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# Measurement of Four Tumor Marker Antigens in the Sera of Pregnant Women

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We sought to determine the maternal serum levels of four tumor-associated antigens during the three trimesters of pregnancy in healthy women. CEA, CA 228, CA 15-3, and Her2/neu oncogene product p105 assay values were determined for 90 healthy pregnant women during the three trimesters of pregnancy at five participating evaluation sites. Results were compared to means and cut-off values determined for healthy nonpregnant women. Differences in assay values in the 1st and 3rd trimester were analyzed for statistical significance (Student's *t*-test). CEA, CA 228 and CA 15-3 assay values in general were found to be within the normal range. CA 15-3 and Her2/neu p105 serum assay values were above the cut-off (3.3% and 8.2%, respectively) and were significantly elevated in the 3rd trimester as compared to the 1st trimester of pregnancy ($P < 0.05$ and $P < 0.001$, respectively). CEA and CA 228 may be of potential value in monitoring pregnant women with malignant disease. Normal elevations in 3rd trimester serum Her2/neu p105 and CA 15-3 assay values should be considered when monitoring a pregnant patient with malignant disease. J. Clin. Lab. Anal. 13:35–39, 1999. 

**Key Words:** tumor-associated antigens; pregnancy; immunoassay; tumor markers; healthy women; Muc 1 mucin; NCA 50/90; carcinoembryonic antigen; c-erbB-2

## INTRODUCTION

Tumor markers are currently used to provide an indication of response to therapy and disease progression or recurrence in patients with cancer. A number of biological factors can induce fluctuations in the levels of serum tumor markers. Elevations in serum tumor markers have been reported during pregnancy and menstruation (1). During pregnancy, the proliferation of embryonic tissue and the effect of hormonal influences on the mammary glands can cause significant variations in maternal serum tumor marker levels.

Cancer is a leading cause of death in American women between the ages of 15 and 34, accounting for 19% of deaths, and in women ages 35–54, cancer accounts for 41% of deaths (2). The occurrence of cancer during pregnancy is not rare and it is estimated that 1 in 1,000 pregnant women will develop cancer (2). At present, there is a trend for delaying pregnancy into the later reproductive years and it is expected that cancer in pregnancy will be encountered more commonly in clinical practice (3). Therefore, it is important that the specificity of a tumor marker be evaluated during pregnancy to avoid misinterpretation in the follow-up of a pregnant cancer patient.

The CA 15-3™ assay (Centocor, Inc., Malvern, PA) is a promising tumor marker assay for use in the management of breast cancer (4). CA 15-3 assay values correlate with the extent of disseminated mammary carcinoma (5). Carcinoembryonic antigen (CEA) has found widespread use as a marker protein for monitoring of digestive tract cancers and mammary carcinoma (6,7). The CA 228 assay measures a protein product of the CEA gene family, nonspecific cross reacting antigen 50/90 (8). Elevated levels of serum CA 228 have been found in patients with breast, colon, and lung cancer and patients with leukemia (8). At present, the clinical utility of CA 228 in the longitudinal monitoring of cancer patients is under investigation. The Her-2/neu (c-erbB-2) oncogene encodes a 185 kd protein (p185) which has sequence similarities with the epidermal growth factor receptor (9). Several studies have indicated amplification of the Her-2/neu oncogene and overexpression of the Her-2/neu encoded p185 protein in carcinomas of the breast, ovary, colon and prostate (10,11,12).

As small increases or decreases in tumor marker levels can be indicative of disease progression or early recurrence of disease, fluctuations in tumor marker levels can result in false-positive or false-negative assay values in a given patient. The purpose of our study, therefore, was to determine the normal

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serum maternal levels of these four tumor-associated antigens during the three trimesters of pregnancy, and to determine which tumor markers can be recommended to monitor pregnant patients with previous malignant disease. We chose to evaluate these tumor markers during pregnancy as they have shown potential value in monitoring a variety of cancers that could likely inflict pregnant women.

## MATERIALS AND METHODS

### Tumor Marker Assays

Tumor marker assay values were determined from magnetic separation sandwich immunoassays designed to perform on the fully-automated, random access Bayer Immuno 1™ system. The Bayer Immuno 1 CA 15-3 assay uses two monoclonal antibodies: 115D8 as the capture antibody, and DF3 as the tracer antibody. The Bayer Immuno 1 CEA assay utilizes two monoclonal anti-CEA antibodies in a capture and tracer format. In the Bayer Immuno 1 CA 228 assay, a monoclonal antibody designated 228.2 is the capture antibody and a polyclonal anti-CEA is the tracer antibody. Serum Her-2/ neu p105 values were determined from a method developed by Oncogene Science Diagnostics Inc. (Cambridge, MA). For this assay, two monoclonal antibodies, NB-3 and TA1, are used to quantify p105 antigen, the proteolytic breakdown product corresponding to the extracellular domain of oncoprotein p185. Serum assay results were obtained from singlicate determinations using the four tumor marker assays. Statistical analysis was performed using Student's *t*-test (two-tailed). All assays were performed according to manufacturer's instructions.

### Evaluation Protocol

The study was performed using serum samples from healthy pregnant women at five external evaluation sites: M.D. Anderson Cancer Center (Houston, TX), Memorial Sloan Kettering Cancer Center (NYC, NY), Johns Hopkins Hospital (Baltimore, MD), Hartford Hospital (Hartford, CT), and San Francisco General Hospital (San Francisco, CA). Enrollment of the subjects was both prospective and retrospective. Pregnancies were normal with one fetus in each gestation, and yielded live deliveries without complications or deaths with the exception of three patients whose pregnancies were terminated post sample draw in the first trimester of pregnancy. The ages of the women enrolled in the study ranged from 16 to 42. The subjects were grouped according to the trimesters of pregnancy; 32 women in the 1st trimester, 5 women in the 2nd, and 53 women in the 3rd. The study population consisted of different women in each trimester of pregnancy. Due to sample availability, not every subject was evaluated by each tumor marker assay. Blood samples were obtained at the time of routine examinations and serum samples were kept at –20°C until assayed. The serum samples used for the study were collected under a protocol reviewed and approved by the Institutions Review Board.

## RESULTS

Figure 1 (A through D) presents the tumor marker assay results of the individual pregnant women in relation to the trimester of pregnancy. The means and recommended cut-off values previously calculated from a population of healthy nonpregnant women for the tumor marker assays by the manufacturer are indicated in each graph.

The CA 15-3 assay values determined for the pregnant women were, in general, within the normal range (Fig. 1A). In three patients, CA 15-3 assay values were elevated above the cut-off value of 35 U/mL in the 3rd trimester of pregnancy. The mean CA 15-3 assay values in the 1st and 3rd trimester of pregnancy were 16.76 U/mL and 20.78, respectively. The 1st and 2nd trimester serum CA 15-3 assay levels were similar. CA 15-3 assay values were significantly elevated in the 3rd trimester of pregnancy as compared to the 1st trimester of pregnancy ($P < 0.05$).

The mean Her-2/neu oncoprotein fragment p105 assay values in the 1st and 3rd trimester of pregnancy were 1513 and 2291 HNU/mL, respectively. There was no difference between the 1st and 2nd trimester serum levels. Four patients' Her-2/neu p105 values were elevated above the cut-off value of 2787 HNU/mL (Fig. 1B). These elevations occurred during the 3rd trimester of pregnancy. Her-2/neu oncoprotein p105 serum levels were significantly elevated in the 3rd trimester of pregnancy as compared to serum values in the 1st trimester of pregnancy ($P < 0.001$).

In contrast to the Her-2/neu p105 and CA 15-3 assay values, CEA concentrations in the 3rd trimester of pregnancy were significantly lower than CEA concentrations determined in the 1st trimester of pregnancy; mean values of 0.82 and 1.16 ng/mL respectively ($P < 0.05$). All CEA values, however, were well below the cut-off value of 5 ng/mL (Fig. 1C). CA 228 assay levels were not elevated above the cut-off value of 33 U/mL and similar values were observed for each trimester of pregnancy (Fig. 1D).

The values of these markers showed no correlation with maternal age, and no correlation was found between these markers in the same women.

## DISCUSSION

Tumor markers are currently used to provide an indication of recurrence of disease and response to therapy in the cancer patient. Physiological maternal changes associated with pregnancy and proliferation and differentiation of the fetus can cause elevations in tumor markers (13). These elevations can result in false-positive measurements. Thus, we sought to determine which tumor markers could be of potential value in determining disease status in pregnant women with malignant disease.

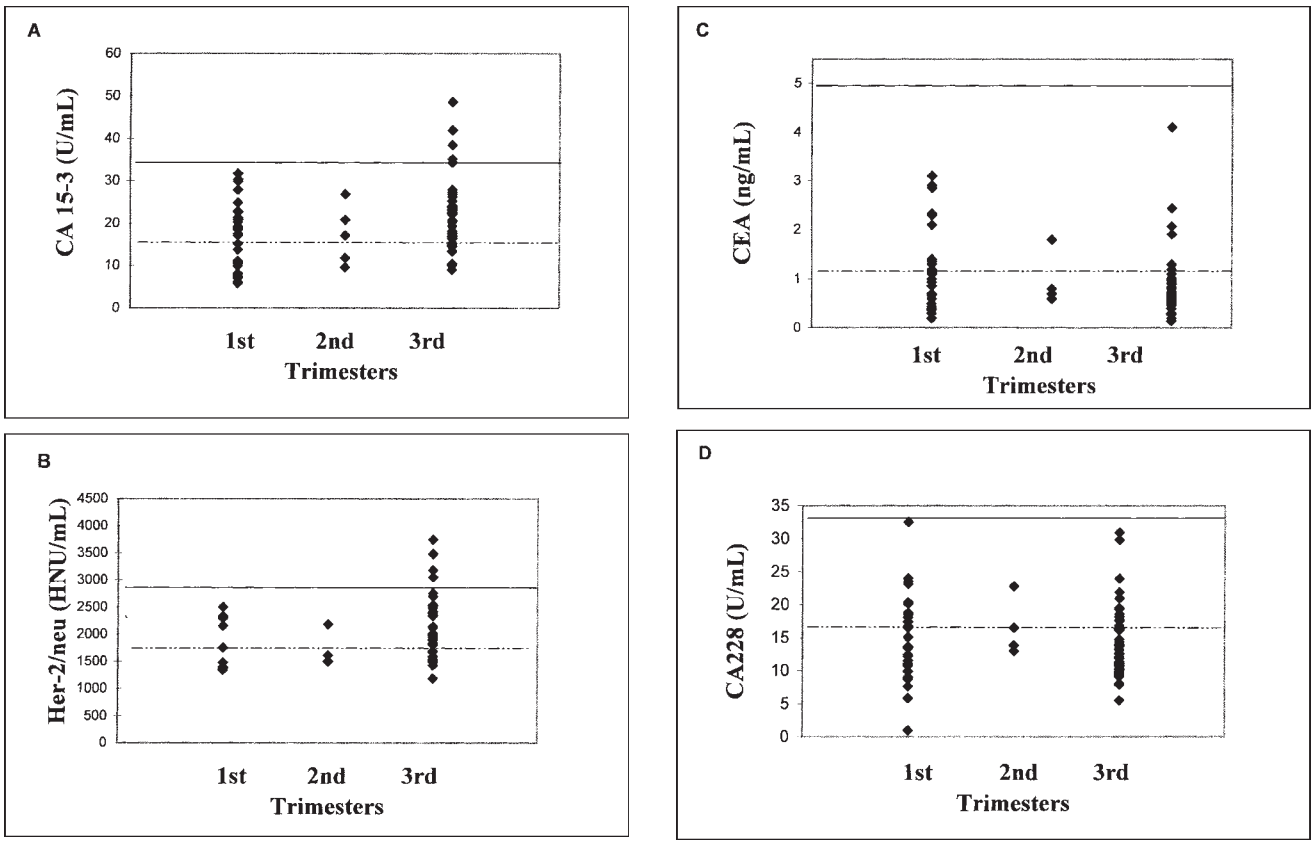


**Fig. 1.** Results of CA15-3 (**A**), Her-2/neu p105 (**B**), CEA (**C**), and CA 228 (**D**) assay measurements in healthy pregnant women during the three trimesters of pregnancy. The means (dashed line) and cut-off (solid line) values are indicated for a population of healthy nonpregnant women. [1 HNU (Her2/neu unit) = 0.05 fmol p105]; 1st trimester, n = 27 for CA 228, n = 32 for CA 15-3 and CEA, n = 11 for Her2/neu; 2nd trimester, n = 5 for CA 228, CA 15-3, and CEA, n = 4 for Her2/neu; 3rd trimester, n = 53 for CA 15-3, CEA, and CA 228, n = 34 for Her2/neu.

Our results demonstrated that 3.3% of the pregnant women evaluated had elevated serum CA 15-3 assay values. Her-2/neu oncoprotein fragment p105 assay values were elevated in 8.2% of the total pregnant women evaluated in the study. These elevations were found during the 3rd trimester of pregnancy. The assay values for these two tumor markers were significantly elevated in the 3rd trimester as compared to the 1st trimester of pregnancy. Tumor marker levels could also be elevated during the 2nd trimester of pregnancy; however, the number of patients studied in this report was too small to determine whether tumor markers are elevated during this time.

Similar findings have been reported regarding the relationship of serum CA 15-3 assay values in pregnancy. Lelle et al. (13) evaluated 107 serum samples from pregnant women and found a 9.4% increase above the cut-off for CA 15-3 assay values; the highest concentrations were observed in the 3rd trimester of pregnancy. Furthermore, Correale et al. (14) studied 65 pregnant women and found that 11% had elevated levels above the cut-off for CA 27.29 (this antigen is believed to be the same antigen encoded by the MUC1 gene); assay values were markedly higher in the 3rd trimester of pregnancy. Touitou et al. (15) found CA 15-3 assay values increased significantly with the stage of pregnancy, although all values were within the normal range. Hayes et al. (16) did not observe an elevation in CA 15-3 assay values in pregnant women, however, the study was small (n = 20), and CA 15-3 assay values were not reported by trimester. Schrocksnadel et al. (17) compared tumor marker values in 50 healthy nonpregnant females with 50 pregnant females. They found that serum CA 15-3 assay values were significantly lower in nonpregnant women as compared to pregnant women.

Studies have also been performed which evaluated tumor marker concentrations in amniotic fluid (13,18). In these studies, CA 15-3 assay values in amniotic fluid were found to be very low throughout gestation. In contrast, we and others have demonstrated that serum CA 15-3 assay values are elevated during the 3rd trimester of pregnancy. These data suggest that elevations of CA 15-3 assay values in maternal serum may result from proliferation of maternal mammary gland epithelium late in pregnancy with enhanced secretion of mucin, as opposed to placental transfer of the mucin.

Her-2/neu p105 results for women in the three trimesters of pregnancy have been reported to give similar results as we observed. Meden et al. (19) in their study of 62 pregnant women found that the highest serum concentrations of the Her-2/neu oncoprotein fragment p105 were in the 3rd trimester of pregnancy. Significant elevations in p105 values were observed in pregnant women as compared to a group of nonpregnant women. In a larger subsequent study, Meden et al. (20) compared p105 serum concentrations in nonpregnant women with serum concentrations in women during the three trimesters of pregnancy. They found a significant decrease in p105 serum levels in the 1st and 2nd trimester of pregnancy compared to nonpregnant women followed by a significant increase in serum concentrations in the 3rd trimester of pregnancy. Additionally, they found that postpartum serum values returned to the levels found in nonpregnant women. Similarly in our study, Her-2/neu p105 assay values were significantly elevated in the 3rd trimester as compared to the 1st trimester of pregnancy. Furthermore, although the number of serum sample measurements was small, we also found that the mean serum Her2/neu oncoprotein p105 serum levels in the 1st trimester of pregnancy were lower than mean assay values calculated for a group of healthy women. The significance of these findings is at present unclear. The 3rd trimester elevations may reflect differentiation and proliferation of embryonic tissue during fetal development. Meden et al. (20) suggests that the early gestational decrease in Her2/neu oncoprotein fragment p105 values may be related to increased levels of maternal estrogen during this period inhibiting p185-expression of maternal epithelial cells.

In our study, maternal serum CEA levels were well below the cut-off value of 5 ng/mL throughout gestation. In agreement with our findings, Lelle et al. (13) in their study of 107 pregnant women found no significant elevations in maternal serum CEA levels throughout gestation; all values were within the normal range. Touitou et al. (15) found no differences in maternal CEA levels for the three stages of pregnancy and all levels were within the normal range. Schrocksnadel et al. (17) reported no differences in serum CEA levels among 50 healthy pregnant and nonpregnant women. However, an early study described a 12% increase in serum CEA values in 86 pregnant women above normal limits for serum of healthy blood donors (21).

CA 228 levels showed no significant changes in pregnant women, irrespective of trimester. These results are similar to those observed with CEA and may be expected since NCA 50/90 is a member of the CEA gene family.

The present study suggests that CEA, CA 228, and the CA 15-3 assays can be used to monitor disease if a pregnant woman has cancer. Normal elevations in CA 15-3 assay values in the 3rd trimester of pregnancy should be taken into consideration when evaluating patients to avoid misinterpretation of patient disease status. However, fluctuations in Her-2/neu p105 assay values throughout pregnancy were observed in this study and those of others. These findings suggest that the variability of this tumor marker during pregnancy can confound interpretation of patient disease status and therefore cannot be recommended to monitor disease in the pregnant cancer patient.

In this report, we have analyzed the results of four tumor marker assays determined from the same serum samples in healthy pregnant women. Our findings are in agreement with previous reports which evaluated CA 15-3, Her2/neu, and CEA tumor marker assays in healthy pregnant women. We reported, for the first time, the results of pregnant serum samples using the CA228 tumor marker assay. Furthermore, this report has summarized the current status of these four tumor marker antigens and their potential value in determining disease status in the pregnant cancer patient. Future studies should evaluate serum tumor marker levels in pregnant subjects with malignant disease to elucidate the real value of these tumor markers in determining disease status in the pregnant cancer patient.

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