nin antibody is cytotoxic to malignant cells *in vitro* in concentrations of picograms/cell. In a 20-year study involving several hundred physicians and three independent laboratories in the U.S., and three hospitals and one laboratory in the U.K., we have found that the concentration of antimalignin in serum, in μg/ml, (1) of normal healthy non-tumor-bearing humans increases moderately each decade between the third and the seventh, as the risk of cancer increases ($p < 0.001$; $N = 1972$), (2) increases earlier and more markedly in as yet apparently unaffected members of high-risk cancer families ($p < 0.001$; $N = 1106$), and (3) is markedly increased in concentration in human serum within weeks of the occurrence of malignant transformation and returns to normal within 3 months of successful treatment ($p < 0.001$; false positives and false negatives <5% on first determination, <1% on repeat determination; $N = 600$).

Quantitative determination of serum antimalignin antibody is therefore of interest for use as a non-invasive biomarker to indicate successful results in breast cancer chemoprevention trials.

In addition, purified antimalignin antibody (MTAG), due to its demonstrated specificity in fluorescent and other chromogen staining of cell membranes with exposed malignin epitopes, is applicable for use alone or as part of a battery of pre-dysplasia or dysplasia-based surrogate endpoint biomarkers in both individual and computerized cytometry.

Validation of the Use of C-2/C-16α Estrogen Metabolites as Markers for the Action of Chemopreventive Agents in the Prevention of Breast Cancer

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Abstract Studies from this laboratory have demonstrated that negative modulation of the C-2/C-16α ratio of estradiol metabolites serves as a marker of the action of oncogenes and carcinogens which increase tumorigenicity, while positive modulation of this ratio measures the preventive effects of chemotherapeutic and chemopreventive agents on tumors and tumor cells. In order to facilitate human studies on chemopreventive agents and facilitate the measurement of this ratio, we have validated an ELISA assay using monoclonal antibodies developed by Immunocare, Inc., coated to 96-well plates.

Urine samples (10λ) were diluted in buffer and hydrolyzed with mixed glucuronidase and sulfatase to cleave the conjugates. Aliquots of the hydrolysate were added to ELISA plates coated with the C-2 and C-16α antibodies respectively and the appropriate labeled antigens were added. After incubation the plates were washed, the color reagent added, and the plates read kinetically to determine the amount of compound present. A standard curve is run on each plate along with high and low standards. All samples were run in triplicate and the mean values determined. The ratios were computed automatically by the reader.

Blind comparisons of duplicate urine samples showed a mean r value of 97%. Mean intra-assay variability was under 8% and inter-assay variability was under 10%.

Studies involving diet modification and the differences between breast cancer patients and controls are underway.
