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COMPARATIVE ANALYSIS OF COMPLEXED PROSTATE SPECIFIC ANTIGEN, FREE PROSTATE SPECIFIC ANTIGEN AND THEIR RATIO IN DETECTING PROSTATE CANCER

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ABSTRACT

Purpose: We evaluate the diagnostic use of total, free and complexed serum prostate specific antigen (PSA), and their ratios for enhancing the specificity in detecting prostate cancer.

Materials and Methods: A total of 354 nonconsecutive men undergoing prostate biopsy were eligible for this retrospective and prospective study. Cancer was found in 122 of these 354 men (34%). Receiver operating characteristics curve analyses were used to calculate and compare the performance of total PSA (Hybritech, San Diego California and Bayer, Tarrytown, New York), complexed PSA (Bayer), percent complexed PSA and percent free PSA. In addition, sensitivity and specificity were calculated and compared.

Results: The area under the receiver operating characteristics curve was highest for percent free PSA, followed by percent complexed PSA, complexed PSA and the 2 total PSA assays (Hybritech and Bayer). The cutoff value of 3.45 ng./ml. for complexed PSA detected the same number of cancers and resulted in 1 additional false-positive case compared with a Hybritech total PSA threshold of 4.0 ng./ml. At sensitivities of 80% to 95%, there were no significant differences for detection comparing the corresponding specificities between Hybritech total PSA and complexed PSA for all 354 men. Complexed PSA alone did not enhance the overall diagnostic accuracy compared with percent free PSA in the Hybritech total PSA range between 4.01 and 6.00 ng./ml., between 6.01 and 10.00 ng./ml., and between 2.50 and 6.00 ng./ml. At sensitivities of 80% to 95% specificity of percent complexed PSA was almost identical to that of percent free PSA except for the Hybritech total PSA range less than or equal to 4.00 ng./ml.

Conclusions: This study suggests complexed PSA is equivalent to total PSA for the early detection of prostate cancer. Percent free PSA outperforms complexed PSA and percent complexed PSA performed equivalently to percent free PSA in all total PSA ranges analyzed between 2.5 and 10 ng./ml.

KEY WORDS: prostate, prostate-specific antigen, prostatic neoplasms

Since the initial reports describing the measurement of the complexed isoforms of prostate specific antigen (PSA),[1,2] several studies have assessed the clinical usefulness of PSA and α1-antichymotrypsin in the early detection of prostate cancer. However, the first assays for PSA-α1-antichymotrypsin gave disappointing results except for the study reported by Leinonen et al,[3] and failed to demonstrate any enhanced cancer detection compared with use of the ratio of free-to-total PSA.[4,5] Rittenhouse and Chan demonstrated that PSA-α1-antichymotrypsin provided information that was clinically equivalent to that provided by free PSA, because total PSA is essentially equivalent to the sum of free PSA and PSA-α1-antichymotrypsin.[6] In 1998 a novel assay for complexed PSA, which avoided the use of antibodies to α1-antichymotrypsin, was developed.[7] Using this new assay, several studies have demonstrated that complexed PSA and its ratio enhance cancer detection compared to total and percent free PSA. In contrast to several favorable retrospective and prospective studies,[8–11] other reports concluded that complexed PSA was less useful in enhancing prostate cancer detection compared to total and/or free PSA.[12–14]

Due to these discrepant reports on the usefulness of complexed PSA, a combined prospective and retrospective multiinstitutional study was undertaken to examine its clinical use as a tool to detect prostate cancer. In addition, we compared the diagnostic performance of total PSA, complexed PSA, free PSA and their respective ratios (complexed-to-total PSA and free-to-total PSA) to clarify the optimum total PSA range for each test.

MATERIALS AND METHODS

Archival serum samples obtained during screening were obtained before prostatic manipulation or biopsy and tested

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retrospectively at 6 sites, including the Northwest Prostate Institute, University of Washington, Seattle, Washington, The Johns Hopkins Hospital, Baltimore, Maryland, Memorial Sloan-Kettering Cancer Institute, New York, New York, Brigham and Women's Hospital, Boston, Massachusetts and The University of Texas, M. D. Anderson Cancer Center, Houston, Texas. A total of 3,268 men were enrolled in this protocol from the 6 sites. Participants with a personal history of prostate cancer or symptoms of acute prostatitis and/or current urinary tract infection were excluded from study.

Of these 3,268 men 363 underwent transrectal ultrasound guided prostate needle sextant biopsy. The indication to perform a biopsy was either PSA 4.0 ng./ml. or greater and/or an abnormal digital rectal examination. Biopsies were performed according to standard practice of the respective institutions at the time, but the sextant strategy was the minimum. Of these 363 men, 9 were excluded from study because of incomplete sample collections. Consequently, a total of 354 men 50 to 84 years old (median age 66) were eligible for study.

A blood sample was drawn and analyzed at each institution to measure total PSA by either Hybritech Tandem-R, Tandem-E or Tandem-MP as well as by the Bayer assay (Technicon Immuno 1 PSA method), free PSA by either the Hybritech Tandem-R or Tandem MP assay, and complexed PSA by the Bayer Immuno 1 assay. All serum samples were drawn no more than 1 month before enrollment digital rectal examination, or greater than or equal to 1 week but less than 1 month after digital rectal examination. Within 24 hours of collection, all samples were centrifuged and stored at −70C. The minimum specimen volume was 0.6 ml. To determine free-to-total PSA, we used the Hybritech free and total PSA assays. The complexed-to-total PSA ratio was determined using Bayer complexed and total PSA assays.

The chi-square test was used for each statistical comparison between 2 specificities at the same sensitivity. For continuous variables a t-test or analysis of variance was used to compare groups. Receiver operating characteristics (ROC) curves were generated by plotting the sensitivity versus (1-specificity) as previously described.[15] Area under the ROC curve was likewise calculated for each assay, and their ratio. The area under the curve calculated from the ROC curve for each variable was compared using the Tukey Honestly Significant Difference (Tukey HSD) multiple range test[16] and published tables at $p = 0.05$ and $p = 0.01$ using custom programs PIWAY and RANGER 2 (free from www.odin.mdacc.tmc.edu). All statistical calculations were performed by or under the direct supervision of a statistician from M.D. Anderson Cancer Center (D. A. J.) who has no relationship with the sponsor. The SAS (SAS Institute Cary, North Carolina) or SPSS 10.0 (SPSS Inc, Chicago, Illinois) software package was used, and $p < 0.05$ was considered statistically significant.

RESULTS

Of the 354 men with complete sample collection 122 (34%) had positive biopsy results for prostate cancer while 232 had no evidence of malignancy on biopsy including histologic confirmation of prostatitis (13) and prostatic intraepithelial neoplasia (6). Comparing the patient populations from each institution, there was a significant difference in mean ages varying from 62.1 to 68.4 (pooled standard deviation 6.98, $p < 0.001$) as well as a significant difference in cancer proportion ($p = 0.004$) with the incidence rate varying from 23.9% to 53.6%. However, there was no significant differences in the 2 total PSA assay values (Hybritech and Bayer total PSA) among the 6 institutions.

The total number of free-to-total PSA ratios available for analysis was 351 because the Hybritech total PSA was less than 0.1 ng./ml. in 3 cases (the lower limit of detection of Hybritech total PSA 0.1 ng./ml.). The correlation of the Bayer total PSA with the Hybritech total PSA was excellent within the assay range (354 cases, $r^2 = 0.996$; and slope = 1.14 with Hybritech = x and Bayer = y). The correlation of these 2 assays for total PSA in the range between 2.0 and 10.0 ng./ml. was also good ($r^2 = 0.896$) with a slope of 1.012. As a result of the limitations of detection limits and imprecision particularly for free PSA in men with total PSA less than 2.0 ng./ml., we will limit our discussion of free-to-total and complexed-to-total ratio to men with PSA 2.5 or higher.

Cutoff value of complexed PSA. Table 1 shows distribution of cancer and patients with no evidence of malignancy stratified by Hybritech and Bayer total PSA. Hybritech and Bayer total PSA ranged to 835.7 and 955.5 ng./ml., respectively (median 6.6 and 6.28, respectively, log PSA $p < 0.001$). In 12 of 81 patients with Hybritech total PSA 4.00 ng./ml. or less prostate biopsies were performed based on family history of prostate cancer despite normal results of digital rectal examination. There were no significant differences for free PSA and patient age between men with and without prostate cancer (table 2). Comparing the p values of the other 4 variables, free-to-total and complexed-to-total PSA differed the most significantly ($p < 0.0001$) between men with and without cancer, followed by complexed ($p = 0.0051$) and total PSA (Hybritech $p = 0.009$ and Bayer $p = 0.0111$).

We compared the numbers of patients with a cancer missed or a false-positive result based on the 2 total PSA assays (Hybritech and Bayer) and complexed PSA (table 3). The numbers of cancers missed and false-positive results using a Hybritech total PSA cutoff value of 4.0 ng./ml. were 17 and 168, respectively. When the Bayer total PSA (greater than 4.0 ng./ml.) was used 18 cancers were missed and there were 176 false-positive cases. The number of cancers missed with a 4.0 ng./ml. Hybritech total PSA threshold was equivalent to a complexed PSA cutoff value of 3.45 ng./ml. At the same 4.0 ng./ml. threshold for Bayer total PSA and 3.45 ng./ml. for complexed PSA cutoff, complexed PSA missed 1 less cancer and detected 7 fewer false-positive cases. In comparison to the scenario of the Hybritech total threshold of 4.0 ng./ml. and a 3.45 ng./ml. cutoff for complexed PSA, the latter missed the same number of cancers and detected 1 additional false-positive case. A complexed PSA cutoff value of 3.75 ng./ml. missed 9 and 8 more patients with cancer compared with a Hybritech and Bayer total PSA threshold of 4.0 ng./ml., re-

TABLE 1. *Distribution of total PSA values in 354 men with and without prostate cancer detected by biopsy*

	No. With Ca	No. Without Ca	Total No.
Hybritech total PSA (ng./ml.):			
0.00–4.00	17	64	81
4.01–6.00	29	66	95
6.01–10.00	42	73	115
Greater than 10.01	34	29	63
Totals	122	232	354
Bayer total PSA (ng./ml.):			
0.00–4.00	18	56	74
4.01–6.00	25	64	89
6.01–10.00	44	80	124
Grater than 10.01	35	32	67
Totals	122	232	354