Part II: Tumor markers from A to Z

By David Plaut

In our final article on “Tumor markers from A to Z,” we will discuss the clinical utility of alpha-fetoprotein (AFP), CA 125, CA 19-9, and prostate-specific antigen (PSA), free PSA (fPSA), complexed PSA (cPSA), and delta PSA. We begin with AFP and the diagnosis of hepatocellular carcinoma (HCC). While HCC is a major public health issue worldwide, it is rare in the United States where the incidence is about two per 100,000 with a male to female ratio of 3:1 and approximately 4,000 deaths per year. Two common HCC risk factors are cirrhosis of the liver due to increased alcohol consumption and/or hepatitis.

One problem with AFP assays in the diagnosis of HCC is the low sensitivity when coupled with high specificity, or the low specificity when asking for high sensitivity (a problem that is not unique to AFP). Increased levels of AFP are sometimes found in some other tumors (i.e., stomach, biliary tract, and pancreas), although the levels are much lower. Over the past several years, studies have indicated that AFP exists in three isoforms or sub-species — L1, L2, and L3 — based on their different affinities to lectin. Measurement of total AFP (tAFP) and AFP-L3 may provide more clinically useful information than tAFP alone. While tAFP may indicate tumor burden, AFP-L3 may be a marker to measure malignant potential of the liver-cancer cells. For example, while a liver tumor <2 cm in diameter may be considered small, it may behave quite aggressively, with the potential for rapid growth and distant metastasis. This may be detected with the AFP-L3: tAFP ratio.2

HCC patients had free AFP (fAFP) values above the 20 ng/mL cutoff in 44% of cases (22/50), and AFP-IgM IC values above the 120 AU/mL cutoff in 60% of cases (30/50). The occurrence of the free and IgM-complexed forms of circulating AFP did not overlap, and 82% of patients (41/50) were positive for at least one marker. The results indicate that AFP-IgM IC is a complementary serological marker to fAFP and that the combination of these biomarkers may be useful in the diagnosis of liver cancer.1-5

CA 125 and CA 19-9

CA 125 has been used mainly to detect ovarian cancer, although there are some data on its use in detecting pancreatic cancer.6-8 As with the other markers, CA 125 generally has a lower level of clinical sensitivity, especially in Stage I, suggesting that CA 125 is not adequate for detecting cancer. It also has a rather low level of specificity, leading to a high false-positive rate. This marker has been used to monitor treatment. Ovarian cancer has been one of the areas in which markers have been combined to increase sensitivity. For example, one such study used CA 125 with OVX1 (found increased in 47% of patients whose CA 125 was not elevated) and M-CSF. In this study, CA 125 was increased in 69% of the patients with Stage I ovarian cancer compared to an elevation in any one of the three in 84% of the patients. This gain in sensitivity came, however, at the expense of specificity.

CA 19-9 has been used mainly in detecting and monitoring pancreatic cancer, the fourth leading cause of cancer death.9,30 In pancreatic carcinoma, elevations of CA 19-9 are found in 70% to 80% of patients. Compare this to 15% and 35% sensitivity for CEA in similar patients. In 14% to 22% of patients with stomach cancer and in 18% of those with colon cancer, increased levels are found. Elevations of CA 19-9, like elevations of CEA, are found in benign diseases, such as acute hepatitis, chronic active hepatitis, pancreatitis, and inflammatory diseases.11

PSA; delta PSA (PSA velocity or PSA change)

Used as a tumor marker for several decades, PSA was first used to monitor patients, then used “off label” to aid in the diagnosis of and even screening for cancer. Early on, three issues with PSA were encountered. It was insensitive for the early detection of prostate cancer. It was not very specific [as many as 25% of the patients with benign prostatic hypertrophy (BPH) gave values greater than 4.0 ng/mL]. The fact that prostate cancer was considered a disease most men were more likely to die with than from raised the questions of whether the disease should be detected and whether screening for prostate cancer was an acceptable use of finite resources. Because of the first two issues with PSA, researchers looked for better ways to diagnose prostate cancer.

As men age, the prostate tends to enlarge, resulting in a “normal” increase in serum PSA. In the process of using this increase to separate prostate cancer from BPH, it has been suggested that an increase of less than 0.8 ng/mL per year may be considered “normal,” while a change greater than 0.8 ng/mL in one year is “suspect.” It is also suggested that at least three samples (over two years) be used, especially if the first-year change is borderline. This assumes that the prostate tumor is slow growing. Indeed, such tumors are generally slow growing, but for reasons not understood at this time, some tumors will quickly become quite rapidly growing. Measuring the change in PSA at yearly intervals assumes that at the time of the first sample the tumor is slow growing and does not become more rapidly growing without being detected until the treatment.12

Isoforms of PSA

It has long been known that PSA exists in both bound (or complexed) as well as free forms. Assays have been developed for both cPSA and fPSA to accompany the widely used assays for total PSA (tPSA). TPSA equals the sum of cPSA plus fPSA. Generally, none of these newer assays is requested until the tPSA has been found to be above 3 ng/mL or 4 ng/mL (and less than 10 ng/mL) — then, a free or complexed measurement may be requested. Numerous articles on these assays indicate that the measured value for tPSA is compared to the measured value for fPSA or cPSA; thus, the answer is reported as a ratio (percent) of free or complexed. Some studies have compared several of these assays on the same group of patients. Using any of a number of ratios following a PSA of 4 ng/mL to 10 ng/mL does not substantially add to what the tPSA level has indicated. Currently, there is no consensus as to which of these newer assays, if any, are especially useful in detecting prostate cancer at an early stage.13

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CLINICAL ISSUES

Age-related reference levels

Another issue is the use of age-related reference values. This idea is coupled with the fact that the prostate gland increases in size (and releases more PSA) over time. While increasing the sensitivity, the question must be asked whether the specificity, which is nominally 75% for tPSA, is even that high with these ranges.

For a number of years, whether PSA should be ordered for men at all — and, if so, at what age — has been the center of debate. As of this writing, the Centers for Disease Control and Prevention and the American Cancer Society suggest that men between ages 45 and 50 be considered for the PSA test and counselled as to the meaning of the results, possible treatments, and the “pros and cons” of treatments — but neither group recommends general screening.14,15

BPSA and proPSA

Recently, several intriguing articles16,17 described two distinct molecular forms of fPSA in serum: BPSA and proPSA. BPSA is a form of fPSA that is associated with BPH. The inactive precursor of PSA, proPSA, is associated with prostate tumors. ProPSA consists of native proPSA as well as truncated proPSA forms, [-2]pPSA and [-4]pPSA, which have been shown to be more cancer-associated than native proPSA. Although each form of PSA can range from 0% to more than 50% in individual samples, fPSA in prostate cancer serum contains a median of 28% BPSA and 32% proPSA. Early studies revealed that proPSA significantly increases the specificity for prostate cancer, especially in the 2-ng/mL to 4-ng/mL PSA range. It is estimated that 20% to 30% of men with PSA values from 2 ng/mL to 4 ng/mL have prostate cancer. At 90% sensitivity, the specificity for proPSA was 25%, compared to 10% for %fPSA and cPSA. ProPSA was superior to %fPSA and cPSA in the 4-ng/mL to 10-ng/mL PSA range. Perhaps proPSA represents a more cancer-specific form of PSA that better discriminates prostate cancer from benign prostatic hypertrophy.

The future with proteomics

Early in the “genomic era,” new technologies were evolving with the possibility that significant tools would be developed for detection — patient-specific targeted therapeutics with reduced toxicity and increased efficacy. To that end, technologies such as laser capture microdissection, or LCM, may provide unparalleled access to the purified diseased human cells directly from tissue specimens. Proteomics (the use of rapid, high-throughput mass spectrometric-based fingerprints of widely to globally expressed peptides and proteins) may prove to be valuable for new molecular classification of human tumors and disease stages. For cancer detection and drug development, the important biomarkers found by surface-enhanced laser desorption and ionization time of flight (SELDI-TOF)-based pattern-recognition analysis are mostly low molecular-weight fragments produced at the specific tumor microenvironment.

As an example, in one recent study, results derived from the serum analyses from 76 patients with breast cancer and 54 unaffected women were analyzed by two-dimensional (2-D) electrophoresis and matrix-assisted SELDI-TOF. One marker, HSP27, was found up-regulated, while a second (14-3-3 sigma) was down-regulated in the serum of breast-cancer patients. The two protein biomarkers were then used to classify an independent set of 104 masked serum samples. The results showed that the protein pattern on 2-D gels can completely segregate the serum of breast cancer from noncancer. The discriminatory pattern correctly identified all 69 breast-cancer cases in the masked set. Of the 35 cases of nonmalignant disease, 34 were recognized as noncancer. These encouraging findings suggested to the authors that a prospective population-based assessment of proteomic technology as a screening or diagnostic tool for breast cancer in high-risk and general populations should be undertaken.18 Lung cancer is presently the number one cause of cancer death in the United States, and no biomarker is yet available to detect early lung cancer in serum samples. In an independent set of masked serum samples from 15 lung cancer patients and 31 healthy individuals, SELDI analysis yielded a sensitivity of 93% and a specificity of 97%. This study also suggests that serum is a capable resource for detection of specific lung-cancer biomarkers. SELDI technique, combined with an artificial intelligence classification algorithm, can facilitate the discovery of better biomarkers for lung cancer and provide a useful tool for molecular diagnosis in the future.19

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Over the past few decades, beginning with CEA and then PSA, the application of serum markers for tumors has grown considerably. New markers have been developed that add to the clinicians' armamentarium and have often led to an earlier diagnosis and better treatment. As the laboratory continues to work with clinicians and researchers, expectations remain high for newer assays and newer ways to perform them. The decoding of the human genome and proteomic technology will be at the forefront of these new, exciting developments.20

David Plaut is a clinical chemist and statistician who has worked in and written about the clinical laboratory science field for more than 40 years. Part II concludes the three-part series “Tumor markers from A to Z.”

References