

Successful oxytocin-assisted nipple aspiration in women at increased risk for breast cancer

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Published online: 5 May 2010

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Abstract The high rate of interval malignancies urges for new screening methods for women at high risk for breast cancer. Nipple aspiration provides direct access to the breast tissue and its DNA, and therefore is a likely candidate, but clinical applications have been limited by the failure to obtain nipple aspiration fluid from most women. We performed oxytocin-assisted nipple aspiration in 90 women at increased risk for breast cancer based on family history or genetic test results ($n = 63$) and/or previous breast cancer ($n = 34$). Nipple fluid was obtained from 81/90 women (90%) and bilaterally in 77%. Mean discomfort rating was 0.6 (on a 0–10 scale), which was significantly lower than for mammography or MRI. These findings suggest that a new tool for biomarker detection in oxytocin-assisted nipple fluid of women at high risk for breast cancer is at hand.

Keywords Nipple aspiration · Breast cancer · BRCA1 · BRCA2 · Screening · Early detection

Abbreviations

DCIS	Ductal carcinoma in situ
NAF	Nipple aspirate fluid
QM-MSP	Quantitative multiplex methylation specific PCR
RPFNA	Random periareolar fine needle aspiration

Introduction

Breast cancer is the most prevalent cancer for women in the Western world, accounting for 519,000 deaths worldwide each year [1]. Approximately one in eight women will eventually develop breast cancer, but for some women this risk is even higher. A hereditary predisposition is the most important risk factor, with a lifetime risk of developing breast cancer up to 80% in women known to carry a *BRCA1* or *BRCA2* germline mutation [2].

Several screening and prevention options are available to these women, in order to either prevent the development of breast cancer or detect it at an early stage. However, until now, screening programs are only partly effective and in one out of four in high-risk women interval malignancies are detected [3]. Prophylactic mastectomy and salpingo-oophorectomy have been proven to effectively decrease the risk in women at high risk, but the morbidity associated with this procedure discourages many women to choose this option.

A screening method that gives access to the tissue in which breast cancer arises could add value to the existing methods and increase the rate of breast cancer detection at an early stage [4]. Nipple Aspirate Fluid (NAF), containing breast epithelial cells, free DNA, secreted proteins and hormones is produced in the breast ducts of non-lactating

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women. In the last decades, multiple techniques have been developed to obtain this fluid, such as non-invasive nipple aspiration, ductal lavage and Random Periareolar Fine Needle Aspiration (RPFNA). Nipple aspiration was previously reported to be only feasible in a small part of the population [5, 6]. The other two techniques showed higher success percentages, but their invasive nature is a serious drawback.

From a previous pilot study we reported that, using oxytocin nasal spray, we were able to obtain nipple fluid in 94% out of 67 healthy volunteers, of which 75% yielded fluid bilaterally [7]. The procedure was very well tolerated. Here we report the initial results of a prospective study on oxytocin-assisted nipple aspiration in women with a moderate to high breast cancer risk.

Materials and methods

Study population

This study is part of a project that aims to explore the possibilities of monitoring development of epigenetic changes in nipple aspirates, with the ultimate goal to optimally schedule preventive (or refrain from) breast surgery in high-risk women. To this end we are assessing promoter hypermethylation in nipple aspirates of high-risk women, of which some will undergo preventive or therapeutic surgery. This will enable assessing the correlation between methylation patterns and the development of (pre)malignant lesions in this group. Oxytocin-assisted nipple aspiration is performed annually. Besides, participants will undergo regular radiological and/or clinical examination as they were used to. Timing of breast surgery will be agreed between patient and oncologist based on usual clinical and radiological findings, blinded to the results of the nipple aspirate analyses. The study protocol of this study was approved by the Hospital's Ethics Committee and written informed consent was obtained from all participants. This report is a summary of the baseline nipple aspiration experience of the first 90 subjects enrolled between August 2008 and July 2009.

Eligibility criteria

Women were considered at increased risk and included in this study if they were carriers of a *BRCA1* or *BRCA2* germline mutation, had a pedigree-based risk $\geq 20\%$, or had a history of Ductal Carcinoma in Situ (DCIS) or invasive breast cancer. Pregnancy and bilateral mastectomy were exclusion criteria. We did not exclude women that previously underwent other forms of breast surgery, radiotherapy, chemotherapy or hormonal treatment.

Nipple aspiration procedure

Anesthetic cream (Emla) was applied onto the nipple while the breasts were warmed for 10 min. The subjects were administered 4 IE of oxytocin into both nostrils. The nipple was gently wiped with a dekeratinizing agent in order to remove keratin plugs and subsequently cleansed with ethanol. A suction cup (aspirator) was placed over the nipple. Repeated gentle suction by a syringe (10–50 cc) drew fluid to the nipple surface, where droplets were collected by capillary tubes. The entire procedure was repeated at the other breast. Quantitative Multiplex Methylation Specific PCR (QM-MSP) for *RASSF1A*, *SCGB3A1* and *APC* was performed on 50 randomly selected nipple fluid samples obtained in this study as described before [8].

Questionnaires

A questionnaire, addressing breast cancer risk factors and factors that might influence the success of the procedure was filled out before nipple fluid aspiration. After aspiration participants rated the experienced discomfort during different stages of the procedure and other screening procedures from 0 (“no discomfort at all”) to 10 (“the worst I can imagine”).

Statistical analysis

A paired *T*-test was used to compare the discomfort scores of different procedures. Fisher's exact and Chi-square tests were used to examine the association between NAF yielding and potential predictive values. For this analysis, subgroups from Table 1 were pooled because of low numbers. A selection of independent variables (see Table 3) was used in a logistic regression analysis using backward selection procedure, retaining covariates with $P \leq 0.10$, for the right and the left breast separately. SPSS 15.0 for Windows was used for statistical analysis, regarding two-sided $P \leq 0.05$ as significant.

Results

A total of 90 women at increased risk were included in this study. Sixty-three women had a family history of breast cancer, six of them were known carriers of a germline *BRCA1* mutation and six had a *BRCA2* mutation. Twelve women had a history of DCIS and 22 women previously had invasive breast cancer. Participants were 21–72 years old (mean 48 years). All baseline characteristics are shown in Table 1.

The procedure was very well endured. Mean discomfort rating for the total procedure was 0.6, which was

Table 1 Patient characteristics of 90 patients at increased risk for invasive breast cancer undergoing oxytocin-assisted nipple fluid aspiration

	<i>n</i> (%)
Age (years)	
< 40	22 (24%)
40–49	25 (28%)
≥ 50	43 (48%)
BRCA germline mutation	
None detected	28 (31%)
BRCA1	6 (7%)
BRCA2	6 (7%)
BRCA1 in family ^a	2 (2%)
BRCA2 in family ^a	1 (1%)
Pedigree-based risk	
None	27 (30%)
Moderate (20–30%)	12 (13%)
High (30–40%)	17 (19%)
Known mutation (> 40%)	18 (20%)
Personal history of breast cancer	
No	56 (62%)
DCIS	12 (13%)
Invasive carcinoma	22 (24%)
Spontaneous nipple discharge	
Yes	11 (12%)
No	79 (88%)
Number of live births	
0	30 (33%)
1–2	43 (48%)
≥ 3	17 (19%)
Age at first birth (years)	
< 25	18 (20%)
25–29	27 (30%)
≥ 30	15 (17%)
Age at menarche (years)	
< 12	27 (30%)
12–14	29 (32%)
> 14	32 (36%)
Menopausal status	
Premenopausal	42 (47%)
Postmenopausal	48 (53%)
Menstrual cycle day ^b	
1–7	12 (13%)
7–14	4 (4%)
14–21	9 (10%)
> 21	11 (12%)
Age at menopause (years)	
< 45	16 (18%)
45–50	16 (18%)
> 50	16 (18%)

Table 1 continued

	<i>n</i> (%)
Previous breast feeding	
Yes	51 (57%)
No	39 (43%)
Current oral contraceptive use	
Yes	14 (16%)
No	76 (84%)
Current hormonal replacement therapy	
Yes	2 (2%)
No	88 (98%)
Size breast	
Small (A–B)	24 (27%)
Normal (C–D)	58 (64%)
Large > D	8 (9%)
Prophylactic oophorectomy	
Yes	18 (20%)
No	72 (80%)
Previous chemotherapy	
Yes	12 (13%)
No	78 (87%)
Previous radiotherapy	
Yes	23 (26%)
No	67 (74%)
Breast surgery	
None	50 (56%)
Excision biopsy	6 (7%)
Breast conserving	16 (18%)
Ablation	17 (19%)
Other	1 (1%)

^a These women have a 50% risk of being a carrier, but have chosen not to undergo testing (yet)

^b Six premenopausal women did not have a menstrual cycle, due to contraceptives (hormonal Intrauterine Device etc.)

Table 2 Discomfort of nipple aspiration compared to other screening procedures for 90 patients at high risk for invasive breast cancer undergoing oxytocin-assisted nipple fluid aspiration

	Number of women	Mean score (SE)	<i>P</i> -value ^a
Nipple aspiration	90	0.6 (0.1)	–
Breast feeding	51	1.8 (0.3)	<0.001
Physical examination	83	1.1 (0.2)	0.004
Mammography	83	4.9 (0.3)	<0.001
MRI	31	2.6 (0.4)	<0.001

^a Paired samples *T*-test

significantly lower than breast feeding (1.8), physical examination by a medical oncologist or surgeon (1.1), MRI (2.6) and mammography (4.9; Table 2). No side effects were reported.

Nipple fluid was obtained from 81 out of 90 women (90%) and bilaterally in 77%. QM-MSP for *RASSF1*, *SCGB3A1* and *APC* was possible in all 50 samples that we have analyzed. From the 15 women that were known to carry a *BRCA1* or *BRCA2* mutation or that have family members that are known to be carriers, 14 women yielded bilaterally upon aspiration; in one woman nipple aspiration was only successful in one breast. Characteristics that made women significantly less likely to yield nipple fluid upon aspiration, were not having a family history of breast cancer ($P = 0.002$), postmenopausal status ($P = 0.03$) and previous surgery (excision biopsy, breast conserving surgery or other surgery of the breast) on the right side of the breast ($P = 0.002$; Table 3). Age did not significantly influence the success of the procedure ($P = 0.08$).

Using logistic regression, no factor could be identified that significantly contributed to the success of nipple aspiration in the left breast in multivariate analysis. For the right breast, only an increased pedigree-related risk (B 2.6; $P = 0.02$) correlated to the success rate of the aspiration in a multivariate model.

Discussion

Here we describe our findings of performing oxytocin-assisted nipple aspiration in a cohort of 90 high-risk women. We were able to obtain nipple fluid in 90% of high-risk women not selected for age, hormonal factors or previous therapy. A negative effect of surgery of the right breast on NAF yielding was shown, but this effect was not significant in the left breast. Furthermore, in 56% of women that previously underwent surgery of the right breast aspiration was still successful. Therefore, previous surgery is not a reason to exclude women from our study. Similarly, although postmenopausal status and not having a family history for breast cancer decreased the likelihood to yield NAF, 83% of postmenopausal women and 78% of women without a family history of breast cancer still yielded NAF.

A recent article by Loud et al. [9] reported failure to obtain nipple fluid (not supported by oxytocin) in the large majority of women from BRCA families. In this study, only 45 out of 171 women from BRCA families (26%) yielded NAF, 63% (107/171) could have a duct cannulated for ductal lavage, while cytologically evaluable samples were obtained in only 41% of these women. Women without a prior breast cancer history had an increased likelihood of yielding NAF and the presence of NAF was

Table 3 Predictive factors for successful oxytocin-assisted nipple aspiration in 90 patients at increased risk for invasive breast cancer undergoing nipple fluid aspiration

	Aspiration procedure outcome		P-value
	Unsuccessful (n)	Successful (n)	
Pedigree-based risk			
Not increased	6	21	
Increased	0	47	0.002^a
Spontaneous nipple discharge			
Yes	0	11	
No	9	70	0.59 ^a
Parity			
Nulliparous	2	28	
Parous	7	53	0.71 ^a
Menopausal status			
Premenopausal	1	41	
Postmenopausal	8	40	0.03^a
Previous breast feeding			
Yes	7	44	
No	2	37	0.29 ^a
Current oral contraceptive			
Yes	0	14	
No	9	67	0.35 ^a
Previous chemotherapy			
Yes	2	10	
No	7	71	0.34 ^a
Previous radiotherapy			
Yes	3	20	
No	6	61	0.69 ^a
Breast surgery left^b			
Yes	6	16	
No	8	51	0.15 ^c
Breast surgery right^b			
Yes	7	9	
No	7	59	0.002^c

^a Fisher's exact test

^b Ablation cases were excluded for this analysis (analysis for left and right breast separately)

^c Chi-square test

an independent correlate of ductal lavage cell count adequacy. From these data, the authors concluded that ductal lavage is unlikely to be useful in breast cancer screening among *BRCA1/2* mutation carriers.

The present study provides quite different results. We believe that vacuum-assisted nipple aspiration, if feasible, is to be preferred over ductal lavage because of its non-invasive nature. Unlike most previous studies, we do not assess cytology, but are focusing on methylation aberrations in our nipple fluid samples. As reported by Loud et al. [9], multiple studies have questioned the reproducibility and clinical value of cytology in nipple fluid. Moreover, Fackler et al. [10] showed that, compared to cytology, analyzing promoter methylation in ductal lavage cells doubled the detection rate of breast cancer. In a previous study, Dooley et al. [11] were able to obtain nipple fluid in a comparable cohort in 84% of women, but in this study only 27% yielded adequate samples for diagnosis.

In conclusion, from our experience oxytocin-assisted nipple aspiration is a very well tolerated non-invasive procedure to obtain nipple fluid in virtually all women, including *BRCA1* and *BRCA2* mutation carriers. The high success rate of the procedure is not restricted by age, reproductive factors or previous therapy and suggests that a new tool for biomarker detection in nipple fluid of women at increased risk for breast cancer is at hand.

Acknowledgments *Financial support:* This work was supported by the Dutch Cancer Society (UU 2007-3977 and UU 2008-4217), the “ Integraal Kankercentrum Midden-Nederland (IKMN)”, the “Ata Visser Stichting”, the American Women’s Club of The Hague and A Sister’s Hope/Pink Ribbon.

Conflict of interest statement We declare no conflict of interest for any of the authors.

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