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Ductal lavage provides a nonsurgical means of evaluating risk based on cytopathologic evidence of abnormal proliferative activity in the breast.

Ductal Lavage for Breast Cancer Risk Assessment

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Background: *Effective chemoprevention is available for breast cancer, but it is associated with the potential for life-threatening adverse events. Accurate identification of women facing increased risk of breast cancer is therefore essential. Atypical hyperplasia is a histopathologic pattern that has been consistently associated with an elevated future risk of breast cancer.*

Methods: *The literature was reviewed to assess the strength of the association between atypical hyperplasia and breast cancer. The rationale for developing a nonsurgical modality to document the presence of atypia is discussed.*

Results: *Ductal lavage identifies atypical hyperplasia by retrieving epithelial cells shed into the ductal system with a specially designed catheter. Women with clinical evidence of increased breast cancer risk may consider ductal lavage as a means of determining whether abnormal proliferative activity is occurring in their breasts at a given point in time from ducts yielding fluid.*

Conclusions: *Ductal lavage is a minimally invasive procedure that facilitates the detection of atypia via retrieval of breast ductal fluid that can be evaluated cytologically. It can facilitate the selection of women who may benefit from breast cancer risk reduction intervention.*

Introduction

Breast cancer risk reduction strategies are currently available in the form of medical and surgical interventions, all of which are associated with possible life-threatening complications. Prophylactic mastectomy is clearly a disfiguring and extreme maneuver; oophorectomy in premenopausal women results in the health risks of premature menopause, and chemoprevention with tamoxifen may result in thromboembolic phenomena or uterine cancer.¹ Therefore, it is essential that a woman subjected to this potential morbidity

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Submitted June 11, 2002; accepted September 5, 2002.

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has been reliably identified as having a substantially elevated risk of breast cancer. Unfortunately, established breast cancer risk assessment models based on a woman's gynecologic and family history have well-recognized limitations. Ductal lavage provides the clinician with a nonsurgical means of evaluating risk based on cytopathologic evidence of abnormal breast tissue proliferative activity.

Ductal Lavage vs Aspiration

Sporadic nipple discharge (usually studied as nipple aspirates following manual breast massage) has been reported to be a risk factor for breast cancer for several decades.^{2,4} Nonlactational secretory activity in the breast may actually be a surrogate marker for proliferative changes in the ductal tissue. These proliferative changes may in turn indicate an increased risk for breast carcinogenesis. However, the yield of cellular material from a direct nipple aspirate is generally too low to permit a meaningful cytologic analysis. A major benefit of ductal lavage is the substantially improved yield of fluid characterized by a significant cellular content.

Dooley et al⁵ conclusively demonstrated the increased cytology yield resulting from ductal lavage compared to nipple aspirates in a multicenter study involving more than 500 women who were identified as being at high-risk for breast cancer on the basis of family history, prior personal history of breast cancer, and/or other features, resulting in a 5-year Gail model risk estimate of at least 1.7%. Ductal lavage was 3.5 times more successful at producing cytologically evaluable fluid compared to nipple aspirates (72% vs 21%, respectively; $P < .001$) and the median yield with ductal lavage was 13,500 epithelial cells compared to 120 cells for the subset of nipple aspirates that were evaluable.

The ductal lavage procedure involves the application of a topical anesthetic cream to the skin of the nipple and removal of keratin formation. Following breast

massage, a suction apparatus is applied to the nipple, and any fluid-yielding ducts are candidates for lavage. An attempt is made to cannulate these ducts with a specially designed catheter that is attached to two separate ports, one for infusion and the other for aspiration. A selected duct is catheterized, approximately 5 to 10 mL of saline is infused into the cannulated ductal system, and the fluid is aspirated and sent for cytologic analysis. The procedure is repeated in a stepwise fashion for any additional fluid-yielding ducts, using a separate catheter for each. The sites for each cannulated duct should be recorded on a grid or map representing the nipple to use for comparisons with any future attempts at ductal lavage. Alternatively, a segment of suture material can be left partially inserted into each cannulated ductal orifice, and a photo is taken to document these sites. The cytology report is standardized to stratify results as follows: (1) inadequate cellular material for diagnosis (fewer than 10 epithelial cells), (2) benign cells, (3) mildly atypical cells, (4) markedly atypical cells, or (5) malignant cells. An example of a lavage specimen characterized by atypical cells is shown in Fig 1.

Ductal Lavage as a Risk Assessment Tool

In the multicenter study reported by Dooley et al,⁵ 84% of participants had fluid-yielding ducts that were amenable to lavage, and 82% of these fluid-yielding ducts were successfully cannulated. Of the patients who were successfully lavaged, 23% were found to have atypia (17% mild and 6% marked atypia), 54% had benign cytology, and <1% had frankly malignant cells identified. Of the lavaged participants, 22% had an inadequate specimen, compared with a 73% inadequacy rate for the nipple aspirate specimens. Furthermore, ductal lavage was more likely to yield a diagnosis of atypia compared to nipple aspirates. The subset of patients found to have atypia is particularly important because this category of elevated risk may be associated with increased benefits from chemoprevention.

The presence of atypical hyperplasia in breast tissue is an established risk factor for future breast cancer development.^{2,3,6-17} The prevalence of atypical hyperplasia in the breasts of an unselected patient population is not fully defined, but it is probably less than 15% and is likely to vary according to method of ascertainment. An autopsy series from Australia reported by Bhathal et al¹⁸ detected atypical hyperplasia in 12.6% of breasts examined histopathologically in 207 forensic postmortem examinations. Lee et al¹⁹ and Wrensch et al^{2,3} identified atypia in nipple aspirates in 2% to 3.4% of mammographically screened women and in 0.7% of unselected volunteers from the west coast of the United States.

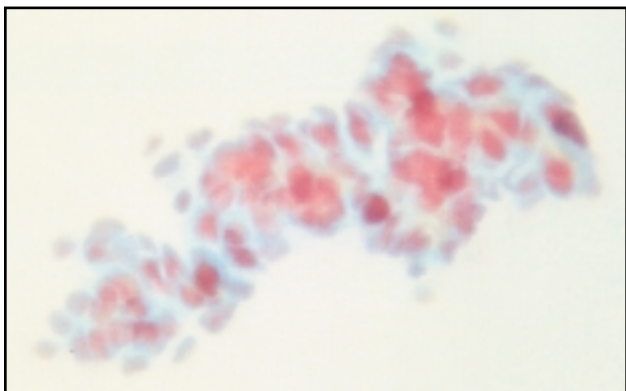


Fig 1. — Example of a lavage specimen characterized by atypical cells.

Association of Atypical Hyperplasia With Relative Risk for Breast Cancer: Selected Studies

Author	No. of Patients	Selection Criteria	Median Follow-up	% Atypia	Method of Detection	Breast Cancer Relative Risk (95% Confidence Interval)
Fabian et al ⁶ 2000	480	University of Kansas patients with high risk via FH/PH breast cancer or history of atypical hyperplasia/ductal carcinoma in situ	45 mos	12.0*	Periareolar FNAs for cytology	5.02 (2.01-12.56)
Wrensch et al ³ 2001	3,633 (Group 1) 3,271 (Group 2)	Group 1: UCSF BCDDP participants Group 2: UCSF volunteers	21 yrs (Group 1) 9 yrs (Group 2)	2.4	Nipple aspirate fluid	2.4 (1.6-3.7) 2.8 (1.5-5.5)
Wrensch et al ² 1992	2,343	General screening population	12.7 yrs	2.0	Nipple aspirate fluid	4.9 (1.7-13.9)
Dupont et al ⁷ 1999	9,494	Vanderbilt University patients with biopsy-proven benign breast disease	20 yrs	3.0	Surgical biopsy specimens	3.58 (2.6-5.0)
Bodian et al ⁸ 1993	1,799	Haagensen patient population from Columbia-Presbyterian Medical Center with biopsy-proven benign breast disease	20.6 yrs	19.0	Surgical biopsy specimens	3.0 (1.5-6.0) (moderate/severe atypia) 2.3 (1.6-3.4) (mild atypia)
Hutchinson et al ⁹ 1980	1,053	Private practice patient population with biopsy-proven benign breast disease	12.9 yrs	3.1	Surgical biopsy specimens	2.85 (0.34-10.28)
Carter et al ¹⁰ 1988	16,692	BCDDP participants with history of biopsy-proven benign breast disease	8.3 yrs	7.8	Surgical biopsy specimens	3.0 (2.1-4.1)
Dupont et al ¹¹ 1985	3,303	History of biopsy-proven benign breast disease	17 yrs	3.6	Surgical biopsy specimens	All: 5.3 (3.1-8.8) With FH of breast cancer: 8.9 (4.8-17) Without FH of breast cancer: 3.5 (2.3-5.5)
London et al ¹² 1992	121 (cases) 488 (controls)	Nurses Health Study participants with either cancer or biopsy-proven benign breast disease	9 yrs	22.3 (cases) 9.6 (controls)	Surgical biopsy specimens	All: 3.7 (2.1-6.8) With FH of breast cancer: 7.3 (1.1-50.1) Without FH of breast cancer: 3.7 (1.9-7.0)
McDivitt et al ¹³ 1992	433 (cases) 261 (controls)	Cancer and Steroid Hormone Study participants with either cancer or biopsy-proven benign breast disease	N/A	15.9 (cases) 10.0 (controls)	Surgical biopsy specimens	Odds ratio 2.6 (1.6-4.1)
Palli et al ¹⁴ 1991	62 (cases) 315 (controls)	Women from Florence, Italy, breast cancer screening program	N/A 2.2 (controls)	17.7 (cases)	Surgical biopsy specimens	Odds ratio 13.0 (4.1-41.7)
Krieger and Hiatt ¹⁵ 1992	2,731	San Francisco, California, women with biopsy-proven benign breast disease	16 yrs	12.0	Surgical biopsy specimens	Rate ratio 7.2 (Black-Chabon score 5/severe atypia)
Dupont et al ¹⁶ 1993	95 (cases) 227 (controls)	BCDDP mammography screening program participants	N/A	14.7 (cases) 4.4 (controls)	Surgical biopsy specimens	Odds ratio 4.3 (1.7-11)
Byrne et al ¹⁷ 2000	133 (cases) 610 (controls)	Nurses Health Study participants with either cancer or biopsy-proven benign breast disease	N/A	25.6 (cases) 11.8 (controls)	Surgical biopsy specimens	Odds ratio 3.6 (2.0-6.4)

* Atypia found in 12% of FNAs from single aspirates; sequential FNAs over 6 and 12 months resulted in 21% atypia prevalence for pooled samples.
 FNA = fine-needle aspirate UCSF = University of California San Francisco BCDDP = Breast Cancer Detection and Demonstration Project
 FH = family history PH = prior history N/A = not applicable

One model for breast tumorigenesis features the evolution of breast ductal cells from normal to hyperplastic, followed by the development of atypical hyperplasia. Further accumulation of genetic abnormalities as ductal cells proceed through the cell cycle leads to the development of carcinoma in situ and ultimately invasive cancer.²⁰ However, the pathogenesis of breast cancer may be heterogeneous. Not every case of breast atypical hyperplasia is committed to progressing through the complete sequence resulting in invasive cancer; some invasive phenotypes may develop without passing through the full spectrum of premalignant phases; and the chronology for these carcinogenic stages may vary.²⁰ Nonetheless, several studies have demonstrated that a diagnosis of atypical hyperplasia is associated with a relative risk for breast cancer that ranges from 3 to 5 over the following 5 years (Table). This risk may double if the patient also has a family history of breast cancer,^{11,12} it appears to be unaffected by history of estrogen replacement therapy,⁷ and it may begin to decline back to the baseline general population risk after 5 to 10 years if no intervening risk-related events occur.²¹ Ma and Boyd²² conducted a meta-analysis of 18 studies involving the strength of the association between atypical hyperplasia and subsequent breast cancer reported between 1960 and 1992. This pooled analysis resulted in a total sample size of over 180,000 patients, and the summary odds ratio for atypical hyperplasia as a breast cancer risk factor was 3.67 (95% confidence interval, 3.16-4.26). The authors rigorously applied the Bradford Hill criteria (eg, temporal relationship, strength of association/dose-response correlation, and biologic plausibility) for assessing the validity of a risk factor,²³ and they conclude that these guidelines "indicated strongly that atypical hyperplasia is a risk factor for breast cancer."

Seminal to discussions regarding the potential value of ductal lavage as a risk assessment tool is the fact that mode of detecting atypia appears to be irrelevant to the strength of the observed association between its presence and likelihood of future breast cancer development. Similar relative risks for breast cancer have been reported following atypical cells detected on open surgical biopsy specimens,⁷⁻¹³ fine-needle aspirates,⁶ and nipple aspirate material.^{2,3} It has been assumed that atypical cells identified from ductal lavage fluid confer the same degree of increased breast cancer risk in the affected woman. It should be noted, however, that there have not yet been any long-term studies reported regarding outcome of women undergoing ductal lavage. Therefore, the relative risk for atypical hyperplasia detected in this setting has not yet been conclusively documented, although the presumption of a similar measure of effect seems reasonable. Furthermore, breast cytopathology has evolved into a specialized field, and any medical institution that is

developing a ductal lavage program should ensure the availability of a skilled, experienced cytopathologist.

Risk Assessment Models

The established breast cancer risk assessment models have several limitations resulting in increased motivation to investigate more accurate alternative means of identifying high-risk individuals. The widely used Gail breast cancer risk model²⁴ is utilized to determine eligibility for chemoprevention trials.^{25,26} The Gail model is a logistic regression model that was developed by analyzing breast cancer risk factors from a case-control subset of participants of the Breast Cancer Detection and Demonstration Project (BCDDP), a mammography screening program. This model estimates the likelihood that an individual woman will develop breast cancer over a 5-year period and over her projected lifetime by accounting for the relative risks conferred by age at evaluation, expected longevity, first-degree family history of breast cancer, age at menarche, nulliparity or age at first live birth, and number of prior breast biopsies. The Gail model has been modified²⁷ since the original version was published in 1989²⁴ to estimate risk for invasive disease only and to account for ethnicity (African American vs white American) and whether atypical hyperplasia was present in any prior biopsies. A relative risk of 1.8 is included in the model calculation for a woman with a history of a breast biopsy demonstrating atypical hyperplasia.

The Gail model has been validated as being accurate in predicting numbers of cancer cases likely to develop in a given cohort of patients,²⁷⁻³⁰ yet its generalizability has been challenged because of several issues. Until recently,³¹ the model was not validated in a cohort of non-white American women; it does not account for risk conferred by the paternal or extended family history; and it does not account for well-known pathologic indices of increased breast cancer risk such as lobular carcinoma in situ. Furthermore, although hormone replacement therapy (HRT) was a significant breast cancer risk factor in the BCDDP patient population from which the model was derived, the number of appropriately exposed women was insufficient for this feature to be included into the model.²⁴ Recently published results from the placebo-controlled phase III study of HRT in the Women's Health Initiative³⁰ demonstrated that after a median follow-up of 5.2 years, exogenous hormones were associated with a hazard ratio of 1.26 (95% confidence interval, 1.00-1.59). These findings raise the potential concern that the model may not be adequately accounting for the degree of risk conferred by an exposure that has increased in prevalence since the era of accrual to the BCDDP.

As demonstrated by Rockhill et al,³¹ the model's discriminatory accuracy at the individual level is particularly suboptimal. Detection of proliferative breast hyperplasia, especially with atypia, may be a useful means of refining short-term risk estimates for the individual patient based on epidemiologic models. In the only study where the model's validity was assessed in a non-white patient population, its accuracy was questioned in a subset of young African American women (ie, younger than 45 years old).³² This study also demonstrated weakness of the model at the individual level.

Tamoxifen Use

The need for an accurate risk assessment tool is even more compelling in light of the available data regarding the efficacy of tamoxifen for breast cancer risk reduction. The National Surgical Adjuvant Breast and Bowel Project's Breast Cancer Prevention Trial (BCPT) randomized more than 13,000 high-risk women to receive either tamoxifen or a placebo for 5 years.²⁵ Eligibility criteria to participate in this study as a high-risk patient included a 5-year Gail model risk estimate of at least 1.66%, age over 60 years, and a history of lobular carcinoma in situ. The study was unblinded early, after approximately 4½ years, because of the magnitude of difference in breast cancer incidence for the two arms of the study. The participants taking tamoxifen had a 49% lower breast cancer incidence compared with the participants assigned to the placebo arm.

Unfortunately, the BCPT confirmed the previously demonstrated potential adverse effects associated with tamoxifen use: statistically significant increases in incidence of uterine cancer, thromboembolic events, and vasomotor symptoms among the tamoxifen users. These adverse effects are generally rare, and the risk of experiencing them is easier to justify in a woman with an established breast cancer diagnosis, where the primary concern is to address and eradicate micrometastatic disease. However, in otherwise healthy women, the decision to take tamoxifen for pure prophylaxis and face the risk of medication-associated morbidity is more difficult. Port et al³³ recently documented the fact that even among high-risk women, there is substantial reluctance regarding a commitment to tamoxifen chemoprevention therapy. Forty-three patients at increased risk for breast cancer (including 23% with lobular carcinoma in situ and 61% with 5-year Gail model risk estimate of at least 1.7%) were provided with risk reduction counseling and education. Although all 43 patients were offered tamoxifen on the basis of their risk profiles, 15 patients (35%) declined definitively; and 26 (61%) were undecided, and only 2 (4.7%) accepted this recommendation. A clear assessment of the underlying risk of

breast cancer and the parallel understanding of benefits gained by chemoprevention would facilitate the decision-making process for the undecided patients.

Detection of atypia is a marker of risk that may clarify the risk-benefit ratio for the individual patient. Among the BCPT participants, the women with a history of atypia experienced the greatest benefit from tamoxifen use. In this subset, chemoprevention resulted in a risk reduction of 86%.²⁵ Nipple aspirate fluid is currently being investigated for levels of expression of *HER-2/neu*.³⁴ It may therefore be speculated that a variety of proteins and molecular markers will ultimately be identified in breast ductal lavage fluid, and some of these may become important for risk assessment as well.

Appropriate follow-up management of patients electing to undergo ductal lavage is an evolving area of study. Patients wishing to undergo serial lavage for ongoing risk assessment should understand that standards for this management strategy have not yet been developed, although annual or biennial studies might be considered. Morrow et al³⁵ have proposed a surveillance strategy that addresses these options. One possible algorithm for incorporating ductal lavage and its findings into clinical risk assessment practice is shown in Fig 2.

Evaluation of Lavage Fluid

When the lavage fluid yields frankly malignant cells, it is prudent to confirm the assessment with a second opinion cytopathology evaluation. Repeat breast imaging with mammography and ultrasound followed by a ductography and/or ductoscopy might also be useful, and repeat lavage to determine whether the abnormality is reproducible should also be considered. Breast MRI³⁶ can also be highly sensitive for detecting an otherwise occult breast tumor. If the entire workup is negative, a terminal duct excision would intuitively seem to be a rational diagnostic approach, but the yield for this strategy is unknown. In summary, the optimal and most definitive means of managing the patient found to have malignant cells on ductal lavage and localizing the site of breast pathology is unclear at present. Fortunately, this condition is unusual, occurring in less than 1% of high-risk women who undergo ductal lavage as a risk assessment procedure.⁵

Many high-risk women are seeking some measure of individualized evidence to determine the appropriate timing for risk reduction intervention. The decision to commit to 5 years of tamoxifen use or to undergo prophylactic mastectomy is a difficult one for the individual patient. Ductal lavage offers a promising opportunity to clarify risk for these women at any given point

in time. However, ductal lavage has not yet been fully integrated into the healthcare third-party payor system. Insurance companies are not universally cognizant of the value and indications for ductal lavage as a risk assessment tool. Reimbursement policies have not been standardized, which limits the availability of the procedure. A patient who wishes to undergo the procedure must be counseled that prior approval with her health insurance carrier will be necessary to minimize out-of-pocket expenses.

As ductal lavage is evaluated further by the oncology research community, we may develop other applications for its use. For example, ductal lavage may be incorporated into neoadjuvant chemotherapy protocols to monitor response. Also, ductal lavage findings may be analyzed in long-term tamoxifen users to study molecular changes and perhaps to identify patients developing tamoxifen resistance. The procedure may even become useful in assessing candidates for breast conservation therapy. At present, these possibilities are

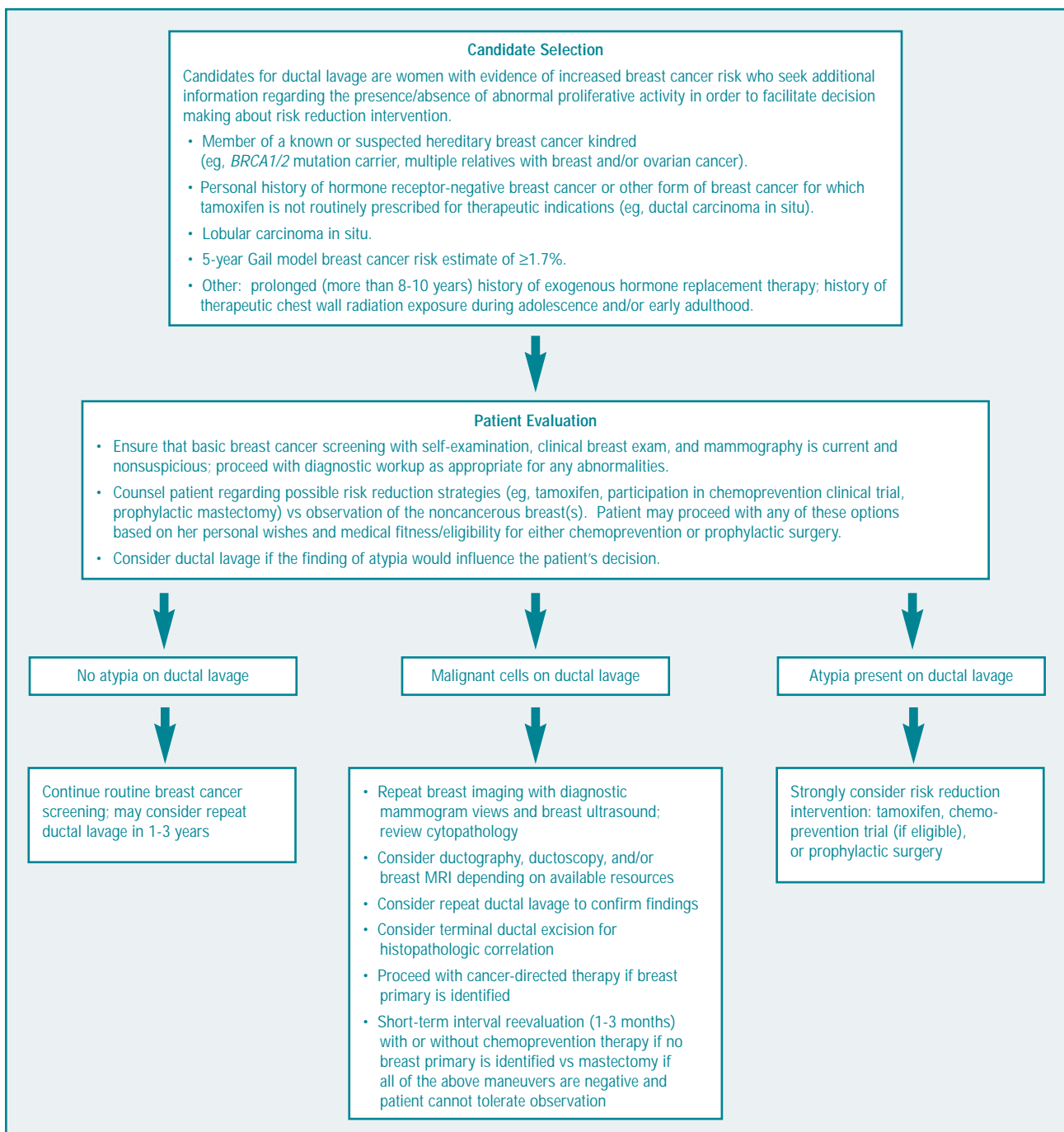


Fig 2. — Algorithm for incorporating ductal lavage into risk assessment strategies.

speculative and will require investigation in the context of carefully designed clinical trials.

Conclusions

Ductal lavage is promising as a risk assessment adjunct. It is a minimally invasive procedure that is well-tolerated by patients, and it is an additional mechanism for identifying women who harbor atypical hyperplasia in their breast ductal system. Ductal lavage is therefore a potentially valuable method of estimating individualized risk, and it can identify women who may derive a significant benefit from risk reduction strategies such as chemoprevention. As we develop a better understanding of the molecular changes that occur during breast cancer pathogenesis, we are likely to identify other molecular markers that may be detected in ductal fluid, and these markers may become important in assessing risk as well as in monitoring response to treatment for patients with an established breast cancer diagnosis.

The authors gratefully acknowledge the assistance of Shashan Madan, MD, Department of Pathology, Wayne State University, for cytology and photography support in this project.

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