ORIGINAL PAPER

# Optimum PSA reflex-range.

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**Summary**

*Objective: Aim of this study was to evaluate different decision strategies based on variations in the cut-off value of percent free PSA and in the range of total PSA values (reflex range) in which free PSA testing was applied. We compared these strategies to conventional total PSA testing by determining which strategies would provide a maximum decrease in unnecessary biopsies with a minimum number of additional undetected cancers. Materials and Methods: This retrospective study was conducted with 807 patients who were referred to transrectal ultrasound biopsies for elevated serum PSA levels or for abnormal digital rectal examination. Overall 156 patients were affected by primary prostate cancer (CaP), 651 were controls without prostate cancer (benign prostatic hypertrophy, prostatic intraepithelial neoplasm, prostatitis or normal prostatic gland). Results: Total PSA was significantly higher (F=4.93; p<0.0001) and percent free PSA was significantly lower in cancer patients than in controls (F=2.16; p<0.0001). Sensitivity, specificity and the positive likelihood ratio (LR+) of PSA and percent free PSA have been calculated for several total PSA intervals: PSA 4-10 ng/ml, 3-10 ng/ml, 3-20 ng/ml, 2-10 ng/ml, 2-20 ng/ml. These data suggest that in the reflex range 2-10 ng/ml there are the best results. On ROC comparison restricted to men with total PSA between 2 and 10 ng/ml, percent free PSA also had a higher area under the curve than total PSA (AUC 0.7452 for free percent PSA; 0.6267 for total PSA: p = 0.0059). Conclusions: The PSA revolution that occurred over the previous 2 decades has positively impacted the detection of prostate cancer. Percent free PSA improves specificity, at the beginning the percent free PSA was used only in the gray zone 4-10 ng/ml. Analyzing our data, we confirm that the usefulness of percent free PSA in prostate cancer diagnosis increases, enlarging the reflex range. Our best result is obtained in the reflex range 2-10 ng/ml using a percent free PSA cut-off of 22%.*

KEY WORDS: *Prostate cancer; Prostate Specific Antigen; PSA; Free PSA; Percent free PSA; Free/total prostate-specific antigen ratio.*

## INTRODUCTION

Prostate cancer will become a social emergency in the near future in Western countries because it is one of the leading causes of cancer death, and it tends to increase with age more rapidly than many other malignancies. However, the conventional strategy for PSA screening, which calls for biopsies in all men with total PSA greater than 4 ng/ml, leads to many false positive results and, therefore, is associated with a high cost in terms of unnecessary biopsies.

The cost is not only economic, but also psychological and emotional, because anxiety on the part of the patient and his family can be of considerable detriment to his well being.

Considerable efforts are currently under way to improve its performance, and emphasis has been directed primarily at enhancing specificity by reducing false positive results.

The slow-growing and indolent nature of prostate cancer, coupled with the fact that a man probably will be tested more than once in his lifetime, makes a false negative test of less importance (1).

In general, whenever efforts are made to enhance the specificity of a diagnostic test, the sensitivity (the identification of patients with the disease in the population) is reduced. This inverse relationship of sensitivity and specificity assesses that increasing the PSA threshold enhances specificity, but does so only with a reduction in sensitivity. A lot of methods have been used to enhancing the specificity of PSA: PSA velocity, PSA density, PSA transition-zone density, Age -specific PSA level, ratio of

***Table 1.***

*Baseline characteristics of patients with prostate cancer and controls. ANOVA one-way: *p<0,0001.*

| | 156 CaP | 651 Controls |
|---|---|---|
| Median age (range) | 68.82 (48.8-88.3) | 68.14 (45-92.7) |
| 50-69 years (%) | 53.8% | 58.6% |
| Score Gleason 2-4 | (4.13%) | |
| Score Gleason 5-6 | (42.9%) | |
| Score Gleason 7-10 | (52.8%) | |
| Median PSA ng/ml (range) | 9.7 (2.26-77.1)* | 5.25 (0.12-51.6)* |
| Median free PSA (range) | 1.02 (0.12-10.8)* | 0.91 (0.03-6.14)* |
| Median % free PSA (range) | 10.81 (2.77-44.51)* | 20.18 (3.51-65)* |
| Median Prostate Volume(range) | 36.6 (10 - 130)* | 51.8 (14 - 155)* |
| Median PSA density (range) | 0.26 (0.07 - 1.84)* | 0.12 (0.01 - 3.04)* |
| % positive DRE | 60.4%* | 21.5%* |

free PSA to total PSA and level of alfa1-antichymotrypsin complex PSA. Despite early enthusiasm for PSA velocity, PSA density, and age-specific PSA cutoff, the enhanced performances suggested by the initial investigators may not be reproducible (1).
Christennson et al (2) found that they could significantly enhance the specificity of total (tPSA) by measuring the ratio of free PSA to total PSA instead of measuring total PSA alone. Numerous other investigators have reproduced these findings. The ratio of free PSA was used in screening program for prostate cancer in different countries with different cutoffs (1-4).

## Aim Of The Study

Aim of our study is to evaluate different decision strategies based on variations in the cutoff value of percent free PSA and in the range of total PSA values (reflex range) in which free PSA testing was applied. We compared these strategies to conventional total PSA testing in order to determine which strategies would provide a maximum decrease in unnecessary biopsies with a minimum number of additional undetected cancers.

## Materials And Methods

This retrospective study was conducted with 807 patients who were referred to transrectal ultrasound for elevated serum PSA levels or for abnormal digital rectal examination.
Inclusion criteria was as follows: male older than 45, without a history of prostate cancer. All men underwent a detailed clinical examination that included digital rectal examination (DRE) and lower urinary tract ultrasound.
Exclusion criteria: Patients were excluded from analysis due to concomitant finasteride treatment or recent ureteral catheterisation, which may distort tPSA value. Patients who rejected prostatic biopsy were excluded.
Patients underwent clinical rectal examination followed by transrectal ultrasound of the prostate gland (TRUS). Scanning was performed in the transverse and saggital planes. Cases with suspicious clinical or sonographic findings or elevated PSA level or rapidly increasing of PSA level underwent transrectal biopsy under ultrasound control. Before august 2003 systematic sextant biopsies using transrectal ultrasonography were performed in patients with positive or doubtful rectal examination as well as those with PSA higher than 4 ng/ml. After September 2003 we increased biopsy cores to 12, in total 128 patients underwent extended 12 cores prostate biopsy. 78 patients underwent a second prostate biopsy, 27 underwent 3 or more prostate biopsies.
The analytical descriptions is reported in Table 1.
Overall, 156 patients were affected by primary prostate cancer (CaP), 651 were controls without prostate cancer (benign prostatic hypertrophy, prostatic intraepithelial neoplasm, prostatitis or normal prostatic gland). The absence of prostatic cancer was histologically confirmed in 375 cases, whereas in 156 cases diagnosis was carried out on clinical and TRUS findings.
Serum was obtained before any diagnostic procedure. Both total immunoreactive (third generation) and free

***Figure 1.***

*Scatterplot of total PSA versus percent free PSA.*

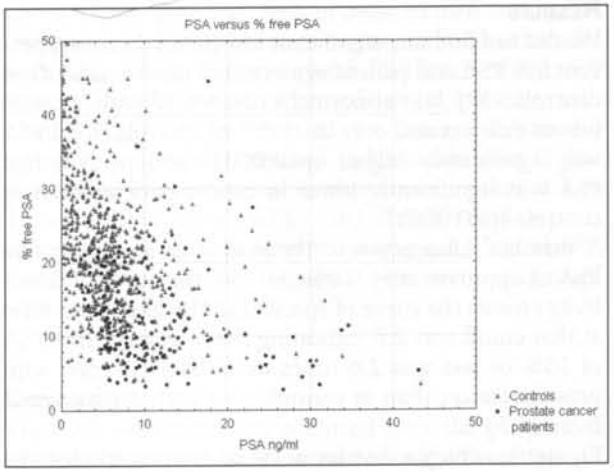


***Table 2.***

*Sensitivity, specificity and positive likelihood ratio (LR+) with reference to different reflex range of total PSA. The signed cells indicate a 95% sensitivity or more.*

| | PSA 2-20 ng/ml | | | PSA 2-10 ng/ml | | | PSA 3-20 ng/ml | | | PSA 3-10 ng/ml | | | PSA 4-10 ng/ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sens. | Spec. | LR+ | Sens | Spec. | LR+ | Sens. | Spec. | LR+ | Sens. | Spec. | LR+ | Sens. | Spec. | LR+ |
| % Free PSA <25 | 95.51 | 35.94 | 1.49 | 96.795 | 34.255 | 1.4723 | 93.59 | 40.86 | 1.5825 | 94.872 | 39.171 | 1.5596 | 92.308 | 46.083 | 1712 |
| % Free PSA <24 | 94.87 | 38.71 | 1.55 | 96.154 | 36.713 | 1.5193 | 92.949 | 43.625 | 1.6488 | 94.231 | 41.628 | 1.6143 | 91.667 | 48.541 | 1.7813 |
| % Free PSA <23 | 93.59 | 42.55 | 1.63 | 96.154 | 40.092 | 1.605 | 91.667 | 47.158 | 1.7347 | 94.231 | 44.7 | 1.704 | 91.667 | 50.691 | 1.859 |
| % Free PSA <22 | 91.67 | 45.62 | 1.69 | 95.513 | 42.704 | 1.667 | 89.744 | 50.077 | 1.7976 | 93.59 | 47.158 | 1.7711 | 91.026 | 52.227 | 1.9054 |
| % Free PSA <21 | 91.67 | 48.85 | 1.79 | 92.949 | 45.776 | 1.7142 | 87.821 | 53.303 | 1.8806 | 91.667 | 50.077 | 1.8362 | 89.103 | 54.378 | 1.9531 |
| % Free PSA <20 | 85.26 | 54.22 | 1.86 | 89.744 | 50.538 | 1.8144 | 83.974 | 57.757 | 1.9879 | 88.462 | 54.071 | 1.926 | 85.897 | 58.218 | 2.0559 |
| % Free PSA <19 | 82.05 | 58.22 | 1.96 | 87.821 | 53.917 | 1.9057 | 80.769 | 60.522 | 2.0459 | 86.538 | 56.221 | 1.9767 | 83.974 | 60.061 | 2.1026 |
| % Free PSA <18 | 80.13 | 62.52 | 2.14 | 85.897 | 57.45 | 2.0187 | 78.846 | 64.209 | 2.203 | 84.615 | 59.14 | 2.0709 | 82.692 | 62.519 | 2.2063 |
| % Free PSA <17 | 78.85 | 66.67 | 2.37 | 84.615 | 60.676 | 2.1517 | 78.205 | 68.356 | 2.4714 | 83.974 | 62.366 | 2.2313 | 82.051 | 65.438 | 2.374 |
| % Free PSA <16 | 75.64 | 72.50 | 2.75 | 82.051 | 65.284 | 2.3635 | 75 | 73.579 | 2.8387 | 81.41 | 66.359 | 2.42 | 80.128 | 68.971 | 2.5823 |
| % Free PSA <15 | 73.718 | 75.269 | 2.9808 | 80.769 | 67.742 | 2.5038 | 73.718 | 76.344 | 3.1163 | 80.769 | 68.817 | 2.5902 | 79.487 | 70.968 | 2.737 |
| % Free PSA <14 | 69.872 | 78.495 | 3.249 | 77.564 | 70.353 | 2.6163 | 69.872 | 79.416 | 3.3945 | 77.564 | 71.275 | 2.7002 | 77.564 | 73.272 | 2.902 |
| % Free PSA <13 | 65.385 | 82.028 | 3.6381 | 73.718 | 73.118 | 2.7423 | 65.385 | 82.642 | 3.7668 | 73.718 | 73.733 | 2.806555 | 73.718 | 75.115 | 2.9624 |
| % Free PSA <12 | 59.615 | 84.639 | 3.881 | 69.872 | 75.269 | 2.8253 | 59.615 | 85.253 | 4.0427 | 69.872 | 75.883 | 2.8972 | 69.872 | 76.959 | 3.0324 |
| % Free PSA <11 | 54.487 | 88.326 | 4.6673 | 66.667 | 78.495 | 3.1 | 55.128 | 88.633 | 4.8498 | 67.308 | 78.802 | 3.1752 | 67.308 | 79.416 | 3.2699 |
| % Free PSA <10 | 50 | 91.244 | 5.7105 | 64.103 | 80.645 | 3.312 | 50 | 91.398 | 5.8125 | 64.103 | 80.799 | 3.3385 | 64.103 | 81.106 | 3.3927 |

PSA (fPSA) were assayed using the chemiluminescent immunoassay Immulite (Diagnostic Products Corp.), according to the manufacturer's instructions. The assays are solid-phase, two-site, sequential chemiluminescent immunometric test that are automatically performed on an automated analyzer with detection limits of 0.02 and 0.03 microg/L for fPSA and tPSA.

Percentage of free PSA was calculated as the ratio of free PSA to total PSA multiplied by 100.

ANOVA one-way test was used to assess the differences in PSA among different groups (Statistica 6.0). Total PSA and percent free PSA were evaluated. The receiver operating characteristic (ROC) curve was generated by plotting sensitivity versus 1-specificity (GraphROC ver 2.0). We compared results by comparing the AUCs according to Hanley and Mc Neil (5, 6).

## Results

We did not find any significant association between percent free PSA and patient age in either cancer patients or controls. DRE was abnormal in 60.4% of patients with prostate cancer and only in 21.5% of controls. Total PSA was significantly higher ($p<0.0001$) and percent free PSA was significantly lower in cancer patients than in controls ($p<0.0001$).

A maximal efficency was obtained with a percent free PSA of approximately 9.9%. At 15% the curve of sensitivity crosses the curve of specificity. The likelihood ratio at that cutoff was 2.6 indicating that a percent free PSA of 15% or less was 2.6 times more likely in men with prostate cancer than in controls, but with a poor sensitivity of 73%.

Figure 1, which provides a visual framework for the analysis, shows a scatterplot of total versus percent free PSA between 0.1 to 50 ng/ml of total PSA. Cancer and controls are illustrated with different markers. In the upper left quadrant most of the cases are controls, while in the lower right quadrant are prostate cancers.

The reflex range has been defined as the optimal range of total PSA in which percent PSA determination is indicated. Sensitivity, specificity and the positive likelihood ratio (LR+) of PSA and precent free PSA have been calculated for several total PSA intervals: PSA 4-10 ng/ml, 3-10 ng/ml, 3-20 ng/ml, 2-10 ng/ml, 2-20 ng/ml. Results are reported in Table 2.

These data suggest that enlarging the reflex range the sensitivity increased. The target value of 95% sensitivity can be reach for example with 25 percent free PSA in 2-20 total PSA range and with 22 percent free PSA in 2-10 total PSA, in this case obtaining the best specificity value of 42,7% (Table 2).

On ROC comparison restricted to men with total PSA between 2 and 10 ng/ml, percent free PSA had a higher area under the curve than total PSA. Figure 2 shows ROC curves comparing total PSA and percent free PSA in 2 to 10 ng/ml PSA range, AUC for percent free PSA is higher than total PSA (AUC is 0.7452 for free percent and 0.6267 for total PSA, $p = 0.0059$).

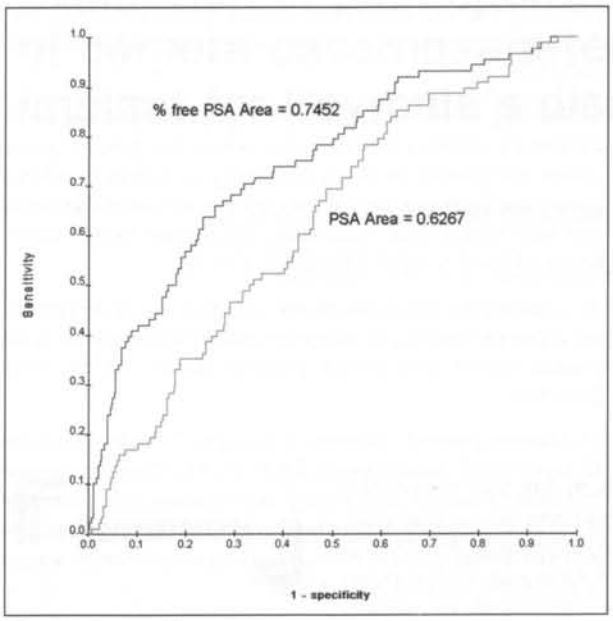
Percent free PSA also provided a significantly better discrimination of cases and controls in this range (2-10 ng/ml PSA) with a sensitivity of 95.51% and a likelihood ratio of 1.667 for percent free PSA greater than 22%.

## Discussion

The ultimate goal for prostate cancer screening is to reduce mortality and this can only be achieved by

***Figure 2.***

*Sensitivity, specificity and positive likelihood ratio (LR+) with reference to different reflex range of total PSA. The signed cells indicate a 95% sensitivity or more.*




detecting prostate cancer at an early curable stage. However since PSA is not a prostatic cancer-specific protein, considerable overlap exists between the levels of serum PSA concentration in patients with benign prostatic enlargement and those in prostate cancer patients (7). The predominant molecular forms of PSA in serum are free-PSA, PSA complexed with alfa1-antichymotrypsin and PSA complexed with alfa2-macroglobulin (8, 9).

Gann measured total and percent free PSA in blanked plasma samples in 430 men who were later diagnosed with prostate cancer and 1642 age matched controls who were not diagnosed with prostate cancer during a 12-year observation period (10). In this study prostate cancer that would be missed by combined free and total PSA strategies due to relative high percent free PSA tended to have a longer interval between blood sampling and diagnosis, and a greater likelihood of being confined to the prostate. This observation implies that subsequent testing would provide multiple opportunities to detect missed cancer at potentially curable stages, which encourages the use of lower percent free PSA cutoffs to increase the elimination of unnecessary biopsies. In a screened US population, a percentage of free PSA of 23.4% or less allowed a reduction of 31.3% in prostatic biopsies, still maintaining a 90% sensitivity (3). Morote et al. utilizing the same assay in a Spanish population with prostatic lower urinary tract symptoms, gave a percentage of free PSA of 25% or less, which reduces unnecessary biopsies by 26.9% maintaining a 95.5% sensitivity (13). In a screened US population with normal digital rectal examinations and total PSA between 2.6 and 4 ng/ml, a cut point of percent free PSA of 27% would reduce unnecessary biopsies by 18%, while still diagnosing 90% of cancers (4). A large series (773 patients), evaluating the percentage of free PSA for enhancing the differentiation of prostate cancer from benign prostatic disease in patients with benign-feeling glands and total PSA 4-10 ng/ml, showed that a 25% free PSA cutoff detected 95% of cancers while reducing unnecessary biopsies by 20% (14). The cancer associated with greater than 25% free PSA were more prevalent in older patients, and generally were less threatening in terms of tumor grade and volume. This was a multicenter clinical trial performed by 7 university medical center across the US and probably included referred and highly selected patients. The high proportion of cancer patients (49%) encountered in this study suggest than the study population cannot be considered representative of real screened population (13).

Our study adds additional information about the usefulness of percentage free PSA in the diagnosis of prostatic cancer. The extrapolation of result of a hypothetical screening done in our cases with different strategies can help in the organization of a screening program in a greater population. We are conscious that the population of our study cannot be considered equal to screened population, in particularity the incidence in our population of prostate cancer probably is greater than in a screened population. This implies that our sensitivity is probably overestimated, but on the contrary the specificity should not be overestimated, due to the high percentage of cancer prostate cases in our selected population. The aim of our study is a comparison of different strategies, therefore even an overestimation of sensitivity or an underestimation of specificity cannot invalidate our results because the errors in estimation are equally reported in our subpopulation and do not interfere in comparison.

Our results reflects what Vashi reported that enlarging the reflex range the percentage free PSA specificity increased (8). In our opinion the urological tradition of considering the 4-10 ng/ml grey zone should be changed. The specificity can be increased by enlarging the grey zone from 4-10 to 2-10.

A large amount of prostate cancers can be found in PSA levels less than 4 ng/ml. Noldus and Stamey reported on 187 men with a PSA levels of less than 4 ng/ml who underwent radical prostatectomy, but only 30% of these patients had normal DRE findings (14). Schroder et al. reported on a screened population of men diagnosed with prostate cancer with PSA values less than 4 ng/ml. Of the cancer detected, 84% of those in men with a PSA levels of 2 to 3.9 ng/ml were organ confined compared with 62% detected in those with PSA levels of 4 to 9.9 ng/ml (15). Krumholtz reported on 94 patients with clinical stage T1c prostate cancer, using a 2.6 to 4.0 ng/ml PSA threshold resulted in detection of a higher percentage of pathological confined tumors than 4.1 to 10 ng/ml (88% versus 63%) (16). Babaian et al. reported on a series of men screened using a 2.5 ng/ml PSA cutoff to recommend biopsies, 24% were diagnosed with prostate cancer (17).

## Conclusion

The PSA revolution that occurred over the previous 2 decades has positively impacted the detection of

prostate cancer. Percent free PSA improves specificity, at the beginning the percent free PSA was used only in the grey zone 4-10 ng/ml. Analyzing our data, we confirm that the usefulness of percent free PSA in prostate cancer diagnosis increases enlarging the reflex range. Our best result is obtained in the reflex range 2-10 ng/ml using a percent free PSA cut-off of 22%.

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