Total Prostate Specific Antigen Stability Confirmed After Long-Term Storage of Serum at −80C

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Purpose: We characterized the long-term stability of total prostate specific antigen in serum samples after storage at −80C from 2001 until 2007.

Materials and Methods: From the San Antonio Biomarkers of Risk biorepository we chose serum samples from white men 55.2 to 80.5 years old (median age 63.3) in which prostate specific antigen was measured in 2001. These men were not diagnosed with prostate cancer by 2007. A blocked randomization scheme was used to randomly select 47 serum samples with prostate specific antigen values spread over the reference ranges 0.0 to 0.4 (10), 0.5 to 0.9 (10), 1.0 to 1.9 (10), 2.0 to 3.9 (11) and 4.0 to 10.0 ng/ml (6) for repeat measurement by the same assay in 2007. Spearman's correlation coefficient was used to calculate the correlation and the paired t test was used to test the null hypothesis of no average difference between prostate specific antigen measured in 2001 and 2007.

Results: Median prostate specific antigen values were 1.20 ng/ml (range 0.20 to 7.20) in 2001 and 1.30 (range 0.20 to 6.70) in 2007. In 2001 and 2007 mean ± SD prostate specific antigen was 1.76 ± 1.59 and 1.84 ± 1.64 ng/ml, and the coefficient of variation was 0.91 and 0.89, respectively. The correlation between prostate specific antigen values in 2001 and 2007 was high (0.995). Prostate specific antigen values in 2007 were consistently and statistically significantly higher than in 2001 (mean 0.08 ng/ml, $p = 0.005$). Systematic and random error increased slightly with increasing prostate specific antigen.

Conclusions: Agreement between total prostate specific antigen values measured from serum samples in 2001, stored at −80C for 7 years and then remeasured was highly correlative.

Key Words: prostate, prostate-specific antigen, freezing, serum

Prostate specific antigen is a 35 kDa single chain serine protease of the kallikrein family.[1] A 237 amino acid glycoprotein, PSA circulates in several forms, including fPSA, an inactive precursor of PSA with additional amino acids (pro-PSA), PSA complexed to ACT and PSA complexed to α2-macroglobulin as well as other PSA complexes.[2,3] PSA was first described and purified in 1979 by Wang et al.[4] Shortly thereafter Papsidero et al identified PSA in the serum of patients with metastatic prostate cancer.[5] Since that time, PSA testing has evolved and been integrated into urological practice to identify prostate cancer while most curable, assess the stability of disease after diagnosis and help monitor the success of treatment for prostate cancer.[6]

Since the inception of PSA screening for prostate cancer in the 1980s, PSA has become the most widely used biomarker in the world. As our understanding of the clinical implications of PSA have increased, clinicians have attempted to create new ways to make PSA screening more clinically applicable to various patient groups. Information on factors that can affect the value of PSA is essential for research investigations and clinical evaluations. Many studies of the usefulness of PSA as a biomarker for prostate cancer are now retrospective and performed in clinical samples that have been stored frozen for years under varying storage conditions in biorepositories created for this purpose.

Sample handling and protein stability information are essential for the proper interpretation of clinical results. Sample handling and sample storage might influence the stability of PSA in serum.[7,8] Several investigators have reported the stability of tPSA in serum samples.[9–11] However, few groups have attempted to document the long-term stability of tPSA protein in serum after long-term (more than 2 years) storage. Such analysis is essential to validate any retrospective study using this biomarker. We characterized the long-term stability of tPSA in serum samples after storage at −80C from 2001 until 2007.

MATERIALS AND METHODS

Study subjects were obtained from SABOR, a Clinical and Epidemiologic Center of the Early Detection Research Network, National Cancer Institute. The institutional review

Submitted for publication December 11, 2007.

Study received approval from the institutional review board at University of Texas Health Sciences Center at San Antonio.

Supported by San Antonio Center of Biomarkers of Risk for Prostate Cancer U01 CA86402, San Antonio Cancer Institute P30 CA54174 and a University of Texas Health Science Center at San Antonio Institute for Integration of Medicine and Science Mentored Career Development Award (DJP).

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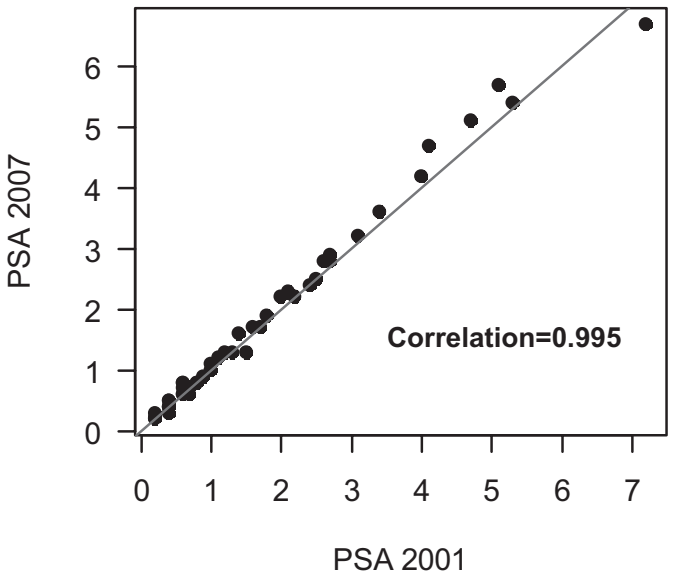

Correlation between 2001 and 2007 PSA values

board at University of Texas Health Sciences Center at San Antonio approved the SABOR study. SABOR is a 10-year prospective cohort study with a current enrollment of 3,716 men. Participation includes the collection of biological specimens for genetic, protein and trace mineral profiling, extensive demographic and dietary history, medical symptoms, including American Urological Association symptom scores, and information about prostate related medications and procedures. In addition, participants are queried annually about a family history of prostate cancer. Digital rectal examinations and PSA measurements are performed annually.

Inclusion criteria for this analysis included participants not diagnosed with prostate cancer as of 2007. Samples were first identified with PSA measured in 2001 as belonging to men who never underwent a positive prostate biopsy at the annual monitoring that occurred as part of SABOR in the next 6 years. Hence, on these grounds the men could be considered prostate cancer-free in 2001. To decrease confounding factors only samples from white men older than 55 years were considered. All men selected for inclusion had PSA measured in 2001 and serum available in the biorepository in 2007. A further subselection of 47 samples measured in 2001 covering a range of PSA values from low to high was chosen as the final set to be remeasured in 2007. PSA ranges were created, including 0 to 0.4, 0.5 to 0.9, 1.0 to 1.9, 2.0 to 3.9 and 4.0 to 10.0 ng/ml, and serum frozen since 2001 from 10, 10, 10, 1 and 6 participants, respectively, was randomly selected within these ranges for analyses, yielding 47 samples, for remeasuring PSA. Median age of the participants from whom samples were chosen was 63.3 years (range 55.2 to 80.5) in 2001.

The samples used to determine PSA stability were originally processed within 6 hours of collection. The samples were aliquoted and stored at −80C in cryotubes. Another aliquot was immediately processed for tPSA measurement. Samples that were reevaluated in 2007 had never been thawed during the storage period and they remained in cryotubes at −80C until repeat assay. The samples were thawed at 4C and re-assayed as a single batch to avoid interassay vs intra-assay variability.

For the 2007 PSA analysis the assay used was identical. The methodology is a 2 site sandwich immunoassay using direct chemoluminescence technology. The samples were assayed using a Centaur® Chemiluminometer with constant antibody concentrations with Advia Centaur PSA Reagents. The detection limits of the assay were 0.01 to 100 ng/ml. In 2007 the technician was blinded to the results of the first reading.

The mean, median, SD, range and CVs are reported separately for 2001 and 2007 PSA values. Spearman's correlation coefficient was used to calculate the correlation and the paired t test was used on logarithmically transformed PSA values to test the null hypothesis of no average difference between PSA values measured in 2001 and 2007. The systematic error, which assesses whether PSA values in 2007 were consistently higher or lower than those in 2001, was graphically shown by a scatterplot of 2001 vs 2007 values and estimated by the mean of the differences between 2007 and 2001 PSA values with random error estimated as the SD of these differences. These 3 parameters were also investigated by 2001 PSA ranges, including less than 1.0, between 1 and 2.0, and greater than 2.0 ng/ml.

RESULTS

There was a high correlation (0.995) between total serum PSA levels determined in 2001 and 2007 (see figure). Accordingly PSA summary statistics for the 2 years were also in agreement (table 1). The paired t test indicated that levels measured in 2007 were consistently and statistically significantly higher ($p = 0.005$), although the increase in 2007 (mean 0.08 ng/ml during 2001) was small in magnitude (table 2). The systematic error increased as the PSA level increased (see figure and table 2). However, this trend was expected since the variability of any marker, including PSA, increases with the magnitude of the marker. For the same reason the random error between the 2 measurements also increased with the PSA level (table 2).

DISCUSSION

These data must be interpreted by the clinician with caution. Although the differences from 2001 to 2007 attained statistical significance, we believe that they are of limited clinical significance to the practicing urologist. Review of the raw data showed that measured PSA values in the 2007 samples were higher but the magnitude of the difference was too small to have prompted any change in clinical decision making.

Until recently no published studies have systematically assessed the stability of PSA in frozen serum samples stored for prolonged periods. Several groups have investigated the

TABLE 1. *PSA in 2001 and 2007 in 47 subjects*

	2001	2007
Mean ± SD PSA (ng/ml)	1.76 ± 1.59	1.84 ± 1.64
Median ng/ml PSA (range)	1.20 (0.20–7.20)	1.30 (0.20–6.70)
CV*	0.91	0.89

* SD/mean.