The Reliability of Nipple Aspirate and Ductal Lavage in Women at Increased Risk for Breast Cancer—a Potential Tool for Breast Cancer Risk Assessment and Biomarker Evaluation

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Abstract

Purpose: **Ductal lavage, a technique used to sample epithelial cells from breast ducts, has potential use in risk assessment and biomarker evaluation among women at increased risk for breast cancer. However, little is known about the reliability of the procedure.**

Methods: **We evaluated the reliability of nipple aspirate (NAF) and ductal lavage at two time points 6 months apart in women at increased risk for breast cancer. Eligible women had a 5-year Gail risk ≥1.66% or lifetime risk of >20%, and/or a family history or personal history of breast cancer. All ducts that produced NAF were cannulated. The κ statistic was used to evaluate reliability of NAF production, cellular yield, and cytologic diagnosis.**

Results: **Sixty-nine women (mean age, 47 years) were enrolled over 35 months. Forty-seven returned for a second visit. At baseline, 65% of premenopausal and 41% of postmenopausal women produced NAF (P = 0.05), of which 72% underwent successful lavage of at least one duct. Samples of inadequate cellular material for diagnosis were significantly more likely in postmenopausal women than in premenopausal women (P = 0.04). Of the women who returned for a second visit, 18 of 24 who produced NAF had at least one duct successfully cannulated. Twenty-four ducts in 14 women were lavaged twice. Among these ducts, cellular yield for the two time points was inconsistent (κ = 0.33 ± 0.13), and only fair cytologic agreement was observed (κ = 0.32 ± 0.15). Ductal lavage was associated with moderate discomfort.**

Conclusion: **Currently, the use of ductal lavage is limited by technical challenges in duct cannulation, inconsistent NAF production, a high rate of inadequate cellular material for diagnosis, fair cytologic reproducibility, and low participant return rates.** (Cancer Epidemiol Biomarkers Prev 2007;16(5):950–5)

Introduction

Ductal lavage is a minimally invasive technique that has the potential of sampling premalignant and malignant ductal epithelial cells. The procedure involves the insertion of a small microcatheter into a breast duct followed by the gradual infusion of saline (1). After each infusion, the effluent is collected in the same microcatheter by massaging the breast. In a multicenter trial of 507 high-risk women (57% with a prior breast cancer and 39% with a Gail risk of ≥1.7%), Dooley et al. (1) showed that (*a*) adequate cytologic samples of epithelial cells can be obtained from ductal lavage in a large number of women at high risk for breast cancer; (*b*) cytologic samples could be classified into five distinct categories [benign, mildly atypical, markedly atypical, malignant, and having inadequate cellular material for diagnosis (ICMD; <10 cells)]; and (*c*) ductal lavage could be successfully done on fluid-producing ducts in an outpatient setting using topical anesthesia with minimal complications and discomfort.

Data are limited on the reproducibility of nipple aspirate (NAF) and the ductal lavage procedure in women at increased risk for breast cancer. In one study, 23 women with mild or markedly atypical cells at their baseline visit underwent repeat lavage a median of 8.3 (range, 2.3-14.3) months later (2). The diagnosis of atypia was only reproducible in 8 of 42 (19%) fluid-producing ducts. In this article, we report results of a detailed assessment of the reproducibility and acceptability of NAF and ductal lavage in women at increased risk for breast cancer.

Materials and Methods

Study Population. Women at increased risk for developing breast cancer were recruited from the risk assessment/genetic counseling and the breast screening services at Johns Hopkins to take part in this study. Women were considered eligible if they had (*a*) an estimated National Cancer Institute Gail model lifetime risk of developing breast cancer of >20% or a 5-year risk of ≥1.66%; (*b*) a prior history of invasive or noninvasive breast cancer; (*c*) a prior history of breast hyperplasia with or without atypia; or (*d*) a family history of breast cancer defined as a first-degree relative or two second-degree relatives on the same side of the family with a history of breast cancer. All study participants had to be ≥18 years of age, provide written informed consent, have had a normal clinical breast exam, and, for women ≥40 years of age, a nonsuspicious mammogram for malignancy in the 12 months before enrollment. Exclusion criteria included women who were pregnant or lactating within the last 12 months; had an active infection or inflammation of the breast; had received chemotherapy within the past 3 months; had surgery within 2 cm of the nipple; had breast implants or silicone injections; or had a known allergy to lidocaine, marcaine, or prilocaine.

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Study Design. At the time of enrollment, study participants consented to undergo ductal lavage in all fluid-producing ducts from both breasts at two time points 6 months apart and be followed for 2 years. Figure 1 outlines the study design. To minimize the effect of operator variability on the outcome, ductal lavage was completed by the same physician assistant, with the exception of four occasions. The remaining was done by one physician. Follow-up calls were routinely made within 72 h after each procedure to discuss any complications. Participants and their primary care physician were given a copy by mail of their results within 8 weeks of the procedure and abnormal results were discussed by phone by one of the investigators (K.V.). For participants with mildly atypical cells, we suggested a review of mammograms done in the last 6 months, if not a repeat mammogram, ultrasound, and/or breast magnetic resonance imaging. For any participant with markedly atypical cells, they had the option of undergoing a repeat ductal lavage within 3 months of the visit and, in consultation with their physician, were recommended to consider a repeat mammogram, ultrasound, and/or breast magnetic resonance imaging and ductoscopy. Up to four calls were made to schedule the return appointment at 6 months. Study visits took place in the outpatient setting at the General Clinical Research Center at John Hopkins Hospital. The Committee of Human Research at the Johns Hopkins Bloomberg School of Public Health approved the study.

Nipple Aspiration and Ductal Lavage Procedure. Study participants were instructed to drink fluids the day before the procedure. Nipple areas of both breasts were dekeratinized using NU Prep Gel (Weaver & Co.) and sterilized with alcohol. Nipple covers with EMLA cream (Astra Zeneca), a topical anesthetic, was then applied to the areolar complex and a microwaved warm bean bag was placed over both breasts for 45 min. Women were then instructed on how to massage their breasts from the base upwards keeping equal pressure on all sides. If fluid was not obtained by the study participant, the physician or physician assistant would attempt massage. If this was also unsuccessful, nipple aspiration was done using the Cytyc aspirator (Cytyc, Inc.). Ductal lavage was then done in fluid-producing ducts under sterile conditions as previously described by Dooley et al. (1). Where possible, 18 mL of normal saline were infused in 2- to 3-mL increments. As the microcatheter was being removed, a suture thread was temporarily inserted into the duct to mark its position and a photograph taken and the location of the duct was recorded on an 8×8 grid. This was done to ensure proper location for the 6-month repeat lavage. A separate catheter was used to cannulate each duct.

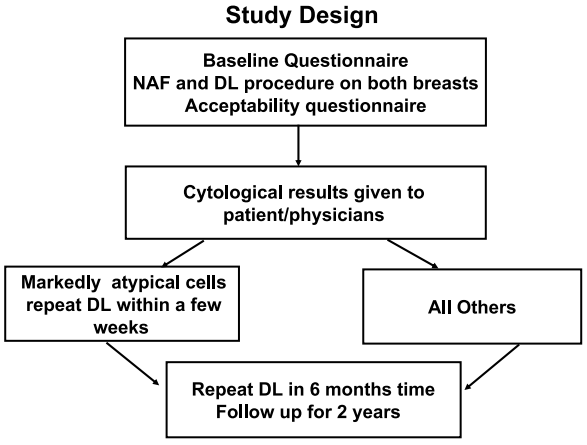

Figure 1. Study design.

Acceptability Questionnaire. Pain and discomfort related to the collection of NAF and the ductal lavage procedure were assessed by an interview-administered acceptability questionnaire at the end of each visit by a nurse not involved in the procedure. Two prior validated pain scales (3) were administered. One rated the pain into five discrete categories: 0, no pain; 1, mild; 2, discomforting; 3, distressing; 4, horrible; and 5, excruciating. The second used a visual analogue scale from 0 to 10 with 0 being no pain and 10 the worst possible pain. The women were also asked how the pain level from the ductal lavage procedure compared with that of mammography and whether they would have the procedure again or use it as a screening test if it was recommended.

Cytologic Processing and Evaluation. The collected fluid was centrifuged for 10 min at 2,160 rpm. The supernatant was poured off and the pellet was used to make two cytospin slides using Thermo Shandon Cytospin 4 (Thermo Electron). The slides were fixed in 95% ethanol and subsequently stained with Papanicolaou stain. Pairs of slides were categorized and reviewed by the same cytopathologist, who is trained in ductal lavage, into one of five predetermined categories based on the higher estimated total cell count of the two slides: <10 cells (ICDM); ≥10 and <100 cells; ≥100 and <1,000 cells; ≥1,000 and <10,000 cells; and ≥10,000 and <100,000. Histiocytes, inflammatory cells, and foamy macrophages were not included in the calculations. Slides with ≥10 cells were categorized according to histology into benign, mildly atypical, markedly atypical, and malignant, based on the most severe alteration identified using criteria described by Dooley et al. (1).

Statistical Analysis. Means or proportions were calculated for several descriptive characteristics. T tests were used for continuous variables and χ^2 or Fisher's exact test was used for categorical variables to compare the baseline distribution of selected characteristics in those women who returned for a second visit. A two-sided P value of ≤0.05 was considered statistically significant. The ductal lavage cytology results were described in two ways: (*a*) per duct, that is the cytologic diagnosis given to each duct sampled at visit 1 and visit 2; (*b*) per person, the potentially most serious diagnosis of the ducts sampled from each individual. A diagnosis of malignant was considered the most serious followed by markedly atypical, mildly atypical, or benign. If there were <10 cells in all the ducts samples for that individual, they would be considered as having ICMD. The κ statistic (4) was used to calculate reliability of NAF production, cellular yield, and cytologic agreement between the same ducts lavaged in visits 1 and 2.

Results

Characteristics of Participants. Sixty-nine women were recruited to the study between May 2002 and April of 2005. The baseline characteristics of these women are shown in Table 1. In five women, ductal lavage could only be done on one breast due to prior breast surgery. Forty-seven women returned for a repeat ductal lavage procedure within an average of 6.1 months (range, 5.4-8.9 months) after the first lavage. Four women were ineligible to return: one woman had a bilateral mastectomy after the baseline visit, another had surgery for a benign breast lump, the third was pregnant, and the fourth developed erythema that persisted for 1 week after her first lavage. Eighteen women who were still eligible at 6 months did not come back for a second visit. One declined due to an overseas assignment, whereas another planned to have bilateral mastectomies. The distribution of baseline characteristics in the subset of participants who did not return for a second visit was not significantly different from the baseline distribution of all the participants enrolled in the study (Table 1). Figure 2 is a flow chart that illustrates the process women underwent once

Table 1. Selected characteristics of participants at baseline and of those who did not return

Characteristics	Baseline (N =69), n (%)	Women still eligible* who did not return (N = 18), n (%)	Two-sided P
Age (y)	46.6 (SD, 7.9)	45.7 (SD, 8.0)	0.67
Ethnicity			
Caucasian	66 (95.7)	18 (100.0)	
African American	3 (4.3)		1.00
Marital status			
Single	3 (4.3)	1 (5.6)	
Married	65 (94.2)	17 (94.4)	1.00
Missing	1 (1.4)	—	
Education			
≤High school	4 (5.8)	—	
College	38 (55.1)	12 (66.7)	
Postgraduate	27 (39.1)	6 (33.3)	0.59
Cigarette smoking			
Never	47 (68.1)	15 (83.3)	
Former	18 (26.1)	3 (16.7)	0.55
Current	4 (5.8)		
Body mass index			
<25	34 (49.3)	7 (38.9)	
≥25	34 (49.3)	11 (61.1)	0.56
Missing	1 (1.4)	—	
Oral contraceptive			
Never	15 (21.7)	3 (16.7)	
Former	40 (58.0)	11 (61.1)	
Current	12 (17.4)	4 (22.2)	0.88
Missing	2 (2.9)	—	
Hormone replacement			
Never	42 (60.9)	11 (61.1)	
Former	16 (23.2)	5 (27.8)	
Current	5 (7.2)	1 (5.6)	
Missing	6 (8.7)	1 (5.6)	0.97
Age at menarche (y)			
<12	17 (24.6)	5 (27.8)	
≥12	49 (71.0)	13 (72.2)	
Missing	3 (4.4)	—	1.00
Menopausal status			
Premenopausal	41 (59.4)	9 (50.0)	
Postmenopausal	28 (40.6)	9 (50.0)	0.59
Mean age at 1st birth (y)	27.8 (SD, 5.7)	28.7 (SD, 5.5)	0.55
Breast-feeding			
No	29 (42.0)	6 (33.3)	
Yes	38 (55.1)	11 (61.1)	
Missing	2 (2.9)	1 (5.6)	0.48
Parity			
Nulliparous	18 (26.1)	3 (16.7)	
Parous	51 (73.9)	15 (83.3)	0.54
Family history breast cancer			
No	16 (23.2)	4 (22.2)	
Yes	53 (76.8)	14 (77.8)	1.00
Gail 5-y risk ++			
<1.66	20 (29.0)	7 (38.9)	
≥1.66	38 (55.1)	8 (44.4)	
N/A	11 (15.9)	3 (16.7)	0.63
Genetic testing			
No	55 (79.7)	11 (61.1)	
Yes	14 (20.3)	7 (38.9)	
BRCA mutation carrier	2	0	0.13
Prophylactic oophorectomy	8 (12.1)	2 (11.1)	1.00
Breast biopsies			
Never	31 (44.9)	12 (66.7)	
1	19 (27.5)	4 (22.2)	
>1	19 (27.5)	2 (11.1)	0.22
Abnormal breast biopsies			
No	49 (71.0)	15 (83.3)	
Yes (atypia, LCIS, DCIS, invasive breast cancer)	20 (29.0)	3 (16.7)	0.38
Prior breast cancer (DCIS/ invasive breast cancer)	11 (15.9)	3 (16.7)	1.00
Treatment for breast cancer			
None	62 (89.9)	17 (94.4)	
Chemotherapy alone	1 (1.4)	1 (5.6)	
Radiation alone	2 (2.9)		
Radiation/chemotherapy	4 (5.8)		0.51
SERMS			
Never	43 (62.3)	10 (55.6)	
Former	12 (17.4)	4 (22.2)	
Current	8 (11.6)	2 (11.1)	
Don't know/missing	6 (8.7)	2 (11.1)	0.92

NOTE: P values were determined by t test for continuous variables and by χ^2 test for categorical variables. The Fisher exact test was used when there were <10 observations per category.

Abbreviations: SERMS, selective estrogen receptor modulators; LCIS, lobular carcinoma *in situ*; DCIS, ductal carcinoma *in situ*.

*Four women were ineligible to return; one woman had a bilateral mastectomy, another had surgery for a benign breast lump, the third was pregnant, and a fourth developed erythema that persisted for 1 wk post lavage. Gail 5-y risk was calculated from National Cancer Institute Gail model (http://bcra.nci.nih.gov/brc/q1.htm).

enrolled in the study. Twelve of the eighteen women who did not return for a second visit did not undergo a successful ductal lavage procedure on their initial visit because either they did not produce NAF or we could not cannulate the duct that produced NAF. There was only one report of an adverse event during the study. Persistent erythema for 7 days surrounding the lavaged duct was observed in one study participant. The erythema improved after aspiration of a complex cyst in close proximity to the lavaged duct.

NAF Procedure. Thirty-nine of the 69 women produced NAF at baseline and were significantly more likely to be younger (P = 0.03) and premenopausal (P = 0.05) compared with nonproducers. The median number of fluid-producing ducts per person was 2 (range, 1-4) for visit 1 and 2 (range, 1-6) for visit 2. The number of fluid-producing ducts did not significantly differ by menopausal status. Forty-seven percent of women who produced NAF in visit 1 also produced NAF in visit 2 but not always from the same duct. Twenty-two new fluid-producing ducts were identified in 17 women at visit 2.

Ductal Lavage Procedure. At visit 1, 57 ducts were successfully cannulated in 28 of the 39 women who produced NAF. At visit 2, 47 ducts were successfully cannulated in 18 of the 24 women who produced NAF. Twenty-four ducts in 14 women were lavaged twice. Ductal lavage was unsuccessful in 18 fluid-producing ducts that were identified, 7 at visit 1 and 11 at visit 2. In 13 ducts the microcatheter could not be correctly positioned despite the use of microdilators, and in 5 ducts, a perforation was suspected, leading to removal of the microcatheter.

Cytopathology Results. The cellular yield most frequently ranged from >10 to ≤100 ductal epithelial cells per slide for both visits 1 and 2. However, it was not consistent for the same duct at visits 1 and 2 (κ = 0.31 ± 0.12). The histologic classifications per duct for ductal lavage specimens collected in visits 1 and 2 are shown in Fig. 3A. ICMD was more common in postmenopausal women (66%) than in premenopausal women (26%; P = 0.04). No duct lavaged at both visits was found to have atypical cells on both occasions, but one woman had a diagnosis of markedly atypical cells in different ducts 6 months apart. Atypical cells were more likely to be identified on slides with increased cellularity in the range of >100 to ≤1,000 ductal epithelial cells. Poor cytologic agreement was

observed for the 24 ducts with repeated samples from visits 1 and 2 ($\kappa = 0.32 \pm 0.15$). Figure 3B classifies each woman who underwent lavage based on their potentially most serious cytologic diagnosis from the ducts that were sampled. For example, although >35% of ductal specimens at both visits were diagnosed as ICMD, this was the sole diagnosis in only 29% of women in visit 1 and in 17% of women in visit 2.

In accordance with study protocol, two women underwent an additional lavage of specific ducts diagnosed as containing markedly atypical cells within a month of visit 1 or visit 2. For one woman, the same duct was lavaged thrice over the 6-month period with a diagnosis of benign at baseline, markedly atypical at 6 months, and ICMD at 7 months. In the second woman, both the baseline and repeat lavage a month after the first visit showed markedly atypical cells. She did not return for her 6-month visit and remained cancer-free for 2 years.

Acceptability. The mean pain score attributed to nipple aspiration was 1.0 (mild pain) on a scale of 0 to 5 at both visits 1 and 2 and independent of whether fluid was produced. The mean pain score reported for the ductal lavage procedure was 2 (discomforting) in visits 1 and 2. Using a visual analogue scale (ranging from 0 to 10), the mean pain associated for NAF was 0.7 (range, 0-6) at visit 1 and 0.9 (range, 0-7) at visit 2. Mean pain associated with the ductal lavage procedure was 4 (range, 0-8) at visit 1 and 3 (range, 0-9) at visit 2. Fifty-two percent of the women in whom ductal lavage was attempted at visit 1 felt that the pain level associated with the ductal lavage procedure was worse than that of a mammogram, whereas 56% had the same response at visit 2. Nevertheless, 70% of women who underwent the ductal lavage procedure at visit 1 reported they would have the test again and, if recommended, would agree to use ductal lavage as a routine screening test for early breast cancer detection. Of these women, only 52% returned for a second visit.

Clinical Management of Women Diagnosed with Atypical Cells. During visit 1, eight women had a diagnosis of mildly atypical cells and two had a diagnosis of markedly atypical cells. At visit 2, three women had a diagnosis of mildly atypical cells and one of markedly atypical cells. After a diagnosis of markedly or mildly atypical cells, two women underwent bilateral magnetic resonance imagings, which were normal, one woman underwent a needle biopsy of a suspicious area on mammogram, which showed benign changes, and two women underwent preventive mastectomies with no malignant changes detected. One woman had an inconclusive ductoscopy following ductal lavage. None of these women began chemoprevention with tamoxifen.

Discussion

Whereas this study showed the ability to repeat the ductal lavage procedure on the same duct at two time points within 6 months, it also raised questions about its feasibility and accuracy in women at increased risk for breast cancer. Factors such as the low return rate of participants, inconsistency in NAF production from the same duct over time, low cellular yield reflected by a high ICMD rate, 10% to 20% procedure-related failure rate, and moderate cytologic reproducibility were identified as significant limitations to the use of ductal lavage in its current form in the clinical or research setting. Although these limitations were observed more frequently in postmenopausal than in premenopausal women, they need to be resolved before ductal lavage can be viewed as a highly useful tool in either group.

The rationale behind performing ductal lavage on only fluid-producing ducts is based on a large prospective study showing a 2-fold increase in breast cancer risk among producers of NAF compared with nonproducers (5). From a practical standpoint, this approach also enabled us to identify the ductal orifices for cannulation. However, given the inconsistency of NAF production in our study, this type of sampling approach may not adequately reflect an individual's breast cancer risk. Although atypical cells have recently been shown to be present in ductal lavage obtained from non–fluid-producing ducts in *BRCA* mutation carriers (6-8), the cellular yield from these ducts may be lower than fluid-producing ducts, perhaps limiting the feasibility of this approach (6, 8).

The low participant return rate is of concern and most likely to be multifactorial in origin. Among the 18 women who did not return, 50% did not produce NAF and the ductal lavage catheter could not be seated in three women. No

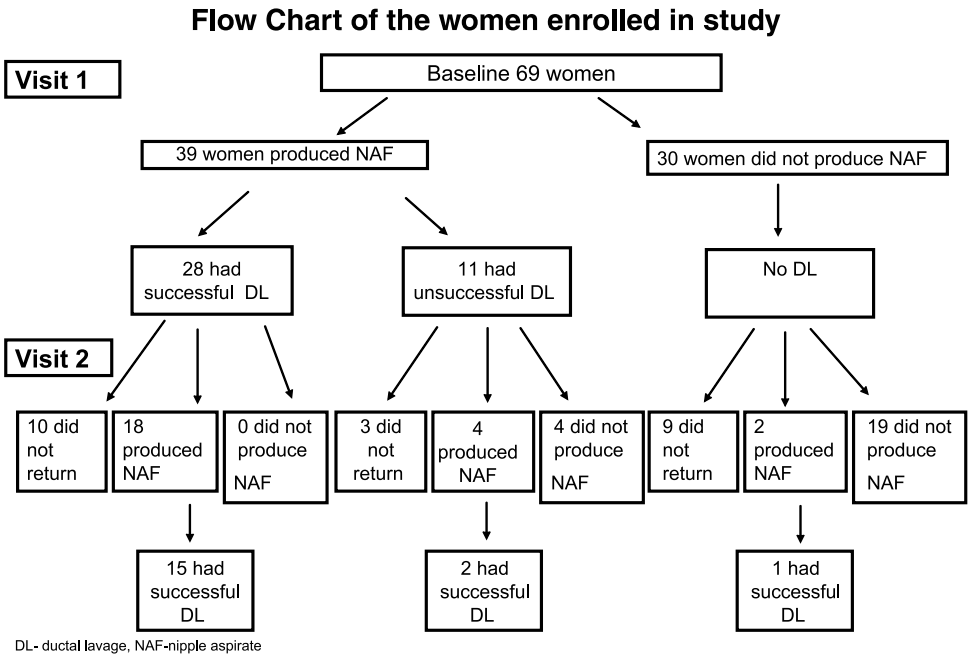

Figure 2. Flow chart of the women enrolled in study.

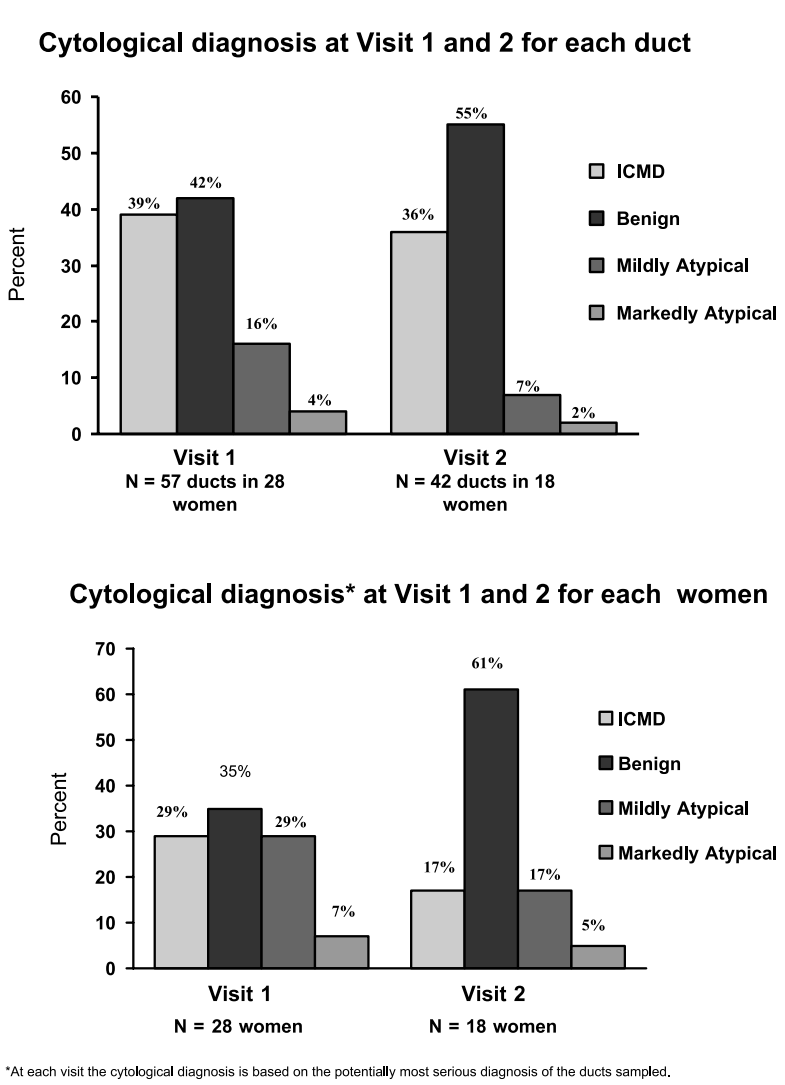

Figure 3. A. Cytologic diagnosis at visits 1 and 2 for each duct. **B.** Cytologic diagnosis at visits 1 and 2 for each woman. At each visit, the cytologic diagnosis is based on the potentially most serious diagnosis of the ducts sampled.

difference in procedure-related discomfort was reported between those who did and did not return, and the average pain reported for the ductal lavage procedure by the study participants was equivalent or higher than that reported for core breast biopsies (9). Pain of a similar magnitude was reported in a study evaluating ductal lavage in *BRCA* mutation carriers (7). The fact that 70% of women in this study did report that they would undergo ductal lavage for screening if recommended, despite 50% of them finding it more uncomfortable than a mammogram, illustrates the need for new and effective screening options in this group. The use of agents such as nitroglycerin (10) to dilate the ducts may decrease the pain of the procedure, making it more acceptable, and increase the likelihood of placing the catheter and enhancing cellular yield.

The reported prevalence of markedly and mildly atypical cells as well as the percentage of ICMD in premenopausal women in this study was similar to those studies that have evaluated similar populations (1, 2). Overall variability in cytology and cellular yield between ductal lavage studies could be due to differences in patient population characteristics, specimen processing, or individual variations in technique. Whereas other studies used Thin Prep or Millipore filtration, in this study we used cytospin, as did Kurian et al.[6] These three approaches to obtain thin layer cytologic preparations for ductal lavage samples have not been compared from the same individuals. In one study, Nayar et al. (11) reported a median epithelial cell count of 1,700 in 78 samples using cytospin and an epithelial cell count of 4,500 when using a Thin Prep–based method. It was unclear at what point the switch was made. In urine samples, Cytcc Thin Prep (Cytyc) has shown to be associated with increased cellularity (12) and reduced cellular debris compared with cytospin (13). However, cytospin preparations were found to be superior to Thin Prep with respect to cytomorphologic details and preservation of cell architecture (14, 15).

In our study, the fair cytologic reproducibility observed can be partly explained by poor intra-rater agreement ($\kappa = 0.60 \pm 0.11$), which was subsequently identified when all abnormal slides and random samples of normal slides were re-reviewed (16). Cytologic reproducibility was low for benign, mildly atypical, and markedly atypical cells. In a study examining the reproducibility of the diagnosis of atypia using Thin Prep (Cytyc) rather than cytospin, the results were similar to the present study (2). Poor reproducibility of atypia may contribute to higher false-positive or false-negative tests; as seen in our study, a cytologic diagnosis of atypical cells can lead to additional tests and anxiety despite communication to both the physician and study participant about the lack of scientific data associating atypical cells and breast cancer development.

In conclusion, the results of this study dampen the enthusiasm for using ductal lavage in its current form for risk

[6] Personal communication.

classification, early detection, or biomarker evaluation. Reproducibility may be improved by optimizing cytologic reproducibility, altering approach to duct sampling, and possibly evaluating drugs to dilate the ductal system. Lastly, whether atypical cells from ductal lavage are associated with increased breast cancer risk still needs to be determined.

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