Assay Standardization Bias: Different Prostate Cancer Detection Rates and Clinical Outcomes Resulting from Different Assays for Free and Total Prostate-Specific Antigen

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OBJECTIVES Numerous commercial assays are available for measuring total and free prostate-specific antigen (PSA) levels in serum. These assays can be referenced to different laboratory standards, and interassay variability occurs. Patients and physicians might be affected by the variability between PSA assays that results from the use of different PSA standards.

METHODS We prospectively compared the free and total PSA measurements obtained using two commercially available PSA assays in 103 participants from a prostate cancer screening program in Caracas, Venezuela. We recommended biopsy to men with a total PSA level of 3 to 10 ng/mL and a free/total PSA ratio of 20% or less with either assay. We compared the sensitivity, specificity, and concordance index between the two assays to assess the effects of interassay variability on the cancer detection rate and clinical outcomes.

RESULTS Although the total PSA results were similar between the assays, the free PSA level was significantly greater with one assay. Therefore, the free/total PSA ratio was discordant between the two assays, resulting in different biopsy recommendations and cancer detection rates.

CONCLUSIONS Using a free/total PSA ratio of 20% or less as the threshold for biopsy, the differences in assay sensitivity and specificity for detecting prostate cancer are significant. Commercially available assays for PSA and its derivatives are not necessarily interchangeable, and these differences might lead to different clinical outcomes. When using free and total PSA measurements to make clinical decisions, patients and physicians should be aware of the potential standardization bias and which assay is being used. UROLOGY 69: 1143–1146, 2007. © 2007 Elsevier Inc.

Many different assays are commercially available for the measurement of free and total prostate-specific antigen (PSA) in serum. Most of these assays are not interchangeable. Conflicting evidence has been published concerning the degree of interassay variability. Some reports comparing two or more assays showed no significant differences. For example, Leewansangtong *et al.*[1] found that total PSA levels of 0 to 2.49, 2.5 to 3.99, 4.0 to 9.99, and 10 to 25 ng/mL were similar using the Tandem-R and AxSYM assays. Roehrborn *et al.*[2] similarly found no significant difference in total PSA levels or sensitivity for prostate cancer detection among the Hybritech Tandem-E, Abbott IMx, and Tosoh AIA-600 assays. However, other studies have shown considerable differences.

Nixon *et al.*[3] compared the free PSA levels measured with three different assays (Hybritech, Dianon, and Chiron) and concluded that they were not interchangeable. In participants from a screening program, Link *et al.*[4] found that the total PSA level was significantly greater using the Hybritech Access assay than using the Centaur assay. Recently, Stephan *et al.*[5] compared the results of five commercially available assays (AxSYM, Access, Immulite, Elecsys, and Centaur) in the archived serum samples of 282 men with negative prostate biopsy findings and 314 men with prostate cancer. They found

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considerable differences in the total and free PSA measurements among the assays.

Interassay variability owing to standardization bias could have important implications for clinical outcomes. In the modern PSA era of prostate cancer detection, the accuracy of the PSA measurement is critical. If different assays yield different PSA concentrations for the same serum sample, either a larger or smaller proportion of men might be advised to undergo prostate biopsy. This could have life-altering consequences for individual patients.

In this study, we prospectively measured the total and free PSA concentrations in serum using the AxSYM and Immulite assays in a cohort of Latin-American men undergoing screening for prostate cancer. Our screening protocol called for a prostate biopsy for men with a total PSA level of 3 to 10 ng/mL, only if the free/total (F/T) PSA ratio was 20% or less, which has been shown to significantly increase the specificity in this total PSA range.[6,7] We used the biopsy results to calculate the apparent sensitivity and specificity of the AxSYM and Immulite assays and to examine their concordance for prostate cancer detection.

MATERIAL AND METHODS

From July to December 2001, men from the metropolitan area of Caracas, Venezuela, were invited through brochures, written press releases, and radio announcements to participate in a prostate cancer screening study. The inclusion criteria were age 50 years old or older and a total PSA level within 3 to 10 ng/mL. The exclusion criteria were a previous diagnosis of prostate cancer, symptoms of urinary tract infection, and a history of abdominoperineal resection.

All participants provided informed consent. The total PSA level was measured on the day of collection using both assays: the AxSYM (Abbott Laboratories Northbrook, Ill) and Immulite (Diagnostic Products, Los Angeles, Calif). The specimens were immediately placed on ice because of the lability of free PSA, and a single lot of Immulite was used. For tests using the AxSYM assay, more than one lot of reagents was used.

AxSYM total PSA is referenced against the World Health Organization (WHO) reference material, and AxSYM free PSA is standardized to the Stanford 90:10 reference material. Immulite total PSA is standardized against the WHO National Institute for Biological Standards and Control First International Standard (NIBSC 1st IS) 96/670 and Immulite free PSA is standardized against WHO NIBSC 1st IS 96/688. Both of these assays have been independently demonstrated to have acceptable within-run and between-day precision.[7,8]

According to our study protocol, men with a F/T PSA ratio of 20% or less using either assay underwent 10-core prostate biopsy (including the standard sextant pattern and two additional cores on each side laterally directed in the anterior horns). A single pathologist reviewed all biopsy specimens.

All statistical analyses were performed using EPI 6.04 software (epidemiology, version 6.04, Centers for Disease Control, Atlanta, Ga). The mean values from both assays were compared using the Student's *t* test for paired specimens. The sensitivity and specificity of each assay were calculated, and their concordance for prostate cancer detection was determined by McNemar's test.

RESULTS

A total of 103 patients meeting the inclusion criteria were enrolled. Their mean age was 69 years (range 50 to 88). Their mean total PSA concentration was 5.30 ng/mL using the AxSYM assay and 5.42 ng/mL with the Immulite assay ($P = 0.64$). The mean free PSA level was significantly greater with the AxSYM assay than with the Immulite assay (1.12 versus 0.95 ng/mL, $P = 0.03$), as was the F/T PSA ratio (AxSYM 21.1% versus Immulite 17.9%, $P = 0.005$).

Using the AxSYM assay, 52 (51%) of 103 men had a F/T PSA ratio of 20% or less. In contrast, with the Immulite assay, 72 (70%) of 103 men had a F/T PSA ratio of 20% or less ($P < 0.001$). In 28 men, the F/T PSA ratio was greater than 20% with both assays, and in 49 men, the F/T PSA ratio was 20% or less with both assays. However, in 26 men, only one of the two assays would have led to a recommendation for biopsy. Overall, the concordance index was 0.74 between the AxSYM and Immulite assays for a F/T PSA ratio of 20% or less.

All men with a F/T PSA ratio 20% or less using either technique underwent a 10-core prostate needle biopsy, except for 2 men in the Immulite group who refused the biopsy. Using a F/T PSA ratio of 20% or less as the threshold to recommend biopsy, prostate cancer was detected in 10 (19%) of the 52 patients identified by the AxSYM assay. Of the 72 patients identified by the Immulite assay, prostate cancer was detected in 15 (21%).

Overall, 73 biopsies were performed to detect a total of 16 prostate cancer cases. Of 16, 10 (63%) would have been diagnosed using the AxSYM assay and 15 (94%) would have been diagnosed with the Immulite assay. Thus, using our biopsy protocol, the AxSYM assay had 63% sensitivity and 25% specificity for prostate cancer detection, and the Immulite assay had 94% sensitivity and 4% specificity, according to the biopsies performed and the cancers detected.

For 26 patients, the F/T PSA threshold was reached using only one of the two assays. Of these 26 patients, 24 underwent prostate biopsy, and cancer was detected in 7. Of the 7 cases, 6 were identified only by Immulite and only 1 was identified on the basis of the AxSYM assay alone. This difference approached statistical significance ($P = 0.058$, McNemar's test).

Receiver operating characteristic analysis was also performed to determine the F/T PSA threshold that would optimize the sensitivity and specificity for prostate cancer detection (Fig. 1). A greater overall area under the curve was found for Immulite (0.676) than for AxSYM (0.625). The corresponding F/T PSA thresholds were 16.3% and 12.6%.

COMMENT

With recent advances in immunoassay development, the number of total and free PSA assays available commercially has increased considerably. Although the concept of a single universal PSA standard was proposed more

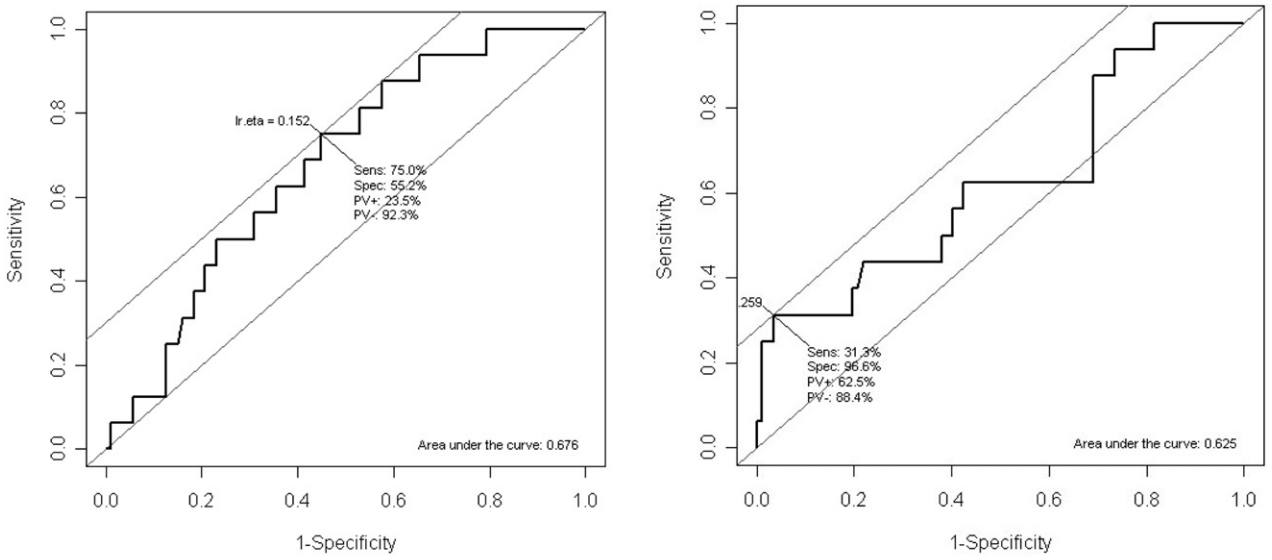

Figure 1. Receiver operating characteristic (ROC) analysis for prostate cancer detection using the **(A)** Immulite and **(B)** AxSYM assays.

than a decade ago,[9] to date no such standard has been accepted by the international medical community. Despite growing evidence that many of the commonly used PSA assays differ considerably from one another, many practitioners use the final measurement to guide clinical decisions without consideration of which assay was used to make the measurement.[4]

In screening protocols that use total PSA thresholds as an indication for biopsy, the mere use of discordant assays would change the number of men recommended for biopsy. This was demonstrated by Blijenberg *et al.*,[10] who estimated the effect of interassay variability on biopsy rates in 17,334 serum samples from the European Randomized Study of Screening for Prostate Cancer. Using a total PSA level of 3 ng/mL or more (as measured by the Tandem-E assay) as the cutoff for biopsy, they calculated the number of men who would no longer meet the biopsy threshold using a hypothetical "assay X." If the measurement using "assay X" differed from the Tandem-E level by 5%, 10%, or 20%, this would have altered the recommendation for biopsy in 155, 504, and 1133 men, respectively.

In our screening study, we recommended that men with a total PSA level of 3 to 10 ng/mL undergo biopsy only if the F/T PSA ratio was 20% or less. Thus, similar to the findings of Blijenberg *et al.*,[10] we examined the effect that interassay variability would have on medical decision-making in protocols involving the total PSA level and the F/T PSA ratio. Specifically, we compared the AxSYM and Immulite assays in participants from a city-wide PSA screening campaign in Caracas, Venezuela.

We found a significant difference between the two assays in free PSA and, as a result, in the F/T PSA ratio, that was the indication for prostate biopsy in our study ($P < 0.001$). The mean free PSA result was 9.09% greater with the AxSYM assay than with the Immulite assay. As a result, a considerable discrepancy was found in the biopsy recommendation rate and, consequently, the cancer detection rate, depending on which assay was used. Differences in standardization, nonequimolarity, and nonlinearity could all factor into the disparity between the free PSA assay results.

A screening test with the maximal sensitivity would be positive in all patients who have the disease for which the screening was being performed. To reach 100% sensitivity, the F/T PSA threshold would have to be set at 23% or less for the Immulite assay and 29% or less for the AxSYM assay. However, the ultimate choice of a threshold level depends on a tradeoff between a decrease in cancer detection and an increase in the number of unnecessary biopsies performed. On receiver operating characteristic analysis, the threshold that optimized sensitivity and specificity was 16.3% for the Immulite and 12.6% for the AxSYM assay. Rather than recommending a specific threshold value, our goal was merely to illustrate how any given cutoff can have very different clinical implications, depending on which assay was used.

Our results could have additional implications that warrant further evaluation. For example, in our study, the total PSA level was 1.38% greater with the Immulite assay than with the AxSYM assay, although this difference did not reach statistical significance. We suspect that the similar total PSA values obtained resulted because both total PSA assays are standardized to the WHO reference materials. Substituting another assay that was instead standardized to a different reference standard would likely result in greater disparity. That notwithstanding, the PSA velocity is often used to help distinguish between PSA elevations resulting from prostate

cancer and those resulting from benign conditions.[11,12] If the total PSA level was measured 1 year using AxSYM and the following year with Immulite, the mere differences in assay results could confound the use of PSA kinetics to recommend biopsy.

One limitation of our study was that we do not have long-term follow-up data for the study population. Future prospective research is needed to determine whether such assay differences will affect the ultimate clinical outcomes.

CONCLUSIONS

Both the free PSA levels and F/T PSA ratios were significantly different using two commercially available PSA assays, resulting in a discrepancy in biopsy recommendations and cancer detection rates. Overall, one assay for free PSA was less sensitive but more specific than the other for prostate cancer detection. We believe that these assays are not interchangeable and that caution should be exercised when comparing results from different commercial assays. Patients and physicians should be aware of which assay was used each time a PSA measurement is performed, and an effort should be made to use the same assay at the next screening visit. In addition, studies of PSA kinetics over time using different assays should be interpreted with caution.

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