COMPARABILITY OF THE TANDEM-R AND IMx ASSAYS FOR THE MEASUREMENT OF SERUM PROSTATE-SPECIFIC ANTIGEN*

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ABSTRACT—**Objectives.** To assess the comparability of the Tandem-R and IMx serum prostate-specific antigen (PSA) assays across levels of the ratio of free-to-total serum PSA found in a community-based population of healthy men.

Methods. Banked serum samples from the baseline component of the Olmsted County Study of Urinary Symptoms and Health Status Among Men were thawed and analyzed using the Tandem-R and IMx PSA assays. Serum levels also were determined for the free, noncomplexed form of PSA, PSA complexed to alpha-1 antichymotrypsin, and total PSA with a research-based immunofluorometric assay.

Results. The results of the Tandem-R and IMx assays were strongly correlated at all levels of the ratio of free-to-total serum PSA. The Spearman correlation coefficients ranged from 0.87 to 0.98 (all $p < 0.001$). The relationship between the Tandem-R and IMx assays, however, differed at low levels of free-to-total serum PSA compared with high levels. In the lowest 10th percentile of the ratio of free-to-total serum PSA (0.04 to 0.18), the IMx assay read lower than the Tandem-R (slope $\pm$ standard error = 0.92 ± 0.04, intercept $\pm$ standard error = 0.21 ± 0.14); whereas in the upper 10th percentile of free-to-total ratio (0.46 to 0.65) the IMx assay yielded values higher than the Tandem-R assay (slope = 1.21 ± 0.07, intercept = 0.14 ± 0.05). In the middle 90%, the slope did not statistically differ from 1.0.

Conclusions. For the majority of men, results of the Tandem-R and IMx PSA assays were virtually identical. The small differences found would not be of clinical significance for most men but should be considered when comparing results of different assays in sequential determinations for a specific man.

Prostate-specific antigen (PSA) is a 33-kDa serine protease produced by the epithelium of the prostate gland.[1] Serum levels of PSA have been noted to be elevated in men with an enlarged prostate[2-4] and in men harboring prostate cancer.[2,5] In men with enlarged prostates, elevated PSA levels have been assumed to be due to increased epithelial mass. Local tissue destruction and concomitant leakage of PSA into the systemic circulation are also thought to contribute to elevated levels of PSA in men with cancer.[6] The measurement of serum levels has

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become extremely widespread, since serum PSA has been proven valuable in monitoring the response of prostate cancer to therapy.[7-11] Serum PSA determinations have also been used for case finding and diagnosis,[12-14] and some investigators have even advocated the use of PSA for screening for prostatic cancer.[15-18] This view, however, is not universally held.[19,20]

The measurement of PSA concentration in the serum is not as straightforward as for many other substances. In the peripheral circulation, PSA exists primarily in two forms: as a free, noncomplexed form and complexed to alpha-1 antichymotrypsin. It has also been suggested that PSA may exist in a form complexed to alpha-2 macroglobulin.[21,22] The free form of PSA and PSA complexed to alpha-1 antichymotrypsin are both measurable by various commercial and research-based assays. The PSA complexed to alpha-2 macroglobulin is not immunodetected by these assays.[21-25] Some of the commercially available assays have reported different sensitivities to the different forms of PSA in the peripheral circulation, which has caused some investigators to suggest that the two most widely used assays (Tandem-R and IMx) may not be comparable.[26,27] In particular, it has been suggested that differences between the two assays vary greatly among samples with disparate relative concentrations of free PSA. Because the signal from free PSA is a major determinant of PSA level with the IMx assay,[25] the IMx could report relatively higher values for total PSA levels as the ratio of free to complexed PSA increases. The few data available to assess the magnitude of the potential bias in total PSA determination are limited. Some have been derived from select samples of patients[26] or based on in vitro experiments performed at PSA concentrations 50 to 100 times greater than physiologic levels.[27]

In this report, we present the results of multiple serum PSA determinations on samples from a community-based cohort of men free of prostate cancer. Banked serum samples were used to determine PSA levels using the Tandem-R and IMx assays, and the concentration of total PSA (free and complexed) and selective measurement of free PSA were determined by research-based immunofluorometric assays (IFMA). These determinations were used to provide greater insight into the comparability of the Tandem-R and IMx assays, especially with regard to the ratio of free to complexed PSA.

MATERIAL AND METHODS

Study Subjects

Many of the details of the design and implementation of this study have been described in detail elsewhere.[3,28-30] The resources of the Rochester Epidemiology Project[31] were used to identify an age- and residence-stratified random sample of 5135 men aged 40 to 79 years from the Olmsted County, Minnesota population. Men with a documented history of prostate cancer, prostatectomy, or specified conditions (other than benign prostatic hyperplasia [BPH]) that would interfere with voiding function were excluded from the study (n = 1261). The remaining 3874 (75%) were invited to participate in a prospective cohort study of the natural history of prostatism. Of those identified, 2115 (55%) completed a previously validated questionnaire that assessed urologic and general health. From these men, a random sample of 537 (25%) was invited to the Mayo Clinic for a detailed clinical examination that included obtaining a serum specimen for PSA determination, digital rectal examination, and ultrasonographic examination of the prostate. All 3 diagnostic tests were completed by 475 men (88%). The results prompted 52 men (11%) to undergo an ultrasound-guided biopsy of the prostate, and prostate cancer was found in 4 (8%) of these men. These 4 were excluded from subsequent analyses.

PSA Determinations

Prior to the clinical examination, study subjects were phlebotomized at a central clinic facility in the morning hours in a nonfasting state. Approximately 15 mL of venous blood were obtained from each subject and divided into 4 aliquots of 3 to 4 mL each. Each aliquot was labeled and logged, and all but 1 placed in frozen storage at –70°C. The unfrozen aliquot was processed immediately in a central laboratory to determine the serum PSA concentration with the Tandem-R PSA assay (Hybritech Inc., San Diego, CA). The results of this determination have been published previously.[3]

In September 1993, 1 aliquot of serum was withdrawn from frozen storage for 395 (84%) of the 471 men to determine serum PSA levels with several assays. The frozen samples were transported on dry ice to Turku, Finland, where samples were thawed. There the total and free serum PSA concentrations were determined by research-based immunofluorometric assays.[21,32-34] Total serum PSA concentrations were determined with a monoclonal-monoclonal assay similar to the previously described assay.[21] The H117 anti-PSA monoclonal antibody was used as the capture antibody and europium-labeled 2H50 anti-PSA as the detection antibody. The H117 and H50 antibodies detect different epitopes available on the noncomplexed PSA molecule as well as PSA complexed to serine

TABLE I. *Distribution of serum PSA determinations and differences: clinic cohort Olmsted County study of urinary symptoms and health status among men*

	Median	Q_1	Q_3	Min	Max
Age (yr)	54	47	64	40	79
PSA (ng/mL)					
Tandem-R	0.9	0.5	1.6	0.1	9.7
IMx	1.0	0.7	1.7	0.2	11.3
Difference*	0.13†	0.02	0.26	−2.0	2.4
Free/total ratio‡	0.29	0.23	0.37	0.04	0.65

KEY: Q_1, 25th percentile; Q_3, 75th percentile.
**Determination by IMx − determination by Tandem-R.*
†$p < 0.001$, sign rank test.
‡Ratio of free PSA to total PSA by research-based immunofluorometric analysis.

protease inhibitors such as alpha-1 antichymotrypsin. This assay provides an equimolar detection of free, noncomplexed PSA and PSA complexed to alpha-1 antichymotrypsin. The concentration of free PSA was selectively determined with a monoclonal-monoclonal assay similar to the previously described assay,[21] except that H117 anti-PSA monoclonal antibody was used as the capture antibody and 5A10 as the detection antibody. The 5A10 antibody is specific to the free, noncomplexed form of PSA.[21]

The remaining serum was refrozen and transported on dry ice to Eastern Virginia Medical School, where the serum PSA concentration was determined using both the Tandem-R and IMx (Abbott Laboratories, North Chicago, IL) PSA assays.[35] The Tandem-R assay is a dual monoclonal immunoradiometric assay. The IMx PSA assay, however, is a polyclonal-monoclonal microparticle capture enzyme immunoassay formatted for the IMx system. Serum PSA determinations were made according to manufacturer's instructions with standard reagents and methods of quality control. For this report, analyses are restricted to the more recent determinations from frozen specimens.

Statistical Analyses

The distributions of serum PSA determinations by Tandem-R, IMx, and the ratio of free-to-total PSA by immunofluorometric analysis were described in terms of the median and 25th and 75th percentile. Systematic differences between the Tandem-R and IMx assay were tested with the sign rank test. Graphic techniques were used to examine the relationship between the difference between the two assays and PSA level and ratio of free-to-total PSA and total PSA determined by the IFMA.[36,37] The association was quantified with the Spearman rank correlation coefficient. Linear least-squares regression analyses were performed within strata defined by the 10th, 25th, 50th, 75th, and 90th percentiles of the distribution of the ratio of free-to-total PSA. Regression models were constructed with the IMx result as the dependent variable and Tandem-R result as the independent variable. Estimates of the slope and intercept for these regression models were used to describe the functional relationship between the two assays. If the Tandem-R and IMx assays were in complete agreement, the estimated slope from the regression model would be 1.0 and the intercept would be equal to 0. When the 95% confidence interval about the regression coefficient excludes the null hypothesis (1 for slope and 0 for intercept), the coefficient is significantly different from the null value ($p < 0.05$). All analyses were performed using SAS Statistical Software (SAS Institute, Cary, NC).

RESULTS

The distributions of serum PSA determinations are presented in Table I. For this community-based sample of men, the distribution of PSA level, whether determined by Tandem-R or IMx assay, is strikingly similar. The median difference between assays was 0.13 ng/mL, suggesting that, on average, the IMx assay determinations were slightly higher than Tandem-R ($p < 0.001$, sign rank test). Both the IMx and Tandem-R assays yielded comparable results with the IFMA (Fig. 1). Overall, the specificity for the IMx, Tandem-R, and IFMA assays were 93.8%, 93.8%, and 94.9%, respectively, with the cutpoint set at 4.0 ng/mL. However, the specificity decreased with increasing age. With the age-specific reference ranges,[3] the corresponding specificities were 95.6%, 95.9%, and 96.7%. The ratio of free-to-total PSA ranged from 0.04 to 0.65 with a median of 0.29. If the cutpoint of abnormality for free-to-total ratio was set at 0.18, 91.7% of the sample would be labeled normal (specificity) and 8.3% labeled abnormal.

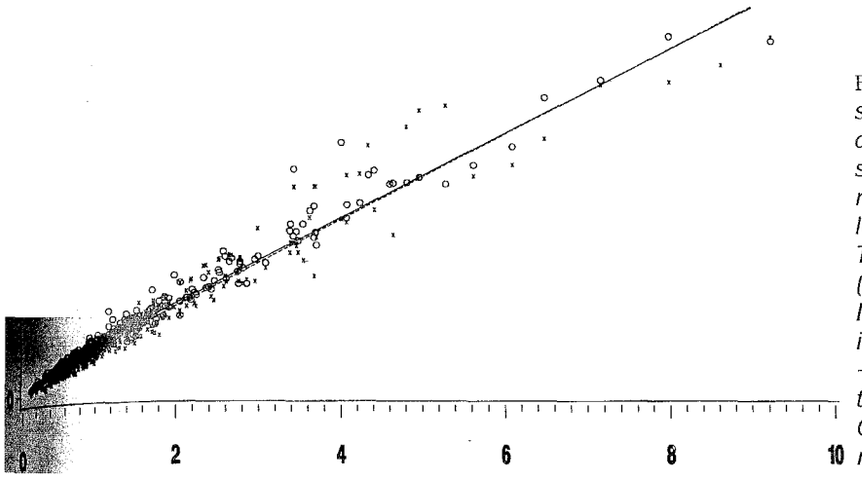

FIGURE 1. *Scatterplot of total serum PSA determination by Tandem-R (x) and IMx (o) by research-based immunofluorometric analysis. Lines represent least-squares regression line of Tandem-R (dashed line) and IMx (solid line) determination by IFMA. The correlation, slope, and intercept were 0.98, 1.07, and −0.04 for Tandem-R, respectively; for IMx 0.99, 1.10, and 0.06, respectively (adapted from ref. 30).*

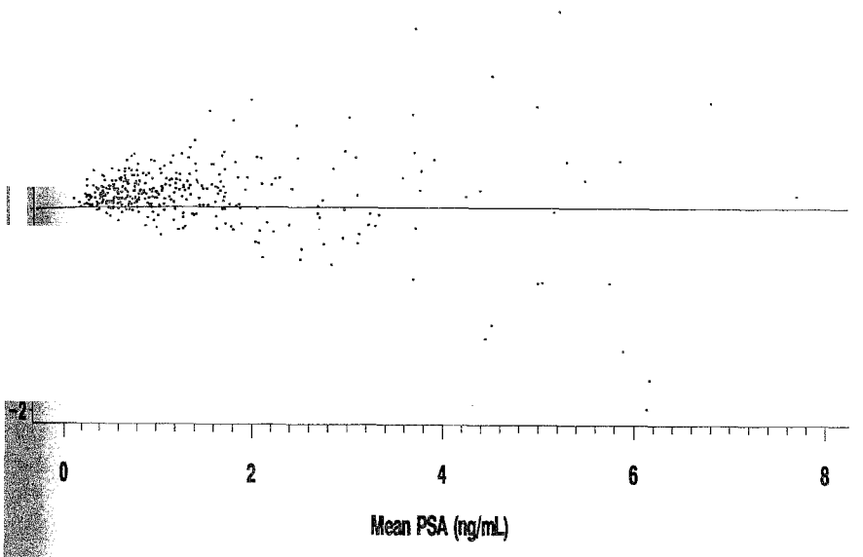

FIGURE 2. *Scatterplot of difference in determination of serum PSA concentration by IMx and Tandem-R assay (IMx minus Tandem-R) by averaged determination. Points above line indicate samples in which IMx determinations were higher than Tandem-R determinations. Spearman correlation coefficient = 0.03, p = 0.61.*

The difference between serum PSA determination by IMx and Tandem-R is plotted against the average of the 2 determinations in Figure 2. Although the majority of points were above 0, there did not appear to be a systematic upward or downward bias with increasing PSA levels ($r_s = 0.03$, $p = 0.61$). In contrast, serum PSA levels, whether determined by Tandem-R or IMx, tended to be higher in samples with lower ratios of free-to-total PSA (Fig. 3) and lower at higher levels of the ratio ($r_s = -0.49$ and −0.42, respectively, both $p < 0.001$). When the difference between the 2 determinations was plotted against the ratio of free-to-total PSA (Fig. 4), however, there was an indication of a slight increase in bias. At low levels of the free-to-total ratio, the IMx determinations were slightly lower than Tandem-R; at higher ratios of free-to-total PSA, determinations by IMx were slightly higher than by Tandem-R ($r_s = 0.20$, $p < 0.001$).

The stratified regression analyses provide further insight into the relationship between Tandem-R and IMx assays at different levels of free-to-total PSA ratios (Table II). In the groups with cutpoints determined by the 10th, 25th, 50th, 75th, and 90th percentiles, the estimates of slope in the regression of IMx determination on Tandem-R determination were significantly different than 1.0 in only the upper and lower 10th percentiles. Furthermore, the estimates of intercept were significantly different than 0 in only the higher levels of free-to-total ratio. This suggests that for the majority of men the difference between IMx and Tandem-R determinations is fairly constant, with only a slight upward shift in determination by IMx assay.

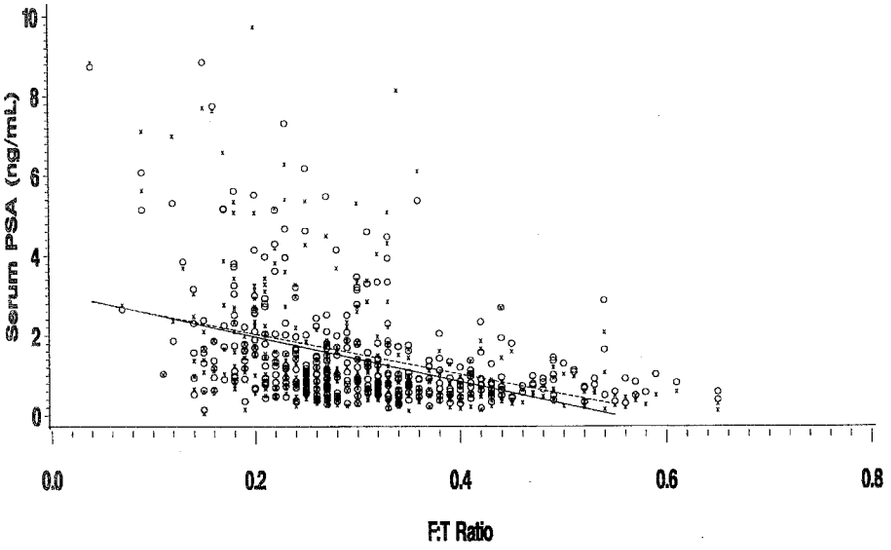

FIGURE 3. *Scatterplot of total serum PSA determination by Tandem-R (x) and IMx (o) by ratio of free/total PSA. Lines represent least-squares regression line of Tandem-R (solid line) and IMx (dashed line) determination on free/total ratio. The correlation, slope, and intercept were −0.41, −5.61, and 3.10 for Tandem-R, respectively; for IMx −0.36, −5.02, and 3.05, respectively.*

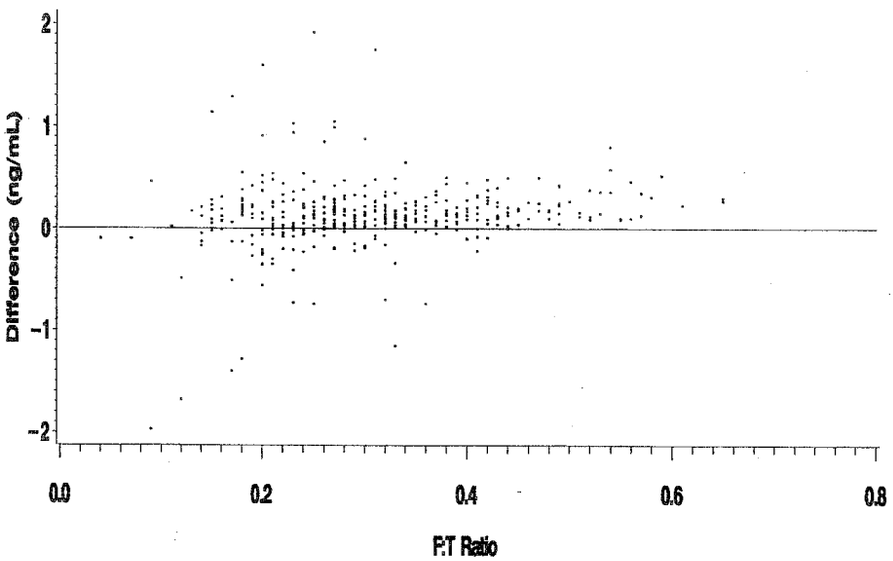

FIGURE 4. *Scatterplot of difference in determination of serum PSA concentration by IMx and Tandem-R assays (IMx minus Tandem-R) by ratio of free/total PSA determined by research-based immunofluorometric analysis. Points above line indicate samples in which IMx determinations were higher than Tandem-R determinations. Spearman correlation coefficient = 0.20; $p < 0.001$.*

TABLE II. *Relationship between Tandem-R and IMx serum PSA assay results, by ratio of free/total PSA: Olmsted County study of urinary symptoms and health status among men*

			Regression of IMx on Tandem R*			
			Slope		Intercept	
F/T ratio†	n	r_s‡	est§	95% CI‖	est§	95% CI‖
0.04–0.18	43	0.98	0.92	0.86, 1.00	0.21	−0.07, 0.49
0.19–0.23	61	0.97	1.06	1.00, 1.12	−0.02	−0.17, 0.13
0.24–0.29	88	0.96	1.06	0.99, 1.13	0.08	−0.02, 0.18
0.30–0.37	99	0.97	1.04	0.98, 1.10	0.11	0.01, 0.21
0.38–0.45	56	0.92	1.02	0.93, 1.11	0.15	0.08, 0.22
0.46–0.65	37	0.87	1.21	1.08, 1.34	0.14	0.05, 0.23

**Least-squares regression analysis of IMx PSA determination on Tandem-R determination.*
†F/T ratio; ratio of free/total serum PSA by immunofluorometric analysis. Cutpoints determined by 10th, 25th, 50th, 75th, and 90th percentiles.
‡Spearman correlation coefficient (all $p < 0.001$).
§Point estimate.
‖95% confidence interval for estimate.

COMMENT

The results of this study provide important information on the comparability of the Tandem-R and IMx PSA assays. This is timely and important given the widespread determination of PSA levels with different assays, each with different sensitivity to various biochemical forms of PSA in the serum.[25] We found that despite the different antibodies used in the two assays, it is only at the extremes of free-to-total PSA ratios that the difference between the Tandem-R and IMx assays changes with PSA level. At low free-to-total ratios, the IMx assay reads lower than the Tandem-R assay, and this decrement is greater at higher PSA levels. In contrast, at higher free-to-total ratios the IMx assay provides higher readings than the Tandem-R assay, and the difference is greater at higher PSA levels. These differences are likely to be due to the different combinations of antibodies used in the various assays, the use of monoclonal versus polyclonal antibodies, different laboratory methodologies, and the relatively greater signal from the free form of PSA in the IMx assay.[25] However, it must be remembered that the higher free-to-total PSA ratios tend to be found in men with lower total PSA values.[38] Thus the net effect is that the combination of upward bias in the higher free-to-total ratios is counterbalanced by the overall generally lower total serum PSA levels in this group. For example, the median PSA level for men in the upper tenth percentile of free-to-total ratio as determined by Tandem-R is 0.51 ng/mL. Based on the regression analyses, the expected IMx value would be 0.76 ng/mL. In the second quartile of free-to-total ratio, the median PSA level is 0.92 ng/mL, and the expected IMx level would be 1.05 ng/mL. These differences would not be clinically significant.

In interpreting these results, several potential limitations must be kept in mind. First, the PSA values from this community-based sample of men without prostate cancer were concentrated at the lower end of the spectrum; there are relatively few men with serum PSA determinations at higher levels. However, it is at these levels where the important clinical decisions are being made: whether or not to undergo further invasive and minimally invasive diagnostic tests. Our results suggest that the Tandem-R and IMx assays are probably comparable for this purpose. A second potential limitation is in the generalizability of the findings. The exclusion criteria for assembling the sample, the 55% response rate, and exclusively white composition may make generalizability uncertain. Although the analysis of frozen banked samples may not represent normal clinical practice, both short-term and long-term comparisons of PSA determinations on frozen serum samples suggest that PSA is stable when handled properly, as in this study.[30]

In summary, these data provide important reassurances about the comparability of the Tandem-R and IMx assays for serum PSA levels. For most men, they provide nearly identical levels. There are situations, however, in which they are not quite so close, especially at extremes of the ratios of free-to-total serum PSA. This must be kept in mind when assessing men thought to have BPH in whom the free-to-total ratio may be elevated.[38] In these men, the IMx assay may provide slightly higher determinations than the Tandem-R, which might yield slightly more false-positive results for prostate cancer. In men with prostate cancer (in whom the ratio may be relatively lower), the IMx may provide determinations slightly lower than the Tandem-R, potentially resulting in slightly more false-negative results for prostate cancer. Most important, however, the small differences should be accounted for in sequential determinations made in a given patient with the two assays. At this time, however, it is not apparent which of these assays may provide the most useful, predictive information in the management of prostate disease. Further study will be required to provide a better understanding of the role that determination of serum levels of PSA, measured in the free, complexed, or total form can play.

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