[-2]Proenzyme Prostate Specific Antigen is More Accurate Than Total and Free Prostate Specific Antigen in Differentiating Prostate Cancer From Benign Disease in a Prospective Prostate Cancer Screening Study

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Purpose: Due to the limited specificity of prostate specific antigen for prostate cancer screening, there is an ongoing search for adjunctive biomarkers. Retrospective studies have suggested that an isoform of proenzyme prostate specific antigen called [-2]proenzyme prostate specific antigen may enhance the specificity of prostate specific antigen based screening. We examined the usefulness of this isoform in a prospective prostate cancer screening study.

Materials and Methods: From a population of 2,034 men undergoing prostate cancer screening we examined the relationship between the measurement of the [-2]isoform of proenzyme prostate specific antigen (p2PSA) and prostate cancer detection. Specifically we compared the usefulness of total prostate specific antigen, the ratio of free-to-total prostate specific antigen, the ratio of p2PSA-to-free prostate specific antigen, and a formula combining prostate specific antigen, free prostate specific antigen and p2PSA (the Beckman Coulter prostate health index or phi®) to predict prostate cancer in men from the study undergoing prostate biopsy with a prostate specific antigen of 2.5 to 10 ng/ml and nonsuspicious digital rectal examination.

Results: Despite similar total prostate specific antigen ($p = 0.88$), percent free prostate specific antigen ($p = 0.02$) and %p2PSA ($p = 0.0006$) distinguished between positive and negative biopsy results. On ROC analysis %p2PSA (AUC 0.76) outperformed prostate specific antigen (AUC 0.50) and percent free prostate specific antigen (AUC 0.68) for differentiating between prostate cancer and benign disease. Setting the sensitivity at 88.5%, p2PSA led to a substantial improvement in specificity as well as positive and negative predictive values. The Beckman Coulter prostate health index (AUC 0.77) had the best overall performance characteristics.

Conclusions: This is the first prospective study to our knowledge to demonstrate that p2PSA provides improved discrimination between prostate cancer and benign disease in screened men with a prostate specific antigen of 2.5 to 10 ng/ml and a negative digital rectal examination.

Key Words: prostate-specific antigen, protein isoforms, early detection of cancer

Abbreviations and Acronyms

BPH = benign prostatic hyperplasia
DRE = digital rectal examination
fPSA = free prostate specific antigen
%fPSA = ratio of free-to-total prostate specific antigen × 100
p2PSA = assay that measures [-2]proenzyme prostate specific antigen concentration in serum
%p2PSA = ratio of p2PSA-to-free prostate specific antigen × 100
proPSA = proenzyme prostate specific antigen
[-2]proPSA = isoform of proenzyme prostate specific antigen
PSA = prostate specific antigen

PROSTATE specific antigen is the most widely used serum marker for the early detection of prostate cancer, and its introduction in clinical practice has revolutionized contemporary management of this disease.[1] However, since the se-

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rum PSA concentration is often increased in benign conditions such as BPH and prostatitis, the test lacks specificity, especially at lower concentrations.[2] Accordingly only 20% to 30% of men with a serum PSA of 2 to 4 ng/ml and 30% to 45% with a serum PSA of 4 to 10 ng/ml have prostate cancer diagnosed on prostate needle biopsy.[3–5] To address these limitations of PSA, adjunctive measurements including the ratio of free-to-total PSA (%fPSA) have been investigated and have been shown to significantly improve cancer detection rates in the 4 to 10 ng/ml range.[6–9]

More recently distinct molecular forms of fPSA have been characterized and found to be differentially associated with BPH or prostate cancer.[10,11] These precursor forms of PSA are enzymatically inactive, and include 1) proPSA, which is increased in cancer tissue[12] and serum,[10,13,14] as well as 2) benign PSA and 3) intact PSA, which are associated with BPH.[15,16] Several isoforms of proPSA exist and are named based on the length of the pro-leader peptide. The 7 amino acid pro-leader peptide form, [-7]proPSA, is cleaved by human kallikrein 2 and trypsin to yield active PSA. Truncated forms containing leader sequences of 5, 4 or 2 amino acids are also present and can be measured in serum using immunoassays. These highly specific immunoassays have been tested clinically.[13–15,17,18] The [-2]proPSA (p2PSA) has emerged as a promising marker for prostate cancer detection because it is preferentially concentrated in cancerous tissue on histochemical staining and, compared to other isoforms, it demonstrates superior accuracy in the detection of prostate cancer.[19] (The analyte is referred to as [-2]proPSA and the assay is referred to as p2PSA. For simplicity we use p2PSA throughout the remainder of this article.[20])

In men with PSA between 2 and 10 ng/ml we previously demonstrated in large retrospective studies that the ratios of total proPSA and p2PSA to fPSA (%proPSA and %p2PSA, respectively) were more cancer specific than combinations of total PSA and fPSA.[20,21] Other research groups have similarly reported improved accuracy for cancer detection using p2PSA compared to free and total PSA.[15,17,18] Moreover, subsequent studies have demonstrated a correlation between p2PSA levels with clinically significant cancer including more advanced pathological stage, higher tumor volume and higher tumor grade.[21] In addition, p2PSA has been shown to improve discrimination in specific patient populations with particular diagnostic uncertainty such as those with %fPSA greater than 25 and a total PSA of 2 to 4 ng/ml.[13]

However, all of these studies were retrospectively performed using archived serum samples and were limited by selection bias. In addition, more recently Beckman Coulter, Inc. developed a mathematical formula combining total PSA, fPSA and p2PSA called the Beckman Coulter prostate health index, or phi, that showed encouraging results in preclinical studies but requires clinical validation. Therefore, we performed the first prospective study of p2PSA and Beckman Coulter phi for prostate cancer screening in men undergoing prostate biopsy for a total PSA from 2.5 to 10 ng/ml with benign findings on DRE.

MATERIALS AND METHODS

To evaluate the performance characteristics of p2PSA in real-world conditions we conducted a prospective screening study of 2,034 men in Chicago, Illinois. During 1 week in April 2007 men of all ages with no prior history of prostate cancer were offered screening. Each visit began with a focused medical history, after which serum samples were obtained by venipuncture for PSA, fPSA and p2PSA testing. Lastly DRE was performed by a urology resident or faculty urologist. Because of the grassroots nature of the screening study our population was heterogeneous in that a variable proportion of the population had prior PSA values available for comparison or had undergone previous prostate biopsy. Informed consent was obtained under institutional review board approved, and Health Insurance Portability and Accountability Act compliant protocols.

Our study protocol recommended biopsy for a serum PSA of 2.5 ng/ml or greater, or any abnormality suspicious for cancer (ie induration, nodule or irregularity) on DRE. For the purposes of this analysis we focused on men with a PSA between 2.5 and 10 ng/ml, and a nonsuspicious DRE. Although biopsies were performed at the institution selected by the patient, all participants were contacted for up to 2 years after a biopsy recommendation to follow up on the biopsy results and any subsequent treatment received.

Specimens were analyzed in blinded fashion for PSA, fPSA and p2PSA concentrations on a Beckman Coulter ACCESS® 2 immunoassay system, which involved dual monoclonal immunoenzymatic, sandwich, paramagnetic particle, chemiluminescent automated Hybritech® assays. Beckman Coulter phi was calculated for each patient as phi = (p2PSA/fPSA) × sqrt(PSA). We performed ROC analysis, and compared the sensitivity, specificity, and positive and negative predictive values of total PSA, %fPSA, %p2PSA and phi for cancer detection. All statistical analyses were performed using SAS®, version 9.2.

RESULTS

In April 2007 a total of 2,034 men participated in the screening study. The demographic features of the population are listed in table 1. Median patient age was 57 years with a median PSA of 1.05 ng/ml and most men were white. Of the men screened 322 were recommended to undergo biopsy for PSA 2.5 ng/ml or greater, or abnormality on DRE. Those men recom-