Part I: Tumor markers from A to Z

By David Plaut, PhD

The applications of CA 15-3, CA 19-9, CA 27-29, CEA, the estrogen receptors, and HER2/neu are the focus of our continuing discussion of tumor markers and how assays have been used clinically to aid practicing physicians. Keep in mind that different reagent sets may give different results in the same patient, that cancer cells almost by definition are mutant cells and may continue to mutate, producing various isoforms of markers, and that tumors grow at different rates and, therefore, the tumor burden can change rapidly.

CA 15-3, CA 27-29, and CEA

As yet, the etiology of breast cancer is not known, but significant advances have been made in the area of early detection and treatment monitoring with cancer antigens (CAs). The CA 15-3, CA 27-29, and CEA serum markers, together with HER2/neu, are often used as markers for breast cancer. Although CA 15-3, CA 27-29, and CEA assays are not recommended for the early detection of breast cancer because of their comparatively low sensitivity and specificity levels, they do have applications for monitoring and detecting recurrence in patients treated for Stages II and III.

The main reasons CA 27-29 is not recommended as a screening test are its low clinical sensitivity (ability to correctly identify patients with disease) for early detection and its low clinical specificity (ability to correctly identify disease-free patients). The clinical specificity of CA 27-29 is as low as 90%, yielding a high number of false-positive results. False-positives in cancer-detection assays can lead to more invasive diagnostic techniques and often to unnecessarily heightened concern on the patient’s part.

According to Bast, et al, CA 125 has been applied in ovarian cancer; data also indicates it has some utility in pancreatic cancer. As with the other markers, CA 125 generally has a relatively low level of clinical sensitivity, especially in Stage I, suggesting it is inadequate for detecting early-stage cancer, but useful in monitoring treatment.1

Combinations of markers

Although combinations of markers have been used, the rather high frequency of false-positive results (low specificity) is the most likely reason that more tumor-marker profiles are not used. If a profile of tests A, B, and C is considered, with each test having a false-positive rate of 6%, 83% would be true positives on all three tests; the remaining 17% would be false positives on one or more of the other tests. Even if the number of true positives were increased, the numbers would not be additive because of the fact that some patients would be positive for two or three markers. On the other hand, the advent of advanced molecular-biology techniques, mapping of the human genome, and the availability of high-throughput genomic and proteomic strategies open up new opportunities and will potentially lead to the discovery of novel biomarkers for early detection and prognostication of breast cancer.

Currently, many biomarkers — particularly the hormonal and epidermal growth-factor receptors — are being utilized for breast-cancer prognosis, none of which, unfortunately, has sufficient diagnostic, prognostic, and/or predictive power across all categories and stages of breast cancer. More useful information can be generated if tumors are interrogated with multiple markers. Choosing the right combination of biomarkers is challenging, because multiple pathways are involved (up to 62 genes and their protein products are potentially involved in breast-cancer-related mechanisms). And, both time and cost increase as more markers are evaluated.

Bast, et al, points out that ovarian cancer has been one of the areas in which markers have been combined to increase sensitivity. 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, which is 99% for CA 125 alone, compared to 89% for the combination.1

CA 19-9 has been used mainly in detection and monitoring of pancreatic cancer. In pancreatic carcinoma, CA 19-9 elevations will be found in 70% to 80% of patients, compared 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 will be found. CA 19-9 elevations, like CEA elevations, are found in noncancerous conditions, such as acute hepatitis, chronic active hepatitis, pancreatitis, and inflammatory diseases.

Serum HER2 elevation

Winston, et al, write that the HER2/neu oncogene is located on chromosome 17q and encodes a transmembrane glycoprotein with intracellular tyrosine kinase activity. Several studies have shown an association of HER2 gene amplification or protein overexpression with prognosis and predictor of therapeutic response. Most important, the presence of...
amplification or overexpression is the basis of eligibility for trastuzumab therapy, which blocks the effects of the growth-factor protein HER2/neu, which, in turn, transmits growth signals to breast-cancer cells.

Presently, a number of commercially available assays are available for serum HER1 and HER2 (sHER1 and 2) — two members of a family of proto-oncogenes, also known as Erb-B1 and Erb-B2. While HER2/neu gene amplification and/or protein overexpression is detected in approximately 25% of primary breast cancers, according to Luftner, et al, serum HER2/neu levels are elevated beyond the upper limit of normal in 50% to 60% of Stage IV breast-cancer patients.

Post-treatment sHER2 is an independent prognostic factor enabling the identification of patients likely to benefit from aggressive adjuvant treatments. As an example of its utility, in a median follow up of 7.7 years, sHER2 levels of 701 consecutive patients with Stage I, II, and III tumors were assayed by an ELISA. The pre-treatment sHER2 concentration range was 3.15 ng/mL to 82.00 ng/mL. Forty-seven patients (6.7%) had sHER2 concentrations >12 ng/mL (cutoff level). The pre-treatment level of sHER2 correlated positively with CA 15-3 as well as with the pathological tumor size, the number of invaded lymph nodes, and histological grading.

A statistical analysis indicated that pre-treatment sHER2 was of prognostic value for contralateral breast cancer, metastasis-free survival — particularly lung and liver metastases — and overall disease-specific survival. When combined with estradiol or progesterone-receptor status, patients with elevated sHER2 and receptor-negative tumors had a significantly shorter disease-specific survival for both receptors.

Saghatchian, et al, state that serum HER2 elevation in early breast cancer correlates with the principal criteria of tumor aggressiveness, thus permitting selection of patients with a high risk of visceral metastases and contralateral breast tumors. These data suggest that post-treatment sHER2 is an independent prognostic factor enabling the identification of patients likely to benefit from aggressive adjuvant treatment.

In a recent study by Fehm, et al, of 120 metastatic breast-cancer patients, 39% had elevated sHER2 levels. The positivity rate of CA 15-3 was 51%.

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Immunohistochemical analysis and FISH assays

It appears that the most useful markers, especially to predict response to hormone-therapy markers in breast cancer, are the estrogen and progesterone receptors (ER and PR, respectively). Duffy, et al, point out that both American and European expert panels have recommended routine determination of these steroid hormone receptors in all patients with breast cancer. A number of characteristics of breast cancer — including tumor burden, pathologic category, and axillary node status — are useful in assessing differences in the growth rates and metastatic potentials. In addition to these are the steroid receptors that are helpful as predictive factors of response to therapies such as tamoxifen. In general, those patients with ER-positive tumors have, at least over the short term, a better prognosis. Approximately 75% of ER-negative and as many as 30% ER-positive patients will relapse with metastatic disease and die within 10 years. Recent work has found that these receptors exist in multiple forms (as many as 20 such variations exist). In order to assess the clinical potential for these, new assays will have to be developed.

Winston, et al, note that several methods of determining HER2 status exist, each measuring a different aspect such as DNA content, gene copy number, protein expression, expression of RNA, and circulating HER2 extracellular domain protein, but no consensus exists with regard to the optimal test for HER2 assessment. The most widely used assays are immunohistochemical-analysis and fluorescence in situ hybridization (FISH), which measure protein expression and gene amplification, respectively. Based on current data, FISH is the most accurate and reproducible test with a better correlation with prognosis and response to therapy.

These immunohistochemical-analysis and FISH assays may become potential markers for breast and ovarian cancers, especially in the detection of pre-clinical cancer, in patients at risk for cancer, in the diagnosis of a detectable mass, in monitoring therapy, and in predicting response to and outcome of the disease.

Effective biomarkers for clinical lab effectiveness

The factors that make serum markers useful can be classified according to their involvement in the main alterations characterizing tumor cells: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis, as shown in Coradini and Daidone. Despite relevant research efforts and the identification of many reputedly good prognosticators, few of these factors are proving clinically useful for identifying patients at minimal risk of relapse, patients with a worse prognosis, or patients likely to benefit from specific treatments. Coradini and Daidone point out that novel findings derived from gene-expression analysis indicate that the simultaneous consider-
ation of molecular alterations contributing to the hallmarks of cancer might provide clinically useful prognostic, and perhaps therapeutic, information.

"Most of (the markers)," say Coradini and Daidone, “such as HER2/neu, epidermal growth factor receptor, cyclin E, p53, bel-2, vascular endothelial growth factor, urokinase-type plasminogen activator-1, and the recently discovered anti-apoptosis protein survivin, are suggested for possible inclusion in the category of biomarkers with a high level of clinical-laboratory effectiveness. No single biomarker, however, was able to identify those patients with the best (or worst) prognosis or those which would be responsive to a given therapy.”

**Note:** A recent set of guidelines for the application of tumor markers can be found at the American Society of Clinical Oncology website, [www.asco.org](http://www.asco.org).

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 of “Tumor markers from A to Z” will appear in MLO’s November 2004 issue.

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### References


**Correction:** In the “Introduction to Tumor Markers from A to Z” in MLO’s August 2004 issue, the measurement (1*10^-9m) should have read (1*10^-9m). We apologize for any confusion this error caused.