# PSA Isoforms' Velocities for Early Diagnosis of Prostate Cancer

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**Abstract.** *Free prostate-specific antigen (fPSA) and its molecular isoforms are suggested for enhancement of PSA testing in prostate cancer (PCa). In the present study we evaluated whether PSA isoforms' velocities might serve as a tool to improve early PCa diagnosis. Our study population included 381 men who had undergone at least one ultrasound-guided prostate biopsy whose pathologic examination yielded PCa or showed no evidence of prostatic malignancy. Serial PSA, fPSA, and proPSA measurements were performed on serum samples covering 7 years prior to biopsy using Beckmann Coulter Access immunoassays. Afterwards, velocities of PSA (PSAV), fPSA% (fPSA%V), proPSA% (proPSA%V) and the ratio proPSA/PSA/V were calculated and their ability to discriminate cancer from benign disease was evaluated. Among 381 men included in the study, 202 (53%) were diagnosed with PCa and underwent radical prostatectomy at our Department. PSAV, fPSA%V, proPSA%V as well as proPSA/PSA/V were able to differentiate significantly between PCa and non-cancerous prostate. The highest discriminatory power between cancer and benign disease has been observed two and one year prior to diagnosis with all measured parameters. Among all measured parameters, fPSA%V showed the best cancer specificity of 45.3% with 90% of sensitivity. In summary, our results highlight the value of PSA isoforms' velocity for early detection of PCa. Especially fPSA%V should be used in the clinical setting to increase cancer detection specificity.*

The use of prostate-specific antigen (PSA) as a screening tool for prostate cancer (PCa) has revolutionized detection and monitoring of PCa, which is the leading cancer entity in men (1-3). In general, PSA is an organ-specific but not cancer-specific marker and, consequently, associated with difficulties in discriminating PCa from non-cancerous prostate diseases, including benign prostatic hyperplasia or prostatitis. Moreover, PSA elevation can also occur with prostate manipulation, such as ejaculation, prostate examination, urinary retention or catheter placement and prostate biopsy (4, 5).

In general, 70-90% of total PSA is present as a complexed form in serum associated with numerous different endogenous protease inhibitors, which prevent damage by the protease activity of PSA. However, 10 to 30% of PSA is present in an uncomplexed form. This, so called, free PSA (fPSA) is composed of three different types of enzymatically inactive PSA: benign PSA (BPSA), intact inactive PSA and proPSA (6, 7). The use of percentage of free PSA (fPSA%) for PCa prediction has successfully improved the accuracy of cancer detection compared to PSA measurement alone (8). Moreover, fPSA% has been proposed to differentiate benign from malignant prostate disease (9). Lee *et al.* for example were able to show in a population of 617 men that fPSA% is useful in predicting PCa in a repeat biopsy (10). Other studies investigated the impact of proPSA levels in PCa detection: Catalona *et al.*, showed that proPSA% is able to improve the specificity for PCa detection in a PSA range between 2 and 4ng/ml, thereby decreasing the number of unnecessary biopsies (11). Several other publications showed that integration of proPSA in risk calculation systems improved PCa prediction compared to standard prediction markers PSA, fPSA% and age. Also, our group recently described proPSA as predictor for aggressive forms of PCa already four years prior to diagnosis (12).

In recent years, measurement of PSA dynamics (velocity and doubling time) has been adjunct to total PSA measurements. Thus, the concept of using PSA kinetics, including PSA velocity (PSAV), defined as the rate of change in serum PSA level in a specific period, has received considerable attention in PCa prediction and monitoring. PSAV, for example, was shown to be associated with short survival in men with castration-resistant PCa (13, 14). In addition, Loeb *et al.* described PSAV as a significant marker for PCa aggressiveness (15). Similar data were also reported by another group showing that PSAV risk count was significantly associated with the development of high-risk

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PCa in a study comprising of 717 patients (16). In contrast to these findings, others were unable to demonstrate an incremental value beyond total PSA alone (17, 18).

In the present study, we measured the velocities of PSA isoforms in the years prior to PCa diagnosis. We describe, for the first time, the high discriminatory power of fPSA%V, proPSA%V and proPSAV/PSA/V between PCa and benign prostate. In summary, our data clearly show that PSA isoforms' velocities differentiate PCa from the non-cancerous prostate years before diagnosis. fPSA%V has the best discriminatory power of all velocities tested.

## Materials and Methods

Our study population included 381 men (benign (n=173) and cancer (n=208)) who had undergone at least one ultrasound-guided prostate biopsy at the Department of Urology, Medical University of Innsbruck, between 1993 and 2006 due to elevated PSA levels. All patients diagnosed with PCa included in the study underwent open radical retropubic prostatectomy at our Institution followed by a histopathological examination of prostate specimen by an experienced uropathologist at the Department of Pathology, Medical University Innsbruck. As described previously, a screening project using PSA as the only screening test was launched in the Federal State of Tyrol, Austria. Age-referenced PSA levels in combination with fPSA% of less than 18% were used as biopsy criteria in the PSA range up to 10 ng/ml (19). Additionally, patients with PSA levels ≥10 ng/ml or suspicious findings on digital rectal examinations were advised to undergo biopsy. Due to changes in the biopsy protocol, the number of obtained biopsies increased from 6 cores (January 1993 to October 1995) to 10 (November 1995 to March 2000) and then to 15 cores (March 2000 to July 2006).

Serum specimens collected between January 1993 and July 2006 were frozen immediately after measurement of serum PSA and stored frozen at –70˚C until use. Serum samples obtained up to seven years before prostate biopsy were retrieved for the study cohort and PSA, fPSA and proPSA measurements were performed using the Beckman Coulter Access PSA immunoassays as described previously (12).

The rate of PSA change using first and last values only (FL method) and the equation: $PSA_n - PSA_1/T_n - T_1$ (n=number of years, T=time) has been used to calculate velocities of PSA, fPSA% (fPSA/PSAx100) and proPSA% (proPSA/PSAx100) and various ratios of these markers (20).

Statistical analysis was performed using the SPSS for Windows (SSPS, Chicago, IL, USA) or Statistica (StatSoft, Tulsa, OK, USA) software packages. The Student's t-test was applied to calculate the statistical significance of differences between treatment groups. All p-values below 0.05 were considered significant (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

## Results

Among 381 men included in the study, 53% (n=202) were diagnosed with PCa and underwent open radical retropubic prostatectomy at our Department between 1993 and 2006.

Evaluating the PSAV over time in both cancer and non-cancerous prostates, we found that, in patients with PCa (n=202), PSAV increased 7 years before diagnosis with a significant increase in the last year before diagnosis. In contrast to this finding, men without PCa (n=179) showed only smaller PSAV changes over time. The highest difference of PSAV between PCa and benign prostatic diseases was in the year prior to diagnosis ($p < 0.001$) (Figure 1).

Next, we evaluated the differences of percent fPSA velocity (fPSA%V) in PCa patients compared to patients who showed no cancer in the biopsy specimens. As shown in Figure 2, we observed a negative trend of fPSA%V in PCa, whereas the fPSA% velocity mostly increased in benign patients in the years prior to diagnosis. In the second year prior to diagnosis, cancer fPSA%V was significantly different from benign fPSA%V ($p < 0.05$) with a cancer specificity of 45.2% and 90% of sensitivity (Figure 2).

We recently showed that proPSA is a reliable marker for differing PCa from benign prostatic diseases several years prior to diagnosis (12). Therefore, we also calculated the velocity of proPSA in both PCa and benign prostatic diseases. As shown in Figure 3, the proPSA%V differed significantly in the second ($p < 0.001$) and the first year ($p < 0.01$) prior to diagnosis between PCa and non-cancerous prostate disease (Figure 3). The best specificity of 26.6% with a sensitivity of 90% was reached two years before diagnosis.

In line with these data, also proPSA/PSA/V was a reliable early marker for PCa with the highest predictive value two and one years prior to diagnosis ($p < 0.01$) (Figure 4). In the second year before diagnosis the ratio proPSA/PSA/V showed a specificity of 26.6% with a sensitivity of 90%.

## Discussion

In the present study we evaluated, for the first time, the impact of PSA isoforms' velocities in the early diagnosis of PCa. All measured velocities are able to differentiate PCa from the non-cancerous prostate. Free PSA and proPSA velocities and the ratio proPSA/PSA/V were significantly different already one year before tumor biopsy based on the combination of total PSA and fPSA%. Out of all parameters calculated, fPSA%V had the best predictive value with a cancer specificity of 45.2% at 90% of sensitivity. Therefore, fPSA%V is suggested as an additional marker for early detection of PCa in a clinical setting.

In recent years, the concept of PSA kinetics has been investigated intensively with contradictory results. Most studies described a coherence of PSAV with PCa incidence, aggressiveness and mortality. For example, a recent long-term follow-up study of 20 years showed an improved classification of PCa risk and mortality with PSAV in addition to baseline PSA measurement (21). Another large multi-center study, including 24,709 men, also evidenced that PSAV has additional value over a single PSA determination in identifying men with PCa. Moreover, the authors of the
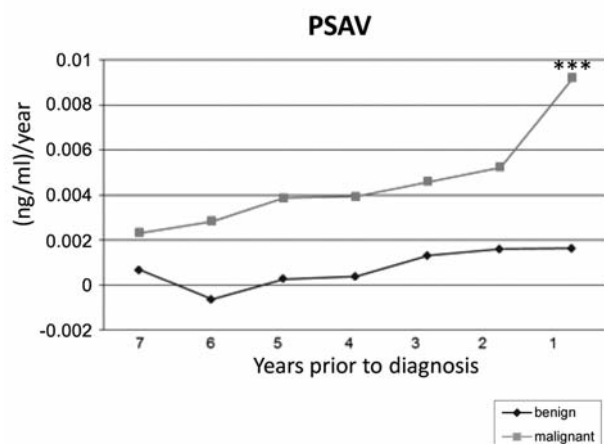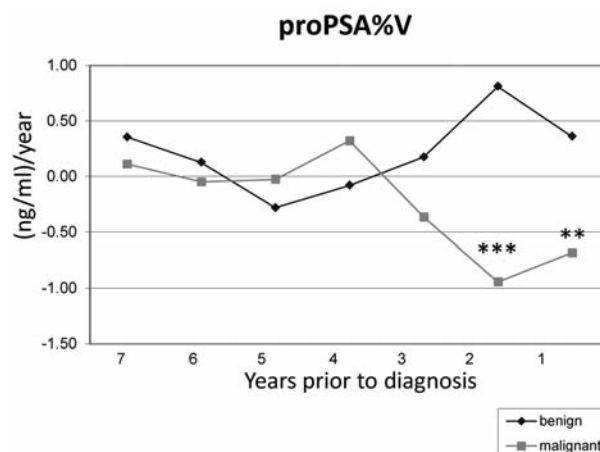
Figure 1. *PSAV history ((ng/ml)/year) 7 to 1 year prior to diagnosis in benign prostate and prostate cancer. Data are presented as mean values, p-values below 0.05 were considered significant (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001).*



Figure 3. *proPSA%V history ((ng/ml)/year) 7 to 1 year prior to diagnosis in benign prostate and prostate cancer. Data are presented as mean values, p-values below 0.05 were considered significant (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001).*
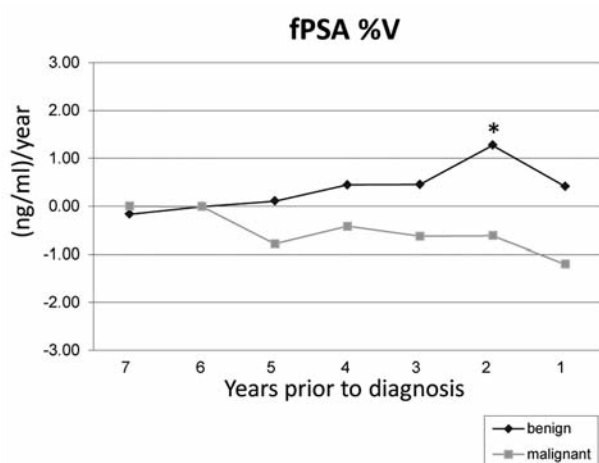


Figure 2. *fPSA%V history ((ng/ml)/year) 7 to 1 year prior to diagnosis in benign prostate and prostate cancer. Data are presented as mean values, p-values below 0.05 were considered significant (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001).*
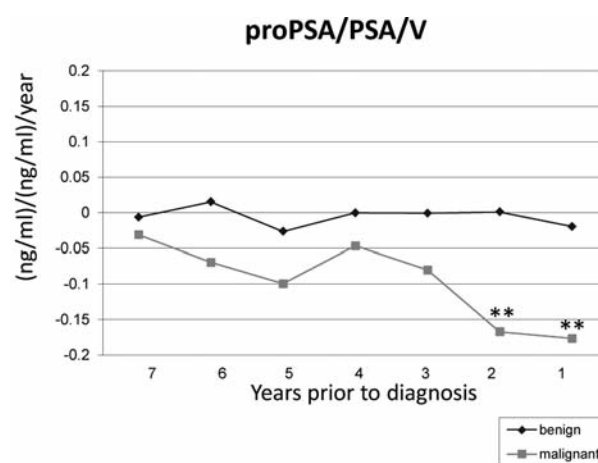


Figure 4. *proPSA/PSA/V history ((ng/ml)/(ng/ml)/year) 7 to 1 year prior to diagnosis in benign prostate and prostate cancer. Data are presented as mean values, p-values below 0.05 were considered significant (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001).*

latter study showed a positive correlation of PSAV with tumor grade and overall survival when the PSAV was >2.0 ng/ml/year rather than below that (20). The results of the present study provide evidence that PSAV is capable to differentiate significantly between PCa and benign prostate one year prior to diagnosis in a data set of 381 patients.

The present study shows that, in addition to PSAV, also proPSA%V, fPSA%V and proPSA/PSA/V have a high discriminatory power between cancerous and non-cancerous prostates. To the best of our knowledge, this is the first study reporting the impact of PSA isoforms' velocities in differentiating PCa and non-cancerous prostates. Among proPSAV%, fPSA%V and proPSA/PSA/V fPSA%V showed the highest discriminatory power with a cancer specificity of 45.2% at a sensitivity of 90%.

Our study has important clinical implications. For example, PSA velocities and especially fPSA% could provide an approach to determine the need of biopsy in patients with

elevated PSA levels and support early diagnosis of prostate cancer and, thus, increase the chance of curative treatment. Strengths of this study are the long-term follow-up character and the large patient collective. However, the present study also has several limitations such as the lack of a multi-center character and, therefore, randomized controlled multi-center studies are urgently needed to confirm the present findings.

In summary, the present study shows, for the first time, the impact of PSA isoforms' velocities in the early diagnosis of PCa. fPSA%V showed the highest predictive value two years prior to diagnosis. This finding justifies further determinations of fPSA%V in clinical routine. Moreover, this study indicates that early diagnosis of PCa should not be limited to measurement of single parameters.

## Conflicts of Interest

None to declare.

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