

PROSPECTIVE EVALUATION OF A NOVEL QUANTITATIVE GALACTOSE OXIDASE-SCHIFF'S REACTION IN NIPPLE ASPIRATE FLUID FROM WOMEN WITH INVASIVE BREAST CARCINOMA

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Background: The galactose oxidase-Schiff's reaction (GOS) is positive in a number of malignant solid tumors. Its clinical value has been limited by subjective interpretation of color. It was our goal to develop a novel technique of quantifying GOS reactivity in nipple aspirate fluid (NAF) of women with invasive breast carcinoma, and to assess its clinical utility in this setting. Methods: Patients with biopsy proven unilateral invasive breast cancer were eligible for entry into this IRB-approved prospective investigation in which NAF was obtained from the breast with cancer and the patient's normal contralateral breast (which served as an internal control) prior to definitive surgery. 16 women had aspirates yielding > 25 uL that was accepted as the minimum required for this test. Nipple aspirate fluid was diluted with phosphate buffered saline with a mixture of protease inhibitors. 10 uL samples were applied to a glass fiber membrane, and incubated with 100 uL of galactose oxidase and 1 mL Schiff's reagent. The stain was developed and the color reaction quantitated by measuring hue (shade) and chroma (intensity) using a spectrophotometer and QA Lite software. GOS testing was done blinded to clinical diagnosis. Statistical analysis comparing NAF from the cancerous breast to the contralateral healthy one was done using SPSS Version 10.1 (Wilcoxin signed rank test). Results: All patients had stage I or II invasive breast cancer, and age of 53.5 ± 14.6 years. We quantified GOS reactivity using two parameters of color, hue and chroma. As chroma varies with concentration, this measurement was adjusted for the concentration of NAF in each sample. The absolute values of hue and chroma were independent of each other, and of protein concentration in NAF. In addition, the hue and chroma values from the cancerous breast did not depend on patient age, race, tumor size, nodal status, tumor grade, histology or ER status. Chroma adjusted for NAF concentration was found to be statistically significantly different between the breast with cancer, and the healthy contralateral internal control, $p=0.026$. Comparisons of NAF parameters between the breast with cancer and the normal contralateral breast are shown in the table. Conclusions: We define the use of a quantitative measure of GOS reactivity based on spectrophotometric measurement of intensity of color, chroma, which is found to be significantly different in the cancerous versus non-cancerous breast in our population of early stage breast cancer patients. Our preliminary results support further exploration of this novel quantitative test in breast cancer screening or early detection of recurrent disease.

Factor	Healthy breast	Cancerous breast	p
Median volume (uL)	60.0 ± 26.0	99.0 ± 23.6	0.056
Protein concentration (mg/mL)	62.6 ± 11.4	60.0 ± 16.4	0.820
Hue	326.5 ± 0.5	327.3 ± 0.5	0.083
Chroma (adjusted for % NAF)	2.9 ± 1.1	1.8 ± 0.3	0.026