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Free / Total Prostate Specific Antigen Ratio for Prostate Cancer Detection: A Prospective Blind Study

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Abstract. *Background: We conducted this prospective study to evaluate the ability of the percentage of free PSA (% f/t PSA) to improve the specificity of prostate cancer (PC) detection. Materials and Methods: Seven hundred and eighty-five patients with serum PSA levels between 4 and 25 ng/ml underwent sextant biopsies of the prostate. The % f/t PSA was analyzed for the sensitivity, specificity and positive predictive values. Results: The mean % f/t PSA of the 169 patients with PC was 18.0±7.7 and was significantly ($p<0.0001$) lower than that of the 616 patients who had histologically benign biopsy specimens, with a mean % f/t PSA of 26.6±9.7. Using 30% f/t PSA as the cut-off eliminated 34.4 % of the negative biopsies while still detecting 93.4% of the carcinomas. Conclusion: The optimal cut-off value of 30% f/t PSA may be applied as reference using the Cis bio international assay for PC detection.*

Measurement of serum prostate specific antigen (PSA) has been widely used as an aid for the early detection of prostate cancer (PC) (1, 2). Because PSA is produced by all types of prostate tissue, the reference range of a normal serum PSA concentration of 4 ng/ml has resulted in a high false-positive rate generated by benign prostate hyperplasia (BPH) (2, 3).

Recently, many encouraging studies in enhancing the performance of PSA testing have involved measuring the different forms of PSA in the bloodstream (4). Many investigative results have indicated that the serum free to total PSA ratio may be advantageous for use in the discrimination of benign from malignant disease of the prostate (5, 6). Measurement of the ratio of free to total PSA may also increase the specificity of PSA tests and decrease the number of negative biopsies, while not significantly compromising the sensitivity of PSA testing (6-8). However, the majority of reports have been retrospective studies and the proposed cut-off value for the free to total PSA ratio for reference in biopsy has varied from study to study (9, 10). The wide variation may be due to different sample sizes, immunoassay methods and interpretations of the receiver operating characteristic (ROC) curve, or to the racial factor of PC risk (9, 10). This prospective study was designed to evaluate the application of free PSA in the discrimination of PC from benign disease in Taiwan, which is considered to have one of the lowest incidences of PC worldwide. In addition, we wanted to determine the optimal cut-off value of the percentage of free PSA for enhancing the PSA specificity using our available immunoassay kits.

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Materials and Methods

Samples. From September 1996 through October 2000, patients with PSA levels between 4 and 25 ng/ml undergoing transrectal ultrasonography of the prostate (TRUS) and six systemic biopsies were included in this prospective study. Patients with urinary tract infections, urinary retention or recent instrumentation or catheterization of the urethra, and those receiving finasteride treatment, were excluded. Before the TRUS and prostate biopsy, another serum sample was obtained from each patient and stored at –70˚C within 2 hours, or immediately stored at 4˚C and then stored at –70˚C within 16 hours for determination of free and total PSA.

PSA detection. The serum free PSA and total PSA were measured with the FPSA-RIACT kit and TPSA-RIACT kit (Cis Bio International, France) as a solid phase two-sided immunoradiometric

Table I. *Results of PC detection following TRUS-guided systemic biopsies based on DRE and PSA levels.*

	PSA ng/ml		
DRE	4-10 PC/Bx (%)	10.01-25 PC/Bx (%)	4-25 PC/Bx (%)
Normal	40/448 (8.9)	25/168 (14.9)	65/616 (10.6)
Abnormal	23/85 (27.1)	54/84 (64.2)	77/169 (45.6)
Total	63/533 (11.8)	79/252 (31.3)	142/785 (17.9)

DRE: Digital rectal examination; abnormal indicates any palpable firm or hard area of prostate.
PC: prostate cancer, Bx: biopsy

assay. The interassay and intraassay variation in our laboratory was less than 10%. The B & K 3535 urologic ultrasound coupled with a biplane transrectal probe (Bruel and Kjer, Denmark) was used. TRUS-guided sextant biopsies were performed with an automatic 18-gauge spring-loaded biopsy cutting needle (Manan Pro-Mag 2.2).

Statistical analysis. For statistical analysis, the percentage of free PSA was calculated as the ratio of free PSA to total PSA and multiplied by 100. An unpaired Student's *t*-test or Fisher's test was used to assess the relationship between or among groups with different ages, and different total PSA and percentage of free PSA values. Receiver operating characteristic (ROC) curves were generated for total PSA and percentage of free PSA, plotting sensitivity *versus* 1-specificity. Areas under the ROC curves (AUC) were calculated for the percentage of free PSA and total PSA. The statistical difference was determined by z test.

Results

In total, 785 patients entered this study. Following the first round of TRUS-guided biopsy, 142 patients (17.9%) had PC detected and the other 643 patients were found to have benign disease, with most being defined as nodular hyperplasia. In patients with serum PSA values between 4.0 and 10.0 ng/ml, the cancer detection rate was 8.9% (40/448) for non-palpable abnormal prostate [digital rectal examination (DRE)-negative] patients and 27.1% (23/85) for palpable abnormal prostate (DRE-positive) patients, as shown in Table I. In patients with PSA values between 10.01 and 25.0 ng/ml, the biopsy positive rate was only 14.9% (25/168) for DRE-negative patients, but as high as 64.2% (54/84) for DRE- positive patients.

Of 643 patients with initial negative biopsy, 195 (30.4%) received a second TRUS-guided biopsy and 108 patients (16.5%) underwent TURP. An additional 27 patients with PC were diagnosed; those included 19 patients (9.7%) detected by rebiopsy and 8 (7.4%) by TURP; thus, a total of 169 patients with a histologically confirmed diagnosis of prostate adenocarcinoma and 616 patients with benign prostate disease were included for analysis.

Table II. *The mean age, total PSA and percent PSA in patients of each subgroup.*

		DRE-negative	DRE-positive	*p* value (benign *vs* cancer)
Age (years)				
PSA 4-10	Benign	68.8±7.8	69.7±6.5	0.001
	Cancer	71.5±5.6	72.4±4.5	
PSA10-25	Benign	70.5±6.7	72.6±6.9	0.526
	Cancer	72.4±5.9	70.8±5.6	
Total PSA (ng/ml)				
PSA 4-10	Benign	6.4±1.8	5.9±1.8	0.125
	Cancer	6.7±1.7	6.7±1.6	
PSA10-25	Benign	14.2±3.8	14.8±4.2	0.001
	Cancer	15.6±4.9	17.2±4.5	
f/t PSA (%)				
PSA 4-10	Benign	26.8±9.7	28.4±8.9	<0.001
	Cancer	18.8±8.5	19.5±8.67	
PSA10-25	Benign	25.1±9.1	27.0±12.6	<0.001
	Cancer	19.5±7.2	15.5±6.3V	

DRE-negative: normal digital rectal examination.
DRE-positive: abnormal digital rectal examination.
*In cancer patients with PSA 10-25 ng/ml, the DRE-positive had significantly lover f/t PSA (%), $p=0.01$

The patients' ages ranged form 40 to 86 years with a mean age of 69.9±7.2 and a median age of 70 years. Free PSA values ranged from 0.14 to 8.08 ng/ml, and the percentage of free PSA values ranged from 2.7 to 65.5%. There was no significant difference in mean age, total PSA and percentage of free PSA among patients with palpable or non-palpable abnormal prostates (Table II). However, in cancer patients with PSA levels between 10.01 and 25 ng/ml, the DRE-positive patients had a lower percentage of free PSA compared to the DRE-negative patients ($p=0.01$). The percentage of free PSA was significantly lower in patients with cancer (18.0±7.7) compared to patients with BPH (26.6±9.7), regardless of the total PSA levels and DRE status ($p<0.0001$).

Comparison of ROC curves for prospective PC detection based on serum total PSA and percentage of free PSA were calculated. The area under the ROC curve (AUC) for the perecentage of free PSA was significantly larger than that for total PSA (0.77 *vs.* 0.33, $p<0.00001$). The probability of PC detection based on the percentage of free PSA was 51, 43, 14 and 5% in those patients with <10, 10 to 20, 20 to 30 and >30% of free PSA. The sensitivity, specificity and positive predictive value of cancer detection using the percentage of free PSA at different cut-off levels is shown

Table III. *The sensitivity, specificity and positive predictive value (PPV) of percentage of free PSA cut-offs in detection of PC based on total PSA values.*

% free PSA cut-off	Sensitivity	Specificity	Positive Predictive Value
PSA 4-10 ng/ml			
≤20	67.5 (54/80)	76.6 (347/453)	33.8 (54/160)
≤25	82.5 (66/80)	56.7 (257/453)	25.2 (66/262)
≤30	90.0 (72/80)	36.2 (164/453)	20.0 (72/361)
≤34	95.0 (76/80)	21.2 (96/453)	17.6 (76/433)
PSA 10-25 ng/ml			
≤20	73.0 (65/89)	74.9 (118/163)	60.9 (67/110)
≤25	88.8 (79/89)	50.3 (82/163)	49.4 (79/160)
≤29	95.5 (85/89)	32.5 (53/163)	43.6 (85/195)
≤30	96.6 (86/89)	29.5 (48/163)	42.8 (86/201)
≤31	98.9 (88/89)	26.4 (43/163)	42.3 (88/208)

in Table III. Given a fixed 95% as the recommended sensitivity, the lower PSA level tends to have a higher cut-off value of percentage of free PSA (34% for PSA values between 4 and 10 *vs.* 29% for PSA values between 10 and 25 ng/ml). For patients with PSA levels from 4 to 10 ng/ml, to achieve 90% sensitivity the optimal cut-off point for percentage of free PSA was 30%, and that had 36.2% specificity and 20.0% positive predictive value. For patients with PSA levels between 10 and 25 ng/ml, a cut-off of 30% for percentage of free PSA produced 96.6% sensitivity, 29.5% specificity and 42.8% positive predictive value.

Discussion

Although the incidence of PC in Taiwan is one of the lowest in the world, the increase in incidence in recent years has been remarkable. Perhaps the most important trend in this country has been the steep increase in the annual death rate from PC. The age adjusted death rate was 6.54/100,000 male population in 2002, which was 4.38 times the rate in 1987 (1.49/100,000 male population) (11, 12). This reflects the fact that the majority of diagnosed patients are in a late stage of the disease. Early detection of PC with PSA testing in combination with digital rectal examination is mandatory to prevent any further increase in mortality rates in the future.

PSA has proven to be an extremely useful tool for urologists for the early detection of PC. In the USA, among men with palpably normal prostates who have serum PSA values in the range from 4 to 10 ng/ml, the probability of detecting PC by a single sextant biopsy has been estimated to be approximately 25% (2). However, there is only a 9% probability of cancer detection by a single sextant biopsy for the same PSA range of patients in this country. Even in those patients with palpable normal prostates and serum PSA values in the range from 10 to 25 ng/ml, the probability of detecting PC is only 15% for a single sextant biopsy. Therefore, the diagnostic gray zone might be considered to include those patients with PSA values up to 25.0 ng/ml in this country with its lower incidence of PC.

This prospective study may reflect clinical practice cases in urologic offices in evaluating the usefulness of percentage of free PSA in those patients with a moderate elevation in PSA level (from 4 to 25 ng/ml). Because of the relatively lower probability of PC detection in this country, enough time is needed to enroll an adequate number of subjects with cancer who would be available for analysis. The number of PC patients and BPH patients in this study met the criteria requested by Partin and Carter (13). They suggest approximately 140 PC subjects are required to establish a 90±5% cut-off point (with a 95% confidence interval) and approximately 370 non-cancer subjects are required to establish that this cut-off point results in a 40% (±5%) specificity or a decrease in unnecessary biopsies. In this study, there was no statistical significance of age and total PSA between DRE-positive and DRE-negative patients, regardless of whether they had PC or a benign condition. Thus, we enrolled both groups of patients together to obtain a sufficient number of cancer patients for statistical analysis. However, only 18% of the population of subjects had cancer in this study, compared to almost 50% of the population having cancer in a recent prospective trial (14).

Similar to the results of most other reports, the percentage of free to total PSA was significantly lower in those patients with PC than in those with BPH. In this study, the mean and median percentage of free PSA for both patients with benign and those with malignant conditions were particularly high compared to almost all the other reports (8, 10, 13-16). This phenomenon might be explained by the different cancer risk population and different assay kits for total and free PSA (15, 17, 18). However, it is also possible that we enrolled all patients with moderately high levels of PSA regardless of the percentage of free PSA values, and that many patients with higher percentages of free PSA could be excluded from biopsy if the study had not been done in a perspective blinded fashion.

Many studies have suggested that the percentage of free PSA is most useful when the PSA level falls in the ranges from 2 to 20, 3 to 15, or 4 to 10 ng/ml (7, 13, 16, 19). The percentage of free PSA may not offer added usefulness to the differentiation of BPH from PC when the total PSA values exceed 10 or 20 ng/ml; the positive predictive value of PSA alone may be as high as 50 or 80% (3, 7). However, this is not compatible to our results from the study population in an area with lower incidence of PC due to lower predictive rate. In the present study, the AUC was

significantly larger for percent of free PSA than for total PSA (0.76 *versus* 0.45 for patients with PSA levels between 4 and 10 ng/ml, 0.77 *versus* 0.36 for patients with PSA levels from 10 to 25 ng/ml and 0.77 *versus* 0.33 for patients with PSA 4-25 ng/ml). These results indicate that the percentage of free PSA is much more predictive of PC than total PSA in this country, and the benefits of this testing can also be obtained for patients with PSA levels between 10.0 and 25.0 ng/ml and a normal palpable prostate.

Current studies have shown that use of the percentage of free PSA can enhance the specificity of PSA testing and decrease the number of unnecessary biopsies. However, the cut-off points for percent free PSA in studies has ranged from 14 to 28% free PSA, with 60 to 100% sensitivity and 19 to 95% specificity (8, 10, 13, 14, 17). Differences in study designs, subject populations and assay manufacturers may account for the wide variability in study results. Prospective multisite clinical trials that take into account influencing factors are needed to set assay-specific cut-off points (13). Recently, Catalona *et al.* reported the first and largest series of prospective multicenter clinical trials for evaluating the use of percent free PSA to enhance the differentiation of PC from benign prostate disease (14). Using the Tandem assay (Hybritech), a cut-off of 25% or less of free PSA is recommended for patients with PSA values between 4.0 and 10.0 ng/ml and a palpably benign prostate, regardless of the patient's age and prostate size. In the present study, a cut-off of 25% could not be recommended because of unacceptable sensitivity (<90%). A 30% free PSA cut-off in patients with PSA levels between 4 and 10 ng/ml would produce 90% sensitivity and 37.4% specificity. The majority of missed cancer patients with serum PSA level less than 10 ng/ml (8 cases) were elderly or had low-grade tumors. In those patients with PSA levels between 10 and 25 ng/ml, we selected 30% as the cut-off point to produce 96.6% sensitivity and 29.5% specificity. To our knowledge, this is highest cut-off point of percentage of free PSA reported to date. The selected optimal cut-off of percentage of free PSA is reflected by the higher mean and median of percentage of free PSA for this study population. Recent studies have also shown that percentage of free PSA cut-offs and clinical performance differ when various combinations of free PSA and total PSA assays from different manufacturers are used (17, 20-22). The median percent free PSA of BPH and PC, which were 26 and 17 in this study, seems higher compared to 18 and 12 in a recent prospective study using the Hybritech Tandem assay (14). Direct comparison of two different kits with the same serum samples in our data demonstrated that the percent free PSA calculated by the Cis bio international assay was significantly higher than by Tandem R assay (24.08±10.09 *vs* 18.72±8.17, $p<0.0001$) (18).

In conclusion, measurement of the percentage of free PSA can reduce the number of unnecessary biopsies in urologic clinic patients with PSA levels of 4 to 25 ng/ml. This test is particularly useful for selecting patients to have biopsy of the prostate when facing a population of patients with BPH in an area of low PC incidence. The appropriate cut-off point for the percentage of free PSA may vary greatly depending on the type of assay from the manufacturers. The optimal cut-off set at 30% in the Cis bio international assay can eliminate 30 to 36% of unnecessary biopsies and maintain a reasonable sensitivity.

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