Improving the Clinical Utility of the Serum PSA Test

The addition of assay information from biological factors, such as prostate volume and serum levels of a PSA precursor protein, significantly increases the sensitivity and specificity of serum PSA determinations

In 1986, the FDA cleared the use of serum PSA testing to monitor the progression of prostate cancer in men already diagnosed with the disease. Not long after, in 1994, the FDA cleared the PSA test for use in conjunction with the digital rectal exam (DRE) to screen asymptomatic men for prostate cancer.[1] What followed was an influx of PSA tests in the clinical diagnostics market, a dramatic surge in PSA testing, and an ensuing increase in the number of prostate cancer diagnoses. Along with the increased number of prostate cancer cases being detected came a wealth of new clinical data to be examined and re-examined in an effort to assess the clinical utility of the PSA test.

The surge in prostate cancer diagnoses was attributable to PSA tests detecting small tumors, most of which were confined to the prostate (local disease). Most of these tumors were asymptomatic, but they resulted in elevated serum PSA levels which prompted tissue biopsies to confirm the presence of cancer. Biopsy of the prostate is not a simple procedure, however, and is associated with a number of complications. Most notable of these are urinary incontinence, problems with bowel function, erectile dysfunction, and infection.[1,2]

Merely 25% of prostate biopsies are positive for cancer so the false-positive rate of the test is rather high. As such, a large number of unnecessary biopsies are performed each year, and the quality of life of a very large number of men is adversely affected.[3,4] The American Cancer Society estimates that approximately 238,590 new cases of prostate cancer will be diagnosed in the U.S. in 2013. This represents only 25% of the total number of biopsies performed, meaning approximately 715,770 men are likely to suffer unnecessarily the complications associated with prostate biopsy. This unfortunate situation has not gone unnoticed, as the U.S. Preventive Services Task Force recently recommended against the use of the PSA test as screening tool for the general male population.[2] (Of note, the recommendation does not apply to the use of the assay for patient follow-up after prostate cancer diagnosis or treatment.)

Of the biomarkers used at present to diagnose and/or monitor various types of cancer, PSA is largely considered to be the best. It does, however, have its shortcomings. Its high false-positive rate is due in part to several benign conditions that can elevate an individual's serum PSA level. The most common such afflictions are benign prostatic hyperplasia (BPH), prostatitis (inflammation due to prostate infection), and urinary tract infection. Serum PSA levels are also affected by various drugs, such as finasteride (Proscar/Propecia) and dutasteride (Avodart), which can cloud diagnostic and prognostic evaluations.[5]

Given the challenges with interpreting serum PSA levels, researchers have been looking for ways to enhance the PSA test in an effort to better stratify men who are at risk for developing prostate cancer. Individuals with PSA <4.0 ng/ml are generally considered at low risk for developing cancer, while those with PSA >10.0 ng/ml are considered at high risk. Men who fall in the intermediate range of PSA 4.1-10 ng/ml, however, are considered to be in a "gray zone" and are more difficult to classify.[6,7,8] To improve the diagnostic and prognostic proficiency of the PSA test, for men in the gray zone in particular, a number of biological parameters are under investigation. Those receiving much of the attention are PSA velocity, percentage of free PSA vs. complexed PSA, PSA precursor proteins (pro-PSA), and PSA density.

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PSA Velocity

PSA velocity (PSAV) is the rate of change of serum PSA over time, after at least three serial measurements. In general, increasing PSAV is thought to be indicative of prostate cancer rather than benign disease.[9,10] PSA levels fluctuate, however, due to the benign conditions referred to earlier, making it difficult to establish threshold values for PSAV that definitively distinguish prostate cancer from benign disease.[11,12,13] As such, PSAV as a screening tool is useful but limited.

Percentage of Free PSA to Complexed PSA

The percentage of free PSA to complexed PSA (Free/Total PSA) is determined by comparing the amount of serum PSA that is bound to a protease inhibitor, primarily α1-antichymotrypsin, with the amount of unbound, free PSA. Although not an absolute, it is generally accepted that individuals with prostate cancer produce a higher percentage of complexed PSA and a lower percentage of free PSA than do individuals with BPH, prostatitis, or urinary tract infections.[14,15] Due to this difference, the ratio of Free/Total PSA has been used for several years to increase the sensitivity of prostate cancer detection when serum PSA is in the normal range of

<4 ng/ml and to improve specificity when total PSA is in the gray zone of 4.1-10 ng/ml.[12,13,16,17]

In a landmark study, Catalona *et al.* found that men with PSA levels in the gray zone and with Free/Total PSA <10% had a 56% likelihood of having cancer. In contrast, men with PSA levels in the gray zone and Free/Total PSA >25% had only an 8% probability of having cancer.[15] As is the case with PSA, however, no single threshold value for Free/Total PSA can distinguish prostate cancer from benign disease with complete accuracy.[12,13] As such, the search continues for a better means of risk stratification.

pro-PSA

pro-PSA is a term used to describe the collection of inactive precursor proteins of PSA. Mature PSA is an enzymatically active 237-amino acid protein, but it is synthesized as a longer molecule, called pre-pro-PSA, which is cleaved to the inactive pro-PSA immediately after translation. There are several isoforms of pro-PSA, each designated by the number of amino acids it contains beyond the 237 of mature PSA.[18,19,20] The pro-PSA isoforms range from containing 2 to 7 additional amino acids, but it is the isoform with 2 extra amino acids, called

[-2]pro-PSA, that has received much attention of late as it has been shown to correlate closely with the presence of prostate cancer.[21,22]

[-2]pro-PSA is part of the Prostate Health Index (*phi*), which is an assay system that takes serum measurements of [-2]pro-PSA and Free/Total PSA, then mathematically calculates the probability of the presence of cancer. The *phi* test received Premarket Approval from the FDA in June, 2012 for the testing of men age 50 and older with serum PSA values in the gray zone and a normal DRE. Several recent studies have shown that *phi* outperformed serum PSA and Free/Total PSA tests in differentiating prostate cancer from benign disease.[20,21,23,24] In addition, the ratio of [-2]pro-PSA to Free PSA has been shown to identify aggressive cancers and to detect cancer in men with PSA 2.5-10 ng/ml and normal DRE findings.[25,26]

PSA Density

PSA density (PSAD) is a measure of serum PSA divided by the prostate volume, as determined by transurethral ultrasound (TRUS). As early as 1992 research suggested PSAD could aid in the differentiation of prostate cancer from benign disease: PSAD values >0.15 ng/ml/ml were found to correlate with individuals with cancer, while lower values were indicative of BPH.[27,28] Subsequent studies, however, failed to show that PSAD could clearly delineate these two patient populations and, as a result, interest in PSAD slowed considerably.[29,30]

These early studies tended to assess the value of PSAD as a replacement for serum PSA in screening for prostate cancer, rather than using it to help stratify patients with elevated PSA who are candidates for biopsy or radical prostatectomy. Of late, PSAD has been evaluated in conjunction with parameters such as Gleason score, extracapsular disease, seminal vesicle invasion, lymph node invasion, and pathologic tumor upgrading.

A number of recent studies have measured the ability of PSAD to predict an upgrade in the clinical stage of prostate cancer, as determined by Gleason scores taken after biopsy and again after radical prostatectomy. The Gleason grading system is the widely accepted standard for evaluating the stage of prostate cancer.

(*See inset at right.*) In short, a numerical score in the range of 2-10 is assigned to a given biopsy specimen, with higher numbers indicating a greater likelihood of advanced disease. It is a reliable predictor of disease progression and is used to direct surgical technique (e.g., whether or not to spare lymph nodes or nerve bundles).[31,32,33] In addition, an upgrade in Gleason score (GS) from initial biopsy to surgical excision of the prostate is a significant predictor of aggressive disease.[34] Due to sampling errors, however, GS is often under-estimated at the time of biopsy, leading to mismanagement of patients with high-risk disease.

Patients with GS ≤6 are an important group clinically because they have the potential to be treated with non-invasive measures. It is also, however, the group in which tumors are frequently under-classified, so a test that can help stratify patients as either high risk or low risk would be valuable.

Oh *et al.* studied 505 patients with GS 6 prostate cancer who underwent radical prostatectomy. Although PSA and PSAD both correlated with tumor upgrading, PSAD was the more accurate predictor and by a statistically significant margin. The authors concluded PSAD should replace PSA in the risk stratification of GS 6 patients.[35]

Corcoran *et al.* studied 1516 patients, categorizing them by their initial biopsy GS and their final pathology GS: Group A had a GS of 3+3=6, later upgraded to ≥7; Group B had a GS of 3+4=7, upgraded to 4+3=7; Group C had a GS of 4+3=7, upgraded to 4+4=8.[36] PSAD was the strongest predictor of GS upgrading in Groups A and B, but not in the more advanced cancers of Group C. The authors suggested the reason for this is that higher grade tumors produce less PSA per volume, reducing the effectiveness of PSAD in later stage disease.

Sfoungaristos *et al.* studied several preoperative clinical and pathological factors in 302 men with localized disease who had undergone radical prostatectomy.[31] Looking for a means to predict tumor upgrading or downgrading after radical prostatectomy, the investigation evaluated preoperative PSA, prostate volume, preoperative PSAD, GS,

the presence of prostatitis, and a number of other factors. The authors found prostate volume to correlate significantly with tumor upgrade in patients with a biopsy GS of ≤6. Similarly, PSAD associated very strongly with tumor upgrading in patients with GS of ≤6, but not with GS ≥7. This is consistent with the other findings presented here, as more advanced cancers likely produce less PSA per volume of prostate. The other factors studied, such as preoperative PSA, benign prostatic conditions, etc., did not correlate with tumor upgrading or downgrading.

PSAD was also shown to be a useful marker when compared to PSAV in predicting tumor upgrading in men on "active surveillance." Active surveillance is a treatment classification for men with prostate cancer that has been identified as low-risk and unlikely to metastasize. Such men are monitored by serial DRE and PSA determinations, in addition to possible repeat biopsies. In a study involving 102 patients, Kotb *et al.* found PSAD to be significantly better than PSAV at predicting tumor upgrading.[37]

Chen *et al.* evaluated PSAD, Total PSA, Free/Total PSA, and PSAV in a study of 212 men with PSA of 4-10 ng/ml who had undergone two biopsies and had normal DRE exams.[38] Of all the markers studied, PSAD was the single best predictor of prostate cancer: a cut-off of 0.18 ng/ml/ml yielded 77% sensitivity and 69% specificity.

In a novel 1999 study, PSAD was determined by measuring the volume of the prostate transition zone instead of the entire prostate as in the earlier work. The transition zone (TZ) is the inner portion of the prostate that surrounds the urethra, and evidence suggests TRUS measurement of the TZ is more accurate than that of the whole prostate.[39]

Two recent studies evaluated PSA transition zone density (PSATZD) in patients who had undergone TRUS and prostate biopsy, categorizing them by their serum PSA levels. Tang *et al.* divided their study population into two groups: those with PSA in the gray zone of 4.0-10.0 ng/ml and those with 10.1-20.0 ng/ml.[40] A PSATZD threshold of 0.37 ng/ml/ml provided 68.8% sensitivity and 72.6% specificity in diagnosing prostate cancer in the gray zone group, while a cut-

off of 0.50 ng/ml/ml gave 70.8% sensitivity and 70.1% specificity in the 10.1-20.0 ng/ml group. The authors concluded that PSATZD measurement can improve the diagnostic efficiency of serum PSA measurements and reduce the number of unnecessary biopsies.

Janane *et al.* used a narrower selection criterion for their study, recruiting men with PSA levels of 2.0-4.0 ng/ml. In addition, they further stratified their study population into groups with PSA values of 2.0-3.0 ng/ml and 3.1-4.0 ng/ml.[41] Using a threshold of 0.23 ng/ml/ml for the 2.0-3.0 ng/ml PSA group and 0.28 ng/ml/ml for the 3.1-4.0 ng/ml group, PSATZD demonstrated significantly better efficiency in identifying the presence prostate cancer than did serum PSA or PSAD. Again the authors concluded that PSATZD is a viable means of reducing unnecessary biopsies.

Lastly, a 2012 study of 285 patients found PSAD to be the most predictive indicator of the key pathologic characteristics associated with prostate cancer: positive surgical margins, extracapsular disease, seminal vesicle invasion, and lymph node invasion after radical prostatectomy.[42] The other diagnostic parameters measured

Gleason Grading System

The Gleason grading system is an evaluation of the physical appearance of prostate tissue by microscopy.

The tissue is assigned two numbers, on a 1-5 scale, for a total score in the range of 2-10. The first number reflects the appearance of the cell type that represents most of the prostate tissue. The second number is representative of the second most common cell type found.

Lower numbers mean the cancer tissue resembles normal prostate tissue and is less likely to spread; higher numbers are predictive of aggressive disease.

An example of a low risk Gleason score is 1 + 2 = 3. An example of a high risk score is 4 + 5 = 9.

were preoperative PSA and GS. The authors concluded that PSAD is a valuable tool to help determine risk of recurrence and to guide the follow-up of prostate cancer patients after radical prostatectomy.

Looking Forward

Despite the recent recommendations against the use of PSA as a screening tool, a wealth of data demonstrates much improved detection of prostate cancer and risk stratification when serum PSA levels are combined with other factors, such as prostate volume (PSAD and/or PSATZD), GS, and [-2]pro-PSA. The combination of these factors appears to be especially useful in the difficult to diagnose population of men with serum PSA in the gray zone of 4-10 ng/ml and GS ≤6. Future study should focus on establishing threshold values that will aid in reducing the number of cancer-negative biopsies and improve clinical outcomes.

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