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# Combined Breast Ductal Lavage and Ductal Endoscopy for the Evaluation of the High-Risk Breast: A Feasibility Study

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**Background and Objectives:** Evaluation of the ductal epithelium of the breast at increased risk for breast cancer is needed to define the carcinogenic pathway, for risk assessment, and to improve selection of women for chemoprevention therapy. We studied the feasibility of combining breast ductal endoscopy with ductal lavage in the high-risk contralateral breast of women with ipsilateral breast cancer for the evaluation of high-risk ducts and acquisition of ductal epithelial cells for analysis.

**Methods:** Breast ducts were studied by ductal lavage and ductal endoscopy, and epithelial cell content studied cytologically and quantitatively.

**Results:** Twenty-five subjects and 44 ducts, including 22 (50.0%) which did not produce nipple aspirate fluid (NAF), were studied. Cellular atypia was present in five subjects. Ductal endoscopy was performed on 1 or more ducts in 24 subjects. Structural changes were noted in 63.6% of the ducts, most commonly fibrous stranding or bridging. Ductal sampling with endoscopic brush and coil sampling devices provided additional cellular samples of relatively pure ductal epithelial content ($\geq$91% purity) in 8/11 subjects.

**Conclusions:** Breast ductal endoscopy combined with ductal lavage represents a feasible approach for characterizing the ducts and ductal epithelium of the high-risk breast, especially in a research setting.

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KEY WORDS: **chemoprevention; breast cytology; high-risk breast epithelium; ductal sampling**

## INTRODUCTION

Breast cancer commonly develops in the epithelial cells lining the milk ducts. Initial genetic changes are thought to occur in stem cells in the suprabasal layer of the duct [1]. Clonal expansion of these mutated cells, with the aid of a variety of promotional agents (for example, estradiol and IGF-1) results in a large cancerized field of phenotypically normal but genetically altered epithelial cells. A growing list of genetic abnormalities ranging from loss of heterozygosity and microsatellite instability to DNA methylation and gene

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overexpression have been identified in these cytologically/histologically normal high-risk cells, confirming this model of carcinogenesis [2–7]. Acquisition of additional mutations and subsequent expansion allows progression through the carcinogenic pathway with the development of hyperplasia, atypical hyperplasia, and in situ carcinoma. The latter group of lesions (intraepithelial neoplasia) can be identified microscopically and are considered obligate precursors of malignancy, with known associated risk estimates for progression to malignancy [8]. Identification and characterization of early cellular and genetic changes in women at increased risk for breast cancer is needed to define the carcinogenic pathway, for risk assessment, for identification of biomarkers, and for selection and monitoring of women for chemoprevention trials.

Several methods have been developed for obtaining ductal epithelial cells for cytologic and molecular analyses, including nipple aspirate fluid (NAF) [9], breast ductal lavage (BDL) [10], and random periareolar fine needle aspiration of breast parenchyma (RPFNA) [11]. NAF consists of approximately 50 μl of fluid containing $\leq$200 epithelial cells, with detection of atypia in 10% of cases from NAF-producing ducts. BDL, which is applied principally to NAF-producing ducts, results in a greater cellular yield (4,000–13,500 epithelial cells depending on the type of catheter used), greater percentage (78%) of adequate cellular material acquired for cytologic diagnosis, and greater detection of atypia (24%) [10]. RPFNA collects random aspirates of breast parenchyma throughout the breast, which are then pooled. Atypia has been identified in 21% of cases [11]. RPFNA generally provides 4 microscope slides with cell counts of 1,000–5,000 cells/slide for specimens of hyperplasia (>5,000 for atypia). The latter two techniques acquire a cellular population which may be heterogeneous, consisting of both epithelial and histiocytic cells, and do not provide visual information about the presence of intraductal lesions to either correlate with positive cytologic findings, or to indicate lesions which were missed by a negative cytology. In addition, while excellent immunohistochemical, proteomic, and chromosomal studies have been conducted on NAF, BDL, or RPFNA specimens [12–17], a more comprehensive characterization of high-risk ductal epithelium will require larger numbers of epithelial cells, encouraging efforts to identify additional methods for ductal analysis.

A recently developed technique, breast duct endoscopy, allows visual examination of the ductal tree, and represents a potentially improved means for more definitive ductal epithelial sampling. Breast duct endoscopy employs a 0.7-mm rigid endoscope with high-resolution optics which allows evaluation of $\leq$10 cm of a single ductal tree including tertiary bifurcations. Ductal endoscopy can readily identify intraductal pathology, including papilloma, atypical hyperplasia, carcinoma in situ, and invasive carcinoma [18]. Ductal endoscopy may, thus, provide important assessment of ducts which contain atypical epithelial cells on lavage, allow identification of lesions which might not have been suggested by ductal lavage alone, and provide the opportunity to lavage the more proximal breast ductal tree. The recent introduction of endoscopic brush and coil sampling devices may also allow a more targeted sampling and collection of epithelial cellular samples of greater cell yield and purity. Breast ductal endoscopy combined with ductal lavage might, therefore, allow improved characterization of the high-risk duct and ductal epithelium. We, therefore, evaluated the feasibility of this approach in the high-risk contralateral breast of women with ipsilateral breast cancer. We now review our findings in 25 subjects.

## SUBJECTS AND METHODS

### Subject Population

Women who had ipsilateral breast cancer and a normal contralateral breast were studied. All women were studied under an NCI-IRB-approved clinical trial and gave written informed consent. For eligibility, the breast cancer must be unilateral, may be invasive or non-invasive, and may be in the present or past. The contralateral normal breast was studied and was normal by physical examination (within 1 month) and mammography (within 12 months). The contralateral breast was without a past history of DCIS or invasive carcinoma (a past history of atypical ductal hyperplasia or lobular carcinoma in situ was permissible), whole breast radiotherapy, and without a breast prosthesis or history of major ductal surgery. Subjects must not be currently taking tamoxifen, and must be off chemotherapy for at least 3 months. For assessment of menopausal status, women were considered postmenopausal if menstrual periods had stopped for at least 2 years. All subjects had a WBC >2,500, platelet count >50,000, a PT/PTT <1.5 times normal, and a negative pregnancy test for premenopausal subjects.

### Breast Duct Lavage

All breast duct lavage and breast duct endoscopy procedures were performed under intravenous sedation in the operating room. All subjects were instructed to massage the normal contralateral breast for 30 min the morning of the procedure. At the time of the procedure, the nipple was cleansed with ethanol and Nu-Prep Gel (Weaver and Co., Aurora, CO) to remove superficial keratin, and each breast quadrant was massaged with

mild pressure in a peripheral to central motion for approximately 1 min with the aid of topical saline [10]. Mild suction was then administered with the FirstyCyte Aspirator (Cytyc Corp., Boxborough, MA). If there was no NAF production then massage was repeated, followed by application of nipple suction. In several recent patients, a drop of 2% nitroglycerine ointment was applied to the nipple surface for 20 min to promote dilatation of the ductal sphincter [19]. NAF-producing ducts were identified and the FirstCyte Ultraslim Dilator inserted. If $\leq$1 duct produced NAF, then a non-NAF-producing duct(s) was identified with the Ultraslim dilator. This was then replaced with a FirstCyte Tapered Dilator, followed by insertion of the FirstCyte Microcatheter. Proper positioning of the catheter was confirmed with a bubble test, and the duct lavaged with approximately 15 ml of saline or until the effluent was clear. The lavage fluid was transferred to polystyrene centrifuge tubes and maintained on ice until processing.

### Breast Duct Endoscopy

Upon completion of ductal lavage, the microcatheter was removed and a 2-0 proline suture passed into the duct. The ductal sphincter was dilated sequentially with a 24G, then 22G, and finally 20G angiocath passed over the proline suture [20]. The latter was then removed and the Viaduct endoscopic Introducer and Microendoscope (Acueity Corp., Palo Alto, CA) introduced into the duct. The endoscope was advanced under direct vision as far proximally as possible into the ductal system, and endoscopy performed with the aid of air or saline instillation. Any abnormality of the ductal lining or ductal lumen was noted. At a selected level of the duct, for brush or coil-targeted sampling, the endoscope was removed from the introducer sheath and the BristleBrush or CoilBrush (Acueity Corp.) inserted. The respective device was then passed over the epithelial lining of the duct, removed, and rinsed in a sterile tuberculin syringe containing 1 ml of saline. The sampling site was then washed with 1 ml saline infused with a Fluid Aspirator, collected, and placed in an empty tuberculin syringe. All syringes were placed immediately on ice until processing. Upon completion of the sampling, the endoscope was reintroduced and the area of sampling inspected. The endoscope was then gradually withdrawn, re-examining the ductal system.

### Processing of Samples

(A) *Ductal lavage*: The volume of the lavage fluid was measured, and 20% of the lavage specimen was removed, and placed in a ThinPrep® vial for cell counting, cytologic, and chromosomal analysis. The remainder of the specimen was centrifuged at 2,000 rpm and the supernatant and pellet frozen at $-70^{\circ}$C for future proteomic studies. (B) *Brush, coil, and aspiration samples*: The contents of each syringe were transferred to an Eppendorf cap and 20% removed and added to a ThinPrep® vial for cytology and cell counting. The remaining sample was either frozen directly for future DNA methylation analysis, or RNA later (Ambion, Inc., Austin, TX) added, the cap incubated overnight at 4°C, and then frozen at $-70^{\circ}$C for future gene expression profile analysis.

### Cytologic Analysis and Cell Counting

Breast ductal cellular specimens were analyzed for cytologic abnormalities by two experienced cytologists trained in interpretation of ductal lavage specimens [21]. A Thinprep® slide was prepared from each ductal sample. The cytologic diagnostic categories were very similar to the 1997 consensus criteria for breast fine-needle aspiration biopsy samples published by the National Cancer Institute (Bethesda, MD) [22]. There were five diagnostic categories: inadequate cellular material for diagnosis (samples with $<$10 epithelial cells per sample or unacceptable technical quality [ICMD]), negative for malignant cells, atypia, suspicious for malignancy, and malignant.

For cell counting, each slide was reviewed for the identification of epithelial cell clusters that had greater than or equal to 10 ductal cells. All cell clusters on the slide were counted directly, with the cell numbers estimated in multiples of 10. These numbers were added together to provide the total count of ductal cells in clusters. There were 330 fields (20× fields) on a Thinprep slide (personal communication, Gary Gill, CT, ASCP). Duct cells, histiocytes, and cells in clusters of less than 10 were counted as follows: 10 consecutive 20× fields were viewed down the middle of the Thinprep® slide. For each 20× field, the number of ductal epithelial cells and histiocytes were counted separately and summed. For the final cell count and cellular proportions, the following formula was utilized:

Number of epithelial cells in clusters (a)
33 (the number of epithelial cells in 10–20× fields) (b)
33 (the number of histiocytes in 10–20× fields) (c)
The total number of cells $a + b + c$
Total number of ductal cells $a + b$

The total number of ductal cells was determined, and the percentage of the sample composed of ductal epithelial cells was calculated [21].

## RESULTS

### Subject Population and Demographic Information

Twenty-five subjects were studied between 2003 and 2005. The demographic data for all subjects are summarized in Table I. The majority of subjects were postmenopausal, and the median age was 57 years. All subjects had carcinoma of one breast, most commonly infiltrating ductal carcinoma, without a past history of atypical ductal hyperplasia or lobular carcinoma in situ in the contralateral breast. Eight subjects had prior adjuvant chemotherapy and tamoxifen, 5 subjects had prior chemotherapy only, and 12 subjects had no prior systemic adjuvant therapy.

### Breast Duct Lavage

Breast ductal lavage was performed in 44 ducts among the 25 subjects, with a median of 2 ducts/subject (Table II). NAF was present in 22 ducts (50.0%; 16 subjects). There were nine subjects without any NAF-producing ducts, and at least one ductal orifice could be identified with the Ultraslim Dilator in each. NAF was present in 10/13 (76.9%) of subjects who previously received systemic therapy.

Ductal lavage from all ducts was analyzed for cellular content and cytologic changes. Adequate cellular material for diagnosis (>10 ductal epithelial cells) was obtained in 21 subjects (84.0%). Adequate cellular material was present in 63.6% (14/22) of non-NAF-producing and 72.7% (16/22) of NAF-producing ducts (Table III). In general, NAF-producing ducts tended to have a lower rate of ICMD, higher incidence of atypia and higher median cell count than non-NAF-producing ducts. Nevertheless, non-NAF-yielding ducts could be easily identified with the Ultraslim Dilator, lavaged, and endoscoped, and could provide adequate cellular material for study. Prior systemic therapy (chemotherapy or tamoxifen) was administered in 66.7% (14/21) of subjects and 63.3% (19/30) of ducts, with adequate cellular material. There was no apparent correlation between previous systemic therapy and the incidence of NAF production, median cell count, or cellular atypia.

Five subjects (six ducts) had mild or moderate cellular atypia on ductal lavage (Fig. 1a); cytologic changes of atypia included nuclear crowding with some nuclear size variation, nuclear contour irregularities, and visible nucleoli. Four of the six ducts were NAF-producing and were associated with cellular yields of >1,000 ductal epithelial cells. All other lavage specimens were cytologically unremarkable. Three additional subjects had, respectively, ICMD (one subject) and negative cytology (two subjects) on ductal lavage; however, each had atypical cells on one endoscopically targeted intraductal sample (see below). There were no specimens which were cytologically suspicious for malignancy.

### Breast Duct Endoscopy

Twenty-four subjects underwent breast duct endoscopy of one duct following ductal lavage, and nine subjects had

**TABLE I. Demographic Characteristics of Subjects**

| Category | Incidence |
|---|---|
| Number of subjects | 25 subjects |
| Age | |
| Median | 57 years |
| Range | 33–68 |
| Menopausal status | |
| Premenopausal | 4 subjects |
| Perimenopausal | 1 |
| Postmenopausal | 20 |
| Stage of breast cancer | |
| 0 (DCIS) | 2 subjects |
| I | 2 |
| II | 21 |
| Pathology | |
| Ductal carcinoma in situ | 2 subjects |
| Infiltrating ductal carcinoma | 20 |
| Infiltrating lobular carcinoma | 3 |
| Previous chemotherapy | 13 subjects |
| Median time since chemotherapy | 115 months |
| Range, time since chemotherapy | 54–147 months |
| Previous tamoxifen therapy | 8 subjects |
| Median time since tamoxifen | 78 months |
| Range, time since tamoxifen | 3–103 months |

**TABLE II. Breast Duct Lavage Characteristics**

| Category | Incidence |
|---|---|
| Ducts lavaged | 44 ducts (25 subjects) |
| Ducts lavaged/subject | |
| Median | 2 ducts |
| Range | 1–3 |
| Nipple aspirate fluid (NAF) | |
| Present | 22 ducts (16 subjects) |
| Absent | 22 ducts (9 subjects) |
| Cell count/duct | |
| NAF-producing ducts | |
| Median | 5,870 cells/duct |
| Range | 15–120,000 |
| Non-NAF-producing ducts | |
| Median | 212 cells/duct |
| Range | 10–24,000 |
| Cytologic analysis | |
| ICMD* (<10 cells) | 14 ducts |
| NAF-producing | 6/22 ducts, 2/16 subjects |
| Non-NAF-producing | 8/22 ducts, 2/9 subjects |
| Adequate material for diagnosis | 21 subjects (84.0%)/30 ducts |
| Negative for malignant cells | 16 subjects/24 ducts |
| Atypical ductal epithelial cells | 5 subjects/6 ducts |

*ICMD, insufficient cellular material for diagnosis.

**TABLE III. Characteristics of NAF-Producing and Non-NAF-Producing Ducts**

| Characteristic | NAF-producing* | Non-NAF-producing |
|---|---|---|
| N | 22 ducts | 22 ducts |
| ICMD | 6 ducts | 8 ducts |
| Adequate material for diagnosis | 16 ducts | 14 ducts |
| Median cell count | 5,870 epithelial cells | 212 epithelial cells |
| Cellular atypia | 4 ducts | 2 ducts |
| Ducts endoscoped | 17 ducts | 16 ducts |
| Normal ducts endoscopically | 3 ducts | 9 ducts |
| Fibrous bridging endoscopically | 11 ducts | 7 ducts |
| Yellow sessile lesions | 2 ducts | 0 ducts |
| Papillary lesion | 1 duct | 0 ducts |

*NAF, nipple aspirate fluid.

endoscopy of two or more ducts. Endoscopy was only performed in ducts which had been lavaged. The decision to endoscope >1 duct was made arbitrarily and without regard to lavage characteristics or NAF production. One subject underwent ductal lavage but not endoscopy because of endoscopic camera failure. The median distance endoscoped was 4.5 cm (range 2.5–9.5 cm). Inability of the sphincter to completely relax, a narrow intraductal lumen, or fibrous bridging were the principle limitations to advancement of the endoscope. The application of nitroglycerine to the nipple surface facilitated ductal lavage and endoscopy by relaxing the ductal sphincter and also potentially by relaxing the ductal wall. The endoscopic findings are summarized in Table IV. Twelve ducts (36.4%) were normal in appearance. Eighteen ducts (54.6%) in 13 subjects had mild to moderate fibrous bridging or stranding across the lumen or along the wall. This occurred more often in the ductal system near the nipple (distal ductal system). In cases of mild fibrous bridging, the scope could usually be passed through the fibrous strands, but more significant bridging occasionally prevented further passage of the endoscope. Thirteen subjects with ductal fibrous bridging had previously received systemic therapy. One subject had diffuse yellow sessile lesions throughout the ducts which were smooth, friable, and non-vascular (Fig. 1d). These were present in several branches of two separate ductal systems. One subject had an irregular friable, papillary-like lesion which was non-vascular and non-adherant, and filled the ductal lumen in the proximal

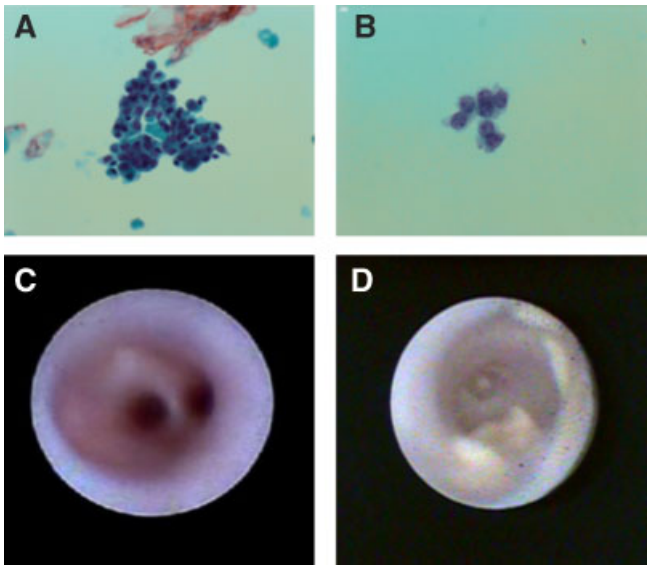


Fig. 1. Cytologic and endoscopic appearance of ductal epithelium. Panel (**A**), cytopathologic appearance of initial ductal lavage specimen: three dimensional cluster of cells shows a minor degree of nuclear crowding with some nuclear size variation, nuclear contour irregularities, and visible nucleoli (Pap, 400×, ThinPrep®). This specimen was from the subject with papillary-like material in the duct on endoscopy. Panel (**B**), follow-up ductal lavage in the same subject: small group of bland, uniform ductal cells (Pap, 600×, ThinPrep®). Panel (**C**), endoscopic appearance of a normal duct. Panel (**D**), endoscopic appearance of a duct containing yellow sessile lesions within a duct; multiple branches of the duct contain sessile, friable, non-vascular yellow lesions.

**TABLE IV. Breast Duct Endoscopy Characteristics**

| Category | Incidence |
|---|---|
| Ductal endoscopy performed | 24 subjects*/33 ducts |
| Ducts endoscoped/subject | |
| Median | 1 duct |
| Range | 1–2 |
| Distance duct endoscoped | |
| Median | 4.5 cm |
| Range | 2.5–9.5 cm |
| Ductal endoscopy findings | |
| Normal | 12 ducts |
| Fibrous bridging | 18 ducts |
| Yellow sessile lesions | 2 ducts |
| Papillary lesion | 1 duct |
| Not done (technical)* | 2 ducts (1 subject) |
| Complications | |
| Perforation | 2 ducts |

*Endoscopy not performed in one subject secondary to camera failure.

branch of the duct. Both NAF-producing and non-NAF-producing ducts were endoscoped. In general, non-NAF-producing ducts were more likely to be normal architecturally, with a lower incidence of fibrous bridging or intraductal lesions (Table III).

### Correlation of Ductal Lavage and Ductal Endoscopic Findings

Thirty-three ducts (24 subjects) were examined by both ductal lavage and ductal endoscopy. These findings were correlated and are summarized in Table V. Among patients with ICMD or cytologically negative ductal cells on lavage, endoscopy was normal in 11 (40.7%) and revealed fibrous bridging in 16 (59.3%) subjects. In subjects with cellular atypia on ductal lavage, 5/6 (83.3%) had ductal lesions, including diffuse yellow sessile lesions, a papillary lesion, and fibrous bridging. One duct with cellular atypia was endoscopically normal.

Among all subjects, there were two ductal perforations among the 33 ducts, and no evidence of infection or bleeding. Postoperatively, subjects had mild soreness of the nipple and mild swelling of the breast for 1–2 days from infusion of fluid, but otherwise the postoperative course was completely unremarkable, including the two subjects with ductal perforation.

### Endoscopic Targeted Intraductal Sampling

In an effort to increase the consistency and quantity of ductal epithelial cellular yield, and to allow targeted analysis of ductal surfaces and intraductal lesions in these subjects, we evaluated the recently introduced endoscopic sampling devices, an endoscopic brush, coil, and aspiration device (Acueity Corp.). We studied 11 subjects in this manner. The findings for representative subjects are given in Table VI, and a summary of all subjects is given in Table VII. The endoscope was passed as far

**TABLE V. Correlation of Ductal Lavage and Ductal Endoscopy Findings***

| Category | Incidence |
|---|---|
| ICMD ductal lavage cytology | 14 ducts (7 endoscoped) |
| Normal endoscopy | 3 ducts |
| Fibrous bridging | 4 |
| Negative ductal lavage epithelial cytology | 24 ducts (20 endoscoped) |
| Normal endoscopy | 8 ducts |
| Fibrous bridging | 12 |
| Atypical ductal lavage epithelial cytology | 6 ducts (6 endoscoped) |
| Normal endoscopy | 1 ducts |
| Fibrous bridging | 2 |
| Yellow sessile lesions | 2 |
| Papillary lesion | 1 |

*Endoscopy was performed on 33 ducts in 24 patients.
ICMD, insufficient cellular material for diagnosis.

**TABLE VI. Findings for Endoscopic Targeted Intraductal Sampling**

| Subject | Category | Lavage | Brush #1 | Brush #2 | Coil #1 | Coil #2 | Aspirate #1 | Aspirate #2 | Aspirate #3 | Aspirate #4 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cell count | 186 cells | 786 cells | 3,340 cells | 4,285 cells | 3,528 cells | 12,540 cells | 10,056 cells | 7,191 cells | 7,674 cells |
| | Cytology | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| | % Ductal cells | 100% | 92% | 96% | 97% | 99% | 97% | 97% | 91% | 99% |
| 2 | Cell count | 5,852 cells | ICMD | 885 cells | 1,320 cells | 14,270 cells | 13,436 cells | 10,224 cells | 12,200 cells | 5,472 cells |
| | Cytology | Negative | | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| | % Ductal cells | 93% | | 100% | 100% | 100% | 97% | 91% | 94% | 91% |
| 3 | Cell count | 11,572 cells | 120 cells | 300 cells | 480 cells | 780 cells | 2,610 cells | 10,098 cells | 6,300 cells | 5,922 cells |
| | Cytology | Negative | Rare | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| | % ductal cells | 98% | Atypical cluster | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 4 | Cell count | 1,633 cells | 200 cells | 660 cells | 150 cells | 300 cells | ICMD | 5,000 cells | | |
| | Cytology | Negative | Negative | Negative | Negative | Negative | | Negative | | |
| | % Ductal cells | 100% | 100% | 100% | 100% | 100% | | 100% | | |
| 5 | Cell count | 100 cells | 100 cells | ICMD | ICMD | ICMD | ICMD | ICMD | | |
| | Cytology | Negative | Negative | | | | | | | |
| | % Ductal cells | | | | | | | | | |
| 6 | Cell count | ICMD | 5,610 cells | 9,900 cells | 8,415 cells | 15,840 cells | 24,250 cells | 3,360 cells | 2,145 cells | 490 cells |
| | Cytology | | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Atypical |
| | % Ductal cells | | 97% | 100% | 97% | 98% | 98% | 96% | 93% | 100% |

Findings for six representative subjects.

**TABLE VII. Endoscopic Sampling Characteristics**

| Category | Incidence |
|---|---|
| Total number of subjects sampled | 11 subjects/11 ducts |
| Number ducts producing NAF | 3 ducts |
| Distance sampled | |
| Median | 7.0 cm |
| Range | 2.5–9.5 cm |
| Number cellular samples/duct | |
| Median | 5 samples |
| Range | 1–8 |
| Cell count/sample | |
| Median | 3,528 epithelial cells |
| Range | 100–33,594 |
| Percent samples >90% epithelial cells | 100% |
| Subjects with ≥1 sample of 5,000 cells | 8 subjects |

NAF, nipple aspirate fluid.

proximally as possible, and two or more samples collected with each of the sampling devices. In eight subjects, the cellular yield by targeted sampling was significantly increased over that obtained by ductal lavage alone. It can be seen, for example in Subject #1, that whereas ductal lavage yielded a total of 186 ductal epithelial cells, targeted ductal sampling yielded a total of 49,400 cells. The yield tended to be greatest with the coil or the aspiration devices. Cytologic evaluation was available for each individual sample. Almost all samples were essentially pure ductal epithelial cell populations, with minimal contamination with other cell types (histiocytes, etc.). In three subjects, cellular yields were low or insufficient. The endoscopic distance at which the samples were taken in the eight successful subjects was ≥6.0 cm (range 6.0–9.5 cm), compared with ≤5.0 cm in the three unsuccessful cases. Among the 11 ducts studied, 8 were non-NAF-producing. Each duct was examined endoscopically after targeted sampling. In one subject, there was mild transient oozing from the ductal surface, otherwise there were minimal ductal changes, no evidence of perforation, and no postoperative complications in any subject. Each of the aliquots was analyzed cytologically. Three subjects, including one on repeat evaluation (see below), had a single targeted sample demonstrating atypical cells where the ductal lavage specimen was cytologically negative. All other targeted samples contained unremarkable ductal epithelial cells.

### Follow-Up Analysis of Atypia

Four of the five subjects with cellular atypia on ductal lavage underwent follow-up re-evaluation with mammography, breast MRI, repeat ductal lavage, and repeat ductal endoscopy at 6–14 months according to the guidelines recommended by O'Shaughnessy et al. [8]. The findings for these four subjects are summarized in Table VIII. The fifth subject was recently studied, and will be re-examined in follow-up in 6 months. Mammography in the subject with the papillary lesion revealed the new appearance of bilateral indeterminate calcifications of both the affected breast (with previous breast cancer) and the normal contralateral breast. A subsequent mammogram in 6 months showed these calcifications to be benign-appearing. In the subject with two ducts containing yellow sessile lesions, MRI revealed non-specific ductal changes in the normal contralateral breast; a subsequent MRI in 3 months was unchanged. At the time of repeat ductal lavage and ductal endoscopy, the location of the involved ducts, which had been previously noted on the Cytyc grid, was readily identified with the Ultraslim Dilator. One of the five ducts was NAF-producing at follow-up evaluation. Repeat ductal lavage and repeat endoscopy were easily performed in 4/4 and 4/4 subjects, respectively. Repeat ductal lavage of the five ducts showed absence of cellular atypia in all cases (Fig. 1b). Repeat ductal endoscopy was performed in the four subjects (one duct each) and showed an essentially normal ductal epithelium and ductal lumen; in the subject with the previously noted yellow sessile lesions, only a single small yellow sessile lesion was noted in one branch. The papillary-like material noted in a second subject had resolved at repeat endoscopy, and the ductal examination was unremarkable. To further evaluate the ductal epithelium, three of the four subjects with cytologic atypia also underwent targeted intraductal endoscopic sampling, providing a more thorough sampling of the duct. In one subject (with previous papillary

**TABLE VIII. Followup Evaluation for Epithelial Cellular Atypia**

| Primary lavage | Primary endoscopy | Follow-up mammogram | Follow-up MRI | Second endoscopy | Second lavage |
|---|---|---|---|---|---|
| Atypical cells | Fibrous bridging | Negative | Negative | Negative | Negative |
| Atypical cells | Fibrous bridging | Negative | Not done (technical) | Negative | ICMD |
| Atypical cells | Yellow sessile lesions | Negative | Non-specific change | Rare single yellow sessile lesion | Negative |
| Atypical cells | Yellow sessile lesions | Negative | Non-specific change | Not done | Negative |
| Atypical cells | Papillary-like material | Indeterminate bilateral calcifications | Negative | Negative | Negative |

lesion), one brush sample showed a single cluster of atypical cells. Subsequent repeat evaluation for this atypia was performed. Ductal lavage was negative for atypia, and ductal endoscopy revealed fibrous strands, with sampling negative for cellular atypia. All other targeted samples (28 samples in total) from the 4 subjects were cytologically unremarkable.

## DISCUSSION

In this study, we determined the feasibility of combining breast ductal lavage and ductal endoscopy to evaluate the architecture and intraductal gross pathology of the high-risk duct, and to improve sampling of ductal epithelial cells for cytologic and molecular analyses. We examined the contralateral breast in women with ipsilateral breast cancer, a well known site of high-risk breast tissue with an annual risk of 0.5–1.0% [23–27].We found breast ductal lavage and ductal endoscopy was easily performed in a variety of women, including premenopausal and postmenopausal, those untreated or previously treated with chemotherapy or tamoxifen, and in non-NAF-producing as well as NAF-producing ducts. The addition of non-NAF-producing ducts potentially expands the application of this approach. Published clinical trials examining breast ductal lavage and endoscopy have studied NAF-producing ducts [10,18,28,29]. The majority of women at increased risk for breast cancer, however, will not have any NAF-producing ducts [30], and among those women who are NAF-producing, only a median of 1.5 ducts (out of 5–9 usual ductal orifices [31]) will produce NAF. Examination of ductal epithelium of non-NAF-producing ducts is thus important for the characterization of the high-risk breast. We found that non-NAF-producing ducts could be easily identified with the Cytyc Ultraslim dilator, lavaged, and endoscoped. In general, NAF-producing ducts were more likely to produce adequate cellular material for diagnosis, have a greater epithelial cell count, higher incidence of atypia, and contain a lower incidence of endoscopically normal ducts than those that were non-NAF-producing. These findings would indicate that, when present, NAF-producing ducts may be preferable for study, but their absence does not preclude evaluation by lavage and endoscopy of the high-risk breast.

Breast duct endoscopy was performed on a lavaged duct in 24/25 subjects, and the duct examined to a distance of 4 cm or more in most subjects. When further advancement of the endoscope was limited, it was most commonly by a narrow intraductal lumen or fibrous bridging across the duct. A noteworthy finding on endoscopy was the many ductal branches present within the ductal system, and the correspondingly large surface area of ductal epithelium which is present. Whereas the rigid ductal endoscope is not able to navigate many of these branches, they should be accessible to lavage fluid, indicating an important benefit of ductal lavage. Breast duct endoscopy identified several intraductal structural abnormalities in these high-risk ducts, including fibrous strands and bridging, raised yellow sessile lesions, and a papillary lesion. Breast duct lavage identified atypical ductal epithelial cells in six ducts (five subjects), and in five ducts, the atypical cells were associated with structural changes in the corresponding duct (fibrous bridging, yellow sessile lesions, a papillary lesion). Whether the ductal abnormalities were the source of the atypia is not known because endoscopic sampling devices were not available at the time to determine the exact nature of the lesions. Four ducts were re-examined in follow-up at 6–14 months, and in all, the atypia had resolved in conjunction with resolution of the ductal changes, suggesting a possible relationship. Evaluation of additional subjects with specific sampling of abnormalities is needed to better define these lesions. At the same time, many of these subjects were previously treated with systemic chemotherapy, which has been shown to cause inflammatory changes, including cellular atypia, in normal breast tissue [32]. It is possible that the endoscopic and cytologic abnormalities are related, at least in part, to the previous therapy. Our findings of the resolution of atypia in the absence of treatment is in agreement with Johnson-Maddux et al. [33]. They performed repeat ductal lavage in 23 women with either mild or marked atypia on initial lavage, and found that the second lavage was classified as atypical in 48%, indicating that while atypia is frequently diagnosed by ductal lavage, the reproducibility of repeat lavage is low, and suggesting that lavage atypia may be physiologic or artifactual rather than pathologic in many cases. These findings emphasize the need to identify additional markers, such as molecular, that characterize high-risk ductal epithelium.

Endoscopy also provided direct access to the duct lining for ductal epithelial sampling. We examined the feasibility of obtaining ductal epithelial samples with endoscopic sampling devices, and their contribution to ductal lavage sampling. We found this sampling technique increased acquisition of ductal epithelial cell samples compared with breast duct lavage alone in the majority of cases, and may potentially expand the research studies performed on ductal epithelium in these subjects. Multiple samples of up to 33,000 cells/sample of relatively pure ductal epithelium could be obtained. Individual cellular aliquots could be collected, and sampling performed with minimal trauma to the duct. Among the 11 cases evaluating intraductral sampling, 8 were successful and 3 were unsuccessful; the latter were

often conducted at a distance of $\leq$5 cm, suggesting sampling might best be performed in the more proximal ductal tree $\geq$6 cm from the nipple. In the three unsuccessful cases, it is also possible that this was a technical problem due to insufficient contact of the sampling device with the ductal wall. Finally, endoscopic sampling may also compliment ductal lavage by allowing additional detection of atypia. In three subjects, cellular atypia was identified in a single endoscopic sample where the ductal lavage specimen was negative.

In conclusion, combined ductal lavage and endoscopy is a feasible approach for the evaluation of the high-risk duct and ductal epithelium. It may be limited, however, by lower cellular yields on ductal lavage, and the limited number of ducts that can be studied. We found that, on ductal lavage, approximately 16% of subjects (31.8% of ducts) had inadequate cellular yield (ICMD). This may have resulted from several factors. Many of the subjects were postmenopausal and previously treated with tamoxifen and chemotherapy, which may have reduced epithelial content of the breast. Non-NAF-producing ducts were included which have a lower cellular yield. Endoscopy revealed stranding or bridging within the ductal lumen which may have provided potential barriers to lavage the duct, especially the more proximal portions. We were able to study 1–2 ducts with combined lavage-endoscopy, and while it is assumed that most of the high-risk breast is a cancerized field [8], the extent to which sampling of 1–2 ducts provides representative material is not known. The time, cost, and requirement for intravenous sedation are additional limitations. This approach does, however, allow improved characterization of a duct and ductal system with, in most cases, increased cellular material for additional studies, which should contribute to our understanding of the high-risk breast carcinogenesis. Combined ductal lavage and ductal endoscopy appear to have the most utility in a research setting. Evaluation of additional patients, including expansion to other high-risk groups, is needed to further define its role.

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## REFERENCES

1. Smalley M, Ashworth A: Stem cells and breast cancer: A field in transit. Nat Rev Cancer 2003;3:832–844.
2. Botti C, Pescatore B, Mottolese M, et al.: Incidence of chromosomes 1 and 17 aneusomy in breast cancer and adjacent tissue: An interphase cytogenetic study. J Am Coll Surg 2000;190:530–539.
3. Euhus DM, Cler L, Shivapurkar N, et al.: Loss of heterozygosity in benign breast epithelium in relation to breast cancer risk. J Natl Cancer Inst 2002;94:858–860.
4. Larson PS, de las MA, Cupples LA, et al.: Genetically abnormal clones in histologically normal breast tissue. Am J Pathol 1998; 152:1591–1598.
5. Allan DJ, Howell A, Roberts SA, et al.: Reduction in apoptosis relative to mitosis in histologically normal epithelium accompanies fibrocystic change and carcinoma of the premenopausal human breast. J Pathol 1992;167:25–32.
6. Forsti A, Louhelainen J, Soderberg M, et al.: Loss of heterozygosity in tumour-adjacent normal tissue of breast and bladder cancer. Eur J Cancer 2001;37:1372–1380.
7. Umbricht CB, Evron E, Gabrielson E, et al.: Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. Oncogene 2001;20:3348–3353.
8. O'Shaughnessy JA, Kelloff GJ, Gordon GB, et al.: Treatment and prevention of intraepithelial neoplasia: An important target for accelerated new agent development. Clin Cancer Res 2002;8: 314–346.
9. 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;135:130–141.
10. 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;93:1624–1632.
11. 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;92:1217–1227.
12. Fabian CJ, Zalles C, Kamel S, et al.: Breast cytology and biomarkers obtained by random fine needle aspiration: Use in risk assessment and early chemoprevention trials. J Cell Biochem Suppl 1997;28-29:101–110.
13. 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;9:1509–1516.
14. Yamamoto D, Senzaki H, Nakagawa H, et al.: Detection of chromosomal aneusomy by fluorescence in situ hybridization for patients with nipple discharge. Cancer 2003;97:690–694.
15. Evron E, Dooley WC, Umbricht CB, et al.: Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR. Lancet 2001;357:1335–1336.
16. Kuerer HM, Thompson PA, Krishnamurthy S, et al.: High and differential expression of HER-2/neu extracellular domain in bilateral ductal fluids from women with unilateral invasive breast cancer. Clin Cancer Res 2003;9:601–605.
17. 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.
18. Sauter ER, Ehya H, Schlatter L, et al.: Ductoscopic cytology to detect breast cancer. Cancer J 2004;10:33–41.
19. Golewale NH, Bryk M, Nayar R, et al.: Technical modifications of ductal lavage to improve cell yield. San Antonio Breast Cancer Symposium 2003; Abstract #1024.
20. Dooley WC: Routine operative breast endoscopy during lumpectomy. Ann Surg Oncol 2003;10:38–42.
21. Abati A, Greene M, Filie AC, et al.: Quantification of the cellular components of breast ductal lavage samples. Diagn Cytopathol 2006;34:78–81.
22. The uniform approach to breast fine-needle aspiration biopsy. NIH Consensus Development Conference. Am J Surg 1997;174: 371–385.
23. Yeatman TJ, Lyman GH, Smith SK, et al.: Bilaterality and recurrence rates for lobular breast cancer: Considerations for treatment. Ann Surg Oncol 1997;4:198–202.
24. Samant RS, Olivotto IA, Jackson JS, et al.: Diagnosis of metachronous contralateral breast cancer. Breast J 2001;7:405–410.
25. Robbins GF, Berg JW: Bilateral primary breast cancer: A prospective clincopathological study. Cancer 1964;17:1501–1527.

26. McCredie JA, Inch WR, Alderson M: Consecutive primary carcinomas of the breast. Cancer 1975;35:1472–1477.
27. Hislop TG, Elwood JM, Coldman AJ, et al.: Second primary cancers of the breast: Incidence and risk factors. Br J Cancer 1984;49:79–85.
28. Khan SA, Wiley EL, Rodriguez N, et al.: Ductal lavage findings in women with known breast cancer undergoing mastectomy. J Natl Cancer Inst 2004;96:1510–1517.
29. Khan SA, Baird C, Staradub VL, et al.: Ductal lavage and ductoscopy: The opportunities and the limitations. Clin Breast Cancer 2002;3:185–191.
30. Love SM, King BL: Ductal lavage in nipple aspirate fluid producing and non-producing ducts in healthy volunteers. Breast Cancer Res Treat 2004;88 Supplement 1:S189–S190 (abstract 5019).
31. Love SM, Barsky SH: Anatomy of the nipple and breast ducts revisited. Cancer 2004;101:1947–1957.
32. Kennedy S, Merino MJ, Swain SM, et al.: The effects of hormonal and chemotherapy on tumoral and nonneoplastic breast tissue. Hum Pathol 1990;21:192–198.
33. Johnson-Maddux A, Ashfaq R, Cler L, et al.: Reproducibility of cytologic atypia in repeat nipple duct lavage. Cancer 2005;103: 1129–1136.