

Ductal lavage: a way of carefully tracing the breast-secreting duct

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Background: Breast cancer is the most commonly diagnosed neoplasia in women after nonmelanoma skin tumors. Unfortunately, present-day diagnostic methods are unable to identify the presence of a cancer until it has been developing for several years. Currently, ductal lavage seems to represent a new method of reaching an early diagnosis of breast cancer. **Materials & methods:** This study analyzed 30 patients with ages ranging from 40 to 55 years; and in 26 of these patients, we were able to obtain a sufficient quantity of material for cytological and biomolecular analysis. **Results & conclusion:** We propose an easy, reproducible method that makes it possible to obtain a detailed map of the nipple, in order to re-identify the duct orifice and take a series of repeated samples from it over a period of time. This procedure is a promising screening and translational research tool since it provides the quantity and quality of ductal fluid required for subsequent cytological and biomolecular analyses.

Breast cancers are the most commonly diagnosed neoplasias in women after nonmelanoma skin tumors and the second cause of death from cancer in this population [1]. Unfortunately, present-day diagnostic methods such as mammography, magnetic resonance, ultrasound and nuclear medicine, are unable to identify the presence of a cancer until it has been developing for several years and has reached a size which can be seen as an anomalous mass within the normal breast tissue. It is therefore necessary to find a new way of reaching an early diagnosis of breast carcinoma – ductal lavage would seem to fulfill this hope [2,3].

It has already been established that most cancers of the breast originate in the epithelial cells covering the ducts of the mammary gland [4]; the lavage of these ducts is a fairly new and simple method of collecting these cells; it is not particularly invasive and above all makes it possible to extract a much larger quantity of material for analysis (an average of 13,500 cells/duct) than that resulting from traditional nipple aspiration (an average of 100 cells/duct) [5]. The sample obtained with this new method consists of ductal fluid, which is mainly made up of proteinaceous secretions of the ductal system and of ductal cells; the fluid thus obtained provides information not only about cell events, but also about the dynamic secretory process of the breast [6] and is therefore to be considered an efficient biological marker for evaluation of risk and the early diagnosis of breast carcinoma. Unlike other diagnostic techniques, ductal lavage makes it possible

to trace premalignant and/or malignant cells by means of the re-examination of the same duct over time [7,8]. This procedure might therefore be useful for the selection of women at high risk who would benefit by more careful monitoring and/or more individual preventive measures [9,10]. Currently, very little is known about the best methods for the reproducible identification of the duct itself. The aim of this study is to try to find a more efficient method of identifying the ductal orifice in patients at high risk for breast cancer and patients who had previously undergone radical breast surgery.

Materials & methods

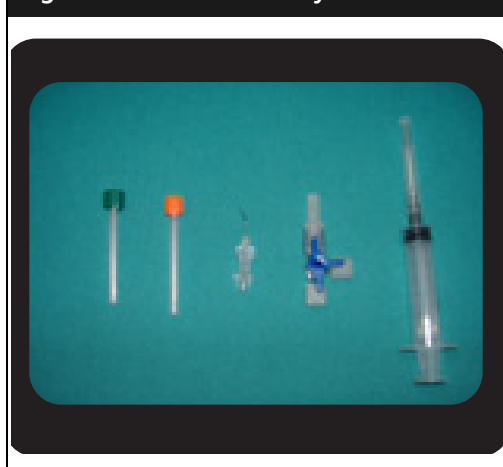
A total of 30 patients between the ages of 40–55 years were selected for a preliminary study on ductal lavage conducted in the Molecular Unit of the Department of Oncology of Palermo University (Palermo, Italy).

Patients were required to have the following requisites: be at high risk for the disease (Gail index > 1.7; previous history of breast cancer; genetic mutation of the *BRCA1/2* genes) have had a mammography and medical examination with negative results during the 12 months prior to lavage. Women who had previously undergone radical breast surgery or mastectomy for a prior breast cancer, but only the contralateral breast, were studied. Patients who had received chemotherapy or tamoxifen/selective estrogen-receptor modulator (SERM) treatment, or who were pregnant or breastfeeding [5,6] were not considered eligible for ductal lavage. Furthermore,

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Figure 1. Microcatheter system.

patients with mucous or purulent secretions were excluded from the study, since the dense secretion in the former cases causes cannulation difficulties, while in the latter patients cannulation may lead to infection subsequent to the injection of the physiological fluid into the ducts. Moreover, women with congenital nipple introflexion were also excluded from the study.

Ductal lavage involves three basic steps: nipple aspiration; cannulation and lavage of the duct; and cytological and biomolecular interpretation of the sample. A local anesthetic containing lidocaine is applied around the nipple approximately 30 min before the start of the procedure. The nipple aspiration step involves the identification of the secreting duct or ducts. Ductal lavage can only be performed on naturally secreting ducts,

since it is extremely difficult to cannulize a non-secreting duct; if necessary, a suction pump may be placed on the nipple or the breast can be massaged to facilitate the emission of the ductal secretions; this study utilized the second method.

After identifying the secreting duct, cannulation and lavage are performed. For this, we used a set of dilators of gradually increasing diameters (SteryLab S.p.A) until the ductal orifice was wide enough to insert a microcatheter linked up to a two-way hydraulic system (Figures 1 & 2), with a connecting stopcock (BRAUN, Melsungen AG D-34209) between the two tubes, one for the entry of the lavage solution and the other for its exit. After inserting the microcatheter into the duct to a depth of approximately 1.5 cm, 5–10 ml of lavage solution (physiological saline) was gradually injected at a pace of 2 ml at a time. The breast was massaged after each step of the infusion to facilitate the exfoliation of ductal epithelial cells. A further breast massage was performed at the end of this insufflation stage and then proceeded to the aspiration stage for the collection of ductal fluid to be used for the subsequent cytological and biomolecular investigation.

It is important to note the advantage of using the two-way stopcock system, which makes it possible for the physician to perform consecutive, step-by-step insufflation and aspiration of small quantities of fluid, since our group observed that when more than 1–2 ml of saline solution was injected at once, several patients complained of breast tension and pain. Furthermore, with the rapid, consecutive insufflation and aspiration of small quantities of fluid, it is possible to prevent the tissues from reabsorbing the insufflated fluid. The two-way stopcock also makes it possible to leave the two syringes (one for the entrance and the other one for the exit of the fluid) in place simultaneously, using them alternately and thus avoiding discomfort for the patient caused by repeated attachment and removal of the syringe from the microcatheter, which may cause pain in the nipple area.

The above-described procedure can be applied to several secreting ducts, either of the same or of both breasts; it is extremely important at this point to be able to accurately identify premalignant and/or malignant cells from the same duct over time. For this reason, a fundamental step in ductal lavage regards the precise location of the cannulated duct. In order to solve this problem, our group has arranged a system of Cartesian axes linked to a goniometric system; the latter was subdivided into four quadrants identified as:

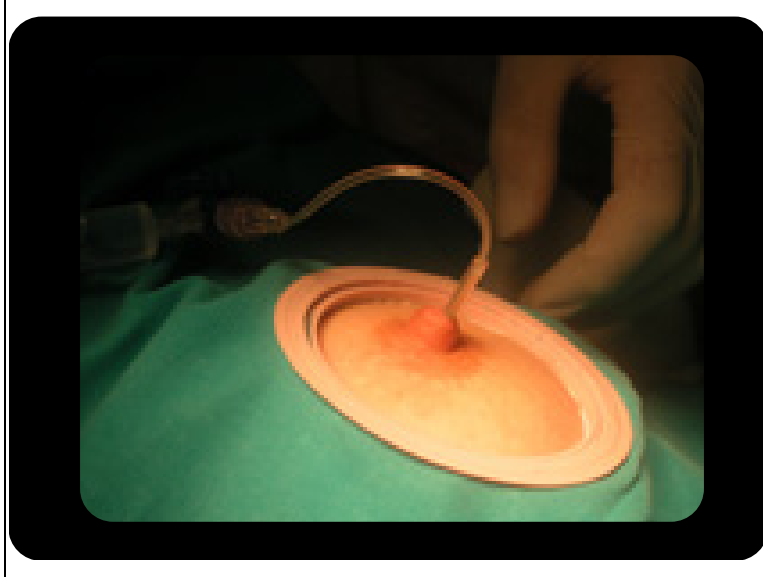
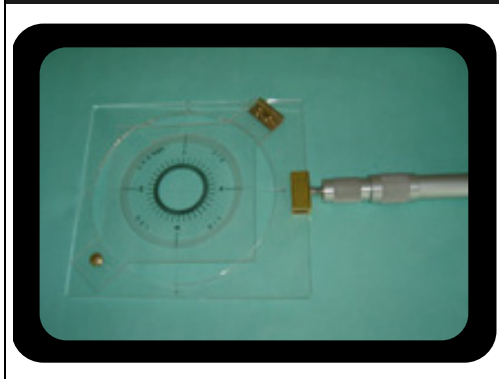
Figure 2. Insertion of the microcatheter system.

Figure 3. System of Cartesian axes linked to a goniometric system.



- Upper inside quadrant: UIQ
- Lower inside quadrant: LIQ
- Upper outside quadrant: UOQ (Figure 3)

This location system is applied to the breast and the exact position of the duct is entered on a grid where it is given a reference number and letter and then photographed in order to document the duct position (Figure 4).

Results

In four (13%) of the 30 selected cases, cannulation proved to be impossible because of a slight ductal secretion and of the reduced size of the duct itself. From one 1–3 ml of material sufficient for cytological and biomolecular analysis was obtained from 26 patients. By means of the Cartesian

localization method, we managed to identify secreting ducts in 14 out of 26 patients on the UIQ within a range of 280–300° and in the remaining 12 out of 26 patients on the LOQ from 120–170°. The relative ease of the technique and the simplicity of identifying the secreting duct made it possible to repeat the same analysis several times, thus guaranteeing the quality of the samples used in the study. The method did not lead to any complications or present any particular difficulties.

Discussion

Ductal lavage is only slightly invasive and is extremely useful for the *in vivo* sampling of ductal epithelial cells. This procedure may prove to greatly assist in the identification of occult malignant lesions in women at high risk of developing a breast cancer and may possibly become a valid method of risk assessment and therefore of early diagnosis [10].

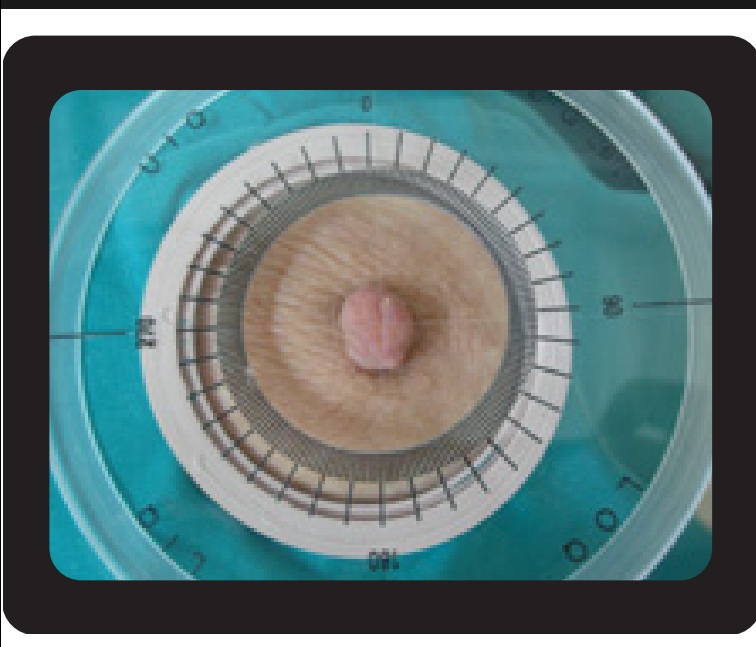
Our method provides a detailed map of the nipple which offers the repeated identification of the ductal orifice involved and the subsequent resampling of the same duct over time, which is absolutely essential if ductal lavage is to become a valid tool for preventive screening. In addition, the application of our technique does not require the injection of a small quantity of anesthetic intraductally, allowing for a higher recovery of cells and protein and a decrease in the amount of pain experienced by patients. Ductal lavage is often performed in women with non-spontaneous secretion where the identification of the secreting duct is reduced to 10–13%.

This procedure is also a promising transitional research tool, since it provides a large quantity of high-quality material needed for the subsequent cytological and biomolecular analyses [11].

The ductal fluid obtained by means of ductal lavage is examined by one or more cytologists who establish the number and morphology of the cells present in the sample, subdividing specimens into five categories as follows: inadequate diagnostic sample (fewer than ten epithelial cells); sample containing benign tumor cells; sample containing cells with intermediary atypia; sample containing cells with severe atypia; sample containing malignant cells [12].

There is considerable interest at present regarding the integration of morphological interpretations with the assessment of biomolecular markers. Studies involving a large number of molecular markers are now underway and the accurate characterization of these is undoubtedly of great importance, since they do not present

Figure 4.



Highlights

- Ductal lavage is an easy, reproducible method that makes it possible to obtain a detailed map of the nipple and is extremely useful for the *in vivo* sampling of ductal epithelial cells.
- This procedure is a promising screening and translational research tool since it provides the quantity and quality of ductal fluid required for subsequent cytological and biomolecular analyses.
- This procedure might therefore be useful in the identification of occult malignant lesions in women at high risk of developing breast cancer and may possibly become a valid method of risk assessment and therefore of early diagnosis.

the subjectivity of cytomorphological techniques; nevertheless, their usefulness with regard to positive or negative prognostic significance is still not fully understood [13].

Evron and colleagues have studied the possibility of identifying the presence of breast tumor cells in ductal fluid by means of methylation-specific-polymerase chain reaction (MSP) [9]. The authors have analyzed the methylation status of the genes *Cyclin D2*, *RAR-β* and *TWIST* in a series of 56 samples of ductal fluid from women considered as clinically healthy. These genes were proven to be methylated in 30% of breast tumors, which does not occur in healthy breast tissues. Of the 56 cases, six were cytologically classified as malignant or with severe atypia; in 66% the three genes examined were methylated; most of the samples (50 samples) contained cells with intermediary or benign atypia and there was methylation in 11% of the cases. These data suggest that methylation might be an early event in the development of a breast cancer and are important since this status can be reverted with the use of dealkylating agents [14].

Isaacs and colleagues have assessed the presence of loss of heterozygosity (LOH) in the *BRCA1* and *FHIT* genes and of alterations of the D310 microsatellite marker and in the mitochondrial DNA (mtDNA) in 26 samples of ductal fluid obtained by means of ductal lavage in 14 women with known *BRCA1* status (nine out of 14 women presented *BRCA1* mutations, five out of 14 had no mutations) but with no clinical signs of neoplastic breast lesions. The authors

found that LOH occurring in several important *BRCA1* sites is an early event occurring in women with mutations and may also be found in nonmalignant cells. Furthermore, they found alterations of the mtDNA in the D310 microsatellite marker in 30% of the cases. These two results taken together suggest that the study of LOH at the *BRCA1* gene and of mtDNA genetic alterations in samples of ductal fluid might become an important tool for the early diagnosis of breast cancer to be associated with more traditional screening methods [15].

Other authors have found instability in chromosomes 1, 8, 11 and 17 (using Interphase Fluorescence *in situ* Hybridization) in 71% of ductal fluid samples from patients with malignant breast lesions and in 11% of samples from patients with benign breast lesions [16]. Other authors have observed the frequent loss of the long arm of chromosome 16 (16q) and of the short arm of chromosome 17 (17p) by means of Comparative Genomic Hybridization, suggesting that the alterations of genes in these chromosomal regions may play an early role in the carcinogenesis of breast cancers [17].

In conclusion, ductal lavage is an extremely new method for the sampling of breast ductal cells; furthermore, the possibility of being able to identify a previously examined duct means that the method might become an extremely useful screening tool. The future identification of new molecular markers in ductal fluid could lead to a great improvement in methods of early diagnosis of breast carcinoma, in the study of breast carcinogenesis and in the monitoring of therapeutic response. Unfortunately, no one has yet developed an inexpensive reproducible technique to add and/or replace traditional cytology for these specimens. Further studies, including the analysis of a major number of cases and considering a list of probably markers, are needed in order to reach a more complete understanding of these aspects.

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