

*While some researchers examine whether prostate-specific antigen (PSA) isoforms will solve PSA's specificity issues, others are turning their attention to potential new proteomic and molecular markers of prostate cancer, hoping to find a test that can significantly reduce the number of unnecessary biopsies that are performed because of false-positive PSA results. This month, Strategies examines what lies ahead for clinical laboratories in the area of prostate cancer detection.*

The use of PSA in prostate cancer screening has always been controversial—the assay's false-positive rate is nearly 75%, and its false-negative rate ranges from 20%–30%. Although the U.S. Preventive Services Task Force (USPSTF) states that it has “found good evidence that PSA screening can detect early-stage prostate cancer,” the assay's lack of specificity, as well as “mixed and inconclusive evidence” that early detection improves health outcomes, has prevented the USPSTF from recommending PSA be used routinely to screen men in the U.S. for this potentially deadly disease.

Nevertheless, the Medicare program has been instructed by Congress to cover PSA as a screening test, and the assay is used routinely by clinicians, who generally follow the recommendations of the American Cancer Society and the American Urological Association (see box) both of which recommend PSA screening. “There's no doubt that using PSA, we have detected a significant number of prostate cancer cases, and that we're now detecting cancers at an early stage where potentially they are more curable than at a later stage,” said Herbert Fritsche, PhD, Professor and Chief of Clinical Chemistry at the University of Texas M.D. Anderson Cancer Center in Houston, Texas.

### Improving on PSA

Although PSA has been a useful marker for prostate

cancer screening, Fritsche believes that there are ways to improve PSA's performance. “We can detect cancers very well with PSA, so the detection rate is high, but the specificity needs to be improved. We need to improve our positive predictive value from 25% to at least 50%,” asserted Fritsche. “Right now, only one cancer is being detected for every four biopsies performed, and we need a testing strategy that will allow us to detect one cancer for at least every two biopsies performed,” he said.

Richard Babaian, MD, Urologist and Professor of Medicine at the University of Texas M.D. Anderson Cancer Center and Director of the M.D. Anderson Prostate Cancer Detection effort, agrees with Fritsche. “We really need to optimize the sensitivity of our prostate cancer markers for clinically significant disease, and we need to minimize their sensitivity for clinically insignificant disease,” he said, speaking at a recent AACC audioconference called “Prostate Disease Diagnosis and Management: Current Practice, Con-

“If the molecules that they are measuring are selected so that they come from cancer cells, it's possible that they may yield results, but they still don't know what they are measuring.”

—Eleftherios Diamandis, MD, PhD

flucting Evidence.” Babaian believes that the specificity and the positive predictive value of PSA should be maximized in an effort to eliminate those unnecessary biopsies that Fritsche referred to. “We also need to be able to use our markers to predict the biologic behavior of prostate cancer and its response to therapy, and we really need to develop some molecular staging strategies,” he added.

### Use of Neural Networks May Reduce Unnecessary Biopsies

In an effort to reduce biopsies, M.D. Anderson has been experimenting with neural networks, which Babaian defines as nonlinear, computational, mathematical models for processing information and looking for pattern classifications. “Our neural network looked at several parameters, which included free PSA, total PSA, CPK, and prostatic acid phosphatase in the 2.5–4 ng/mL range,”

...continued on page 4

Continued from page 5

said Babaian. "Using a neural network, we were able to increase the specificity of PSA from approximately 40% if you use a PSA density measurement alone to 62%, and to save approximately 50% of all unnecessary biopsies compared to 34% with the same PSA density measurement," he noted, adding that free PSA did not perform as well in the group of men studied.

Babaian and a multi-institutional group of researchers further described their work with the use of neural networks in prostate cancer last year (*Cancer* 2003;98(9):1849–54). In the study, the researchers used a database comprised of 3,268 men recently evaluated for the early detection of prostate cancer. Seven clinical features were used in their neural network to determine which men were good candidates for biopsy—these included age, race, family history, International Prostate Symptom Score (IPSS), DRE, and total and complexed PSA results. Three hundred forty-eight subjects in the dataset included men with determined prostate biopsy outcomes and for whom at least 6 of 7 features were available. The dataset was divided randomly into a training set (60%) and a test set (40%), and was modeled with linear and quadratic discriminant function analysis and a neural computational system. After an acceptable model was achieved, reverse regression analysis using Wilks' generalized likelihood ratio test was performed to evaluate the statistical significance of each input variable. The ROC area for the neural computational system in the test set was 0.825, whereas total PSA and complexed PSA alone had ROC areas of 0.678 and 0.697, respectively.

"Each of the seven variables on which the model was based was critically significant to model performance," concluded the investigators, suggesting that clinicians use the model when deciding whether or not to perform prostate biopsy.

### Evaluating PSA Isoforms

In the neural network model, Babaian and his colleagues used total PSA and complexed PSA measurements, but should these be the assays of choice for PSA screening? "I believe that the ratio of complexed PSA to total PSA and the ratio of free PSA to total PSA are basically equivalent, and there's no advantage of using one over the other," Babaian opined. M.D. Anderson switched to using complexed PSA as a first-line screening test in all of its screening activities last year. "If you look at

complexed PSA in a range between 2 ng/mL and 6 ng/mL at a sensitivity of 95%, you will see that the specificity for complexed PSA is 21% and is a little bit better than total PSA, which has a specificity of 13.4%," he said, whereas % free PSA has a 9% specificity, and % complexed PSA has a specificity of 13%.

"The place where we found complexed PSA to be extremely useful potentially is if one was to lower the total PSA threshold from 4.0 ng/mL to 2.5 ng/mL. If you use complexed PSA at a level of 2.2 ng/mL as your cut-off, you could potentially save 42% of all the biopsies and have a sensitivity of 90%," Babaian observed, adding that the use of a free to total PSA ratio in this context would only save 11% of biopsies.

Babaian adds that complexed PSA results can be further enhanced by adjusting them to prostate volume, which would result in saving approximately 59% of biopsies. Earlier this year, Babain, Fritsche, and others from M.D. Anderson published a study in *Urology* (2004 63(3):492–8) that evaluated the efficacy of precursor isoforms of PSA (pPSA) and benign prostatic hyperplasia-associated PSA (BPSA) for the detection of prostate cancer in combination with ultrasound-estimated prostate volume (PV). The

team of investigators discovered that median values for BPSA, free-to-total PSA ratio (f/tPSA), and benign-to-total PSA ratio (B/tPSA) differed statistically between men with cancer and a PV less than 25 mL and men with cancer and a PV greater than 50 mL ( $P < 0.05$ ) and between the men with cancer and a PV less than 25 mL and men without cancer and a PV greater than 50 mL ( $P < 0.005$ ). "In this preliminary study, the

"What makes PSA such a good marker is the fact that it's prostate-specific, so any other marker has got to be prostate-tissue specific, but also must improve cancer specificity, and that has been difficult to do because we have not yet found any really unique proteins that have the cancer specificity and tissue specificity we need. I think that's really the challenge."

—Herbert Fritsche, PhD

level of (-2), (-4), sum pPSA, and BPSA seemed to be PV-related, but only BPSA and B/tPSA and f/tPSA were significantly associated with PV. Therefore, pPSA did not aid in better discriminating cancer from noncancer," the authors concluded.

Why doesn't M.D. Anderson use free PSA to screen for prostate cancer? "In a limited number of cases, free PSA improves specificity and reduces the false-positive rate, but at the same time it misses cancers, and urologists don't want that to happen, especially in younger men," noted Fritsche. "In working up specific cases, it offers some value, but not in general screening."

Lori Sokoll, PhD, Assistant Professor of Pathology at Johns Hopkins University Medical Institutions and Associate Director of Clinical Chemistry at the Johns Hopkins Hospital, described when % free PSA might be

## Current Guidelines for Prostate Cancer Screening

According to the American Cancer Society and the American Urological Association, both prostate specific antigen (PSA) and digital rectal examination (DRE) should be offered annually, beginning at age 50 years, to men who have a life expectancy of at least 10 years. Men at high risk should begin testing at age 45. High-risk groups include men of African descent (specifically, sub-Saharan African descent) and men with a first-degree relative diagnosed at a young age. Risk increases with the number of first-degree relatives affected by prostate cancer. Among men of African descent, age-specific risk increases steadily beginning at age 45. Men at appreciably higher risk of prostate cancer due to multiple first-degree relatives who were diagnosed with prostate cancer at an early age could begin testing at age 40. However, if PSA is less than 1.0 ng/mL, no additional testing is needed until age 45. If PSA is greater than 1.0 ng/mL but less than 2.5 ng/mL, annual testing is recommended. If PSA is 2.5 ng/mL or greater, further evaluation with biopsy should be considered.

Source: *American Cancer Society, Prostate Cancer Screening Guideline, Jan Feb 2001 Issue of CA - A Cancer Journal for Clinicians*

best used. "We know that there is a diagnostic gray zone for total PSA between 4ng/mL and 10ng/mL, where patients with cancer and benign diseases have overlapping PSA values. This is where % free PSA is most helpful," she explained. "However, percent free PSA has a diagnostic gray zone as well in the 10–25% range, where the likelihood of cancer is neither very high nor very low. This could be a potential application for hK2."

Human kallikrein 2 (hK2), said Fritsche, is a potentially useful marker, but he would like to see more studies performed that delineate how it can be used in prostate cancer detection. "There's some who feel strongly that the data for hK2 is positive, but there are others who feel just as strongly that the data is not there yet," he said.

"Perhaps the best application for hK2 at this point in time is in staging men who have cancer to identify more aggressive forms," said Sokoll, who believes that to improve the detection of prostate cancer, the molecular forms of PSA—free PSA and pPSA—as well as hK2 are "the most promising."

### Lowering the Detection Limit of PSA

As lab directors evaluate the usefulness of the isoforms of PSA, they will also likely be evaluating with their clinicians where to set the detection limits, both for PSA and complexed PSA. "I believe we need to be screening in the lower PSA ranges, probably in the 2.0 ng/mL–2.5 ng/mL range, as opposed to the 4 ng/mL currently recommended, as the cutoff value," noted Fritsche. "The positive predictive value is the same in that 2–2.5 ng/mL range as it is in the 4–10 ng/mL range, and somewhere around 70–80 percent of the patients being detected in the 2.5–4 ng/mL range have clinically significant cancer," he said.

Since current guidelines still recommend a cutoff of 4.0 ng/mL for PSA, said Fritsche, lab directors and clinicians may have to make the case themselves for lowering the cutoff. "This is a tough recommendation for guide-

line groups to make because the number of false-positives will increase by at least a factor of two when the detection limit is lowered," noted Fritsche.

If making the switch to complexed PSA, said Babaian, "If you are a believer in 4.0 for total PSA, then your cutoff is going to be 3.4 for complexed PSA. If you want to lower your threshold to 2.5, then it's going to be 2.2."

### Other Potential Markers

Also on the horizon for prostate cancer detection are a series of markers that have been found using protein pattern recognition, a unique approach to disease identification that compares the proteomic patterns of diseased and non-diseased individuals using bioinformatics tools combined with technologies like surface-enhanced laser desorption and ionization time-of-flight (SELDI-TOF) or matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectroscopy.

The *Wall Street Journal* reported in its November 4, 2003, edition that a protein called Pca-24, which is found in tissues taken from men with prostate cancer, but not in similar samples from healthy men, could hold the key to more accurate prostate cancer detection. Harvard University researchers found the protein by comparing cells from 17 men with prostate cancer to cells from 12 men who had enlarged prostates. Pca-24 did not exist in the non-cancerous tissues, but was present in 16 of the 17 cancerous tissues.

Harvard isn't the only institution researching protein pattern recognition for prostate cancer detection—a similar program exists at Memorial Sloan-Kettering Cancer Center in New York, and companies like Correlogic Systems (Bethesda, Md.), Matritech (Newton, Mass.), and CIPHERgen Biosystems are all scrambling to commercialize protein pattern recognition technologies.

"In the NCI-FDA Clinical Proteomics Program, we're pursuing a platform that's based on high- and ultra-high-resolution MALDI-TOF spectrometry, because we know that we can use this platform to look at proteomic frag-

Continued from page 7

ment information that contains the diagnostic information we have seen in our previously published work," noted Emanuel Petricoin, III, one of the original developers of the technology.

In a presentation last year at the 94<sup>th</sup> Annual Meeting of the American Association for Cancer Research, Petricoin provided a glimpse at what his institution has been doing in the area of prostate cancer protein pattern recognition in conjunction with a team of University of North Carolina researchers. He reported that the group ran serum samples from 63 men with PSA levels between 2.5 and 15 ng/mL, 23 of whom had had two or more negative biopsies, 10 of whom had had one negative biopsy and 30 of whom had had biopsy-detected prostate cancer. The serum samples were used to form a protein expression algorithm designed to determine which patients could have been spared biopsy. Using a proteomic algorithm, the researchers ran 91 additional samples from 28 men with prostate cancer and 63 with at least one negative biopsy on a WCX2 ProteinChip Array from CIPHERGEN Biosystems. That method yielded a sensitivity of 100% and a specificity of 67%, suggesting that two of three men without prostate cancer but with suspect PSA scores could have avoided the biopsy.

The technology is very new, however, and some are skeptical about the findings that have been published to date. Eleftherios Diamandis MD, PhD, FRCPC, Head, Section of Clinical Biochemistry in the Department of Pathology and Laboratory Medicine at Mount Sinai Hospital in Toronto (Ontario, Canada), says that while the technology is promising, it currently "has many problems." Diamandis explored the potential pitfalls of this technology in an invited editorial in the March 3 issue of *The Journal of the National Cancer Institute* (2004;96(5):353-6).

Contrary to what Petricoin and other protein pattern recognition researchers say, Diamandis believes that it is extremely important to know what the technology is actually measuring. "If the molecules that they are mea-

suring are selected so that they come from cancer cells, it's possible that they may yield results, but they still don't know what they are measuring," he said, adding that the fragments could be epiphenomena generated by the presence of any type of cancer, therefore affecting the specificity of the assay.

Diamandis recently published a study in conjunction with an international group of researchers that also examines a molecular component of prostate cancer, only his study quantitatively analyzed hepsin gene expression in prostate cancer tissue using real-time PCR, calculating hepsin's relationships with clinicopathological parameters in a large cohort of samples (*J Urol* 2004;171:187-91). "The quantitative analysis of hepsin expression . . . shows strong and significant overexpression in prostate cancer tissue. Hepsin expression may be a new prognostic marker that could be used for assessing prostate cancer aggressiveness,"

Diamandis and his colleagues wrote.

According to Fritsche, coming up with a marker that measures how aggressive a cancer is would be extremely helpful to clinicians. "We definitely need to better diagnose aggressive cancers. We probably don't even need to treat some men who have insignificant cancers, especially if they are older. Any new marker that we're working on really should focus on aggressive cancers, but this is difficult to do because we don't really know which are the aggressive tumors," he said.

Will the new proteomic tests hold the key? "Unfortunately, there's very little data on any of these new markers, and that's what makes it difficult," said Fritsche. "These techniques need a great deal of validation and standardization before they can even be considered for any kind of clinical use."

In the meantime, lab directors should pay attention to studies that look at proPSA, hK2, and other markers that have more data behind them. "The PSA story may get better, but I think we're still years away from really learning how to improve prostate cancer detection with PSA," Fritsche concluded.

"The PSA story may get better, but I think we're still years away from really learning how to improve prostate cancer detection with PSA."

—Herbert Fritsche, PhD

## New Resource Available for Managing Postdocs, New Faculty

The Howard Hughes Medical Institute recently released a free, online publication called *Making the Right Moves: A Practical Guide to Scientific Management for Postdocs and New Faculty*. Topics in the publication include: obtaining and negotiating a faculty position and planning for tenure; the scientific investigator within the university structure; defining and implementing your mission; staffing your laboratory; mentoring and being mentored; time management; project management; data management and laboratory notebooks; getting published and increasing your visibility; and understanding technology transfer. The document, which 250 pages long, may be read on-line or chapters may be downloaded from the publication's Web site at: <http://www.hhmi.org/labmanagement>.