Original Article
Diagnostic accuracy of virtual touch tissue quantification for patients with breast cancer

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Abstract: Breast cancer remains a significant clinical and societal challenge worldwide. The diagnosis of breast cancer plays crucial role in determining the treatments and mean survival time. In recent studies reported that virtual touch tissue quantification (VTTQ) is a novel and promising technique for detecting human cancer tissues. In this study, the sensitivity of VTTQ was determined in diagnosing breast cancer and accurate diagnosis of VTTQ determined by biologic markers was assessed, along with molecular subtypes, and histologic analysis. A total of 128 invasive ductal breast cancer patients including 72 invasive ductal carcinoma cases, 28 ductal carcinoma cases, 8 mucinous carcinoma cases, 14 fibroadenoma cases and 6 intraductal papilloma cases were analyzed in this study. The results demonstrate that VTTQ velocities are statistically higher in malignant cases than in benign cases (P < 0.01). The results showed that the best cutoff value of VTTQ velocity was > 2.94 m/s in malignant cases. Outcomes revealed that VTTQ velocity were statistically significantly correlated with the Ki-67 labeling index (r = 0.405, and P < 0.01). Furthermore, VTTQ velocity negatively correlated with estrogen receptor (r = -0.342, P < 0.01) and progesterone receptor (r = -0.374, P < 0.01) status of invasive breast cancer. High histological grade (P = 0.001) and C-erbB-2 expression (P = 0.029) were significantly associated with VTTQ velocity (r = 0.316, P < 0.01). Multiple linear regressions revealed that breast tumor size was the pathologic indicator determinant of VTTQ velocity (P < 001). In conclusion, these outcomes indicate that VTTQ is markedly associated with clinicopathologic breast nodule, which provide diagnostic information in patients with benign or invasive breast cancer.

Keywords: Breast cancer, VTTQ, diagnosis

Introduction

Breast cancer is one of the most common metastatic tumors, which significantly affects women's health [1]. Human breast carcinoma remains a highly lethal disease due to local invasion and distant metastasis at diagnosis [2, 3]. Although the potential role of circulating tumor cell detection and monitoring in breast cancer has been explored in the clinic, the lymphatic metastasis and recurrence rate are still high [4, 5]. A systematic review has summarized the current treatments for breast cancer-related lymphedema, which provides potential anti-cancer strategies for patients with breast cancer [6]. Currently, breast cancer is a significant public health problem and the treatment of breast cancer has many side effects for patients [7, 8]. Therefore, reports have concluded that the utilization of diagnostic tools would

greatly assist in the modern management of breast cancer.

In recent years, virtual touch tissue quantification (VTTQ) has been widely applied for human cancer diagnosis. Study showed that VTTQ can effectively detect the stiffness of prostate cancer and benign prostatic hyperplasia [9]. A report has indicated that VTTQ could be a valuable clinical tool for estimating breast cancer pathology in a non-invasive fashion [10]. In addition, VTTQ can detect ductal carcinomas in situ and small invasive breast cancers, which may be a useful application for assessing breast tumors [11]. Furthermore, VTQQ were significantly associated with clinicopathologic abnormalities, and may therefore provide prognostic information in patients with invasive ductal breast cancer [12]. However, the association between VTQQ and clinicopathologic breast

nodule has not clearly investigated in patients with breast cancer.

The aims of this randomized study were to analyze the correlation between VTTQ velocities and status of invasive breast cancer, in order to improve early diagnosis. This study also evaluated the relationship between VTTQ velocity and the estrogen receptor and C-erbB-2 expression in patients with breast cancer, which may be one of methods to diagnose the status of breast cancer recurrence or metastasis based on VTTQ velocity.

Materials and methods

Patients

All patients were required to write informed consent for all participants. Between May 2011 and October 2013, we analyzed 128 invasive ductal breast cancer patients including 72 invasive ductal carcinoma, 28 ductal carcinoma, 8 mucinous carcinoma, 14 fibroadenoma and 6 intraductal papilloma using VTTQ. The median age was 43.6 years old (age range, 35.64-54). The study was approved by the Ethics Committee of the Yantai Yu Huang Ding Hospital.

VTTQ assay

In all the patients, VTTQ examinations were performed for diagnosis of breast cancer tissue. VTTQ was used to detect lesions for its elastic properties by using a region-of-interest (ROI) cursor according to manufacturer instrument [13]. Acoustic push pulse and detection pulses in VTTQ were used to analyze the shear wave velocity and calculated by quantitative assessment of the tissue stiffness by wave velocity (m/s). The fastest velocities were defined as the hotspots of these lesions described previously [14].

Histological analysis

Surgical specimens were isolated and fixed in neutral 10% formalin. Breast cancer tissues were then embedded in paraffin and cut into 4 mm sections. Tumor sections were stained using hematoxylin and eosin, and avidin-streptavidin immunoperoxidase method using antibody targeting of estrogen receptor (1:1000, ab32063, Abcam), progesterone receptor (1:1000, ab2765, Abcam), Ki-67 (1:1000, ab156956, Abcam) and C-erbB-2 (1:1000, ab194979, Abcam) in an automated immunostainer (Benchmark System; Ventana). Horseradish peroxidase-conjugated anti-rabbit IgG (Bio-Rad, Hercules, CA, USA) was used at a 1:5000 dilution and detected using a Western blotting luminol reagent. Histopathologic evaluations were performed based on the World Health Organization histologic classification of tumors of breast [15]. Expression levels of progesterone receptor, estrogen receptor, Ki-67, and C-erbB-2 were determined by nuclear staining and was graded from 0 to 8 using the Allred score, with positive cases defined as a score of 3 [16].

Statistical analysis

All data are presented as mean ± standard deviation (SD) of triplicate and analyzed by SPSS 19.0 statistical software (SPSS Inc. Chicago, IL, USA). Comparison of means among multiple groups was performed by one-way analysis of variance (ANOVA) followed by Tukey post hoc pairwise comparisons. The optimal cutoff value for VTTQ to distinguish breast cancer tissue was selected using the histologic subtype of the lesions, by minimizing the sum of the observed false-positive and false-negative errors with the bootstrapping methodology [17]. Linear regression analyses were performed to investigate the degree of correlation between VTTQ and progesterone receptor, estrogen receptor, Ki-67 and C-erbB-2. Statistical significance was defined when $P < 0.05$.

Results

Patient characteristics

A total of 128 invasive ductal breast cancer patients were recruited and measured using VTTQ. Breast cancer patients including 72 cases of invasive ductal carcinoma, 28 cases of ductal carcinoma, 8 cases of mucinous carcinoma, 14 cases of fibroadenoma and 6 cases of intraductal papilloma were analyzed in this study. The mean age for breast cancer patients was 43.6 years old. **Table 1** shows the patient characteristics.

Efficacy of VTTQ on diagnosis of breast cancer

The efficacy of VTTQ on diagnosis of breast cancer was analyzed in this study. VTTQ velocities were statistically higher in malignant cases than in benign cases (**Figure 1**, $P < 0.01$). The

Table 1. Patients characteristic

	Number	Age	Tumor size
Total	128	35.64-54.00	14.2 (0-60)
Histologic types			
Invasive ductal carcinoma	72	36.4-48.2	16.2 (3-56)
Ductal carcinoma	28	40.3-50.2	7.8 (6.4-34.5)
Mucinous carcinoma	8	37.48-52.16	18.2 (10-54.3)
Fibroadenoma	14	41.24-50.62	22.8 (14.4-60)
Intraductal papilloma	6	38.4-47.50	19.2 (13-52.6)
Histopathologic characteristics			
Ki-67 (median)	17 (1-80)		
C-erbB-2	12 (1-50)		
Estrogen receptor	18 (1-60)		
Progesterone receptor	20 (1-70)		

Figure 1. VTTQ velocities can differentiate malignant cases and benign breast cancer cases.

Figure 2. Relationship between VTTQ velocities and tumor size for lesions of invasive breast cancer determined by Mann-Whitney test.

best cutoff value of VTTQ velocity was > 2.94 m/s in malignant cases and it was < 2.74 m/s in benign cases.

Relationships between VTTQ and Ki-67 expression

Figure 2 shows the relationship between VTTQ velocities and tumor size for lesions of invasive breast cancer (Mann-Whitney test, P = 0.0015). VTTQ velocities were positively corrected with breast cancer volume (r = 0.368, and P < 0.01, **Figure 3**). Outcomes revealed that VTTQ velocity were statistically significant correlations with the Ki-67 labeling index (r = 0.405, and P < 0.01, **Figure 4**).
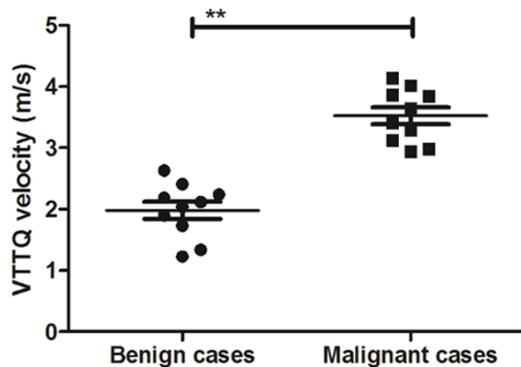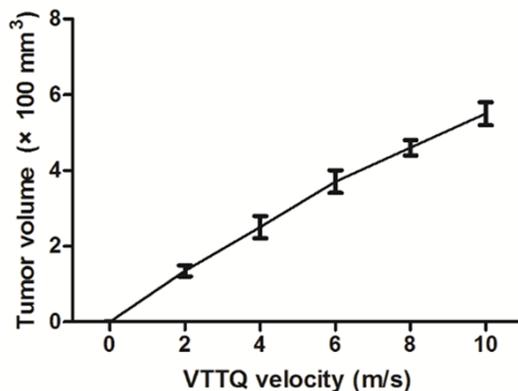
Relationships between VTTQ and breast cancer markers

VTTQ velocity negatively correlated with the estrogen receptor (r = -0.342, P < 0.01) expression in breast cancer cases (**Figure 5**). **Figure 6** demonstrated that VTTQ velocity also negatively correlated with progesterone receptor (r = -0.374, P < 0.01) status of invasive breast cancer.

Relationships between VTTQ and histological grade

The relationships between VTTQ and histological grade were further analyzed. As shown in **Figure 7**, VTTQ velocity is correlated with the histological grade (r = 0.326, P < 0.01). VTTQ velocity was correlated C-erbB-2 expression (r = 0.364, P < 0.01, **Figure 8**).

Discussion

The VTTQ diagnosis of breast cancer plays crucial role in determining treatments and mean survival time. Evidence has shown that VTTQ is highly reliable and reproducible in diagnosing benign and malignant lesions [13]. In this study, the impact of VTTQ on breast imaging was investigated, demonstrating that VTTQ has the value in diagnosing of malignant cases and benign cases. VTTQ was associated with clinicopathologic breast nodule, which may provide diagnostic information in patients with benign or invasive breast cancer. The associations between VTTQ and breast tumor size, as well as Ki-67 labeling index, C-erbB-2, estrogen recep-
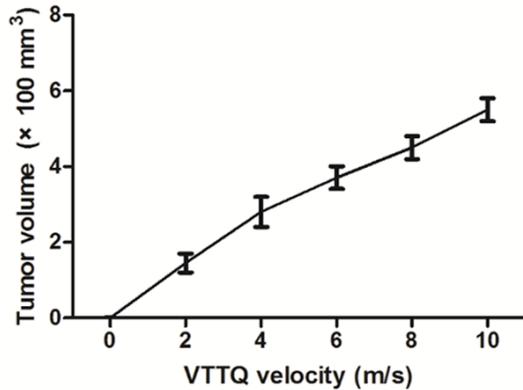
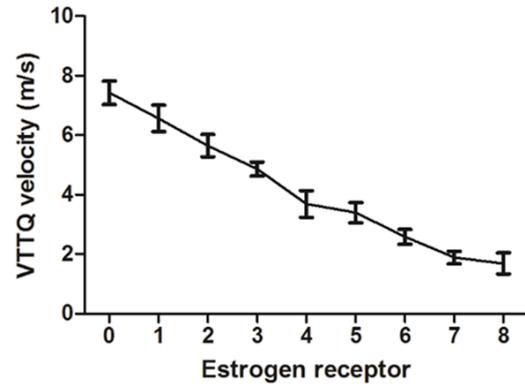**Figure 3.** VTTQ velocities are positively corrected with breast cancer volume.

Figure 5. VTTQ velocity is negatively correlated with the estrogen receptor (r = -0.342) expression in breast cancer cases.
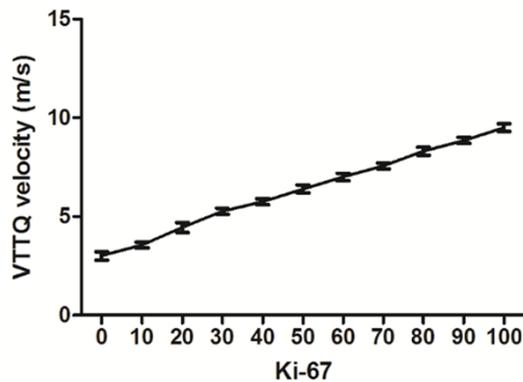

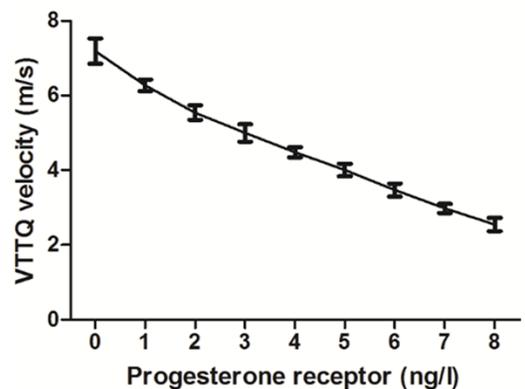**Figure 4.** VTTQ velocity is significantly correlated with Ki-67 labeling index (r = 0.405).

Figure 6. VTTQ velocity is negatively correlated with progesterone receptor (r = -0.374) status of invasive breast cancer.

tor and progesterone receptor were investigated in this clinical study.

Currently, VTTQ is valuable to differentiate benign from malignant breast imaging-reporting and data system category 4 lesions, which might be useful for patient selection before biopsy [18]. VTTQ could detect patients with benign, invasive breast cancer and normal breast tissue. Previous study has showed that VTTQ imaging and quantification is a helpful and reproducible tool for predicting thyroid malignancy [19]. In this study, VTTQ velocity was correlated with the histological grade (r = 0.326, P < 0.01) that is essential for the tumor eradication, treatment and prognostic analysis. In addition, VTTQ imaging quantification was efficient in differential diagnosis of metastatic and non-metastatic cervical lymph nodes [20]. Furthermore, VTTQ imaging can be used to

evaluate thyroid nodules [21]. The best cutoff value of VTTQ velocity was found to be > 2.94 m/s in malignant cases and it was < 2.74 m/s in benign cases.

Ki-67 labeling index, C-erbB-2, estrogen receptor and progesterone receptor are associated with the progression of breast cancer [22-25]. Study has found that Ki-67 is involved in promoting the genesis and development of breast cancer by affecting the proliferation and migration of cancer cells [26]. VTTQ velocity was correlated with the Ki-67 labeling index. Arslan et al. has showed that expression of c-erbB-2 is overexpressed and associated with the progression of patients with breast cancer [27]. VTTQ velocity value was shown here to be positively correlated with estrogen receptor expres-
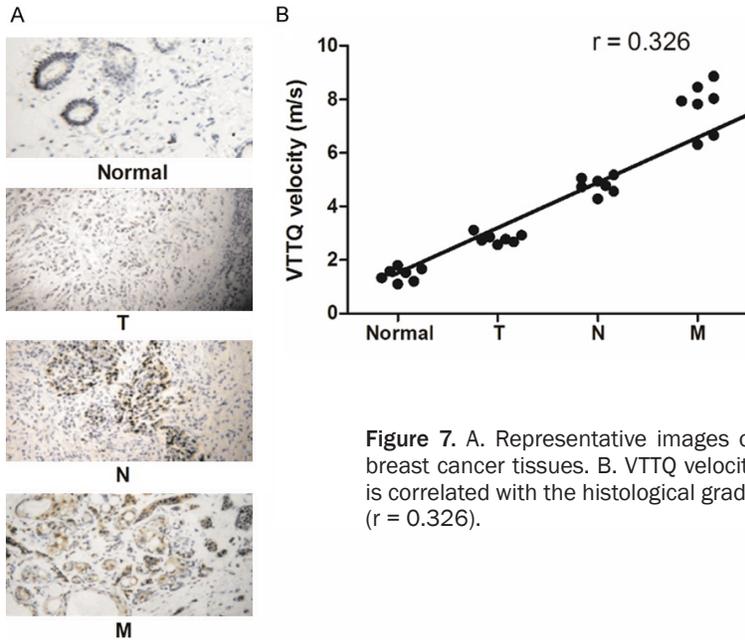
Figure 7. A. Representative images of breast cancer tissues. B. VTTQ velocity is correlated with the histological grade (r = 0.326).

Figure 8. VTTQ velocity is correlated with C-erbB-2 expression (r = 0.364).

sion. Malkov et al. have suggested that estrogen receptor is positively correlated with cancer [28]. Reports also indicated that progesterone receptor loss identifies hormone receptor-positive breast cancer subgroups at higher risk of relapse [29, 30]. In this study, VTTQ velocity was negatively correlated with progesterone receptor in patients with breast cancer. Multiple linear regressions revealed that breast tumor size is the pathologic indicator determinant of VTTQ velocity.

In conclusion, the breast lesion could be quantitatively assessed by VTTQ in patients with invasive ductal breast cancer. VTTQ appears to be a promising method in the differential diag-nosis of the malignant breast lesion. These findings indicate that VTTQ is a potential method in assessing breast tumors in early phase. However, further studies were warranted to improve the sensitivity of VTTQ assessment in breast cancer patients in a large number population.

Disclosure of conflict of interest

None.

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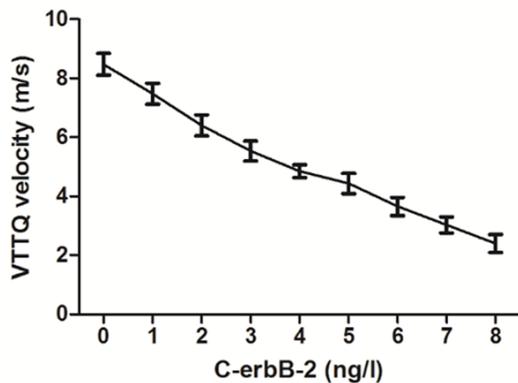
References

[1] Dawood S, Broglio K, Buzdar AU, Hortobagyi GN and Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. J Clin Oncol 2010; 28: 92-98.

[2] Conlon N, Sadri N, Corben AD and Tan LK. Acinic cell carcinoma of breast: morphologic and immunohistochemical review of a rare breast cancer subtype. Hum Pathol 2016; 51: 16-24.

[3] Conlon N, Howard J, Catalano J, Gallagher M, Tan LK and Corben AD. Breast carcinoma in young women: no evidence of increasing rates of metastatic breast carcinoma in a single tertiary center review. Breast J 2016; 22: 287-292.

[4] Cormier JN, Askew RL, Mungovan KS, Xing Y, Ross MI and Armer JM. Lymphedema beyond breast cancer: a systematic review and meta-analysis of cancer-related secondary lymphedema. Cancer 2010; 116: 5138-5149.

[5] Chan DN, Lui LY and So WK. Effectiveness of exercise programmes on shoulder mobility and lymphoedema after axillary lymph node dissection for breast cancer: systematic review. J Adv Nurs 2010; 66: 1902-1914.

[6] Tan PH and Lota AS. Interaction of current cancer treatments and the immune system: implications for breast cancer therapeutics. Expert Opin Pharmacother 2008; 9: 2639-2660.

[7] Sharma M, Lingam VC and Nahar VK. A systematic review of yoga interventions as inte-

grative treatment in breast cancer. J Cancer Res Clin Oncol 2016; 142: 2523-2540.

[8] Zhang T, Wang R, Liu Y, Huang J and Yang Z. Efficacy and safety of doublet versus single agent as salvage treatment for metastatic breast cancer pretreated with anthracyclines and taxanes: a systematic review and meta-analysis. Curr Med Res Opin 2016; 32: 1883-1889.

[9] Zheng X, Ji P, Mao H and Hu J. A comparison of virtual touch tissue quantification and digital rectal examination for discrimination between prostate cancer and benign prostatic hyperplasia. Radiol Oncol 2012; 46: 69-74.

[10] Tamaki K, Tamaki N, Kamada Y, Uehara K, Miyashita M, Ishida T and Sasano H. A non-invasive modality: the US virtual touch tissue quantification (VTTQ) for evaluation of breast cancer. Jpn J Clin Oncol 2013; 43: 889-895.

[11] Tada K, Nishioka K, Kikuchi Y, Niwa T and Seto Y. Virtual Touch tissue quantification cannot assess breast cancer lesions except for ductal carcinomas in situ and small invasive cancers: a retrospective study. World J Surg Oncol 2015; 13: 147.

[12] Xie J, Wu R, Xu HX, Yao MH and Xu G. Relationship between parameters from virtual touch tissue quantification (VTQ) imaging with clinicopathologic prognostic factors in women with invasive ductal breast cancer. Int J Clin Exp Pathol 2014; 7: 6644-6652.

[13] Golatta M, Schweitzer-Martin M, Harcos A, Schott S, Gomez C, Stieber A, Rauch G, Domschke C, Rom J, Schutz F, Sohn C and Heil J. Evaluation of virtual touch tissue imaging quantification, a new shear wave velocity imaging method, for breast lesion assessment by ultrasound. Biomed Res Int 2014; 2014: 960262.

[14] Gong L, He Y, Tian P and Yan Y. Effect of elastic strain rate ratio method and virtual touch tissue quantification on the diagnosis of breast masses. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2016; 41: 729-735.

[15] Kleihues P and Sobin LH. World Health Organization classification of tumors. Cancer 2000; 88: 2887.

[16] Kroger N, Milde-Langosch K, Riethdorf S, Schmoor C, Schumacher M, Zander AR and Loning T. Prognostic and predictive effects of immunohistochemical factors in high-risk primary breast cancer patients. Clin Cancer Res 2006; 12: 159-168.

[17] Mueller JL, Gallagher JE, Chitalia R, Krieger M, Erkanli A, Willett RM, Geradts J and Ramanujam N. Rapid staining and imaging of subnuclear features to differentiate between malignant and benign breast tissues at a point-of-care setting. J Cancer Res Clin Oncol 2016; 142: 1475-1486.

[18] Li XL, Xu HX, Bo XW, Liu BJ, Huang X, Li DD, Guo LH, Xu JM, Sun LP, Fang L and Xu XH. Value of virtual touch tissue imaging quantification for evaluation of ultrasound breast imaging-reporting and data system category 4 lesions. Ultrasound Med Biol 2016; 42: 2050-2057.

[19] Zhou H, Zhou XL, Xu HX, Li DD, Liu BJ, Zhang YF, Xu JM, Bo XW, Li XL, Guo LH and Qu S. Virtual touch tissue imaging and quantification in the evaluation of thyroid nodules. J Ultrasound Med 2017; 36: 251-260.

[20] Zhao Y, Xi J, Zhao B, Xiong W, Jiang D, Yang L, Cai Z, Liu T, Jiang H, Rong S and Jin X. Preliminary evaluation of virtual touch tissue imaging quantification for differential diagnosis of metastatic and nonmetastatic cervical lymph nodes. J Ultrasound Med 2017; 36: 557-563.

[21] Sun CY, Lei KR, Liu BJ, Bo XW, Li XL, He YP, Wang D, Ren WW, Zhao CK and Xu HX. Virtual touch tissue imaging and quantification (VTIQ) in the evaluation of thyroid nodules: the associated factors leading to misdiagnosis. Sci Rep 2017; 7: 41958.

[22] Thangarajah F, Malter W, Hamacher S, Schmidt M, Kramer S, Mallmann P and Kirn V. Predictors of sentinel lymph node metastases in breast cancer-radioactivity and Ki-67. Breast 2016; 30: 87-91.

[23] Yang SX, Loo WT, Chow LW, Yang XH, Zhan Y, Fan LJ, Zhang F, Chen L, Wang QL, Xiao HL, Wu JL, Bian XW and Jiang J. Decreased expression of C-erbB-2 and CXCR4 in breast cancer after primary chemotherapy. J Transl Med 2012; 10 Suppl 1: S3.

[24] Hussein MH, Schneider EK, Elliott AG, Han M, Reyes-Ortega F, Morris F, Blastovich MAT, Jasim R, Currie B, Mayo M, Baker M, Cooper MA, Li J and Velkov T. From breast cancer to antimicrobial: combating extremely resistant gram-negative "Superbugs" using novel combinations of polymyxin B with selective estrogen receptor modulators. Microb Drug Resist 2017; 23: 640-650.

[25] Aktas B, Kasimir-Bauer S, Muller V, Janni W, Fehm T, Wallwiener D, Pantel K and Tewes M. Comparison of the HER2, estrogen and progesterone receptor expression profile of primary tumor, metastases and circulating tumor cells in metastatic breast cancer patients. BMC Cancer 2016; 16: 522.

[26] Yuan P, Xu B, Wang C, Zhang C, Sun M and Yuan L. Ki-67 expression in luminal type breast cancer and its association with the clinicopathology of the cancer. Oncol Lett 2016; 11: 2101-2105.

[27] Arslan C, Isik M, Guler G, Kulac I, Solak M, Turker B, Ozisik Y and Altundag K. Does androgen receptor have a prognostic role in patients with estrogen/progesterone-negative and c-

erbB-2-positive breast cancer? Am Surg 2012; 78: 992-999.

[28] Malkov S, Shepherd JA, Scott CG, Tamimi RM, Ma L, Bertrand KA, Couch F, Jensen MR, Mahmoudzadeh AP, Fan B, Norman A, Brandt KR, Pankratz VS, Vachon CM and Kerlikowske K. Mammographic texture and risk of breast cancer by tumor type and estrogen receptor status. Breast Cancer Res 2016; 18: 122.

[29] Caronongan A 3rd, Venturini B, Canuti D, Dlay S, Naguib RN and Sherbet GV. Neural analyses validate and emphasize the role of progesterone receptor in breast cancer progression and prognosis. Anticancer Res 2016; 36: 1909-1915.

[30] Sun JY, Wu SG, Li FY, Lin HX and He ZY. Progesterone receptor loss identifies hormone receptor-positive and HER2-negative breast cancer subgroups at higher risk of relapse: a retrospective cohort study. Onco Targets Ther 2016; 9: 1707-1713.