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Research article

Cytologic features of nipple aspirate fluid using an automated non-invasive collection device: a prospective observational study

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Abstract

Background: Detection of cytologic atypia in nipple aspirate fluid (NAF) has been shown to be a predictor of risk for development of breast carcinoma. Manual collection of NAF for cytologic evaluation varies widely in terms of efficacy, ease of use, and patient acceptance. We investigated a new automated device for the non-invasive collection of NAF in the office setting.

Methods: A multi-center prospective observational clinical trial involving asymptomatic women designed to assess fluid production, adequacy, safety and patient acceptance of the HALO NAF Collection System (NeoMatrix, Irvine, CA). Cytologic evaluation of all NAF samples was performed using previously described classification categories.

Results: 500 healthy women were successfully enrolled. Thirty-eight percent (190/500) produced fluid and 187 were available for cytologic analysis. Cytologic classification of fluid producers showed 50% (93/187) Category 0 (insufficient cellular material), 38% (71/187) Category I (benign non-hyperplastic ductal epithelial cells), 10% (18/187) Category II (benign hyperplastic ductal epithelial cells), 3% (5/187) Category III (atypical ductal epithelial cells) and none were Category IV (unequivocal malignancy). Overall, 19% of the subjects produced NAF with adequate cellularity and 1% were found to have cytologic atypia.

Conclusion: The HALO system is a simple, safe, rapid, automated method for standardized collection of NAF which is acceptable to patients. Cytologic assessment of HALO-collected NAF showed the ability to detect benign and pre-neoplastic ductal epithelial cells from asymptomatic volunteers.

Background

The majority of breast cancers originate in the epithelium lining the milk ducts. It is believed that most breast cancers are slow growing and progress from precancerous cells, which have cellular and nuclear changes that can be identified microscopically. Finding microscopic evidence of ductal epithelial atypia/atypical ductal hyperplasia (ADH) has been shown in previous epidemiologic studies to be a predictor of future breast cancer development in an individual woman. [1-10] This increased risk has been identified using random peri-areolar fine needle aspiration (FNA), tissue biopsy or nipple secretion samples for assessment of cytologic atypia.

Figure 1
The HALO NAF collection system (photos courtesy of NeoMatrix, Irvine, CA). A. Control Console B. Adjustable Breast Cups with Fluid Reservoir Cassette C. Disposable Sample Collection Cups.

petals. Towards the end of the cycle, the HALO system initiates mild compression of the Breast Cup petals to retrieve any fluid from the ducts. The entire cycle is 5 minutes in duration. The Console indicates when the NAF acquisition cycle has completed. Suction is gently automatically released from the breast cups.

Any collected NAF was transferred from the nipple or sample collection cup(s) to a vial of fixative (CytoLyt, Cytyc Corporation, Boxborough, MA) using a pipette if necessary. If fluid was obtained from either one or both breasts, all samples were combined into a single sample preservative vial. Only one attempt was made to obtain NAF in the five minute session, and if no NAF was produced by either breast, the participant was considered to be a non-producer.

Sample processing and cytologic examination

All samples were shipped to a single reference laboratory (ARUP Laboratories, Inc., Salt Lake City, UT). Microscopic slides were prepared from the entire NAF sample using a Millipore filter technique (Millipore Corp., Billerica, MA), which was chosen due to the low cellularity of the specimens. The filter preps were stained with the modified Papanicolaou stain technique.

Each slide was reviewed by one of a group of three cytopathologists with experience examining breast

Table 1: Nipple aspirate fluid cytology classification*

Classification	Characteristics	Interpretation
Unsatisfactory (Category 0)	<10 ductal epithelial cells.	Unsatisfactory specimen.
Benign (Category I)	Duct epithelial cells within normal limits. Foam cells. Apocrine metaplastic cells.	No malignant cells identified. Benign (non-hyperplastic) ductal epithelial cells present.
Hyperplasia (Category II)	Minimal changes including slight cell and nuclear enlargement. Chromatin remains finely granular and evenly distributed. Small and regular nucleoli sometimes present. Cell distribution predominately in groups and cohesive with >10–50 cells (papillary and apocrine subcategories).	No malignant cells identified. Benign hyperplastic ductal epithelial cells present.
Atypical Hyperplasia (Category III)	Moderate to severe abnormalities with distinct nuclear enlargement, increasing nuclear to cytoplasmic ratio, irregular nuclear borders, and nuclear variation. Coarsely granular chromatin. Prominent chromocenters. Cell distribution in groups with some papillary formations. Increased numbers of single atypical cells (apocrine type subcategory).	Atypical hyperplastic ductal epithelial cells present. Malignancy cannot be completely excluded.
Malignancy (Category IV)	Single cells and groups of cells with unequivocal nuclear features of cancer.	Malignant cells present derived from adenocarcinoma.

* King et al [4]

NAF was obtained from 31% of non-white subjects (p <= 1.0). Thirty-six percent of subjects with no 1st degree family history produced NAF, 46% of subjects with one 1st degree relative with breast cancer, and 75% of subjects who had more than one 1st degree relative with breast cancer (p <= 0.10). Forty-two percent of women with a lactation history produced NAF while 34% of women who never lactated were fluid producers (p < 0.10). Overall, 14% had at least one first degree relative with cancer and 11% had a history of a previous breast biopsy. In summary, none of the differences between fluid producers and non-producers with regards to any of the listed demographics was statistically significant.

Nipple aspirate fluid analysis

Three of the 190 specimens collected from the fluid producers had a container leak during specimen transport and therefore could not be analyzed, with the remaining 187 available for evaluation. The final cytology results are summarized in Table 3. Fifty percent (93/187) of the NAF samples were classified as Category 0, 38% (71/187) Category I (Figure 3), 10% (18/187) Category II (Figure 4), and 3% (5/187) Category III (Figures 5 and 6). No Category IV (unequivocal malignancy) samples were identified. Statistical analysis whereby all patients 55+ are combined into one group in order to strengthen the raw numbers showed there was no significant difference between age groups or cytologic categories (p = 0.27).

Procedure acceptance and adverse events

A total of 419/500 (84%) women were surveyed for procedure acceptance four to eight weeks after their procedure. The average comfort assessment rating immediate post-procedure was 5.0 on a scale of 1–10 (one being most comfortable) and 4.2 at the four to eight week telephone/mail post-procedure survey (Table 4). The nipple, areola, and breast areas were visually assessed by the study nurse immediately following the procedure. Twenty-six percent of the participants had no observed skin redness after the procedure, 59% mild redness, 14% moderate redness and less than one per cent had severe redness reported. No major adverse events were reported. Two participants chose to discontinue the procedure mid-cycle due to discomfort and there were five reported minor events including bleeding or small surface lacerations. These were treated with topical ointment, observation, Keflex for one suspected mild mastitis, and Mycolog for candiasis noted in one participant. All resolved without further intervention. Eighty-three percent of the participants reported that they would have the HALO procedure again and 88% said they would recommend the procedure to others.

Nipple aspirate fluid and Gail score

Gail 5 year risk profiles were obtained for the participants over the age of 35. Overall, no statistical difference was seen with regards to fluid production and calculated Gail profile result (p = 0.2). Comparison of Gail risk (>1.7% vs. <1.7%) and cytology category results, for the 190

Table 2: Participant characteristics, demographics and fluid production status

	Overall, No. (%)	NAF Producers, No. (% of subgroup)	p-value
Total No. of Women Enrolled	500	190 (38.0)	
Age groups, y, No. (%)			p <= 1.0
18–24	63 (12.6)	16 (25.4)	
25–34	93 (18.6)	36 (38.7)	
35–44	115 (23.0)	45 (39.1)	
45–54	155 (31.0)	65 (41.9)	
55–64	71 (14.2)	26 (36.6)	
65+	3 (0.6)	2 (66.7)	
Parity, No. (%)			p <= 0.05 ***
Nulliparous	83 (16.6)	40 (48.2)	
Parous	417 (83.4)	150 (36.0)	
Age at Menarche, years, No. (%)			p <= 1.0
<=12	241 (48.2)	90 (37.3)	
13–14	193 (38.6)	77 (39.9)	
>=15	61 (12.2)	21 (34.4)	
Missing	5 (1.0)	2 (40)	
Ethnicity, No. (%)			p <= 1.0
Caucasian	445 (89.0)	173 (38.9)	
Non-Caucasian*	55 (11.0)	17 (30.9)	
1st Degree Relatives with breast cancer, No. (%)			p <= 0.1
No	429 (85.8)	156 (36.4)	
Yes, 1	67 (13.4)	31 (46.3)	
Yes, >=2	4 (0.8)	3 (75.0)	
History of breast biopsy, No. (%)			p <= 1.0
Yes**	56 (11.2)	22 (39.3)	
Menstrual status, No. (%)			p <= 1.0
Pre-Menopausal	358 (71.6)	137 (38.3)	
Menopausal	142 (28.4)	53 (37.3)	
Lactation history, No. (%)			p <= 0.1
Never lactated	268 (53.6)	92 (48.4)	
History of lactation	232 (46.4)	98 (51.6)	

* 47 total African American, 1 Asian, and 7 Hispanic. **type of biopsy not reported *** If only pre-menopausal women are included in the analysis of NAF production vs. parity there is no significant difference (p <= 1.0)

Table 3: Nipple aspirate fluid (NAF) cytologic findings

Cytologic diagnosis	No. of women/ Total No. fluid producers (%)	18–24 yr, No. (%)	25–34 yr, No. (%)	35–44 yr, No. (%)	45–54 yr, No. (%)	55–64, No. (%)	65 + yrs, No. (%)
Unsatisfactory (Category 0)	93/187 (49.7)	9/93 (9.7)	14/93 (15.1)	22/93 (23.7)	32/93 (34.4)	15/93 (16.1)	1/93 (1.1)
Benign (Category I)	71/187 (38.0)	5/71 (7.0)	15/71 (21.1)	21/71 (29.6)	25/71 (35.2)	5/71 (7.0)	0
Hyperplasia (Category II)	18/187 (9.6)	1/18 (5.6)	6/18 (33.3)	1/18 (5.6)	5/18 (27.8)	5/18 (27.8)	0
Atypical Hyperplasia (Category III)	5/187 (2.8)	1/5 (20.0)	0	1/5 (20.0)	2/5 (40.0)	0	1/5 (20.0)
Malignancy (Category IV)	0/187 (0.0)	0	0	0	0	0	0
Sample Leak	3/187 (1.6)	0	1	0	1	1	0

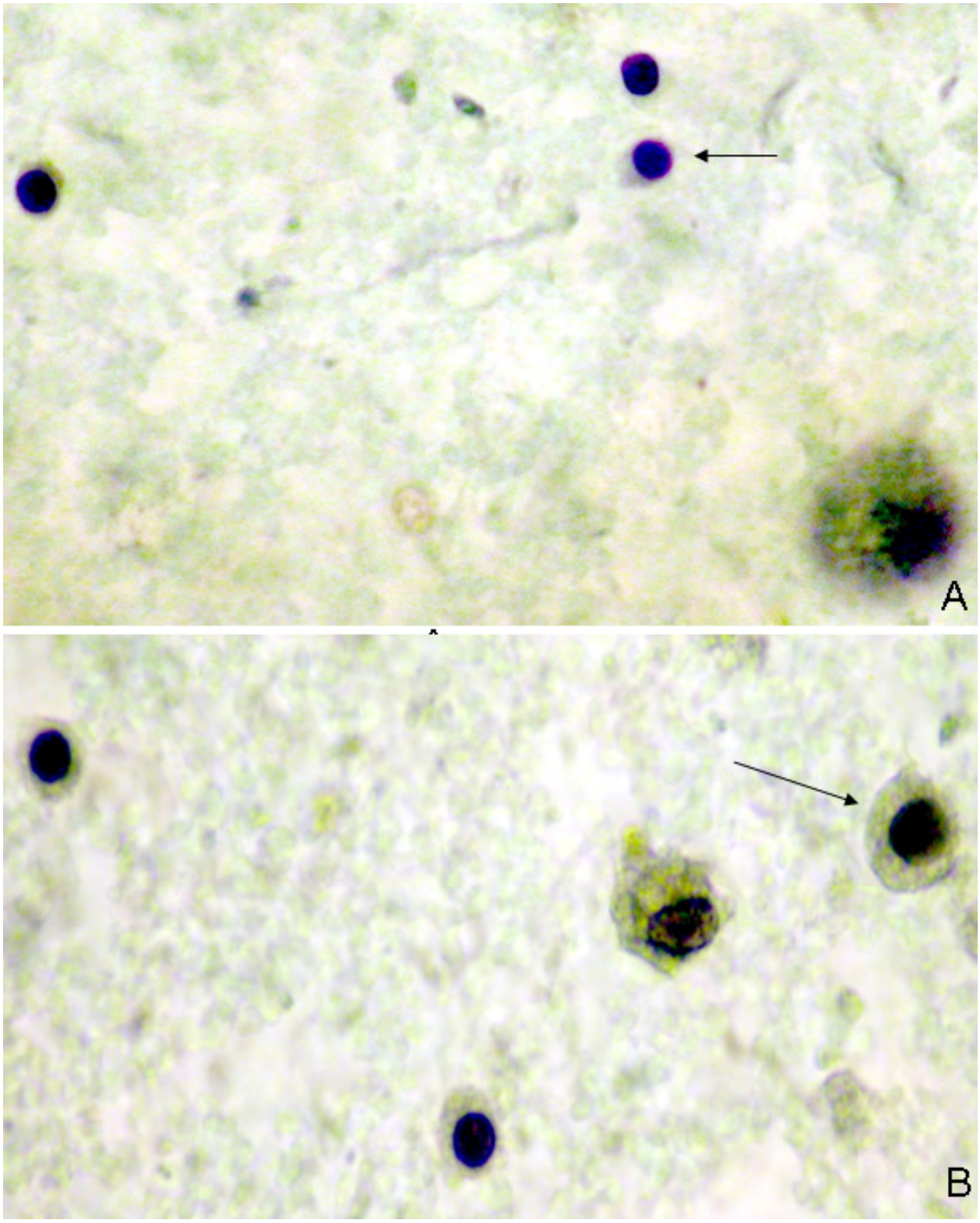

Figure 3
A-B. Category I. Benign (non-hyperplastic) ductal epithelial cells. The breast ductal epithelial cells are single, small, and uniform (arrow-A). Foam cells are a frequent finding (arrow-B). Apocrine metaplastic cells are sometimes identified. Pap 100X.