Meeting abstract

Relation of hormones in ductal lavage fluid to age, tamoxifen treatment, and breast cancer risk

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Breast ductal lavage provides a source of both cells and fluid from the breast in a non-invasive manner. The fluid contains freely diffusible steroid hormones and other factors that may be secreted or released from the epithelial cells that line the alveoli and ducts of the breast as well as from myoepithelial cells, stomal cells, and immune cells that populate the breast. The purpose of the present study was to determine how perturbations of the system are reflected in ductal lavage fluid (DLF). We examined changes associated with menopausal status, tamoxifen treatment, and Gail model breast cancer risk scores.

Methods
Subjects
Subjects were recruited from the Bluhm Family Program for Breast Cancer Early Detection and Prevention, at the Lynn Sage Breast Center of Northwestern Memorial Hospital. Data on known breast cancer risk factors were used to estimate the 5-year breast cancer risk using the Gail model. Eligible women included (a) unaffected healthy women with a 5-year risk estimate of >1.6 and (b) women with breast cancer who had not received treatment. Only the contralateral breast was sampled in the latter group. The number of patients treated with tamoxifen was 29. A ductal lavage (DL) was performed under local anesthesia in the office setting according to established methods. Women were informed of the cytologic findings and allowed to choose tamoxifen therapy at a dose of 20 mg daily, or observation (OBS group). None had taken tamoxifen (TAM) or dietary supplements for prevention. All subjects were asked to return for DL 6 months after the first procedure (follow-up lavage). The day of the menstrual cycle was estimated in premenopausal women based on the last menstrual period and the typical length of menstrual cycles among women who had had regular menstrual cycles of 26 to 32 days.

Samples and laboratory analyses
The lavage effluent was collected in Cytolyte (Cytyc) and was made up to a final volume of 20 ml. Of this 10 ml was taken for analysis of hormones and other analytes in the fluid. The methanol component was removed in a centrifugal evaporator and sample volume was reduced to 4 ml by lyophillization. Unconjugated steroids were extracted into ethyl acetate-hexane (3:2) and the aqueous fraction was kept for steroid sulfates and proteins. Estrogens were separated from non-phenolic steroids by solvent partition. All analytes were measured by immunoassays. TAM and 4-hydroxytamoxifen concentrations in plasma and NAF were determined by liquid chromatography-tandem mass spectrometry. Data are expressed in terms of the content of the lavage.

Statistical analysis
All data except 5-year breast cancer risk estimates were transformed to their natural logarithms. This provided adequate normalization for parametric analyses. The means, ranges, standard deviations were calculated for

pre- and postmenopausal groups and for TAM and control treatments. The intraclass correlation coefficients (ICCs) were calculated for values between subjects (average of both breasts) between breasts and between ducts (both breasts) for the baseline samples. Relationships between DLF analytes and breast cancer risk estimates were determined by a multiple stepwise backward regression procedure with probability of entry or removal of 0.1 using the SYSTAT v11 statistical package, Richmond, CA.

Results
Variability estimates
Variation among data obtained between breasts and between visits was determined for first visits (prior to treatment) of all patients. The ICCs between visits (six months apart) were generally as low as those between breasts, indicating stability of measurements over time. The between-subject variance represented, on average, approximately 50% of the total variance and was not different between pre- and postmenopausal women.

Pre- and postmenopausal comparison
A comparison of the geometric mean contents of the analytes in DLF from pre- and postmenopausal women (initial visits) was made. Values of androstenedione, DHEA, and DHEA sulfate in postmenopausal women were highly significantly lower than those of premenopausal women (Bonferroni adjusted P values were all <0.001). The mean values for E2 and estrone sulfate were lower in postmenopausal than in premenopausal women (17.4% and 10.7%, respectively) but not significantly so. Differences in other analytes were not significant. The range of natural log of E2 values was 0.45 to 6.91. The natural log of E2 increased significantly with age in DLF of the premenopausal women. The equation with its standard errors was: Age = 1.02 ± 0.48 (ln E2) + 41.6 ± 2.2 years (regression, P = 0.04).

TAM concentrations
Tamoxifen concentrations in plasma were approximately 20-times greater than that of 4-OHT. The ratio of 4-OHT to TAM in the DLF was 4-times higher than that in plasma.

Effect of TAM on hormone concentrations
The median age of women who chose TAM was 54 yr; the median BMI was 27.1 kg/m², not significantly different from those who chose OBS. The mean Gail score was also not different between the groups. TAM significantly increased the amount of androstenedione and DHEA in the lavage fluid (Bonferroni adjusted P values are 0.040 and 0.037, respectively). The unadjusted P value for cathepsin D was 0.039 but after adjustment for the number of comparisons, it was not significant. A paired comparison of 10 subjects who had samples from both initial and 6 months following TAM visits revealed a 3-fold increase

(Bonferroni adjusted P = 0.02) in E2 in DLF during TAM treatment. Other analytes were not different from the group comparisons. Comparisons between first and second visits in the OBS group resulted in no significant differences in any analytes.

Relation of analytes to risk estimates
The association between biochemical DLF factors and Gail breast cancer risk estimates were evaluated by multiple stepwise backward regression. In premenopausal women, DLF analytes accounted for 47.1% of the variability in breast cancer risk estimates. Among the variables, cathepsin D was most significantly related to risk in a negative relationship and DHEA sulfate was next in significance as a positive factor. In postmenopausal women 28.7% of the variability in breast cancer risk scores was accounted for by the analytes in the multiple regression model. Here androstenedione was significantly positively related to Gail scores and DHEA was negatively related.

Relation of estrogen precursors to E2 levels
Androgens and estrone sulfate are potential precursors of E2 in the breast. Overall the squared multiple R value was less for pre- than for postmenopausal subjects, 0.269 vs 0.405, respectively. In premenopausal women DLF estrone sulfate was most highly associated with DLF E2; the association with individual androgens was lower. In postmenopausal women the overall association was higher; androstenedione had the highest association and DHEA sulfate contributed as well.

Summary
These analyses of ductal lavage fluid provide us with a picture of the endocrine environment of the breast which is similar to that provided in our previous studies of nipple aspirate fluid, but we do not see any significant advantages of DLF over NAF in the measurement of these endocrine parameters. However, they support the concept that the local breast environment is a rich source of markers of risk, and measures of the efficacy of preventive interventions which in the future will provide the underpinnings of biologically targeted breast cancer prevention.