Trends in Immunoassays of Prostate-Specific Antigen: Serum Complexes and Ultrasensitivity

Multiple reviews and ~1200 papers have appeared in the literature describing prostate-specific antigen (PSA). Without question, this has become the most prominent tumor marker in oncology. Since the introduction of the first commercial PSA immunoassays 7 years ago, the antigen has been an invaluable marker for monitoring patients with prostate cancer. Furthermore, during the past few years, detection of increases (i.e., >4 µg/L) of this unique tissue-specific antigen has been used in screening studies as an adjunct to digital rectal examination to detect prostate cancer in men at risk of developing prostate cancer (typically limited to men of ages >50 years). In fact, many national organizations, including the American Cancer Society, endorse the use of PSA in the early diagnosis of prostate cancer, and a Food and Drug Administration advisory panel has recommended conditional approval of the application by Hybritech, Inc., for the use of the Tandem-E and Tandem-R PSA immunoassays in the early detection of prostate cancer.

This issue of Clinical Chemistry contains three important papers on PSA assays, two describing ultrasensitive assays [Liedke et al. (1) and Yu and Diamandis (2)] and one describing the simultaneous determination of total PSA and PSA complexed with α1-antichymotrypsin (PSA-ACT) [Leinonen et al. (3)]. These three articles highlight the current intense interest in these subjects. The issues are not, however, without controversy.

To adequately discuss the issue of ultrasensitivity, it is necessary to define a few pertinent terms and briefly review the clinical scenarios where PSA determinations in the ultrasensitive range may have clinical value. Although there is no agreed-upon definition of an ultrasensitive PSA assay, those who have previously addressed this subject have focused on immunodiagnostic assays with a biological detection limit for PSA of <0.1 µg/L (4–9). The two articles on ultrasensitive PSA assays in this issue conform to that de facto standard. The term “biological detection limit” may be unfamiliar to many readers but was introduced by us in our initial description of the standard Hybritech Tandem-R PSA assay (10) and used again in our recent evaluation of the ultrasensitive Abbott IMx PSA immunoassay (7).

In describing PSA assays, especially those that have features of an ultrasensitive assay, three terms are encountered that are potentially critical in the monitoring of PSA concentrations after therapy (7). Under ideal analytical conditions, one can determine the concentration of PSA that the assay distinguishes from zero. We call this value “the lower limit of detection” (LLD); others may use the term “analytical sensitivity.” However, we believe that the reporting of clinical PSA values at this level is not reliable because such values do not encompass the interassay precision of the assay. Therefore, we recommend calculating the biological detection limit, which takes into consideration the interassay precision of the assay with clinical specimens at this PSA concentration. The biological detection limit, when calculated as the LLD plus 2 SD from the interassay precision data, provides at least 95% confidence that PSA is present in the specimen and that, upon repeat testing, the resulting value will remain above the LLD. This substantially eliminates the reporting of false-positive results attributed to assay precision and admittedly is a conservative approach. Specimens yielding PSA concentrations below the biological detection limit are reported only as less than this value.

Before the development of ultrasensitive PSA assays, PSA concentrations above the biological detection limit of the standard PSA assays often corresponded to the presence of residual disease (10). This gave credibility to the theory that removal of all normal and malignant prostatic tissue during a radical prostatectomy should result in the absence of PSA in serum specimens. With the emergence of PSA assays that accurately reported values <0.1 µg/L, PSA serum concentrations slightly above the biological detection limit no longer universally corresponded to residual prostate cancer (see below). We previously used the term “clinical threshold” to represent the PSA concentration that clearly indicated persistent disease (7, 10). Likewise, Graves et al. (4, 5, 8) used the term “residual cancer detection limit” to indicate a similar threshold in their clinical evaluation of an ultrasensitive PSA assay; we now encourage the use of their more descriptive term.

Of these three terms, lower limit of detection and biological detection limit are clearly dependent on assay performance, and the values achieved are a reflection of numerous factors, including the choice of antibodies, the means of signal generation and detection, and overall assay design. These and several other contributing aspects relating to PSA immunoassays have been recently reviewed (11). The third term, which relates to the detection of residual disease after radical prostatectomy, depends less on the assay than on the clinical situation.
and the various nuances of PSA production. These terms and their method of calculation were introduced in an attempt to standardize assessments of PSA immunoassay performance and to provide reasonable expectations when testing clinical specimens. Although other approaches and terms may be preferred by some investigators, Liedke et al. (1) and Yu and Diamandis (2) for the sake of consistency included the LLD and biological detection limit values in the descriptive analysis of their respective ultrasensitive PSA assays.

I emphasize that ultrasensitive PSA assays have the potential advantages over standard PSA assays only for monitoring patients after therapy, when the PSA concentrations fall into the undetectable range of standard assays. They offer no advantages in the early detection of prostate cancer among the general population, in the assessment of bony metastasis or in the monitoring of advanced progressive disease. The decline of PSA to <0.1 \( \mu \text{g/L} \) can occur during treatment by hormonal ablation, radiation therapy, or radical prostatectomy. The most interest and debate have surrounded the use of ultrasensitive PSA values after radical prostatectomy. Historically, 40–60% of patients who had a “curative” radical prostatectomy, with removal of all normal and malignant prostatic tissue and subsequently undetectable PSA (e.g., <0.4 \( \mu \text{g/L} \)) at 6 months postsurgery by standard assays, have eventually shown evidence of residual progressive disease. Therefore, ultrasensitive PSA assays capable of measuring <0.1 \( \mu \text{g/L} \) might provide at least four advantages over standard PSA assays: (a) the suggestion of residual disease within the first few months of radical prostatectomy, (b) an enhanced warning of progressive residual disease over other clinical methods (e.g., lead time advantage), (c) assessment of the virulence of the residual disease by assessment of PSA-doubling times within the ultrasensitive range, and (d) assurance that patients with PSA concentrations below a certain level for a prolonged period (e.g., latency period) would have a very low probability of subsequently developing life-threatening, virulent progressive disease.

During the evaluation and potential clinical application of ultrasensitive assays by us (7, 8, 12), Graves et al. (4), Liedke et al. (1), and Yu and Diamandis (2), some of the theoretical advantages just cited were realized. Nevertheless, there are still good reasons to proceed cautiously and to be acutely aware of the controversial issues surrounding the clinical use of ultrasensitive PSA measurements. Three of these issues are as follows.

1. One very important issue relates to the source of PSA in postprostatectomy patients and one’s confidence in attributing detectable PSA to residual cancer. Until recently, PSA was thought to be an exclusive product of the prostatic epithelium. However, it is now known that PSA is also produced by and detected within the perirectal and periurethral glands (13–16). The source of detectable serum PSA in the ultrasensitive range can also be attributed to residual normal prostatic tissue, which is sometimes left intact during radical prostatectomy (17). Finally, given the significant structural homology of PSA with other members of the kallikrein family, especially hGK-1 (18), substantially increased serum concentrations of these potentially cross-reacting substances might confound the accurate measurement of PSA in the ultrasensitive range.

The potential contribution of these additional sources of PSA or of cross-reacting substances to PSA determinations in the ultrasensitive range has not been sufficiently studied. Several observations suggest that this may not be a significant problem at least down to 0.02 \( \mu \text{g/L} \). Parenthetically, we found that PSA from the periurethral glands is present in urine of postprostatectomy and cystoprostatectomy patients (16), thus diminishing the clinical value of such determinations to signify residual localized disease. In serum, non-cancer-derived PSA increases after radical prostatectomy, although rare, can complicate the identification of residual disease either at the initial evaluation (usually within 3–5 months of surgery) or during subsequent long-term follow-up.

We recently reviewed PSA results obtained with the Abbott IMX PSA assay during the initial postoperative period for patients who had had a radical prostatectomy within the past 4 years at the University of Washington and Seattle VA Medical Centers. The vast majority had initially undetectable PSA (<0.06 \( \mu \text{g/L} \)), even though some, on subsequent follow-up, revealed measurable PSA consistent with recurrent disease (>0.1 \( \mu \text{g/L} \)). Then, using a research chemiluminescent assay with lower detection limits (9), we evaluated 35 of these initial postsurgical sera with PSA <0.06 \( \mu \text{g/L} \) and found that 29 had PSA values <0.01 \( \mu \text{g/L} \), including 6 of 7 from patients with minimal stage D disease at the time of surgery.

During long-term follow-up, infrequent (~5%) and transient PSA increases slightly above the biological detection limit and probably not associated with recurrent prostate cancer were noted in the two previously described clinical evaluations of ultrasensitive assays (7, 8), highlighting the necessity of establishing a residual disease detection limit. The source of the detected PSA in these few patients’ sera remains unknown, but certainly the periurethral and perirectal glands and (or) residual minute pieces of normal prostatic tissue are prime candidates. In support of these previous observations, Yu and Diamandis (2) found that 16 of 27 post-radical prostatectomy patients had PSA concentrations <0.02 \( \mu \text{g/L} \) (pathological stage and time after radical prostatectomy not provided) and Liedke et al. (1) confirmed that 6 sera provided by us from long-term post-radical prostatectomy stage B patients had PSA concentrations <0.02 \( \mu \text{g/L} \).

The results from these previous and current studies suggest that in most postradical prostatectomy patients, neither residual normal prostatic tissue nor other PSA-producing glands contribute to detectable PSA in serum. As noted earlier, this does not eliminate the possibility that these structures contribute to transient PSA increases between the biological detection limit.
and the residual disease detection limit. These results also suggest that the detection of residual disease within a few months of radical prostatectomy is not likely with current versions of ultrasensitive assays and that even lower biological detection limits are required for this task. Historically, because nearly all patients with stage D disease who have had a radical prostatectomy eventually have recurrent cancer, most if not all of those six patients in our series with PSA ≤0.01 μg/L have occult residual disease despite having PSA below the level of detection. This is disappointing but not surprising when one realizes that PSA serum concentrations of 0.01 μg/L approximate 10^{12} molecules of PSA in circulation. We may be a long way from detecting the PSA that is produced from occult microscopic tumor foci. Finally, transient increases of PSA within the ultrasensitive range should not be easily mistaken for recurrent disease if one remembers to evaluate trends in data rather than rely on single-point results.

2. The second controversial issue surrounding ultrasensitive PSA immunoassays is how to utilize the data. This is a complicated subject area because currently there is no curative treatment modality for progressive disease after radical prostatectomy. Hormonal ablation, either surgical or pharmaceutical, is the only option for disseminated progressive disease; at best, this provides only a few years of palliation. Many argue strongly that the use of hormonal ablation before overt clinical symptoms is not to the patient's advantage and therefore knowledge of residual disease based on ultrasensitive PSA values can only add to the patient's anxiety. Others counter that intervention at the time of micrometastasis is often more effective in solid tumors than at a point of more burdensome and symptomatic disease and that the former intervention should be the goal in prostate cancer. Although admitting that no curative therapies are at present available, they advocate that much can be learned from monitoring PSA concentrations in the ultrasensitive range and remain optimistic that modified versions of current therapies or new therapies will rely heavily on this knowledge. However, any interventions based on ultrasensitive PSA values should at present clearly be reserved for approved experimental protocols.

3. The third issue also relates to the clinical scenario of progressive disease after radical prostatectomy. In some patients the residual disease manifests a rapid and virulent progression, whereas in others it is much more indolent, with a very slowly progressive pattern. Patients in these two clinical scenarios should probably not be treated similarly and, in fact, those patients with the indolent form might not need treatment at all because the residual disease is not life-threatening. Critics of ultrasensitive PSA immunoassays caution that results (within the ultrasensitive range) that signify residual disease may lead to treatment of patients who have the more indolent form of residual disease. Patients who know their PSA concentrations may advocate such interventions prematurely and further complicate their management. Proponents of ultrasensitive PSA determinations counter that PSA-doubling times or similar "slope adjustments" may well differentiate between these groups of patients, providing substantial advanced warning of progressive virulent disease while alleviating the anxiety in those patients with the more indolent forms.

The reports of neither Liekde et al. (1) nor Yu and Diamandis (2) addressed the clinical issues of latency period or load time, but there is every reason to presume that these newly described ultrasensitive assays will be used to provide new and potentially useful data on susceptible subjects. Using the ultrasensitive chemiluminescent PSA assay previously mentioned, we demonstrated a pronounced improvement in lead time when serially tracking increases in PSA concentrations from 0.01 to 0.1 μg/L (9); we expect future results from other investigators to confirm these findings.

The work of Leinonen et al. (3) in this issue focuses not on ultrasensitivity, but on another highly pertinent aspect of PSA quantification, that of potentially differentiating between benign prostate hyperplasia (BPH) and prostate cancer. In two recent screening studies for the early detection of prostate cancer in men over age 50, the specificity for cancer was low when PSA >4 μg/L was used as the cutoff level. Catalona et al. (19) in a series of 1653 men found that 8% had PSA >4 μg/L, but only 27% of these increased values were subsequently attributed to prostate cancer; the remainder were primarily due to BPH. Brawer et al. (20) reported that 15% of the patients in his series had PSA >4 μg/L, with approximately one-third of the cases being attributed to cancer. Therefore, a means of differentiating between BPH and prostate cancer in men over age 50 years with an increased PSA content would be of considerable interest.

Stenman et al. (21) and Lilja et al. (22, 23) were the first to describe a potential method for facilitating the differential diagnosis of BPH and prostate cancer based on the various forms of PSA that are measured by immunoassay. Because PSA is a serine protease, the enzymatically active form binds to inhibitors in serum, forming complexes. One such inhibitor is α2-macroglobulin, which is thought to engulf PSA, rendering it undetectable by current immunoassays. A second inhibitor is α1-antichymotrypsin, which binds to PSA to form a complex of ~90 kDa (PSA-ACT). This complex is detected by PSA immunoassay. Serum also contains unbound PSA (free PSA), the nature of which is not fully understood, but may represent the zymogen or other enzymatically inactive form. Free PSA is also detected by PSA immunoassay. Thus, for the purpose of discussion here, PSA-ACT and free PSA are considered to constitute the total immunoreactive fraction that is detected by standard PSA immunoassay (total PSA), with PSA-ACT representing the major portion.

For reasons that remain elusive, the percentage of PSA-ACT may be higher in patients with prostate cancer than in patients with BPH or normal controls. This observation was first reported by Stenman et al. (21) and soon thereafter was confirmed by Lilja et al. (22, 23) although they differed in the observed percentages of the PSA-ACT fraction. Wood et al. (24) also confirmed
the presence of PSA-ACT complexes in patients' sera but noted that further study, possibly involving ratio analysis, would be necessary before speculating as to the clinical value. Recently, Christensson et al. (25) extended the initial experiments of Lilja et al. (22, 23), attributing differences between their work and that of Stenman et al. (21) and Wood et al. (24) to antibody selection, composition of the standards, and assay design.

The initial data citing differences among the ratios of PSA forms in BPH and prostate cancer patients were viewed with some reservations because the data sets included serum from cancer patients with PSA concentrations as great as thousands of micrograms per liter when, in fact, discrimination is needed in the 2–20 μg/L range. Also, Stenman et al. (21) had shown a greater proportion of complexation at higher PSA concentrations in serum, an observation not seen recently by Christensson et al. (25). When a preliminary study confined the investigation to PSA <30 μg/L, the percent of PSA-ACT was generally >90% in all groups of patients (26); although a discrimination between groups was not readily apparent, between-patient differences were noted. This study (26) relied in part on column fractionation to separate the PSA forms before assay rather than direct measurement of the free PSA, PSA-ACT complex, and total PSA as performed by Lilja et al. (22, 23, 25).

The work of Leinonen et al. (3) is an extension of the original report by Stenman et al. (21), who measured PSA-ACT and total PSA by two time-resolved immuno-fluorometric assays. In the present report (3), these two fractions are measured simultaneously by using a double-label assay configuration with different reagents (e.g., monoclonal antibody capturing antibodies) from those in their initial work (21). Leinonen et al. also limited their study to samples with PSA concentrations in the range of 2–20 μg/L. In the PSA range 2–4 μg/L, they noted no improvement in sensitivity by use of the PSA-ACT to total PSA ratio. However, in the range of 4–20 μg/L, the use of a PSA-ACT/total PSA ratio (cutoff = 0.70) increased the sensitivity for detecting prostate cancer from 59% to 93% at a specificity of 60%, when compared with the total PSA value alone (cutoff 7.5 μg/L). At this ratio (0.70), the number of false positives due to BPH was reduced by 60%, while only two of the prostate cancers were missed (BPH, n = 49; prostate cancer, n = 29). Receiver-operating curve analysis confirmed the advantage of using a ratio rather than the total PSA. These sensitivity and specificity numbers are not quite as striking as those reported by Christensson et al. (25) in this same PSA concentration range: sensitivity 71%, specificity 95% at a free PSA/total PSA ratio cutoff of 0.18. However, the number of patients included in this subset analysis (4–20 μg/L) was not provided. Another possible contributing difference is that Christensson et al. (25) used three immunoassays that individually quantified free PSA, PSA-ACT, and total PSA followed by calculation of the free PSA/total PSA ratio.

Finally, I offer the following thoughts for consideration. Neither the free PSA/total PSA nor the PSA-ACT/total PSA ratio approach has been tested in a prospective screening population. Such studies are critical and, we hope, will soon be forthcoming. In the US, the cutoff in early detection protocols has been 4 μg/L, not 7.5 μg/L or higher, as used by Leinonen et al. (3). At this cutoff, specificity for prostate cancer will drop to ~55% when used with the 0.70 ratio cutoff for PSA-ACT/total PSA. This is an improvement over the current rate achieved without using ratios, but it remains to be seen whether the advantage is maintained in an equivalent screening population. Also, in both the Christensson et al. (25) and Leinonen et al. (3) BPH populations, the exclusion of cancer was attempted through analysis of tissue removed during a transurethral resection; however, this method of analysis is not accurate because only ~25% of prostatic cancers occur in the resected transitional zone tissue. A preferable approach would have been to complement this with transrectal ultrasound-guided biopsies of the peripheral zone. It would be interesting to compare the two approaches in a blinded set of serum specimens from well-characterized patients. Finally, a more sensitive assay that directly and precisely quantifies free PSA in the range of 0.05–5.0 μg/L would be helpful, especially in assessing clinical utility in the critical total PSA range of 2–10 μg/L. Although speculative, such an assay might also help discriminate between aggressive and slow-growing forms of prostate cancer revealed through early detection protocols if the percentage of free PSA is shown to correlate with virulence.

Readers of the PSA-related articles in this issue should remain acutely aware that all three of them describe research PSA immunoassays that have not been validated for use in clinical decision-making. The role of the Food and Drug Administration in controlling the use of modified or novel immunoassays has been the subject of prior comments in Clinical Chemistry (27). Parenthetically, the position of Hybritoch on the modifications of the Photon-ERA PSA immunoassay as performed by Liedke is clear: "... precludes the use of that assay for clinical purposes." Caution is strongly urged in the reporting and the potential overinterpretation of PSA results in the ultrasensitive range until the appropriate clinical trials are completed. However, the ultrasensitive PSA assays described here join a growing list of assays that will, I speculate, contribute to the database from which the clinical value of ultrasensitive measurements will be demonstrated.

Of course, the clinical validation of novel PSA immunoassays and their comparison with current assays would be made easier if there was an internationally recognized PSA standard (28, 29, and Stamey, ms. in preparation). Until then, differences among assays, including calibrator design, antibody specificity and affinity, and format will complicate the assessment of clinical utility and comparisons among assays. Some specimen discrepancies encountered between assays may reflect the fact that the PSA assays in current use are not necessarily equimolar in their ability to detect free PSA and the PSA-ACT forms (30). Should the ratio
in forms shift, differences between the measurement modes (e.g., kinetic vs equilibrium) or in any one of several aspects of calibrator design could result in noticeable variances in the reported concentration of total PSA in that specimen. This further underscores the cautionary use of data generated by PSA immunoassays that have not been fully validated through appropriate clinical trials.

References
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