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THE IMPORTANCE OF DUCTAL EVALUATION AND BIOMARKERS IN DUCTAL FLUID IN BREAST CANCER

A. Serhat Gür, Marguerite Bonaventura, Bülent Ünal, Atilla Soran

Magee-Womens Hospital of The University of Pittsburgh Medical Center, Department of Surgery, Pittsburgh, ABD

MEME KANSERİNDE, DUKTAL DEĞERLENDİRME VE DUKTAL SIVILARDAKİ BİYOMARKERLARIN ÖNEMİ

ÖZET

Birçok meme kanseri duktal sistemin epitelyal dokusunda başlar. İnvaziv duktal karsinom, duktal karsinoma insitunun malign transformasyonu sonrasında, duktal sistemde segmental bir bolge olarak ortaya çıkar. İnvaziv duktal karsinomun erken tanısı görüntüleme yöntemleriyle mümkün olabilmektedir. Özellikle mamografi bu konuda altın standarttır. Ancak genç kadınlarda, postmenopozal kadınlarda olduğu kadar duyarlı değildir. Bu nedenlerle araştırmacılar duktal sistemi direk değerlendirmek istemişlerdir. Duktoskopi, duktal lavaj ve meme başı aspirasyon sıvısı duktal sistemi değerlendirmenin yollarıdır. Bu yöntemlerle duktal sistem ne duktal sıvıdaki patolojik hücreler görülebilmektedir. Öte yandan, duktal sıvıda kanser tanısını koydurabilecek maddeler saptanmış ve ölçülebilmiştir. Bu derlemede, literatür bilgisi ışığında duktoskopi, duktal lavaj ve meme başı aspirasyon sıvısı konusundaki gelişmeler özetlenmeye çalışılmıştır.

Anahtar sözcükler: meme kanseri, duktoskopi, duktal lavaj, meme başı aspirasyon sıvısı

ABSTRACT

The most of the breast cancer (BC) are initiated in the epithelial tissue of ductal system. Invasive ductal carcinoma occurs in a segmental fashion in the ductal system after the malignant transformation of ductal carcinoma insitu. The early detection of invasive carcinoma of the breast is possible with imaging systems. Especially mammography is gold standard for screening. However it's not as sensitive in young women as postmenopausal women. Based on these reasons investigators interested in evaluating the ductal system. Ductoscopy, ductal lavage and nipple aspiration fluid (NAF) are evaluating methods of ductal system. These methods promise to detect the ductal system and pathologic cells in ductal fluid. On the other hand substances have been identified in ductal fluid which can be used for cancer detection. In this review, the new approaches with ductoscopy, ductal lavage and NAF were summarized in the light of the literature.

Key words: breast cancer, ductoscopy, ductal lavage, nipple aspiration fluid

The diagnosis of breast cancer (BC) has become formalized as triple assessment, involving clinical examination, imaging (mammography/ultrasound/MRI) and pathology. Although mammography is the accepted gold standard in BC screening, it has a sensitivity of 62.9%-87% (1). Mammography is not as sensitive in young women as in postmenopausal women because of increased breast density. Researchers have prompted for alternative approaches to mammography for the diagnosis of BC (2). Most cancers are initiated in the epithelial tissue of the ductal system of the breast (3, 4). Studies suggest that the malignant transformation of ductal carcinoma in situ, which is the accepted precursor of invasive ductal carcinoma, occurs in a segmental fashion in the ductal system rather than diffusely throughout the breast (5, 6). Based on this characteristic of the origin of BC, researchers are interested in evaluating the ductal system.

Ductoscopy

Ductoscopy is used to identify the abnormal duct, especially in patients who have pathological bloody nipple discharge. Ductoscopy may be used to evaluate intraductal lesions in the early detection of BC (7), although ductal lavage and nipple aspiration fluid (NAF) are used to detect atypical epithelium and biomarkers in ductal fluids. The biochemical and cellular content of ductal fluid may be surrogate markers during cancer development (8). In 2007, Hunerbain et al. (9) presented a study of diagnostic ductoscopy and intraductal vacuum assisted biopsy. The biopsy was performed with a needle which was developed for vacuum assisted biopsy through a 7mm micro endoscope. Technical innovation in miniature instruments permits a change from diagnostic to interventional ductoscopy. Autofluorescence ductoscopy is a new imaging technique and studies of this method are ongoing. Gupta et al (10) evaluated the diagnostic potential of N2 laser excited autofluorescence spectroscopy of human breast tissues. They reported that significant changes were observed in the autofluorescence from normal, benign, and cancerous breast tissues, particularly in the spectrally integrated fluorescence intensity. A discrimination parameter based on spectrally integrated intensity

alone provided a sensitivity and specificity of up to 99.6% over the sample size investigated for discrimination of cancerous breast tissues from benign/normal tissue. The authors suggested that a straightforward measurement of the total integrated fluorescence intensity can provide excellent discrimination between cancerous and benign/ normal breast tissues. In addition to diagnosis, laser ductoscopy is a providing the technology to remove intraductal papillomas. Three dimensional intraductal tracking systems are promised in future projects (11). A pilot study of new spectral imaging techniques with ductoscopy has been initiated at Magee-Womens Hospital. Our aim is to identify an intraductal lesion by ductoscopy, to describe the lesion by spectral analysis and to correlate the findings with mammogram, cytology and pathology.

Ductal lavage

Ductal lavage is a minimally invasive method used to evaluate ductal epithelium. The selected duct is cannulated with a small catheter. Each fluid-yielding duct is cannulated a maximum of 1.5 cm in depth and the ductal system is infused with normal saline (12). The amount of infused saline ranges between 2ml and 15ml. The fluid is retrieved and sent for cytopathologic analysis. Nipple aspiration is also a noninvasive technique but drawbacks are insufficient epithelium or insufficient fluid.

In a prospective multicenter study, ductal lavage and nipple aspiration were compared with regard to safety, tolerability and ability to detect abnormal epithelial cells (12). A total of 507 patients were included in one trial and 700 breasts were evaluated. There was a family history of BC (invasive or ductal carcinoma in situ) in 57% of patients and 5-year Gail risk of breast cancer was greater than 1.7% in 39% of the patients. When NAF and ductal lavage were compared, the tolerability and level of comfort were identical. Sufficient NAF samples were taken from 82 breasts and sufficient ductal lavage samples were taken from 284 breasts. The diagnostic efficiency of ductal lavage was 4.7 times more likely than NAF. Visvanathan (13) has presented a study of the value of nipple aspirate and ductal lavage in women at increased risk for BC. Sixty-nine patients were enrolled in the study evaluating NAF and ductal lavage. At baseline, 65% of premenopausal and 41% of postmenopausal women produced ductal fluid. The rate of successful lavage of at least one duct was 72%. However, there was inconsistency between first and second lavage results for the same woman. Ductal lavage was associated with moderate discomfort. The authors concluded that the use of ductal lavage is limited by inconsistent ductal fluid production and insufficient cellular yield.

Nipple aspiration fluid

Except for the cellular content, breast ductal fluid contains a variety of chemical substances and many different proteins (8). Studies have shown that NAF samples include immunoglobulins, estrogen and progesterone, androgens, prolactin, prostate-specific antigen, and carcinoembriogenic antigen (14-17). Several research groups (18-20) report that evaluation of the biomarkers

in the ductal fluid can be useful for risk assessment, diagnosis, treatment monitoring, and recurrence detection in breast cancer. Breast ductal fluid contains a large number of proteins. It was stated that comparisons of ductal fluid from a breast with cancer and the same patient's normal contralateral breast may reveal significant differences in protein expression associated with breast carcinoma. Ductal fluids from breast carcinoma patients with the advances in image analysis, automated mass spectrometry, and bioinformatics may help researchers to discover a biomarker (or panel) to diagnosis and prognosis of BC (21). The most interesting groups of molecules detected in breast ductal fluid are members of the epidermal growth factor family. This family affects the mutagenic activity on human BC cells (22). Epidermal growth factor, transforming growth factor-α, and ErbB-2 (HER-2/neu) were detected in the ductal fluid (23). Epidermal growth factor and transforming growth factor-α have been found in NAF samples from healthy premenapausal women, but HER-2/neu has been detected the NAF samples in cancer patients. In the Kuerer's study (24), the mean NAF volume obtained and the mean NAF protein concentration were found to be no different in the normal vs the affected breast (62.4 μL vs 60.4 μL and 140.9 mg/mL vs 107.8 mg/mL, respectively). Mean serum HER-2/neu level was nearly 50 times less than the mean NAF HER 2/neu level from all patients and all breasts (209.2 ng/mL, range 1.0–3,480.0 ng/mL). NAF HER-2/neu levels were significantly correlated between breasts for each individual patient ($r = .302$, $P=.038$). HER 2/neu-overexpressing tumors produced significantly more HER-2/neu in the affected breast (653.6 ng/mL) than in the unaffected breast (101.7 ng/mL) or serum (3.46 ng/mL) ($P=.016$).

The achievement of nipple aspiration changes due to the patients' age and menopausal status. The successful aspiration is higher in premenopausal women than postmenapausal women. The ability to obtain fluid is greater in specific cohorts: young age (35–50 years), non-Asian ethnicity, increased parity, and prior lactation (25). Breast massage before aspiration affects the success rate as well. However, in the outpatient setting protocols success rate of aspiration, which can be collected more than 1μl, is 48% (26). NAF yielding breasts was 96% among women who underwent nipple aspiration during general anesthesia in the operating room (27). NAF yield is significantly impaired after radiation therapy (12). In our study protocol, we prefer no to collect NAF in patients who is older than 65 years old, younger than 18 years of age, underwent prior breast surgery, breast or chest radiation, prior subareolar intervention, and the ability to obtain fluid is approximately 80% in our institute.

Identification of protein biomarkers in small amounts of NAF is possible because of the recent improvement in molecular technologies. One recently published study concluded that the identification of proteomic profile of NAF samples can be used as a risk assessment tool and/or to monitor the course of disease and response to treatment (2). Another study has concluded that NAF and clinical biomarkers are sensitive predictors of whether a breast contains cancer, and may ultimately guide treatment.

Table 1. Substances identified in breast fluid (Adapted from Lang JE et al (8))

Substance	Normal Breast Tissue	Breast Cancer
bFGF (33)	+	+
CEA (14)	+	+
2,6 Cyclolycopene-1, 5-diol (34)	+	
Cholesterol and cholesterol epoxides (35)	+	
Dehydroepiandrosterone sulphate (DHEAS) (36)	+	+
Epidermal Growth Factor (37)	+	
Estrone and estradiol (38)	+	+
Her-2/neu (23)	+	+
Human Kallikreins 2, 3, 6, 10. (39)	+	+
8-Isoprostane (34)	+	
Immunoglobulin (40)	+	+
Lactate dehydrogenase (LDH) isozymes (41)	+	
Nicotine (34)	+	
Prolactine (42)	+	+
Progesterone (43)	+	+
Prostate-specific antigen (PSA) (16)	+	+
pS2 (44)	+	
Testesterone (45)	+	+
Vascular endothelial growth factor (VEGF) (33)	+	+
Prostaglandin E2 (46)	+	+
Leptin (47)	+	+
YKL-40 (48)	+	+
S-Phase Fraction (29)	+	+
Urokinase-type plasminogen activator (UPA) Plasminogen activator inhibitor (PAI) UPA receptor (49)	+ + +	+ +
Malondialdehyde (MDA), 8-epimer of Prostaglandin F(2alpha) (8-iso-PGF(2alpha) (50)	+	+
Group IIa secretory phospholipase A2 (sPLA2-IIa) (51)	+	+
Cyclin B1, anticyclin B1,MUC1, antiMUC1 (32)	+	+
Tranforming growth factor-α (37)	+	

Kuerer H et al (28) concluded in their prospective study that protein expression patterns are highly conserved between cancerous and noncancerous breasts in women with unilateral invasive BC; unique expression patterns may be associated with extent of disease. High-throughput proteomic methods may reveal biologically relevant proteins involved in carcinogenesis and progression of disease**.** Future studies to determine the optimal combination of predictive markers are warranted (29). The substances identified in breast fluid are shown in the table 1. Cyclins and MUC1 are identified and are the focus of our research.

The detection of cyclin B1, anticyclin B1, MUC1 and, anti MUC1 in NAF may be early indicators of premalignant or malignant changes in the breast tissue.

Cyclins control the progression through the cell cycle. The correlation between breast cancer and Cyclin E, A, and B were studied. One study revealed that cyclin expression is associated with the clinical course of BC but is of limited value in assessing prognosis. Cyclin E and B independently predicted the occurrence of meta-

static disease, but only cyclin E was an independent predictor for survival (30). In BC it was shown that metastatic cells express the highest levels of cyclin B1 (31). It has also been shown that cyclin B1 was frequently expressed in premenopausal women, in estrogen receptor negative, high grade and high mitotic index tumors and was associated with disease specific survival and metastasis free survival (30). However, antibody responses against cyclin B1 were reported in several cancers. Finn et al (31) demonstrated that anti cyclin B1 antibody is present in 43% of BC patients. MUC 1 glycoprotein is produced by normal breast epithelial cells in low levels. During the process of malignant transformation, starting with early premalignant lesions, MUC 1 is overexpressed and underglycosylated and this form can become abundant in ductal fluid. This form of MUC1 is also immunogenic and patients with BC make antibodies against this molecule. The presence of antiMUC1 in patients' serum is correlated with improved disease free and overall free survival. A pilot study to detect cyclin B1, MUC1 and the antibodies to these in ductal fluid was initiated 2 years ago at Magee-Womens Hospital. NAF samples are preserved at -80ºC until measurements and ELISA assay is being used for detection both

of markers in NAF. The preliminary results of the ongoing study were presented at The 13th Annual Multidiciplinary Symposium on Breast Disease (32). Twenty-four patients have been included in this study and in the majority of NAF samples, antibody levels were much lower than those found in serum. This is the first study investigating anti-MUC1 and anti cyclin B1 antibodies in NAF of BC patients. Even tough the sample size is still small and additional samples are being accumulated the finding of the tumor specific IgA in the NAF of the two BC patients is encouraging. Development of more sensitive techniques for antibody detection may allow detection of the low antibody levels in the premalignant lesions and boost the usefulness of NAF as the source for this diagnostic assay.

In conclusion, developments in detecting biomarkers and molecular elements in samples of ductal fluid and the discovery of new technology to obtain this fluid are exciting. The ductal approach could be the most important diagnostic and perhaps therapeutic method in the management of early BC in the near future.

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İletişim

A. Serhat Gür
Tel   : +1 412 6411640
E-Posta   : akifserhatg@upmc.edu