity) and corresponding false positive rates (1-specificity) was generated from which the ROC curves were constructed. The accuracy of a test was measured by the area under the ROC curve (the *c*-index). An area of 1.0 represents a perfect test, while an area of 0.5 represents a non-discriminating test.

4. Results

4.1. uPA and PAI-1 expression in core needle biopsies of the prostate

Figure 1 shows the recovery of both markers from biopsies of the two groups of patients. The mean uPA concentration emerged as 0.22 ± 0.02 ng/mg protein for the BPH patients and 0.30 ± 0.09 ng/mg protein for PCa patients, showing no significant difference ($p = 0.3367$; $R^2 = 0.0085$). The corresponding ranges of uPA content were found to be 0–0.74 ng/mg protein and 0–3.58 ng/mg protein. On the other hand, PAI-1 concentrations for the BPH and PCa groups were 3.52 ± 0.41 and 5.98 ± 1.12 ng/mg protein, respectively, and were significantly different ($p = 0.0403$; $R^2 = 0.0380$), with corresponding ranges 0.17–17.30 ng/mg protein and 0.26–40.28 ng/mg protein. In general, for patient groups, PAI-1 concentrations were 12–16 times higher than uPA concentrations.

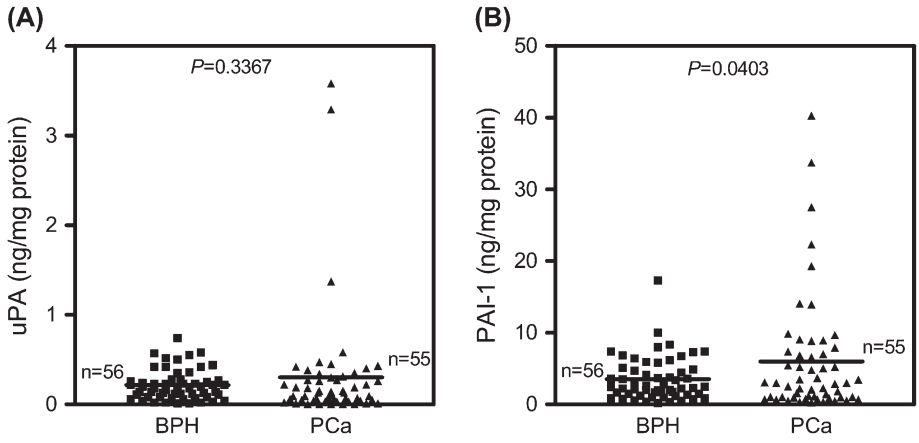


Figure 1. Comparison of marker concentration in BPH and PCa biopsy tissues: (A) uPA and (B) PAI-1.

Note: Horizontal lines represent the mean marker concentration in each group of patients.

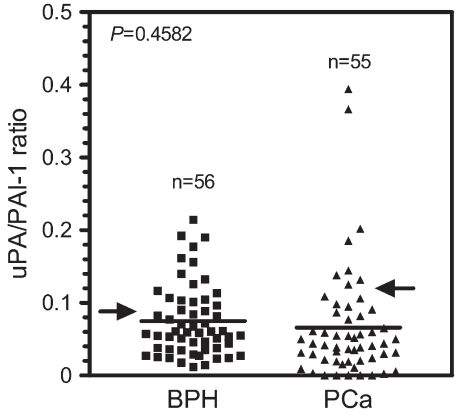


Figure 2. Comparison of uPA/PAI-1 ratios in BPH and PCa biopsy tissues.

Notes: Horizontal lines represent the mean uPA/PAI-1 ratio in each group of patients. Arrows represent the maximum marker levels in TURP tissue [2] and [5].

4.2. Relative abundance of uPA and PAI-1 in prostate biopsies and disease state

The data presented in Figure 2 show similar uPA/PAI-1 ratios of 0.08 ± 0.01 and 0.07 ± 0.01 for the BPH and PCa groups, respectively ($p = 0.4582$; $R^2 = 0.0051$). When marker concentration was plotted against patient age (grouped in 10-year intervals), uPA expression in both groups of patients followed a similar pattern, with no distinguishing features (Figure 3(A)). For PAI-1, a clear trend emerged for patients older than 60 years with marker concentration in PCa patients increasing with

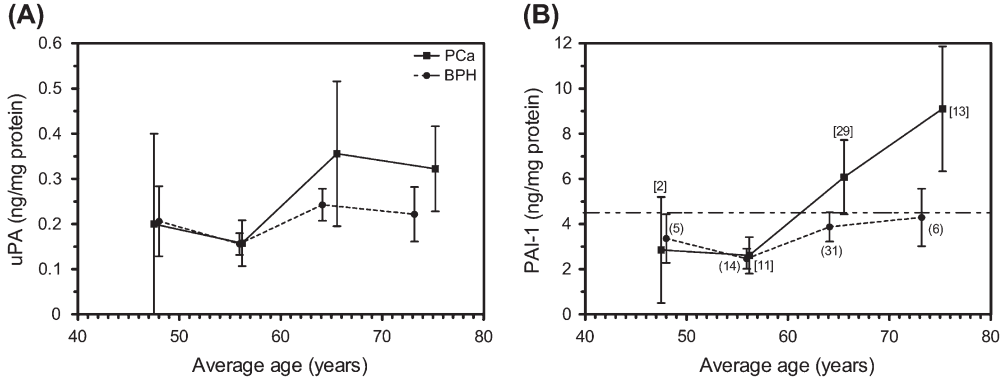


Figure 3. Plot of marker concentration in BPH and PCa biopsy tissues against mean age of patients grouped in 10-year intervals: (A) uPA and (B) PAI-1.

Notes: Numbers in parentheses and square brackets represent the size of each patient subgroup in each age interval for BPH and PCa, respectively. Horizontal dashed-dotted line indicates the cut-off PAI-1 level above which patients older than 60 years may likely have prostate cancer.

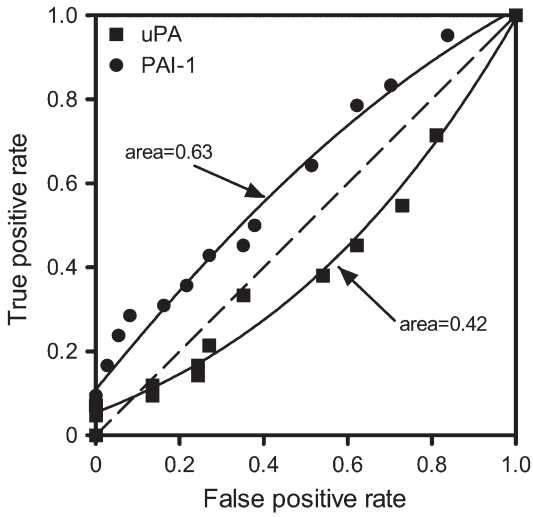


Figure 4. ROC curve of the predictive power of uPA and PAI-1 markers of disease state (BPH or PCa) in biopsy tissues.

Note: PAI-1 expression may have a better ability to correctly classify patients with prostate disease than uPA expression.

age (Figure 3(B)). In PCa patients over 60 years of age, the average PAI-1 level ranged from 4.5 to 9 ng/mg protein. On average, PAI-1 concentrations for all BPH patients were below 4.5 ng/mg protein (Figure 3(B)). Due to the overlap in the data (Figure 1 and 3), it is difficult to assess the utility of

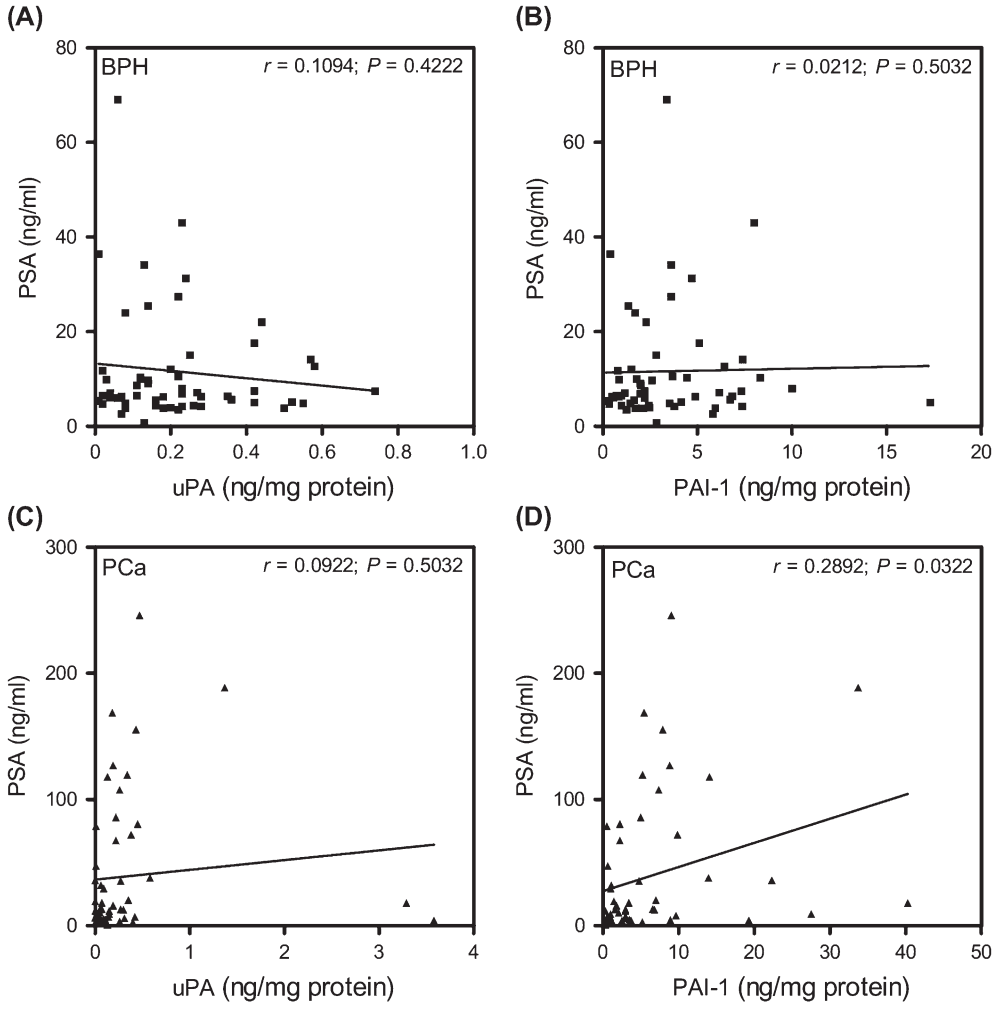


Figure 5. Correlation of the marker pair, uPA and PAI-1, in PCa and BPH biopsy tissues in relation to PSA values.

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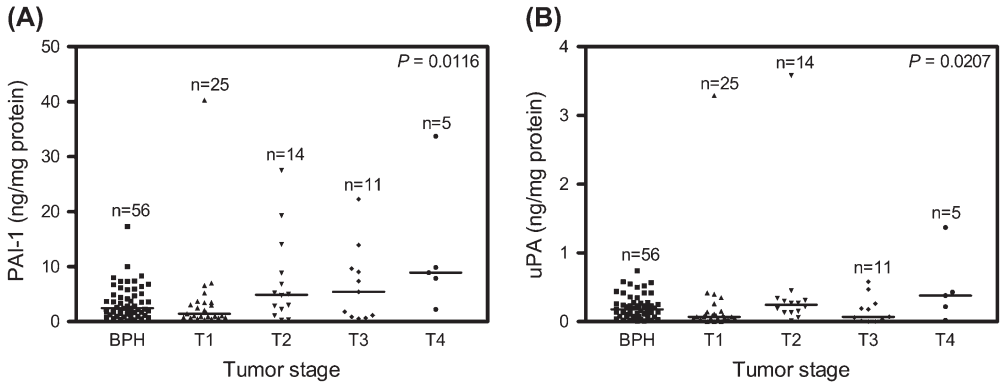

Figure 6. Correlation of the marker pair, uPA and PAI-1, in PCa and BPH biopsy tissues in relation to tumor grade.

PAI-1 and uPA levels as predictive markers. Consequently, we examined the data for patients older than 60 years of age using ROC analysis (Figure 4). The data indicate that PAI-1 expression may have a better ability than uPA expression to correctly classify patients with and without prostate disease. The *c*-indices were 0.63 and 0.42, respectively. No relationship is noted between PSA level and the marker pair uPA and PAI-1, for either BPH or PCa (Figure 5). A positive trend, however, exists for PAI-1 and tumor grade, albeit not strongly (Figure 6).

5. Discussion

We previously demonstrated that the uPA/PAI-1 ratio in transurethral resections of the prostate (TURP) is significantly higher for PCa patients than for BPH patients, and hence may be a useful indicator of prostate disease (Akudugu et al., 2015; Böhm et al., 2013). Establishing a differential in the uPA/PAI-1 ratio in less invasive needle biopsies of the prostate from BPH and PCa patients might have significant clinical implications. The current study evaluated uPA and PAI-1 marker levels in core needle biopsies from a cohort of 111 prostate patients, of which 56 were BPH and 55 were PCa. It must be emphasized that the new data on uPA and PAI-1 are not in conflict with previous measurements in TURP samples, a major difference being the large number of BPH samples in the previous study, and the equal numbers of PCa and BPH samples in the present study. Unlike tissue from transurethral resections, prostate biopsy samples are of small mass and quantification of uPA and PAI-1 protein levels can be challenging. Due to tumor heterogeneity, there are concerns that certain biopsies might miss malignant tissue and would not be representative of an entire tumor (Epstein, 2000; Otto et al., 2014; Puppo et al., 2006; Zigeuner et al., 2003). To address the problem, Thomssen and colleagues compared uPA and PAI-1 values in breast tumor tissue from needle biopsies (weighing 10–30 mg) with those in samples of a larger size (weighing 90–300 mg), and found that their

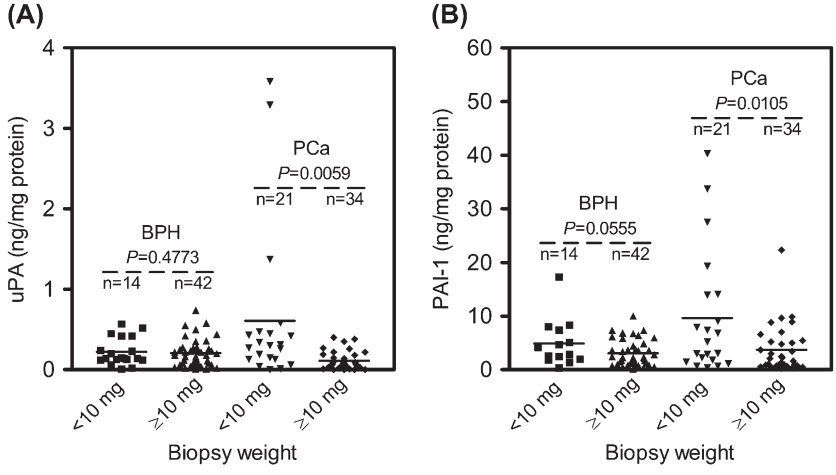

Figure 7. Comparison of: (A) uPA and (B) PAI-1 concentrations in prostate biopsy tissues from 111 patients (56 BPH; 55 PCa), in relation to biopsy size.

Note: All samples < 10 mg were single biopsies. Horizontal lines represent the mean marker concentration in each group of patients.

mean levels were strongly correlated (Thomssen et al., 2009). This study demonstrated that uPA and PAI-1 in needle biopsies can reliably be used to predict primary breast cancer, provided more than one biopsy is assessed to cater for tumor heterogeneity. In contrast, the prostate data presented in Figure 7 demonstrate that prostate biopsy size and number do not seem to impact negatively on uPA and PAI-1 recovery. In fact, the efficiency of marker recovery was similar in single (<10 mg) and multiple (≥10 mg) biopsies (Figure 7(A) and (B)). uPA concentrations from BPH tissue show no mean significant difference, while the statistical difference seen for PCa tissue is likely due to a few outliers (Figure 7(A)). PAI-1 concentrations from BPH and PCa tissues, too, show slight mean differences, due to outliers (Figure 7(B)).

Although a very strong correlation has been shown between prostate epithelial volumes obtained from needle biopsies and TURP tissue (Fukatsu et al., 2003), no data exist demonstrating a relationship for uPA and PAI-1 protein levels in prostate tissue types. Here, it is shown that the ranges of uPA and PAI-1 levels in needle biopsy tissue were comparable to those seen in TURP tissue (Akudugu et al., 2015; Böhm et al., 2013), except for three PCa samples that yielded significantly higher levels (Figure 1(A)). Similarly, the PAI-1 ranges were consistent with those previously reported (Akudugu et al., 2015; Böhm et al., 2013), except for levels in only one BPH and three PCa samples falling above those for TURP tissue (Figure 1(B)). With regard to uPA/PAI-1 ratios in biopsies, the ranges were much wider than for TURP tissue (Akudugu et al., 2015; Böhm et al., 2013). In fact, in as many as 25 biopsies, the ratios were higher than the maxima observed for TURP tissue (Figure 2). This translated into a significant increase in the mean uPA/PAI-1 ratio for the BPH group, thereby nullifying the separation seen between uPA/PAI-1 ratios for BPH and PCa in our previous studies on TURP tissue (Akudugu et al., 2015; Böhm et al., 2013). Also, no link was seen between uPA/PAI-1 ratio and age in PCa patients as previously observed for TURP tissue (Akudugu et al., 2015).

The higher uPA levels seen in needle biopsies relative to that observed in TURP tissue (Akudugu et al., 2015; Böhm et al., 2013) favored biopsies from both BPH and PCa patients, while that for PAI-1 favored only biopsy samples from PCa patients (Figure 1). In fact, the net concentration of uPA in needle biopsies from BPH patients was much lower than that found in TURP samples. This suggests that PAI-1 levels in biopsies might be a more reliable determinant of prostatic disease. To interrogate this assertion, marker levels were plotted against patient age, as illustrated in Figure 3. It is apparent that uPA plays a minor role as a potential marker in prostate biopsy tissue, a feature noted in our recent TURP tissue studies, and elsewhere (Akudugu et al., 2015; Böhm et al., 2013; Plas et al., 2001). Consistent with previous data for TURP, uPA levels did not appear to be influenced by age. On the other hand, PAI-1 levels were found to be age-dependent (Figure 3(B)). A PAI-1 value of ≥ 4.5 ng/mg protein in men of 60 years and older appears to be moderately predictive for PCa, with a sensitivity of 63% (Figure 4). In men below the age of 60 years, no difference in PAI-1 values exists between PCa and BPH patients (Figure 3). However, individuals in this age bracket with PAI-1 values lower than 4.5 ng/mg protein are more likely to have BPH. This analysis furthermore shows that regardless of age, men with a PAI-1 value of less than 4.5 ng/mg protein fall into the BPH category.

6. Conclusions

These findings suggest that PAI-1 levels in core needle biopsies of the prostate from elderly patients might potentially be useful as a predictive marker for prostate disease. Recruitment of a larger patient cohort across different age groups would be needed to further elucidate the role of age in the PAI-1 marker expression. The data further suggest that a single prostate biopsy of about 5 mg may be sufficient for assessing uPA and PAI-1 levels.

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Competing interests
The authors declare no competing interests.

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