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**Learning Outcomes**

Upon completion of this exercise, the participant will be able to:

- Discuss the use of CA 15-3 and CA 27.29 levels in monitoring patients undergoing treatment for breast cancer and for the detection of recurrent and metastatic disease.
- Compare the composition of immunoassays for the measurement of CA 15-3 and CA 27.29.
- List sample handling and storage requirements for specimens to be analyzed for CA 15-3 and/or CA 27.29.

Except for nonmelanoma skin cancers, breast cancer is the most common cancer among women. An estimated 216,000 new cases of invasive breast cancer will be diagnosed among women in the United States this year. Currently there are slightly more than 2 million women in the U.S. who have been diagnosed with and treated for breast cancer, and as many as 40,000 of these women will die in 2004. Early detection and treatment has caused a decrease in breast cancer death rates, but a need for a sensitive and selective breast cancer tumor marker still exists. Many such markers have been investigated with CA 15-3 and CA 27.29 being the markers in blood most currently used.

CA 15-3 and CA 27.29 are carbohydrate-containing protein antigens called mucins. Mucins are large transmembrane glycoproteins classified into 7 families, MUC1 to MUC7. CA 15-3 and CA 27.29 both belong to the MUC1 family. Although the MUC1 gene is found in several tissues, it produces an apparently identical core protein. The variation in the extent of glycosylation (carbohydrate content) is the distinguishing feature between different tissue sources. In breast tissue, the carbohydrate content is approximately 50%. The exact physiological functions of MUC1 proteins are not completely known, but it appears to reduce cell-to-cell interaction and may also inhibit tumor cell cytolysis. The MUC1 gene is frequently over expressed in malignant breast tumors, allowing use of gene products CA 15-3 and CA 27.29 as tumor markers for breast cancer.

The names of these markers are derived from a combination of the molecular structure and the assays developed for their detection. The abbreviation CA originally denoted Carbohydrate Antigen, but has become Cancer Antigen in common usage. The numbers 15-3 and 27.29 refer to the antibodies used in immunoassays for these antigens. The assay for 15-3 includes a monoclonal capture antibody named “115D8” bound to a solid phase and a monoclonal signal antibody name “DF3.” Assays for CA 27.29 are competitive inhibition assays that use CA 27.29 antigen bound to a solid phase and a monoclonal signal antibody named “B27.29” that recognizes the protein core of the MUC1 antigen. CA 27.29 is sometimes referred to as BR 27.29. The commercial immunoassays available differ in the nature of the signal antibody (enzymatic, chemiluminescent, radioisotopic, etc.) employed. Both CA 15-3 and CA 27.29 assays detect products of the MUC1 gene, but the antibodies in the assays recognize different portions (epitopes) of the core protein gene sequence. Thus, the assays are highly correlated but there may be differences in individual patient results due to antibody specificity and other factors.
Assays for both CA 15-3 and CA 27.29 are approved by the FDA for the detection of breast cancer recurrence. Neither assay is approved nor effective for use as a screen for breast cancer. Elevations of these markers are not organ-specific and have been observed in patients with colorectal, lung, ovarian, prostatic, and pancreatic cancer, as well as in patients with hepatitis, cirrhosis, sarcoidosis, tuberculosis, and systemic lupus erythematosus. Also, it has been shown in several studies that preoperative CA 15-3 and CA 27.29 concentrations are rarely elevated in patients with primary breast cancers. Approximately 21% of patients with early breast cancer (stages I-III) have an elevated CA 15-3 concentration and 9% of patients with benign breast disease are positive (using a concentration of >25 U/mL as the cutoff). There is some evidence that CA 27.29 may be a more sensitive but less specific marker than CA 15-3, but this has not been definitively demonstrated and it is generally felt that they are essentially equivalent for most clinical purposes. It has been shown that the serum concentration and the proportion of patients with elevated values of these markers do tend to increase with the severity (stage) of the disease and/or size of the tumor. Because of the low sensitivity and lack of specificity, these assays are not recommended by any group, including the American Society of Clinical Oncology (ASCO), the European Group on Tumor Markers (EGTM), the National Academy of Clinical Biochemistry (NACB), and the French Standards, Options, and Recommendations project (SOR) for screening, diagnosis, or staging of breast cancer.

Although these markers are not recommended for screening, diagnosis, or staging, they do have a role in the monitoring of treatment response and in the detection of recurrences in patients with previously treated stage II and III carcinomas that are clinically free of disease. Although the FDA approved CA 15-3 and CA 27.29 for the detection of breast cancer recurrence, the recommendations of the different groups cited above vary on this use. The ASCO guidelines support use of these markers to suggest treatment failure in the absence of readily measurable disease but do not recommend their use alone for monitoring response to treatment. All of the groups’ guidelines urge caution when using these markers as an aid in monitoring the clinical course of breast cancer patients.

Several studies have demonstrated the use of CA 15-3 and/or CA 27.29 in detecting early recurrence of breast cancer, particularly metastatic disease. Elevated levels have been correlated to subsequent development of metastatic disease. In one study a CA 15-3 level >86 U/mL had a positive predictive value of 100% for detection of metastatic disease. Another study demonstrated that the CA 15-3 levels at the time of first recurrence correlated to patient outcome with patients having a level <30 U/mL surviving significantly longer than those with higher concentrations. The EGTM guidelines suggest that the sensitivity of CA 15-3 for the detection of metastatic breast cancer can be increased by combining its use with that of CEA. There is conflicting evidence, but some groups have reported that the simultaneous use of both CA 15-3 and CEA allows the detection of early recurrence in more patients than the use of CA 15-3 alone. The SOR guidelines stipulate that CEA should only be used when the CA 15-3 is not elevated.

CA 15-3 and CA 27.29 levels are currently used to monitor response to therapy (chemo, hormonal, or radio-). The change in CA 15-3 levels is a better indicator than the absolute value. A decrease of >25% in CA 15-3 is associated with a response to therapy and a 50% decrease was shown to have a positive predictive value of 77% and a negative predictive value of 98%. An increase of >25% is associated with disease progression and a poor response to therapy. An increase or decrease of <25% is associated with stable disease. During the first few weeks of therapy a spike in CA 15-3 levels may occur, but this does not necessarily indicate treatment failure or effectiveness. Clinicians are cautioned to wait a minimum of a few weeks after starting therapy to begin monitoring CA 15-3 or CA 27.29 levels. In one study the duration of this spike ranged from 31-101 days.
Analysis of either CA 15-3 or CA 27.29 should be preformed after prompt separation from the clot. Both are stable in serum for 24 hours when stored at 4°C and it is recommended that samples be stored at -20°C (short-term) or -70°C (long-term) for possible future re-analysis.

Good agreement between the different commercially available CA 15-3 assays has been demonstrated. This is not surprising because they all utilize the same 2 monoclonal antibodies and the same standards. Despite this correlation, values in the literature for the upper limit of normal or cut-off point range from 20-40 U/mL and at least one study has indicated that this point may be method-dependent. The variation or critical difference between successive CA 15-3 determinations may be more important than the exact cut-off point utilized. Based on both analytical imprecision and individual biological variation, one group calculated that successive CA 15-3 levels should differ by at least 30% if they are to be regarded as significantly changed. As previously mentioned, results between CA 15-3 and CA 27.29 correlate well and respond similarly in patients with breast cancer.

In summary, no effective tumor marker for screening, diagnosis, or staging of breast cancer has been discovered. At present CA 15-3 is the most widely used circulating marker for breast cancer and is the assay against which new marker assays are judged. CA 15-3 and CA 27.29 are currently used for the monitoring of treatment response and to monitor patients for recurrent breast cancer, particularly metastatic disease.

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