Ductal Lavage of Fluid-Yielding and Non–Fluid-Yielding Ducts in BRCA1 and BRCA2 Mutation Carriers and Other Women at High Inherited Breast Cancer Risk

Allison W. Kurian,[1] Meredith A. Mills,[1] Margo Jaffee,[4] Bronislava M. Sigal,[2] Nicolette M. Chun,[1] Kerry E. Kingham,[1] Laura C. Collins,[5] Kent W. Nowels,[3] Sylvia K. Plevritis,[2] Judy E. Garber,[4] James M. Ford,[1] and Anne-Renee Hartman[4]

Division of Oncology, Departments of [1]Medicine, [2]Radiology, and [3]Pathology, Stanford University School of Medicine, Stanford, California; [4]Department of Medical Oncology, Dana-Farber Cancer Institute; and [5]Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Abstract

Objective: **Nipple fluid production and atypical breast duct cells in women at high risk of breast cancer have been associated with further increased risk. Most publications on ductal lavage for cell collection report cannulating fluid-yielding ducts only. We report lavage of fluid-yielding and non–fluid-yielding ducts in women at high inherited breast cancer risk.**

Methods: **A pilot breast cancer screening study including ductal lavage was conducted in 75 women at high inherited risk, 56 (74.7%) of whom had BRCA1/2 mutations. Ductal lavage was attempted in any duct identifiable with a catheter.**

Results: **Ducts were successfully catheterized in 60 of 75 patients (80%). Successfully catheterized patients were younger (median age 41 versus 53 years, $P = 0.0003$) and more often premenopausal (51.7% versus 20%, $P = 0.041$). Thirty-one successfully catheterized patients [51.6%, 95% confidence interval (39.4-63.9%)] had non–fluid-yielding ducts only. Seventeen patients [28.3% (18.5-40.9%)] had atypical cells. Twelve of seventeen [70.6% (46.8-87.2%)] samples with atypia were from non–fluid-yielding ducts. Patients with non–fluid-yielding ducts (versus fluid-yielding ducts) were more likely to have had prior cancer (48.4% versus 17.2%, $P = 0.014$) or chemotherapy (45.2% versus 17.2%, $P = 0.027$); this was also true in patients with atypia from non–fluid-yielding ducts.**

Conclusion: **Successfully lavaged women were younger and more often premenopausal. Atypical cells can be found in non–fluid-yielding ducts in patients at high inherited breast cancer risk. Non–fluid-yielding ducts, and atypia from non–fluid-yielding ducts, are more common in patients with prior cancer and chemotherapy. Larger studies are needed to identify risk factors and prognostic significance associated with atypia and non–fluid-yielding ducts in high-risk populations, and define their role as biomarkers.** (Cancer Epidemiol Biomarkers Prev 2005;14(5):1082–9)

Introduction

Women with an inherited predisposition to develop breast cancer are a group at very high risk of the disease, one which has not been well-served by standard screening techniques. It is estimated that 9,000 to 18,000 cases of breast cancer in the U.S. per year are attributable to inherited risk. A large percentage of such cases are related to deleterious mutations in the breast cancer susceptibility genes BRCA1 and BRCA2, of which some 1 in 500 to 800 American women are estimated to be carriers (1, 2). Lifetime risks of breast cancer in women with BRCA mutations have been reported in the 45% to 82% range (3, 4). A minority of such women choose to undergo prophylactic mastectomy, which is the most effective available preventive method (5-10); bilateral salpingo-oophorectomy (generally done to decrease the high risk of ovarian cancer in BRCA mutation carriers) and tamoxifen are also used as breast cancer–risk reducing strategies in this population (11-15).

For the majority of women with an inherited predisposition to breast cancer who do not choose prophylactic mastectomy, intensive screening is an emerging alternative. Standard mammographic screening has been shown to be of inadequate sensitivity in this group of generally young women (16). A high incidence of interval cancers has been reported with mammographic screening in this population (17). Increasingly, breast magnetic resonance imaging (MRI) is being incorporated, both within and outside of research protocols, as a screening technique in these high-risk women, with encouraging reports of high tumor detection rates at early stages (18-23). We have recently reported on the use of mammography, high-quality breast MRI, clinical breast examination, and ductal lavage as a comprehensive screening protocol for women at high inherited risk of developing breast cancer (24). In this population, we have identified high-risk lesions by MRI screen detection and by cytologic assessment of ductal cells (24). Because of the high cancer risk in these patients, there is great need to develop and validate novel breast screening techniques, with the ultimate goal of improving their breast cancer outcomes through early detection.

Most breast cancers start in the breast ducts, and early, potentially premalignant alterations in the ductal epithelium are beginning to be defined (25-27). For these reasons, evaluation of breast duct cells is an emerging technique for breast cancer risk assessment, and for the discovery of potential biomarkers which may serve as intermediate end points in trials of cancer prevention agents (28-34). Various minimally invasive methods exist for collecting breast duct

Received 10/22/04; revised 12/18/04; accepted 2/23/05.

Grant support: Supported in part by grants from the California Breast Cancer Research Program (to A.R. Hartman, J.M. Ford, and S.K. Plevritis), the California Cancer Research Program (to J.M. Ford), the V Foundation (to J.M. Ford and S.K. Plevritis), and the NIH K23 098246-01 (to A.R. Hartman).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This manuscript has not been published elsewhere; preliminary data were presented in abbreviated form at the American Society of Clinical Oncology Annual Meeting, June 5 to 8, 2004, New Orleans, LA.

Requests for reprints: Allison W. Kurian, Division of Oncology, Department of Medicine, Stanford University School of Medicine, 875 Blake Wilbur Drive, Stanford, CA 94305-5820. Phone: 650-736-7252; Fax: 650-498-5150. E-mail: akurian@stanford.edu

cells, including nipple suction aspiration and random periareolar fine-needle aspiration; the finding of atypia in cells collected by each of these techniques has been associated with increased subsequent breast cancer risk (35, 36). A more recently developed cell collection method, ductal lavage, uses a small catheter inserted into the nipple to collect cells lining the breast ducts (37). Advantages of ductal lavage include higher average cell yield than nipple suction aspiration, and relative anatomic specificity, with the ability to resample a specific abnormal duct over time (24, 37, 38). Potential disadvantages include reports of low cancer detection rate in patients with known malignancy, possibly because of duct occlusion by tumor (39, 40); this finding has led to speculation that ductal lavage may be a more appropriate technique for risk assessment than for cancer diagnosis. In a large, multicenter study evaluating ductal lavage for tolerability and cell yield, ductal lavage was attempted only in those ducts which yielded fluid on nipple suction aspiration (fluid-yielding ducts; ref. 37), a strategy based on previous reports that women with fluid-yielding ducts were at higher risk of breast cancer than those with non–fluid-yielding ducts (35, 41, 42).

Our breast screening protocol for women with high inherited risk of breast cancer, combining annual ductal lavage, breast MRI, mammography, and biannual clinical breast exam, was initiated with a goal of improving early detection of cancer and high-risk breast lesions (24, 38). Early in the course of this study, we observed that a higher proportion of our patients than the 16% reported in previous series (37) did not yield fluid on nipple suction aspiration, and we commenced lavage of non–fluid-yielding ducts, as well as fluid-yielding ducts. We now report results of ductal lavage of fluid-yielding ducts and non–fluid-yielding ducts, with associated reproductive and life-style characteristics, in women enrolled in this breast screening protocol.

Materials and Methods

Patient Population. After study approval by Institutional Review Boards at both centers, in accordance with assurances filed and approved by the Department of Health and Human Services, participants were recruited from cancer genetics clinics at Stanford University School of Medicine and the Dana-Farber Cancer Institute. Inclusion criteria and patient enrollment procedures at both centers were similar. Eligibility criteria at both centers included a documented BRCA1 or BRCA2 mutation; at Stanford University School of Medicine, patients were also eligible if they had no BRCA mutation, but had a >10% risk of developing breast cancer at 10 years based on the Claus model, which incorporates only family history of breast cancer (43-45). If patients had a personal history of breast cancer and no mutation in BRCA1 or BRCA2, the Claus model was used to calculate predicted risk for an unaffected sister; if this risk was >10%, the patient was eligible for participation. Only the unaffected breast was eligible for lavage in patients with prior breast cancer history. Participants had to be at least 25 years of age, or 5 years younger than the earliest age at which a relative was diagnosed with breast cancer. Patients with a history of breast cancer or ovarian cancer had to have completed adjuvant therapy at least 1 year previously. Patients who had had prior breast surgery which seemed to distort the duct system, including incisions near or involving the nipple, were not eligible for ductal lavage of that breast, given our concern for potential increase in infection risk under those circumstances. Informed consent was obtained from all patients, and all study procedures were compliant with regulations of the Health Insurance Portability and Accountability Act of 1996. Alternatives to study participation were offered to all patients.

Screening Protocol. Participants were enrolled in a pilot breast screening study incorporating mammography, MRI, and ductal lavage, with the goal of evaluating these combined techniques for their ability to detect high-risk and malignant breast lesions. The breast screening protocol and its preliminary results have been described in detail previously (24, 38, 46). The protocol included twice yearly clinical breast exam, yearly mammogram, MRI, and ductal lavage. Abnormality detected on clinical breast exam required 3 to 4 months follow-up clinical breast exam or biopsy, as determined by clinical features; further imaging, including ultrasound and additional mammographic views, was done as prompted by clinical findings. Abnormal MRI or mammogram required 6 months of follow-up or biopsy, as determined by radiographic features. Atypical cells on ductal lavage required 6-month interval follow-up ductal lavage and 6-month follow-up MRI of the affected breast. Enrollment began in September of 2001, and accrual continues.

Ductal Lavage Protocol. Participants were anesthetized topically with 4% lidocaine cream applied to the nipple 20 to 30 minutes prior to the procedure. Nipple suction aspiration was done to identify any fluid-yielding ducts. Attempts were made to cannulate any duct, regardless of fluid status, which could be identified using a dilator coated in 1% xylocaine gel, and subsequently a catheter (Cytyc Health Corporation, Boxborough MA; Acueity, Palo Alto, CA). If resistance was met on attempt to catheterize a duct, gentle pressure was applied; if further resistance was encountered, or if the patient experienced discomfort, no further attempt was made to catheterize that duct. Once the catheter was inserted into the duct, 3 to 5 mL of 1% lidocaine was injected, followed by approximately 15 mL of normal saline, in aliquots of 5 mL per injection. Following each injected aliquot of normal saline, breast massage was done and fluid collected via the lavage catheter. The location of each lavaged duct was marked in all cases by assigning a location on a two-dimensional grid, and in most cases by inserting a metal clip provided for this purpose (Acueity) and recording its location via photograph. A cytologic diagnosis of normal cells, insufficient cellular material for diagnosis, mild atypia, marked atypia, or malignant cells was made for each specimen. A representation of a benign and an atypical cytologic reading is presented in Figs. 1 and 2. Time constraints limited attempted cannulation to approximately two to three ducts per breast. Both medical oncologists performing the ductal lavage procedure (A.W. Kurian and A.R. Hartman) and both pathologists interpreting the cytologic specimens (L.C. Collins and K.W. Nowels) were trained by the same methods, as published by Dooley et al. (37).

Statistical Analysis. Univariate analysis of patient characteristics associated with the results of ductal lavage was done using Fisher's exact test for categorical data, and the Mann-Whitney U test for continuous data. All P values are two-sided. Logistic regression was used to identify those variables which are most significant independent predictors of fluid-yielding versus non–fluid-yielding duct status.

Results

Patient Characteristics. Patient characteristics are presented in Table 1. A total of 75 patients underwent attempted lavage; 24 patients were enrolled from the Dana-Farber Cancer Institute, and 51 from Stanford University Medical Center. Comparison of baseline clinical characteristics between patients from the two participating centers revealed no statistically significant differences in median age, BRCA1 or BRCA2 mutation status, prior breast or ovarian cancer, prior chemotherapy or radiation therapy, prior breast biopsy, prior

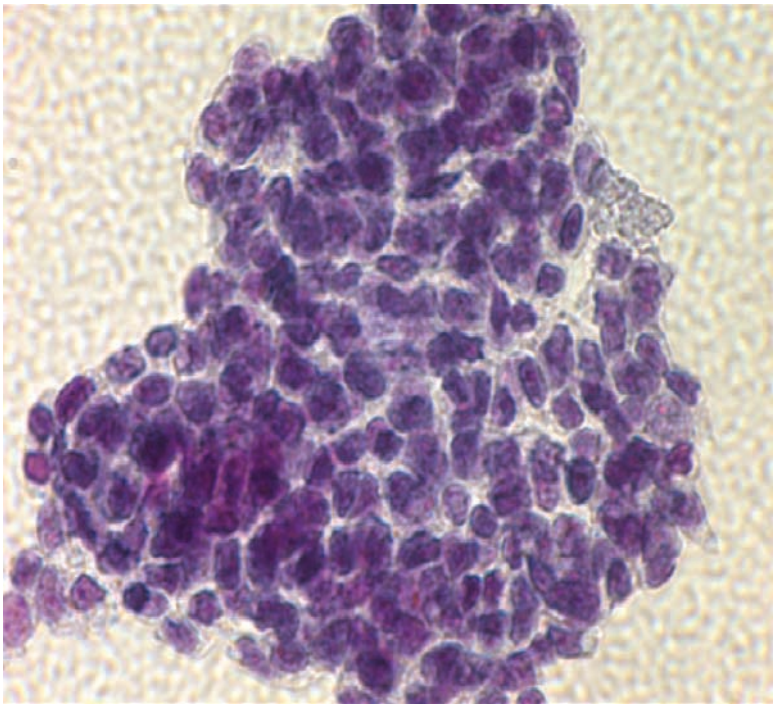

Figure 1. Benign cells from ductal lavage.

use of tamoxifen or other selective estrogen response modulator, hormone replacement therapy, or oral contraceptive pill use, premenopausal status, or fluid yield on nipple suction aspiration. Compared with patients at Stanford University Medical Center, patients at the Dana-Farber Cancer Institute were significantly more likely to be parous (87.5% versus 62.7%, $P = 0.03$), to have breastfed (79.2% versus 43.1%, $P = 0.006$), and to have had a prior bilateral salpingo-oophorectomy (70.8% versus 39.2%, $P = 0.01$, data not shown). A catheter could be inserted into one or more ducts in 60 patients [80%, 95% confidence interval (69.5-87.7%)]. Four patients were African-American, one was Asian-American, and 70 were Caucasian. Given the very small number of patients who were not Caucasian, analyses by race were not done. The median age of all patients in whom ductal lavage was attempted was 43 years. Ductal lavage was considered successful if a catheter could be inserted into a duct, and saline instilled. In all patients who underwent successful

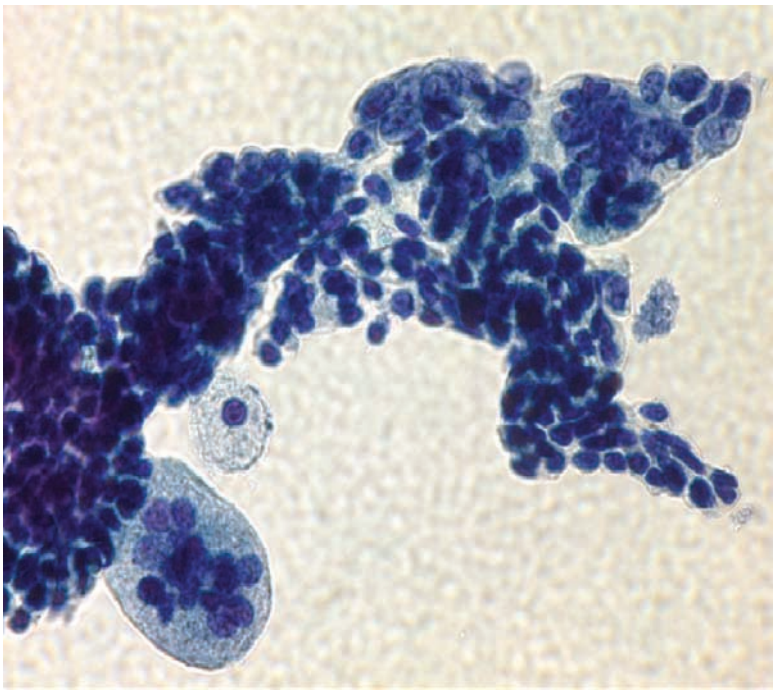

Figure 2. Atypical cells from ductal lavage.

Table 1. Patient characteristics and ductal lavage success

Patient characteristics	Ductal lavage attempted (n = 75)	Ductal lavage successful* (n = 60)	Ductal lavage unsuccessful† (n = 15)	Two-sided P value‡,§
Median age (years)	43	41	53	0.0003
BRCA1	43 (57.3%)	34 (56.7%)	9 (60%)	1.0
BRCA2	13 (17.3%)	11 (18.3%)	2 (13.3%)	1.0
Prior breast cancer	19 (25.3%)	14 (23.3%)	5 (33.3%)	0.51
Prior ovarian cancer	8 (10.7%)	6 (10%)	2 (13.3%)	0.66
Prior chemotherapy	24 (32%)	19 (31.7%)	5 (33.3%)	1.0
Prior breast radiation	12 (16%)	9 (15%)	3 (20%)	0.70
Prior or current selective estrogen response modulator use	15 (20%)	10 (16.7%)	5 (33.3%)	0.16
Prior or current oral contraceptive pill use	62 (82.7%)	50 (83.3%)	12 (80%)	0.72
Prior or current hormone replacement therapy use	20 (26.7%)	15 (25%)	5 (33.3%)	0.53
Parous	53 (70.7%)	43 (71.7%)	10 (66.7%)	0.76
Breastfed	41 (54.7%)	34 (56.7%)	7 (46.7%)	0.57
Premenopausal	31 (41.3%)	31 (51.7%)	3 (20%)	0.041
Bilateral salpingo-oophorectomy before ductal lavage	37 (49.3)	29 (48.3%)	8 (53.3%)	0.78
Prior breast biopsy	40 (53.3%)	32 (53.3%)	8 (53.3%)	1.0
Ever fluid-yielding on suction aspiration	30 (40%)	29 (48.3%)	1 (6.7%)	0.0029

*Ductal lavage considered successful if a catheter could be inserted into one or more ducts, and saline instilled.
† Ductal lavage considered unsuccessful if a catheter could not be inserted into any duct, or saline could not be instilled.
‡ Two-sided P values from Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables.
§For comparison of successful versus unsuccessfully lavaged patients.

catheterization, median age was 41 years, whereas in all patients who could not be catheterized, median age was 53 years (P = 0.0003). Patients who could be successfully catheterized were more likely to be premenopausal than patients who could not (51.7% versus 20%, P = 0.041). Only 1 of the 15 patients who could not be catheterized yielded fluid on nipple aspiration, compared with 29 of the 60 successfully catheterized patients (6.7% versus 48.3%, P = 0.0029). No significant differences in BRCA mutation status, prior breast or ovarian cancer, prior chemotherapy or radiation therapy, prior breast biopsy, prior selective estrogen response modulator use, hormone replacement therapy or oral contraceptive pill use, parity, breastfeeding, or prior bilateral salpingo-oophorectomy were noted between patients who could and could not be successfully catheterized.

Ductal Lavage Cytology. Patient characteristics according to ductal lavage cytology are summarized in Table 2. Eight patients had insufficient cellular material for diagnosis [13.3% (6.7-24.5%)]; compared with the 35 patients with benign cytology [58.3% (45.8-70.0%)], patients with insufficient cellular material for diagnosis were more likely to have had prior breast cancer (62.5% versus 14.3%, P = 0.01), to have had prior chemotherapy (62.5% versus 20%, P = 0.028) or to have taken tamoxifen or another selective estrogen response modulator (50% versus 11.4%, P = 0.028). The median age of patients with insufficient cellular material for diagnosis was 44.5 years, and the median age of patients with benign cytology was 42 years (P = 0.072). Of 8 patients with insufficient cellular material for diagnosis, 1 yielded fluid on nipple suction aspiration, compared with 19 of 35 patients with benign cytology (12.5% versus 54.3%, P = 0.05). Seventeen patients were found to have mildly atypical cytology [28.3% (18.5-40.9%)]. Of 17 patients with atypia, 9 yielded fluid from any duct on nipple suction aspiration [52.9% (31.1-74.0%)]; in 12 of these 17 patients, the ducts which produced atypia were non–fluid-yielding. No significant differences in mean age, BRCA mutation status, prior breast or ovarian cancer, prior chemotherapy or radiation therapy, prior breast biopsy, prior selective estrogen response modulator, hormone replacement therapy or oral contraceptive pill use, parity, breastfeeding, menopausal status, or prior bilateral salpingo-oophorectomy were noted between patients with atypical and benign cytology.

Fluid-Yielding Status. Patient characteristics according to fluid-yielding status are summarized in Table 3. Twenty-nine patients had one or more fluid-yielding ducts on at least one occasion [48.3% (36.2-60.8%)] and 31 patients [51.7% (39.3-63.9%)] had only non–fluid-yielding ducts on all occasions. Patients with non–fluid-yielding ducts were significantly more likely than patients with fluid-yielding ducts to have had prior breast or ovarian cancer (48.4% versus 17.2%, P = 0.014) or prior chemotherapy (45.2% versus 17.2%, P = 0.027). No significant differences in mean age, BRCA mutation status, prior radiation therapy, prior breast biopsy, prior selective estrogen response modulator, hormone replacement therapy or

Table 2. Patient characteristics and ductal lavage cytology results

Patient characteristics*	Insufficient cellular material for diagnosis (n = 8)	Benign cells (n = 35)	Two-sided P value†,‡	Atypical cells (n = 17)	Two-sided P value†,§
Median age (years)	44.5	42	0.072	38	0.63
Prior breast cancer	5 (62.5%)	5 (14.3%)	0.01	4 (23.5%)	0.45
Prior ovarian cancer	0 (0%)	3 (8.6%)	1.0	3 (17.7%)	0.38
Prior breast or ovarian cancer	5 (62.5%)	8 (22.9%)	0.042	7 (41.2%)	0.20
Prior chemotherapy	5 (62.5%)	7 (20.0%)	0.028	7 (41.2%)	0.18
Prior breast radiation	3 (37.5%)	3 (8.6%)	0.067	3 (17.7%)	0.38
Ever fluid-yielding on suction aspiration	1 (12.5%)	19 (54.3%)	0.05	9 (52.9%)	1.0

*There were no significant differences between groups in BRCA mutation status, prior or current selective estrogen response modulator, oral contraceptive, and hormone replacement therapy use, parity, prior breast-feeding, menopausal status, bilateral salpingo-oophorectomy before ductal lavage, and prior breast biopsy.
† Two-sided P values from Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables.
‡ For comparison of patients with insufficient cellular material for diagnosis versus patients with benign cells.
§For comparison of patients with benign cells versus patients with atypical cells.

Table 3. Patient characteristics and fluid-yielding duct status

Patient characteristics	Any fluid-yielding duct (n = 29)	All non–fluid-yielding ducts (n = 31)	Two-sided P value*
Median age (years)	42	40	1.0
BRCA mutation	20 (70%)	25 (80.7%)	0.38
Prior breast cancer	4 (13.8%)	10 (32.3%)	0.129
Prior ovarian cancer	1 (3.5%)	5 (16.1%)	0.196
Prior breast or ovarian cancer	5 (17.2%)	15 (48.4%)	0.014
Prior chemotherapy	5 (17.2%)	14 (45.2%)	0.027
Prior breast radiation	3 (10.3%)	6 (19.4%)	0.47
Prior or current selective estrogen response modulator use	3 (10.3%)	7 (22.6%)	0.30
Prior or current oral contraceptive pill use	25 (86.2%)	25 (80.7%)	0.73
Prior or current hormone replacement therapy use	7 (24.1%)	8 (25.8%)	1.0
Parous	21 (72.4%)	22 (71%)	1.0
Breastfed	15 (51.7%)	19 (61.3%)	0.60
Premenopausal	16 (55.2%)	15 (48.4%)	0.62
Bilateral salpingo-oophorectomy before ductal lavage	13 (44.8%)	16 (51.6%)	0.62
Prior breast biopsy	15 (51.7%)	17 (54.8%)	1.0

*Two-sided P values from Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables.

oral contraceptive pill use, parity, breastfeeding, menopausal status or prior bilateral salpingo-oophorectomy were noted between patients with fluid-yielding ducts and with non–fluid-yielding ducts on univariate analysis. Logistic regression was used to identify independently predictive variables. Forward stepwise selection procedure, starting with the model with no predictors, was used for model-building purposes. Analysis was done using the statistical software S-PLUS version 6.1 for Windows (Insightful Corporation, Seattle, WA). All variables in Table 3 except for premenopausal status were allowed to enter the model; the premenopausal variable was removed because it was perfectly correlated with the bilateral salpingo-oophorectomy variable. As a result of this stepwise procedure, a model with a single predictor, prior breast or ovarian cancer, was selected. This variable had the smallest P value using Fisher's exact test. The same model was selected using backward stepwise selection, starting with a model containing 11 variables (all the variables in Table 3 excluding premenopausal status, prior breast and prior ovarian cancer variables). The variables of prior breast or ovarian cancer and prior chemotherapy were highly correlated: only one woman with prior history of cancer was not treated with chemotherapy, but for all others, a prior history of cancer implied having received chemotherapy. Given this fact, the effects of these two variables were difficult to separate, although both model selection procedures preferred the prior cancer variable.

Atypia and Fluid-Yielding Status. Characteristics of patients with atypia, by fluid-yielding status, are described in Table 4. Of 17 patients with atypical cells, 12 had atypia from non–fluid-yielding ducts only [70.6% (46.8-87.2%)]. Of the remaining five patients, three had atypia from fluid-yielding ducts only [17.7% (5.5-42.1%)] and two had atypia from both fluid-yielding ducts and non–fluid-yielding ducts [11.8% (2.1-35.9%)]. Given the small numbers, patients with atypia from any fluid-yielding ducts [n = 5; 29.4% (13.1-53.7%)] were analyzed as a group. Patients with atypia from non–fluid-yielding ducts only were significantly more likely than patients with atypia from any fluid-yielding ducts to have had prior breast or ovarian cancer (58.3% versus 0%, P = 0.044) or to have had prior chemotherapy (58.3% versus 0%, P = 0.044). No significant differences in mean age, BRCA mutation status, prior radiation therapy, prior breast biopsy, prior selective estrogen response modulator, hormone replacement therapy or oral contraceptive pill use, parity, breastfeeding, menopausal status or prior bilateral salpingo-oophorectomy were noted between patients with atypia from non–fluid-yielding ducts only and patients with atypia from any fluid-yielding ducts.

Discussion

To our knowledge, this is the first characterization of ductal lavage of non–fluid-yielding ducts in high-risk women, with a report on associated atypical cells. The present finding of

Table 4. Patient characteristics and atypia by fluid-yielding duct status

Patient characteristics	Atypia from all non–fluid-yielding ducts (n = 12)	Atypia from any fluid-yielding duct (n = 5)	Two-sided P value*
Median age (years)	39	38	0.96
BRCA mutation†	9 (75%)	2 (40.0%)	0.28
Prior breast cancer	4 (33.3%)	0 (0%)	0.26
Prior ovarian cancer	3 (25%)	0 (0%)	0.51
Prior breast or ovarian cancer	7 (58.3%)	0 (0%)	0.044
Prior chemotherapy	7 (58.3%)	0 (0%)	0.044
Prior breast radiation	3 (25%)	0 (0%)	0.51
Prior or current selective estrogen response modulator use	2 (16.7%)	0 (0%)	1.0
Prior or current oral contraceptive pill use	10 (83.3%)	3 (60%)	0.54
Prior or current hormone replacement therapy use	5 (41.7%)	1 (20%)	0.60
Parous	8 (66.7%)	3 (60%)	1.0
Breastfed	10 (83.3%)	2 (40%)	0.12
Premenopausal	4 (33.3%)	4 (80%)	0.13
Bilateral salpingo-oophorectomy before ductal lavage	8 (66.7%)	1 (20%)	0.13
Prior breast biopsy	7 (58.3%)	1 (20%)	0.29

*Two-sided P values from Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables.
† Of the nine BRCA mutation carriers with atypia from non–fluid-yielding ducts, seven had BRCA1 mutations, and two had BRCA2 mutations; of the two BRCA mutation carriers with atypia from fluid yielding ducts, one had a BRCA1 mutation, and one had a BRCA2 mutation.

atypical cells associated with non–fluid-yielding ducts, at least as frequently as in fluid-yielding ducts, suggests that non–fluid-yielding ducts in women with an inherited predisposition to breast cancer might be associated with higher risk than previously supposed.

Prospective evaluation of outcomes associated with breast duct cytology was first done using nipple suction aspiration (35, 41, 42). Collection of nipple aspirate fluid has been reported in a clinic and population-based sample of women at varying levels of breast cancer risk; women who did not yield nipple aspirate fluid (15% of the studied population) were chosen as the reference group, based on previous observations suggesting that such women would have the lowest risk of breast cancer (47). At a mean 12.7 years of follow-up, relative breast cancer risk of 1.8 was reported in women with normal nipple aspirate fluid cytology, and relative risk of 10.3 was reported in women with atypical nipple aspirate fluid cytology, versus those without nipple aspirate fluid (35). With increased patient numbers and years of follow-up, the authors reported relative breast cancer risk of 1.2 to 1.6 with normal nipple aspirate fluid cytology, and 2.0 to 2.8 with abnormal nipple aspirate fluid cytology, compared with a relative risk of 1.0 in women without nipple aspirate fluid (42). Most subsequent studies of ductal lavage have reported cannulating fluid-yielding ducts only (30, 31, 34), and thus little is known about the prevalence of abnormal cytology from non–fluid-yielding ducts. A recent study reported atypical cells in non–fluid-yielding ducts of lower-risk women (48). Our finding that 12 of 17 patients [70.6% (46.8-87.2%)] with atypical cells produced them from non–fluid-yielding ducts provides evidence that fluid yield is not a prerequisite for cytologic abnormality in high-risk women. It is consistent with a recent report that atypical cells have been collected by random periareolar fine-needle aspiration in patients with non–fluid-yielding ducts on suction (49). It may also provide some explanation for reports of ductal lavage's poor performance as a diagnostic tool in patients with known breast cancer: several of the breast cancer cases considered to have been missed by ductal lavage in a prior publication occurred in patients who had non–fluid-yielding ducts only, in whom ductal lavage was not attempted (40). Given the 2- to 5-fold increase in subsequent breast cancer risk observed in women with atypia in nipple aspirate fluid or on random periareolar fine-needle aspiration (36, 42), our results suggest that non–fluid-yielding ducts should be evaluated when ductal lavage is done in high-risk women. Furthermore, they suggest that reassessment of ductal lavage's performance as a diagnostic tool in women with known breast cancer is warranted, including lavage of non–fluid-yielding ducts as well as fluid-yielding ducts.

One potential explanation for our finding of atypia from non–fluid-yielding ducts might relate to the study population: patients in our breast screening protocol were selected because of their strong inherited predisposition to develop breast cancer. Notably, 7 of the 12 patients with atypia from non–fluid-yielding ducts had mutations in BRCA1, a finding which seems consistent with the high incidence of atypical hyperplasia reported in prophylactic mastectomy specimens of BRCA mutation carriers (50). Patients who carry BRCA1 mutations have a high incidence of estrogen and progesterone receptor–negative tumors; it could be that breast cancer risk in these patients, for which ductal atypia may be a biomarker, is less related to the hormonal factors thought to associate with fluid-yielding ducts than it is in other patient populations. However, one caveat to this hypothesis is the reduction in breast cancer risk seen in BRCA1 mutation carriers after oophorectomy (11-13, 51). It is important to note that the presence of mildly atypical cells collected via ductal lavage has not yet been prospectively associated with increased breast cancer risk, in BRCA mutation carriers or in other patient groups, and that studies with longer follow-up, in larger numbers of women, will be necessary to determine whether such an association exists. It will also be important to determine whether women with atypia from non–fluid-yielding ducts are at risk for different kinds of cancer (for example, a higher incidence of estrogen receptor–negative tumors) than women with atypia from fluid-yielding ducts. If so, then the combination of atypia and fluid-yielding status could have value as a prognostic biomarker, and as a surrogate end point for trials of targeted chemopreventive agents.

On analysis of successfully catheterized patients by fluid-yielding status, factors which differed significantly were: having a history of prior breast or ovarian cancer and having received chemotherapy. History of breast or ovarian cancer remained a significant predictor in multivariate analysis (given the very close correlation between cancer history and chemotherapy, the ability of chemotherapy to add to a model incorporating prior cancer was limited). Consideration of our results and of those previously reported suggests that nipple fluid production may be associated with reproductive and hormonal factors such as ovarian function; our finding of higher breast and ovarian cancer incidence among patients with non–fluid-yielding ducts may reflect the antihormonal maneuvers (selective estrogen response modulator use, bilateral salpingo-oophorectomy, and potential for chemotherapy-induced amenorrhea) used to treat these cancers. The potential relation between fluid-yielding ducts and ovarian function may partially explain the previously observed association of nipple aspirate fluid with increased breast cancer risk (35, 41, 42), given that longer exposure to higher levels of hormones produced by the ovary is likely a mechanism of this observed effect.

Previous authors have reported that age is related to fluid yield (47); a recent Australian study has confirmed the finding of higher fluid-yield and cell count on ductal lavage in premenopausal women (52). Our results show similar trends. The median age of patients who could not be successfully catheterized was 53 years; the median age of patients who could be successfully catheterized, but had only insufficient cellular material for diagnosis, was 44.5 years. Both numbers were larger (in the former case, significantly so) than the median ages of patients who could be successfully catheterized (41 years) or had benign cytology (42 years), respectively. Patients who could not be catheterized were significantly less likely to yield fluid on nipple suction aspiration or to be premenopausal than patients who could. Patients who yielded only insufficient cellular material for diagnosis on catheterization had a higher likelihood of prior breast or ovarian cancer, chemotherapy, selective estrogen response modulator use, and had a nonsignificant trend toward a lower rate of fluid on nipple suction aspiration than patients with benign cytology. Our findings and those of others suggest a decline in patency and fluid production of the ductal system, initially manifested by decreased cellularity of lavage specimens, and associated with falling levels of estrogen and progesterone (which would likely decline after treatment with chemotherapy or selective estrogen response modulators, and with rising age). They are consistent with known proliferative effects of estrogen on the mammary epithelium at various stages in development, as observed in murine models (53). Future studies of high-risk women who are postmenopausal or aged 50 or older should evaluate other methods of assessing ductal cytology, such as random periareolar fine-needle aspiration, which do not rely upon fluid production on aspiration or duct patency. Exploration of methods to increase duct patency, including use of topical nitroglycerin as has been previously reported, might also be effective in cytologic evaluation of this population (54).

Strengths of our study include its prospective, multi-institutional nature. Patients enrolled at the two institutions

had generally similar clinical characteristics; the finding of a higher rate of bilateral salpingo-oophorectomy in the Dana-Farber Cancer Institute group may represent a higher percentage of BRCA1 (70.8% versus 50.9%, $P = 0.14$) and BRCA2 (29.1% versus 11.8%, $P = 0.10$) mutation carriers, although this did not reach statistical significance. The higher rate of parity and breastfeeding among the Dana-Farber Cancer Institute patients might reflect their slightly older median age (45 versus 43 years, $P = 0.22$), a difference which also did not reach statistical significance. Given the overall similarity of populations at both institutions, and the equivalent procedural and diagnostic methods used, these differences seem unlikely to have significantly affected the combined results. One limitation is the absence of a normal control group; future larger studies should include a group of average-risk women who will similarly be evaluated for atypia on ductal lavage, and for subsequent breast cancer incidence. As previously noted, the diagnosis of mild atypia by ductal lavage has not yet been prospectively associated with increased breast cancer risk (unlike atypical findings on nipple aspirate fluid or random periareolar fine-needle aspiration), and artifacts related to the lavage technique cannot be excluded as a contributor to the present findings (35, 36, 42). However, it is notable that a similar atypia rate was found in our population (28.3%) as has been found in other studies of ductal lavage (21%), and random periareolar fine-needle aspiration (24%) among cohorts of high-risk women, which suggests some consistency between these techniques (36, 37). Another limitation is the small number of women of races other than Caucasian, particularly given previous reports of racial differences in nipple aspirate fluid yield (47). Finally, time and technical limitations permitted cannulation of only 2 to 3 ducts per breast, from an estimated total of 6 to 12 (24); uncertainty remains as to whether ductal atypia represents a field effect, involving multiple breast ducts in an affected woman, or is specific to one or a few affected ducts only. With eventual improvements in ductal lavage technology, it is anticipated that the majority of breast ducts in one woman might be lavaged and their cytology compared, which would help to address this question.

The present results show that non–fluid-yielding ducts produce atypical cells in women with an inherited predisposition to develop breast cancer. They suggest that fluid-yielding status is inversely associated with prior cancer and its treatment by chemotherapy (perhaps consistent with an antihormonal mechanism), and that other strategies than ductal lavage may be preferable for cytologic evaluation of postmenopausal women, in whom successful catheterization was less often possible. Future studies of ductal lavage should include evaluation of non–fluid-yielding ducts, and alternate methods, such as random periareolar fine-needle aspiration, for the evaluation of women whose ducts cannot be cannulated, or yield only insufficient cellular material for diagnosis. Longer follow-up of a larger number of patients will be necessary to establish the clinical significance of ductal atypia in women at high inherited risk; we are currently embarked on a prospective, multi-institutional breast cancer screening trial which will address this question.

References

1. Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN. Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst 1995;87:1681–5.
2. Ford D, Easton DF, Peto J. Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 1995; 57:1457–62.
3. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117–30.
4. King MC, Marks JH, Mandell JB; New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643–6.
5. Wagner TM, Moslinger R, Langbauer G, et al. Attitude towards prophylactic surgery and effects of genetic counseling in families with BRCA mutations. Austrian Hereditary Breast and Ovarian Cancer Group. Br J Cancer 2000; 82:1249–53.
6. Meijers-Heijboer H, Brekelmans CT, Menke-Pluymers M, et al. Use of genetic testing and prophylactic mastectomy and oophorectomy in women with breast or ovarian cancer from families with a BRCA1 or BRCA2 mutation. J Clin Oncol 2003;21:1675–81.
7. Bouchard L, Blancquaert I, Eisinger F, et al. Prevention and genetic testing for breast cancer: variations in medical decisions. Soc Sci Med 2004;58:1085–96.
8. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 1999;340:77–84.
9. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. J Natl Cancer Inst 2001;93:1633–7.
10. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2004;22:1055–62.
11. Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002;346:1616–22.
12. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002;346:1609–15.
13. Metcalfe K, Lynch HT, Ghadirian P, et al. Contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. J Clin Oncol 2004;22:2328–35.
14. Narod SA, Brunet JS, Ghadirian P, et al. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Hereditary Breast Cancer Clinical Study Group. Lancet 2000;356:1876–81.
15. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. JAMA 2001;286:2251–6.
16. Tilanus-Linthorst M, Verhoog L, Obdeijn IM, et al. A BRCA1/2 mutation, high breast density and prominent pushing margins of a tumor independently contribute to a frequent false-negative mammography. Int J Cancer 2002;102:91–5.
17. Komenaka IK, Ditkoff BA, Joseph KA, et al. The development of interval breast malignancies in patients with BRCA mutations. Cancer 2004;100: 2079–83.
18. Tilanus-Linthorst MM, Obdeijn IM, Bartels KC, de Koning HJ, Oudkerk M. First experiences in screening women at high risk for breast cancer with MR imaging. Breast Cancer Res Treat 2000;63:53–60.
19. Kuhl CK, Schmutzler RK, Leutner CC, et al. Breast MR imaging screening in 192 women proved or suspected to be carriers of a breast cancer susceptibility gene: preliminary results. Radiology 2000;215:267–79.
20. Warner E, Plewes DB, Shumak RS, et al. Comparison of breast magnetic resonance imaging, mammography, and ultrasound for surveillance of women at high risk for hereditary breast cancer. J Clin Oncol 2001;19:3524–31.
21. Morris EA, Liberman L, Ballon DJ, et al. MRI of occult breast carcinoma in a high risk population. AJR Am J Roentgenol 2003;181:619–26.
22. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. N Engl J Med 2004;351:427–37.
23. Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA 2004;292:1317–25.
24. Hartman AR, Daniel BL, Kurian AW, et al. Breast MRI screening and ductal lavage in women at high genetic risk for breast cancer. Cancer 2004; 100:479–89.
25. Wellings SR, Jensen HM, Marcum RG. An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. J Natl Cancer Inst 1975;55:231–73.
26. Page DL, Dupont WD, Rogers LW. Breast cancer risk of lobular-based hyperplasia after biopsy: ''ductal'' pattern lesions. Cancer Detect Prev 1986; 9:441–8.
27. Stampfer MR, Yaswen P. Culture models of human mammary epithelial cell transformation. J Mammary Gland Biol Neoplasia 2000;5:365–78.
28. Fabian CJ, Kimler BF. Breast cancer chemoprevention: current challenges and a look toward the future. Clin Breast Cancer 2002;3:113–24.
29. Fabian CJ, Kimler BF, Brady DA, et al. A phase II breast cancer chemoprevention trial of oral α-difluoromethylornithine: breast tissue, imaging, and serum and urine biomarkers. Clin Cancer Res 2002;8:3105–17.
30. King BL, Tsai SC, Gryga ME, et al. Detection of chromosomal instability in paired breast surgery and ductal lavage specimens by interphase fluorescence *in situ* hybridization. Clin Cancer Res 2003;9:1509–16.
31. Isaacs C, Cavalli LR, Cohen Y, et al. Detection of LOH and mitochondrial DNA alterations in ductal lavage and nipple aspirate fluids from high-risk patients. Breast Cancer Res Treat 2004;84:99–105.
32. Chagpar A, Evelegh M, Fritsche HA, Krishnamurthy S, Hunt KK, Kuerer HM. Prospective evaluation of a novel approach for the use of a quantitative galactose oxidase-Schiff reaction in ductal fluid samples from women with breast carcinoma. Cancer 2004;100:2549–54.
33. Chatterton RJ, Geiger AS, Khan SA, Helenowski IB, Jovanovic BD, Gann PH. Variation in estradiol, estradiol precursors, and estrogen-related products in

nipple aspirate fluid from normal premenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:928–35.
34. Evron E, Dooley WC, Umbricht CB, et al. Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR. Lancet 2001;357: 1335–6.
35. Wrensch MR, Petrakis NL, King EB, et al. Breast cancer incidence in women with abnormal cytology in nipple aspirates of breast fluid. Am J Epidemiol 1992;135:130–41.
36. Fabian CJ, Kimler BF, Zalles CM, et al. Short-term breast cancer prediction by random periareolar fine-needle aspiration cytology and the Gail risk model. J Natl Cancer Inst 2000;92:1217–27.
37. Dooley WC, Ljung BM, Veronesi U, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. J Natl Cancer Inst 2001;93:1624–32.
38. Hartman AR, Kurian AW, Mills MA, et al. Results from a pilot breast cancer screening trial using a combination of clinical breast exam, mammography, breast MRI, and ductal lavage in a high-risk population. Proceedings of the San Antonio Breast Cancer Symposium [abstract #114]. Breast Cancer Res Treat 2003;82:S1.
39. Brogi E, Robson M, Panageas KS, Casadio C, Ljung BM, Montgomery L. Ductal lavage in patients undergoing mastectomy for mammary carcinoma: a correlative study. Cancer 2003;98:2170–6.
40. Khan SA, Wiley EL, Rodriguez N, et al. Ductal lavage findings in women with known breast cancer undergoing mastectomy. J Natl Cancer Inst 2004; 96:1510–7.
41. Wrensch M, Petrakis NL, King EB, Lee MM, Miike R. Breast cancer risk associated with abnormal cytology in nipple aspirates of breast fluid and prior history of breast biopsy. Am J Epidemiol 1993;137:829–33.
42. Wrensch MR, Petrakis NL, Miike R, et al. Breast cancer risk in women with abnormal cytology in nipple aspirates of breast fluid. J Natl Cancer Inst 2001;93:1791–8.
43. Claus EB, Risch N, Thompson WB. The calculation of breast cancer risk for women with a first degree family history of ovarian cancer. Breast Cancer Res Treat 1993;28:115–20.
44. Berry DA, Parmigiani G, Sanchez J, Schildkraut G, Winer E. Probability of carrying a mutation of breast-ovarian cancer gene BRCA1 based on family history. J Natl Cancer Inst 1997;89:227–38.
45. Parmigiani G, Berry DA, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998;62:145–58.
46. Kurian AW, Mills MA, Nowels KW, et al. Ductal lavage of non-fluid-yielding ducts in BRCA1 and BRCA2 mutation carriers and other women at high genetic risk for breast cancer [abstract]. J Clin Oncol, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 145, 2004;9535.
47. Wrensch MR, Petrakis NL, Gruenke LD, et al. Factors associated with obtaining nipple aspirate fluid: analysis of 1428 women and literature review. Breast Cancer Res Treat 1990;15:39–51.
48. Maddux AJ, Ashfaq R, Naftalis E, Leitch AM, Hoover S, Euhus D. Patient and duct selection for nipple duct lavage. Am J Surg 2004;188:390–4.
49. Sharma P, Klemp JR, Simonsen M, et al. Failure of high risk women to produce nipple aspirate fluid does not exclude detection of cytologic atypia in random periareolar fine needle aspiration specimens. Breast Cancer Res Treat 2004;87:59–64.
50. Kauff ND, Brogi E, Scheuer L, et al. Epithelial lesions in prophylactic mastectomy specimens from women with BRCA mutations. Cancer 2003; 97:1601–8.
51. Pierce L, Levin A, Rebbeck T, et al. Ten-year outcome of breast-conserving surgery (BCS) and radiotherapy (RT) in women with breast cancer (BC) and germline BRCA1/2 mutations: results from an international collaboration. Proceedings of the San Antonio Breast Cancer Symposium [abstract #5]. Breast Cancer Res Treat 2003;82:S1.
52. Antill Y, Murray W, Lindeman G, House C, Phillips G, Mitchell G. Ductal lavage in BRCA1/2 mutation-carriers: initial experience. Proceedings of the San Antonio Breast Cancer Symposium [abstract #4013]. Breast Cancer Res Treat 2004;88:S1.
53. Raafat AM, Hofseth LJ, Li S, Bennett JM, Haslam SZ. A mouse model to study the effects of hormone replacement therapy on normal mammary gland during menopause: enhanced proliferative response to estrogen in late postmenopausal mice. Endocrinology 1999;140:2570–80.
54. Golewale NH, Bryk M, Nayar R, Didwania A, Hou N, Khan SA. Technical modifications of ductal lavage to improve cell yield. Proceedings of the San Antonio Breast Cancer Symposium [abstract #1024]. Breast Cancer Res Treat 2003;82:S1.

Ductal Lavage of Fluid-Yielding and Non–Fluid-Yielding Ducts in BRCA1 and BRCA2 Mutation Carriers and Other Women at High Inherited Breast Cancer Risk

Allison W. Kurian, Meredith A. Mills, Margo Jaffee, et al.

Cancer Epidemiol Biomarkers Prev 2005;14:1082-1089.