

Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease

A Prospective Multicenter Clinical Trial

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Context.—The percentage of free prostate-specific antigen (PSA) in serum has been shown to enhance the specificity of PSA testing for prostate cancer detection, but earlier studies provided only preliminary cutoffs for clinical use.

Objective.—To develop risk assessment guidelines and a cutoff value for defining abnormal percentage of free PSA in a population of men to whom the test would be applied.

Design.—Prospective blinded study using the Tandem PSA and free PSA assays (Hybritech Inc, San Diego, Calif).

Setting.—Seven nationwide university medical centers.

Participants.—A total of 773 men (379 with prostate cancer, 394 with benign prostatic disease) 50 to 75 years of age with a palpably benign prostate gland, PSA level of 4.0 to 10.0 ng/mL, and histologically confirmed diagnosis.

Main Outcome Measures.—A percentage of free PSA cutoff that maintained 95% sensitivity for prostate cancer detection, and probability of cancer for individual patients.

Results.—The percentage of free PSA may be used in 2 ways: as a single cutoff (ie, perform a biopsy for all patients at or below a cutoff of 25% free PSA) or as an individual patient risk assessment (ie, base biopsy decisions on each patient's risk of cancer). The 25% free PSA cutoff detected 95% of cancers while avoiding 20% of unnecessary biopsies. The cancers 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. For individual patients, a lower percentage of free PSA was associated with a higher risk of cancer (range, 8%-56%). In the multivariate model used, the percentage of free PSA was an independent predictor of prostate cancer (odds ratio [OR], 3.2; 95% confidence interval [CI], 2.5-4.1; $P < .001$) and contributed significantly more than age (OR, 1.2; 95% CI, 0.92-1.55) or total PSA level (OR, 1.0; 95% CI, 0.92-1.11) in this cohort of subjects with total PSA values between 4.0 and 10.0 ng/mL.

Conclusions.—Use of the percentage of free PSA can reduce unnecessary biopsies in patients undergoing evaluation for prostate cancer, with a minimal loss in sensitivity in detecting cancer. A cutoff of 25% or less free PSA is recommended for patients with PSA values between 4.0 and 10.0 ng/mL and a palpably benign gland, regardless of patient age or prostate size. To our knowledge, this study is the largest series to date evaluating the percentage of free PSA in a population representative of patients in whom the test would be used in clinical practice.

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MEASUREMENT of serum prostate-specific antigen (PSA) is widely used as an aid in the early detection of prostate cancer.¹ A limitation of PSA testing has been its relative lack of specificity within the 4.0- to 10.0-ng/mL range, a diagnostic gray zone in which prostate cancer is present in only 25% of patients. Most patients with prostate cancer and a PSA level less than 10.0 ng/mL have early-stage disease, whereas more than half of the patients with PSA levels above 10.0 ng/mL have advanced disease.¹ Thus, the detection of prostate cancer in its potentially curable stages requires the use of low PSA cutoffs for screening, which leads to many unnecessary biopsies.

Prostate-specific antigen exists in multiple forms in serum and is predominantly complexed to protease inhibitors; however, one form of PSA, free PSA, is not bound to these proteins.^{2,3} Measurement of PSA forms in serum helps discriminate between prostate cancer and

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Table 1.—Results of Prostate Cancer Detection Testing With PSA and DRE*

DRE Results	Screened Men With PSA/DRE Results, %		
	<4 ng/mL	4-10 ng/mL	>10 ng/mL
Normal	74	9†	2
Indicative of cancer	11	3	1

*Data are from Catalona et al¹ (N = 6630). PSA indicates prostate-specific antigen; DRE, digital rectal examination.

†This group represents the clinical trial population for the present study.

benign prostatic disease.^{4,5} For unknown reasons, the percentage of free PSA is lower in serum samples from patients with prostate cancer than in serum samples from patients with a normal prostate or benign disease. Preliminary evidence also suggests that a lower percentage of free PSA may be associated with a more aggressive form of prostate cancer.⁶⁻⁹

Low percentages of free PSA are well established as indicators of prostate cancer,¹⁰⁻¹⁶ but the selection of cutoffs for use in clinical practice is complicated by the partial dependence of percentage of free PSA on patient age, prostate size, and the total PSA level.^{10-12,16,17}

In the present study, we evaluated the ability of percentage of free PSA to enhance the specificity of PSA testing in prostate cancer detection, developed guidelines for use of percentage of free PSA in clinical practice, and determined the relationships between percentage of free PSA and the histopathologic features of the prostate cancers detected.

METHODS

Study Design

Serum samples were obtained from subjects meeting study entry criteria at 7 university medical centers between July 1994 and December 1996; 91% of the samples were prospective and 9% were from serum banks of recently evaluated patients. Subjects were 50 to 75 years of age and had received no treatment for prostatic disease at the time of the phlebotomy. All subjects had serum PSA concentrations between 4.0 and 10.0 ng/mL and digital rectal examination (DRE) findings that were not indicative of cancer (Tables 1 and 2). All subjects had undergone ultrasound-guided 6-sector needle biopsies of the prostate and, thus, had a histologically confirmed diagnosis prior to determination of free PSA concentrations. In this blinded study, pathologists did not have access to percentage of free PSA values and laboratory scientists did not have access to diagnoses. The study was performed in compliance with the requirements of the respective institutional review boards.

Table 2.—Current Clinical Practice and Proportion of Men With Cancer Based on PSA and DRE Results*

DRE Results	PSA, ng/mL							
	0-2		2-4		4-10		>10	
	Biopsy	% With Cancer	Biopsy	% With Cancer	Biopsy	% With Cancer	Biopsy	% With Cancer
Normal	No	≈1	No	15	Yes	25†	Yes	>50
Indicative of cancer	Yes	5	Yes	20	Yes	45	Yes	>75

*Data are from Catalona et al^{1,27} and Keetch et al.²¹ PSA indicates prostate-specific antigen; DRE, digital rectal examination.

†This group represents the clinical trial population for the present study. For comparison, 20% of women who undergo mammography for breast cancer detection had cancer.

Subjects were enrolled primarily through prostate cancer screening centers, although subjects screened elsewhere and referred for treatment to the study centers were also enrolled. This population of men with biopsies based solely on elevated PSA levels represents the population of interest in which percentage of free PSA would be used in clinical practice.

Serum Sample Collection, Storage, and Assays

Serum samples were processed and refrigerated within 3 hours of blood draw. If the serum sample was to be assayed within 24 hours after collection, the specimen was stored at 2°C to 8°C. Specimens held for longer times were stored at -70°C until analyzed.¹⁸ Concentrations of PSA were determined using the Tandem PSA and free PSA monoclonal antibody assays (Hybritech Inc, San Diego, Calif).¹⁹ The same serum sample was used to determine both total PSA and free PSA concentrations, as would be done in clinical practice. Total PSA testing was performed and the sample was frozen. The free PSA concentration was measured after it was determined that the subject met all study entry criteria. Multiple freeze-thaw cycles do not affect total PSA and free PSA measurements, and both analytes are stable under the conditions described herein.¹⁸

Statistical Analysis

The nonparametric Wilcoxon test was used for comparisons between group medians, and linear regression analysis was used to assess the relationships among percentage of free PSA and PSA, age, and prostate volume. Percentage of free PSA was calculated as the ratio of free PSA to total PSA multiplied by 100.

A multivariate logistic regression analysis,²⁰ with the likelihood ratio χ^2 test, was used to evaluate percentage of free PSA, age, and PSA. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from the logistic regression method using the Wald method.

Receiver operating characteristic (ROC) curves were generated for PSA and percentage of free PSA, plotting

sensitivity vs (1 - specificity). We determined percentage of free PSA cutoffs that would detect 90% and 95% of the cancers (sensitivity), as well as the corresponding percentages of biopsies with negative findings that could be avoided (specificity) by using each of these cutoffs. Areas under the ROC curves (AUC) were calculated for the percentage of free PSA and PSA, and a z test was used to measure differences.

Sample size requirements for the study were calculated to obtain 95% CIs for all possible cutoffs along the percentage of free PSA ROC curve, so that all sensitivity and specificity estimates would be within 5 percentage points or less. This required nearly 400 subjects with cancer and 400 subjects with benign prostatic disease.

However, since sample size requirements were calculated to address the ROC curve analysis, the 50:50 ratio of patients with cancer to patients with benign prostatic disease could not be used for a risk assessment analysis (positive predictive value), which, unlike ROC curve analysis, is susceptible to cancer prevalence. Cancer probabilities based on the 50:50 ratio would inflate the risk estimates,^{11,15} whereas probabilities based on a 25:75 ratio would provide accurate risk estimates appropriate for the group of men in whom this test will be used. Therefore, it was necessary to statistically adjust the 50:50 ratio of subjects with cancer to subjects with benign prostatic disease to a 25:75 ratio, since we have previously shown that a 25% rate of positive biopsy results would be obtained in a population of men with PSA values between 4.0 and 10.0 ng/mL and normal findings on DRE.^{1,21} The bootstrap method^{22,23} was used to repetitively sample the study population and adjust the proportion of subjects with cancer from 50% to 25%. This random sampling process was repeated 1000 times. Median cancer probabilities (risk estimates) and ORs were calculated.

RESULTS

We enrolled 773 men with histologically confirmed diagnoses (379 with prostate cancer and 394 with benign

Table 3.—Demographic and Clinical Characteristics by Diagnosis*

	Benign (n = 394)	Cancer (n = 379)	Total (N = 773)
Median age, y (range)	64 (50-75)	64 (50-75)	64 (50-75)
Median total PSA, ng/mL (range)	5.6 (4-10)	5.9 (4-10)	5.8 (4-10)
Median free PSA, % (range)	18 (4-52)	12 (2-42)	15 (2-52)

*PSA indicates prostate-specific antigen.

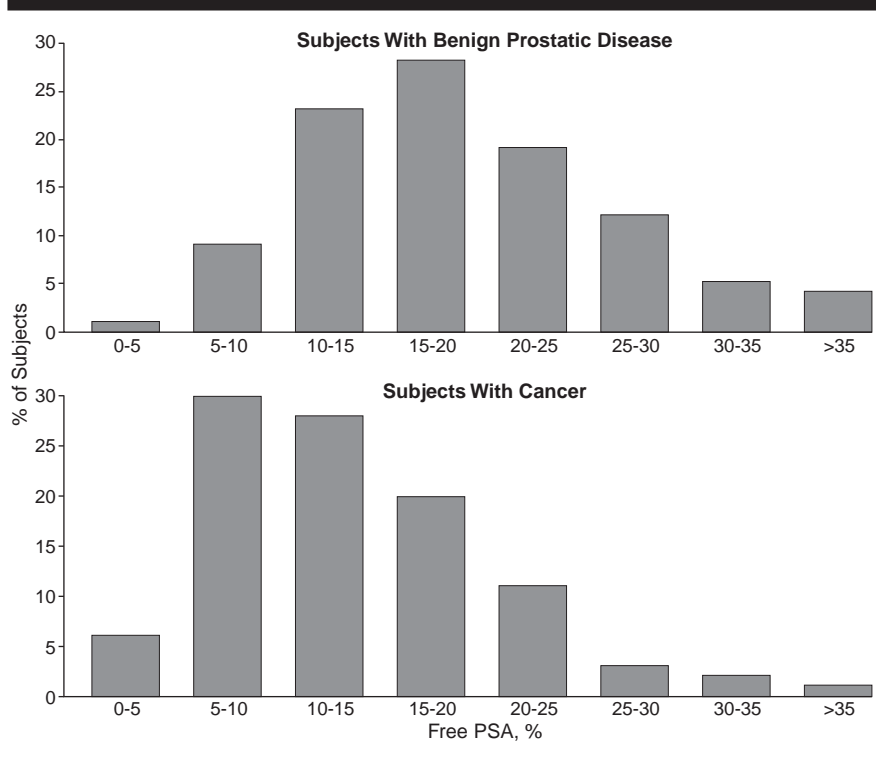


Figure 1.—Distribution of percentage of free prostate-specific antigen (PSA) by diagnosis.

prostatic disease). Age and PSA values were similar for the cancer and benign groups (Table 3). The study population was 86% white, 9% African American, 3% Hispanic, and 2% Asian.

Free PSA values ranged from 0.2 to 5.0 ng/mL, and percentage of free PSA values ranged from 2% to 52%. Median percentage of free PSA values were significantly lower in the cancer group (12%) than in the benign group (18%) ($P < .001$) (Figure 1).

No differences were seen between retrospective and prospective samples in the cancer group ($P = .41$) or benign group ($P = .84$). When controlling for patient age, no differences were seen in percentage of free PSA values for cancer subjects across sites ($P = .11$) or for benign subjects across sites ($P = .17$). No differences were seen between subjects screened at the study centers vs subjects screened elsewhere and referred to the study centers for treatment ($P = .13$). Presence or absence of symptoms did not affect results ($P = .12$).

Percentage of free PSA (AUC, 0.72; 95% CI, 0.68-0.75) was significantly more

predictive of cancer than total PSA level (AUC, 0.53; 95% CI, 0.49-0.57) for this population with total PSA values of 4.0 to 10.0 ng/mL ($P < .001$) (Figure 2).

Cutoffs for percentage of free PSA of 25% and 22% yielded 95% and 90% sensitivity, respectively. Use of these cutoffs (ie, performing biopsies only in patients with percentages of free PSA less than or equal to these cutoffs) could have avoided biopsies in 20% and 29%, respectively, of the patients with benign prostatic disease. Calculations, sample size, and 95% CIs for these cutoffs are shown in Table 4.

There was an inverse relationship between percentage of free PSA and PSA ($r = -0.14$, $P < .001$), and a direct relationship between percentage of free PSA and age ($r = 0.34$, $P < .001$). Although the first correlation is not strong enough to affect the cutoffs within the 4.0- to 10.0-ng/mL range, the latter correlation has a significant effect on the selection of cutoffs, as shown in Table 5 and Figure 3. Because of this increasing trend with age, cutoffs could be adjusted upward as age increases to maintain a

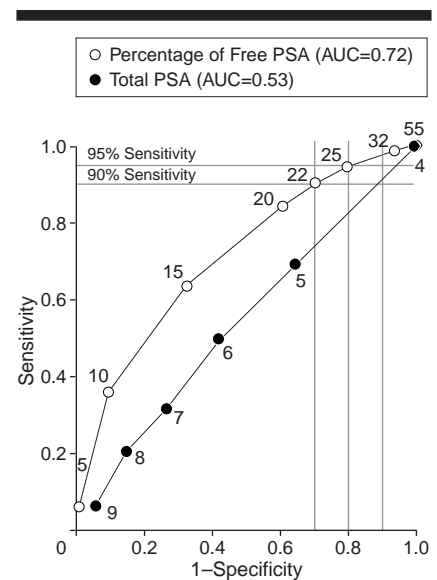


Figure 2.—Percentage of free prostate-specific antigen (PSA) and total PSA receiver operating characteristic (ROC) curve. A cutoff of 25% or less free PSA provides 95% sensitivity and 20% specificity (ie, it spares 20% of men with benign prostatic disease from an inappropriate biopsy). AUC indicates area under the ROC curve.

Table 4.—Percentage of Free PSA Cutoffs, Sensitivity, and Specificity*

Free PSA Cutoff, %	Sensitivity, % (No.) of Cancers Detected [95% CI]	Specificity, % (No.) of Unnecessary Biopsies Avoided [95% CI]
≤22	90 (341/379) [86-93]	29 (115/394) [25-34]
≤25	95 (358/379) [92-97]	20 (80/394) [16-24]

*PSA indicates prostate-specific antigen; CI, confidence interval.

constant 95% sensitivity within each age decade (ie, age-specific cutoffs would miss cancers in 5% of patients within each age decade: 50-59, 60-69, and 70-79 years). Alternatively, a single cutoff (25% free PSA) may be used across all age ranges; in this case, 5% of cancers were missed in subjects aged 50 to 75 years, with more of the missed cancers occurring in older subjects (median age, 68 years). The 25% cutoff detected 98% of cancers for subjects aged 50 to 59 years, 94% for subjects aged 60 to 69 years, and 90% for subjects aged 70 to 75 years.

Of the 379 subjects with cancer, 268 (71%) underwent radical prostatectomy. Analysis (χ^2 testing) showed a statistically significant increase in probability of favorable pathologic findings (Gleason score < 7 , organ-confined [stages T1 and T2], lymph nodes with negative test results for metastasis, tumor volume $\leq 10\%$ of gland) as percentage of free PSA increased (34% with favorable findings for subjects with values $\leq 15\%$ free PSA vs 70% for subjects with values $> 25\%$ free PSA, $P < .001$). Thus, subjects with

Table 5.—Influence of Age on Percentage of Free PSA Cutoffs, Sensitivity, and Specificity*

Age Range, y	Free PSA Cutoff, %	Sensitivity, % (No.) of Cancers Detected [95% CI]	Specificity, % (No.) of Unnecessary Biopsies Avoided [95% CI]
Fixed Sensitivity			
50-59	≤20	95 (105/110) [89%-98%]	22 (21/95) [14%-32%]
60-69	≤26	95 (189/199) [91%-97%]	15 (31/209) [11%-21%]
70-75	≤28	95 (67/70) [86%-99%]	22 (20/90) [14%-32%]
Fixed Cutoff			
50-59	≤25	98 (108/110) [93%-100%]	11 (10/95) [6%-19%]
60-69	≤25	94 (187/199) [89%-97%]	19 (39/209) [14%-25%]
70-75	≤25	90 (63/70) [80%-96%]	34 (31/90) [25%-45%]

*PSA indicates prostate-specific antigen; CI, confidence interval.

cancer with values above the cutoff tended to have less aggressive disease.

Percentage of free PSA was an independent predictor of prostate cancer (OR for 10-point decline in percentage of free PSA, 3.2; 95% CI, 2.5-4.1; $P < .001$) and contributed significantly more than age (OR, 1.2; 95% CI, 0.92-1.55) or total PSA value (OR, 1.0; 95% CI, 0.92-1.11) in this cohort of subjects with total PSA values between 4.0 and 10.0 ng/mL.

Prostate volume estimated by transrectal ultrasound was available for 695 subjects (90%). Percentage of free PSA increased with prostate volume ($r = 0.55$, $P = .001$). Using a 25% free PSA cutoff, sensitivity was 99%, 93%, and 87% for subjects with volumes of 30 cm³ or less, 31 to 50 cm³, and more than 50 cm³, respectively.

Table 6 shows the probability of detecting cancer with needle biopsy, based on PSA and percentage of free PSA results. The PSA results in this table were obtained from a prior multicenter study evaluating the efficacy of total PSA for prostate cancer detection,^{1,21} and percentage of free PSA results were obtained from the current study. It can be seen that rising PSA levels increase the risk of detectable cancer. Percentage of free PSA can further stratify risk for patients with PSA values between 4.0 and 10.0 ng/mL.

Figure 4 shows the probability of cancer based on percentage of free PSA as well as patient age. The risk of cancer was high (55%-56%) when percentage of free PSA was 0% to 10%, regardless of age. The risk decreased as percentage of free PSA increased (eg, cancer probability was 5%-9% when percentage of free PSA > 25%). Older subjects were generally at higher risk than younger subjects.

COMMENT

This prospective study from 7 university medical centers is, to our knowledge, the largest series to date evaluating the usefulness of percentage of free PSA in patients with moderate elevations in PSA levels (4.0-10.0 ng/mL), benign findings on DRE, and histologically confirmed diagnoses.

In prostate cancer detection programs, prostatic biopsy is routinely recommended for patients with abnormal DRE results regardless of PSA level (15% of subjects screened for prostate cancer, Table 1), and for patients with normal DRE results and PSA levels higher than 4.0 ng/mL. Those patients with normal DRE results and PSA levels of 4.0 to 10.0 ng/mL represent the diagnostic gray zone, in which the total PSA value has identified the patients as high risk (25% cancer rate compared with a 4% cancer rate for the general population of men older than 50 years^{1,21}), but specificity could be improved because 75% of biopsy findings are negative.

The current study shows that the use of percentage of free PSA in this group of patients can enhance the specificity of PSA screening and decrease the number of unnecessary biopsies. A cutoff of 25% or less free PSA (ie, perform a biopsy for patients at or below this cutoff) would detect 95% of cancers and spare 20% of patients with benign prostatic disease from biopsy. Since the majority of patients with PSA levels between 4.0 and 10.0 ng/mL have benign prostatic disease, this represents a substantial number of patients who would avoid an inappropriate biopsy. With the widespread use of PSA screening, improvements in specificity are desirable. A recent study found that the benefit-cost equation of prostate cancer detection programs is very sensitive to changes in test specificity, so that minor increases in specificity ($\approx 5\%$) produced marked reductions ($\approx 50\%$) in net cost per individual screened.²⁴ Only 9% of patients tested for total PSA value would be tested for percentage of free PSA, but this represents 35% of all biopsies (Table 1). Prostate cancer screening generates controversy, in part because of specificity concerns, but also because of a lack of long-term studies evaluating the outcome of treatment. However, interim studies have shown that PSA testing has contributed to a shift in detection of earlier-stage disease, from the historically observed 70% rate of advanced cancer at diagnosis to the currently observed rate

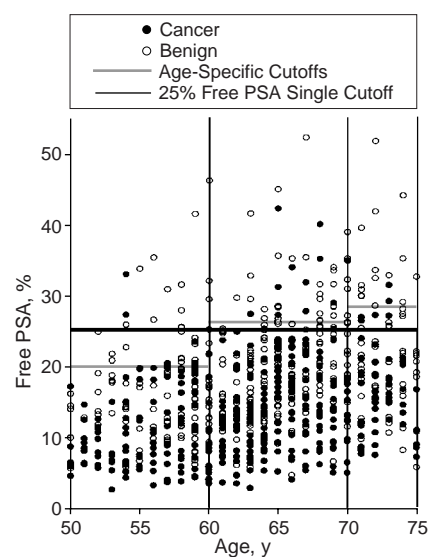


Figure 3.—Percentage of free prostate-specific antigen (PSA) by patient age and diagnosis. Age-specific cutoffs are shown at 20%, 26%, and 28% free PSA for ages 50 to 59, 60 to 69, and 70 to 75 years, respectively. However, a single cutoff of 25% free PSA is recommended across all age ranges.

of 30%.²⁵ It was recently shown that the prostate cancer death rate in the United States declined between 1991 and 1995, providing the first evidence that early detection (probably through transurethral resection of the prostate, since widespread PSA screening did not begin until 1991) may decrease mortality rates.²⁶ Medical policy will continue to be shaped as long-term studies progress, but in the near term, screening test improvements can provide an immediate benefit to patient care.

The large number of subjects in this study provides confidence in the percentage of free PSA cutoffs determined for use in clinical practice. A 25% free PSA cutoff will produce 95% sensitivity (95% CI, 92%-97%) and 20% specificity (95% CI, 16%-24%). In contrast, the cutoffs previously reported in the literature have provided only preliminary estimates with wide CIs.

The AUC was significantly higher for percentage of free PSA (0.72) than for total PSA (0.53), indicating that percentage of free PSA is more predictive of cancer in patients with PSA levels of 4.0 to 10.0 ng/mL. However, in patients with PSA levels of 0 to 50 ng/mL, for example, the AUC would be approximately 0.73 for PSA, because PSA is highly predictive of cancer when a wide range of values is tested.¹ This explains in part why several early studies composed of patients with high PSA levels did not find that percentage of free PSA was effective. Percentage of free PSA is highly effective when used in patients with

Table 6.—Probability of Cancer Based on PSA and Percentage of Free PSA Results*

PSA, ng/mL	Probability of Cancer, %	Free PSA, %	Probability of Cancer, %
0-2	≈1	... †	...
2-4	15
4-10	25	0-10	56
		10-15	28
		15-20	20
		20-25	16
		>25	8
>10	>50

*Data are for men with normal digital rectal examination results, regardless of patient age. Data for prostate-specific antigen (PSA) results are from Catalona et al¹ and Keetch et al.²¹ Percentage of free PSA can further stratify risk for men with PSA values between 4 and 10 ng/mL.

†Ellipses indicate data not applicable.

moderately elevated PSA values, but it cannot improve the considerable specificity of total PSA values in patients with high PSA concentrations. Factors such as this that may affect percentage of free PSA studies are discussed in a detailed review article by Woodrum et al.¹⁷

Percentage of free PSA values decreased as total PSA values increased. This relationship was not sufficiently robust to affect the cutoff in a population with PSA values of 4.0 to 10.0 ng/mL, but could affect cutoffs if percentage of free PSA is useful outside of this range, as suggested by recent studies.^{16,27,28}

The percentage of free PSA values increased as patient age increased. Because of this relationship, age-specific percentage of free PSA cutoffs could be used, but this approach resulted in more undetected cancers in younger subjects. In contrast, a single 25% free PSA cutoff across all age groups resulted in the highest sensitivity (98%) in younger subjects, those most likely to benefit from early detection. Most of the missed cancers occurred in older subjects. Clinically, this finding is advantageous because older subjects (those with less than a 10-year life expectancy) are often not affected by or treated for prostate cancer. For this reason, we recommend a single cutoff (25% free PSA) for patients aged 50 to 75 years.

Other studies have also reported a relationship between percentage of free PSA and age,^{11,12,16} although some studies composed of only healthy subjects have not found this association.²⁹

A significant positive correlation was found between percentage of free PSA and prostate volume. This correlation provides insight into how percentage of free PSA aids in distinguishing prostate cancer from benign prostatic disease. Percentage of free PSA provides information on gland size and total PSA production relative to each other. If PSA is elevated and only a small portion of the total PSA is free PSA, the probability is

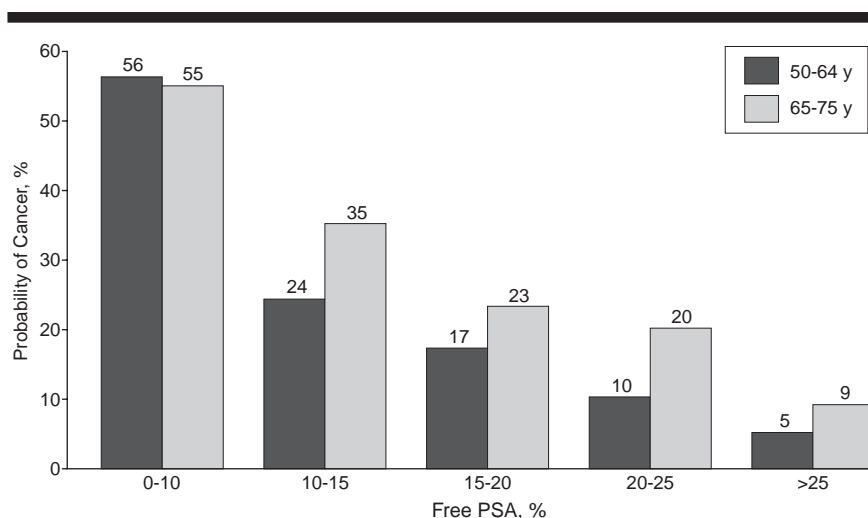


Figure 4.—Probability of cancer by percentage of free prostate-specific antigen (PSA) and patient age for patients with PSA levels between 4.0 and 10.0 ng/mL.

high that the patient has a small gland with cancer. If PSA is elevated and a large portion of the total PSA consists of free PSA, the probability is high that the patient has a large gland without cancer. Thus, use of a single 25% free PSA cutoff is recommended regardless of the patient's prostate size. When a recommendation is made not to perform a biopsy for patients above this cutoff, this is the group with the lowest risk of cancer and the highest probability of benign prostatic disease.

Thus, the cancers that would be missed, occurring in patients with a percentage of free PSA value above the 25% cutoff, are found primarily in older patients with larger glands. These patients also tended to have less aggressive disease. As the percentage of free PSA increased, the probability of favorable pathologic findings increased. The natural history of prostate cancer shows it to be slow-growing; with annual screening, it would be possible to monitor patients and perform biopsies only in patients with increasing PSA levels or decreasing percentage of free PSA.³⁰⁻³²

Our study confirms earlier reports of a relationship between the percentage of free PSA and adverse pathologic features or cancer aggressiveness.^{6-9,14} In contrast, other investigators did not find the percentage of free PSA to be predictive of pathologic results, possibly because of the wide range of PSA values in their study populations,^{33,34} small sample size, or differences in staging techniques or assay systems.

Our data were also analyzed to estimate an individual patient's probability of having detectable cancer based on the percentage of free PSA (Table 6) and the subject's age (Figure 4). Lower percentages of free PSA indicated higher risk,

and older subjects were at higher risk than younger subjects. The risk of cancer ranged from 8% (subjects with percentages of free PSA >25%) to 56% (subjects with percentages of free PSA ≤10%). Thus, percentage of free PSA may be used to stratify individual patients into low-risk to high-risk populations to aid in biopsy decisions.

With this risk assessment approach, it may be possible to actually increase cancer detection (sensitivity). Patients who have undergone 1 biopsy with negative findings might be advised to undergo a second biopsy if the percentage of free PSA indicates high risk (approximately 20% of cancers are missed on the first biopsy specimen).²¹ Thus, cancers that otherwise might be missed would be detected.

Our results confirm earlier reports showing a similar gradient of risk for cancer associated with percentage of free PSA, age, and PSA.^{10,11,15,16} However, preliminary studies often contained a relatively high percentage of subjects with cancer (39% to 44%)^{10,16} that was not adjusted to the known prevalence of detectable prostate cancer (25%) in patient populations with PSA values between 4.0 and 10.0 ng/mL, producing inflated risk estimates.

The protocol for the current study required nearly 400 subjects with cancer and 400 subjects with benign prostatic disease to obtain reliable estimates of cutoffs, sensitivity, and specificity in the ROC curve analyses. It should not be interpreted that the 50% proportion of subjects with cancer in this study means that the population from which they were selected is not representative of a screening population. Subjects with cancer were generally more difficult to enroll because they represented only 25% of the population from which they were

selected. However, if enrollment had been limited to this 25% proportion, an inadequate number of subjects with cancer would have been available for analyses by variables such as age, total PSA, and prostate volume. By enrolling nearly 400 patients in each disease group, it was possible to perform both ROC curve and risk assessment analyses using statistically valid methods; prevalence can be adjusted downward using the bootstrap method, but reliable cutoffs cannot be estimated if the sample size is inadequate.

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