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ORIGINAL ARTICLE

Evaluation of hormone receptor, human epidermal growth factor receptor-2 and Ki-67 with core needle biopsy and neoadjuvant chemotherapy effects in breast cancer patients

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Abstract

Background: We investigated the reliability of core needle biopsy (CNB) in evaluating the status of hormone receptor (HR), human epidermal growth factor receptor (HER)-2, and Ki-67 status, and the effect of neoadjuvant chemotherapy (NAC) on the expression of these immunohistochemical markers.

Methods: Among 177 patients with breast adenocarcinoma, 95 patients underwent NAC and the remaining 82 patients made up the control group. Immunohistochemistry (IHC) was used to evaluate the expression status of estrogen receptor (ER), progesterone receptor (PR), HER-2, and Ki-67 in the specimens obtained by surgical excision or CNB.

Results: In the control group, the expression of ER, PR, HER-2, and Ki-67 was highly consistent between samples from surgical excision or CNB (all $r > 0.8$, $P < 0.05$). In the NAC group, the proportions of samples with changes in ER, PR, HER-2, and Ki-67 expression were 12.7%, 24.1%, 5.1%, and 38.0%, respectively; the figures in the control group were 2.4%, 4.9%, 2.4%, and 7.3%, respectively, which significantly differed in ER, PR, and Ki-67 ($P < 0.05$), but not HER-2 ($P > 0.05$). In the NAC group, pre- and post-treatment ER^+ rates did not significantly differ ($P > 0.05$), although PR^+ and high Ki-67 expression rates did significantly differ ($P < 0.05$).

Conclusion: Neither CNB nor surgical excision samples gave highly consistent results in HR, HER-2, and Ki-67 status. NAC can alter HR and Ki-67 status in breast adenocarcinoma patients. NAC decreased PR^+ rate and Ki-67 expression. The mean ER^+ rate exhibited a decreasing, but insignificant trend after NAC treatment. NAC had no significant effect on HER-2 expression.

Introduction

Expression profiles for hormone receptor (HR), human epidermal growth factor receptor (HER)-2, and Ki-67 have significant implications in the prognosis and choice of treatment for breast adenocarcinoma. Neoadjuvant chemotherapy (NAC) is increasingly used in the management of breast adenocarcinoma. However, the effect of NAC on HR, HER-2, and Ki-67 expression in tumor tissue is unclear. Molecular biology markers reportedly change after NAC, which affects the choice of post-treatment management.[1] The clinical evaluation of biological markers like HR, HER-2, and Ki-67 largely relies on core needle biopsy (CNB). However, consistency in evaluating these markers using CNB or surgical excision samples is controversial.[2,3] This study compared changes in HR, HER-2, and Ki-67 in CNB and surgical excision samples from NAC and non-NAC treatment groups, which aimed at determining the consistency of their detection in CNB and surgical excision samples, and the effect of NAC on the expression profile of the markers.

Materials and methods

Source of samples

Samples were obtained from patients with breast adenocarcinoma who were admitted to the Surgical Division of the Shandong Cancer Hospital, from 1 March 2012 to 31 January 2013. The selection criteria for subjects were: (i) female; (ii)

tumor diameter ≥2 cm; (iii) histological examination of CNB tissue confirmed diagnosis of infiltrative ductal carcinoma or infiltrative lobular carcinoma; (iv) no distant metastasis; (v) aged 18–70 years; (vi) Karnofsky score ≥70%; (vii) no previous chemotherapy, endocrine therapy, radiotherapy or target therapy; (viii) normal cardiac function, with multi-gated acquisition (MUGA) scan or ultrasound scan confirmed left ventricular ejection fraction ≥ 50%; and (ix) patient signed a written consent.

We initially admitted 177 breast adenocarcinoma patients into the study based on the above selection criteria. Their median age was 49 years (range: 27–70 years). Subjects were allocated to the NAC (95 cases) or the control group (82 cases). In the NAC group, no patients developed intolerable adverse effects during or after chemotherapy. No patients discontinued therapy because of any reaction from chemotherapy. However, four patients in the NAC group voluntarily withdrew from the study. The remaining 91 subjects who would receive post-NAC surgery in our hospital became the NAC group in our analysis. Their median age was 48 years (range: 29–64 years). According to the criteria set by the American Joint Committee of Cancer (AJCC, 7th edition, 2010), 35 of the patients were at stage IIA, 40 at stage IIB, 11 at stage IIIA, one at stage IIIB, and four at stage IIIC. The control group subjects were stage IIA–IIIB patients who underwent surgery directly without previous chemotherapy. Their median age was 50 years (range: 27–70 years); 28 were at stage IIA, 40 at stage IIB, 10 at stage IIIA, and four at stage IIIB.

Experimental protocol

Core needle biopsy

Patients lay in the supine position. We used beta-ultrasound for localization of the biopsy site, with routine sterilization and local anesthesia. We obtained two to four tissue strips from each tumor with 16-G needles; the strips were cut into 0.5–2.0 cm, immediately fixed in 10% neutral-buffered formalin, and then sent to the Pathology Department for conventional pathological diagnosis on paraffin sections and immunohistochemical (IHC) analysis.

Immunohistochemical (IHC) analysis

We used the IHC streptavidin-peroxidase (S-P) method. Diaminobenzidine (DAB) and an ultra-sensitive S-P kit were purchased from Fuzhou Maixin Biotechnology Co. Ltd. Ethanol, dimethylbenzene and other reagents were offered by the Shandong Cancer Hospital. The process of IHC strictly followed the instructions of the reagent kit. All batches of reagent kits were tested on both positive and negative controls. Confirmed positive sections were used as the positive controls, while IHC procedure using phosphate buffered saline (PBS) to replace the primary antibody was used as negative control. DAB was used for color development, and hematoxylin was used for counterstaining, followed by covering of the section by neutral resin. The IHC-stained sections were observed under a microscope.

Neoadjuvant chemotherapy (NAC) treatment regimen

Although platinum-based drugs in adjuvant therapy for breast adenocarcinoma are widely used, their use in neoadjuvant chemotherapy is not widely investigated. To determine the role of platinum-based drugs in NAC of breast adenocarcinoma, we designed a clinical trial based on the comparison of two regimens: administration of docetaxel (T) or T plus cisplatin (P), followed by surgery. After surgery, the patients received epirubicin plus cyclophosphamide (EC). The current study was a milestone study on the effect of NAC on molecular marker expression in breast adenocarcinoma.

NAC protocol

Four cycles of TP (docetaxel for injection, Qilu Pharmaceutical Co. Ltd, National Drug Registration No.: H20031244, 75 mg/m^2, intravenous injection; cisplatin for injection [lyophilized], Qilu Pharmaceutical Co. Ltd, National Drug Registration No.: H20023460, 75 mg/m^2, intravenous injection) or four cycles of T (Docetaxel for injection, Qilu Pharmaceutical Co. Ltd, National Drug Registration No.: H20031244, 100 mg/m^2, intravenous injection) were administered before surgery. The post-surgical regimen was four cycles of EC (Epirubicin for injection, Pfizer Pharmaceuticals (Wuxi) Co. Ltd, National Drug Registration No.: H20000497, intravenous injection; Cyclophosphamide for injection, Jiangsu Hengrui Medicine Co. Ltd, National Drug Registration No.: H32026196).

Evaluation of results

The results of estrogen receptor (ER) and progesterone receptor (PR) staining were evaluated based on the criteria in the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines on breast adenocarcinoma hormone receptor IHC detection.[4] Staining in >1% of tumor nuclei was regarded as ER$^+$/PR$^+$, whereas <1% staining was regarded as negative. HER-2 expression evaluation employed the internationally recognized scoring system from the ASCO/CAP guideline.[5] The criteria were: 0 score, no staining of tumor cells; 1+ score, infiltrative tumor cells exhibiting faint, incomplete staining in cell membrane, in any proportion; 2+ score, >10% of infiltrative tumor cells exhibiting weak to moderate, complete but uneven staining in cell membrane, or <30% of infiltrative tumor cells exhibiting strong and complete staining in cell membrane; and 3+ score:

Table 1 ER,PR,HER-2, and Ki-67 expression in patients without/with NAC

Group	Total	CB+ surgical excision+	CB+ surgical excision–	CB– surgical excision +	CB– surgical excision–
Control group					
ER	82	64	0	2	16
PR	82	54	2	2	24
HER-2	82	22	0	2	58
Ki-67	82	49	3	3	27
NAC group					
ER	79	41	9	1	28
PR	79	33	16	3	27
HER-2	79	17	3	1	58
Ki-67	79	9	27	3	40

CB, core biopsy. +: positive (HR [hormone receptor] and HER-2 [human epidermal growth factor receptor-2) OR high Ki-67 expression. –: negative (HR and HER-2) OR low Ki-67 expression. ER, estrogen receptor; NAC, neoadjuvant chemotherapy; PR, progesterone receptor.

>30% of infiltrative tumor cells exhibiting strong and complete staining in cell membrane. A score of 0 to 1+ was regarded as negative, while 2+ was suspiciously positive, and 3+ was positive. All suspiciously positive cases (2+) underwent fluorescence in situ hybridization (FISH). Positive results in FISH would be regarded as HER-2^{+}, while negative results in FISH were regarded as HER-2 negative. Ki-67 expression was evaluated by criteria set at the St. Gallen International Breast Adenocarcinoma Conference, 2011.[6] Ki-67 < 14% was defined as low expression, while Ki-67 ≥ 14% was defined as high expression. Tumor regression was assessed using the Miller–Payer system as follows: grade 1, no change or some alteration to individual malignant cells, but no reduction in overall cellularity; grade 2, a minor loss of tumor cells, but overall cellularity still high, up to a 30% loss; grade 3, between an estimated 30% and 90% reduction in tumor cells; grade 4, a marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain, a more than 90% loss of tumor cells; and grade 5, no malignant cells identifiable in sections from the site of the tumor, only vascular fibroelastotic stroma remains, often containing macrophages.[7] However, ductal carcinoma in situ may be present.

All IHC section observations were carried out independently by two senior pathologists under light microscopy, blinded to the clinical information of the subjects. The data are presented as average values of the observation of the two pathologists.

Statistical methods

Experimental data were analyzed by SPSS 19.0 statistical software. Quantitative data were compared by χ^2 test. The consistency of ER, PR, HER-2, and Ki-67 status was analyzed by Spearman correlation coefficient, Rho > 0.8 was regarded as highly correlated. Test criterion $a = 0.05$.

Results

In the NAC group, two subjects who did not provide tissue samples sufficient for IHC analysis were removed from the group. Of 89 subjects, 10 (11.2%) displayed no cancerous tissues, indicating that the patients had excellent responses to primary treatment (Grade 5). Five (5.6%) subjects did not show any reduction in tumor size after chemotherapy. The tumors showed no regressive changes in the tissue sections, indicating that the tumors had not responded to chemotherapy at all (Grade 1). The remaining 74 (83.1%) patients showed some degree of response to chemotherapy (Grade 2–4).

The consistency of hormone receptor (HR), human epidermal growth factor receptor (HER)-2, and Ki-67 expression

The consistency of HR, HER-2, and Ki-67 expression in the NAC group

As shown in Table 1, the consistency rate of ER expression in samples from CNB and surgical excision was 87.3% (69/79; R = 0.759; $P < 0.001$). The consistency rate of PR expression in samples from CNB and surgical excision was 75.9% (60/79; $R = 0.559$, $P < 0.001$). The consistency rate of HER-2 expression in samples from CNB and surgical excision was 94.9% (75/79; $R = 0.864$, $P < 0.001$). The consistency rate of Ki-67 expression in samples from CNB and surgical excision was 62.0% (49/79; R = 0.250, $P = 0.026$).

The consistency of HR, HER-2, and Ki-67 expression in the control group

As shown in Table 1, the consistency rate of ER expression in samples from CNB and surgical excision was 97.6% (80/82;

Table 2 Changes of HR, HER-2 and Ki-67 status between NAC and control groups [%(*n*)]

Group	NAC ($n = 79$)	Control ($n = 82$)	X^2	*P* value
ER	12.7 (10/79)	2.4 (2/82)	6.092	$P = 0.014$
PR	24 (19/79)	4.9 (4/82)	12.079	$P = 0.001$
HER-2	5 (4/79)	2.4 (2/82)	0.772	$P = 0.379$
Ki-67	38.0 (30/79)	7.3 (6/82)	21.784	$P < 0.001$

ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; HR, hormone receptor; NAC, neoadjuvant chemotherapy; PR, progesterone receptor.

R = 0.928; $P < 0.001$). The consistency rate of PR expression in samples from CNB and surgical excision was 95.1% (78/82; $R = 0.887$; $P < 0.001$). The consistency rate of HER-2 expression in samples from CNB and surgical excision was 97.6% (80/82; $R = 0.941$; $P < 0.001$). The consistency rate of Ki-67 expression in samples from CNB and surgical excision was 92.7% (76/82; $R = 0.842$; $P < 0.001$).

Comparison of changes of HR, HER-2, and Ki-67 status between NAC and control groups

As shown in Table 2, a change in ER status was detected in 12.7% (10/79) of samples in the NAC group, compared with 2.4% (2/82) in the control group, which was significantly lower ($P = 0.014$). A change in PR status was detected in 24.1% (19/79) of samples in the NAC group, significantly higher ($P = 0.001$) than in the control group at 4.9% (4/82). In the NAC group, 5.1% (4/79) of samples showed a change in HER-2 status, compared with 2.4% (2/82) in the control group, which was not significantly different ($P = 0.379$). In the NAC group, 38.0% (30/79) of samples showed a change in Ki-67 status, compared with 7.3% (6/82) in the control group, which was significantly lower ($P < 0.001$).

The change in expression of HR, HER-2, and Ki-67 before and after NAC

In the NAC group, the ER^+ rate changed from 64.6% (51/79) to 53.2% (42/79); the PR^+ rate changed from 63.3% (50/79) to 45.6% (36/79); and the $HER\text{-}2^+$ rate changed from 24.1% (20/79) to 21.5% (18/79). High Ki-67 expression in the specimens changed from 45.6% (36/79) to 15.2% (12/79). Among the four markers, the PR^+ and high Ki-67 expression rates decreased significantly after NAC ($P < 0.05$) However, levels of ER and HER-2 did not significantly change after NAC ($P > 0.05$) (Table 3).

Table 3 Changes in ER, PR, HER-2 and Ki-67 expressions before and after NAC

	Pre-NAC	Post-NAC	X^2	*P*
ER			2.117	0.146
+	51	42		
–	28	37		
PR			5.001	0.025
+	50	36		
–	29	43		
HER-2			0.139	0.710
+	20	18		
–	59	61		
Ki-67			17.236	<0.001
High expression	36	12		
Low expression	43	67		

ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; HR, hormone receptor; NAC, neoadjuvant chemotherapy; PR, progesterone receptor.

Discussion

Whether NAC can change HR, HER-2, and Ki-67 expression profiles is controversial. Detection of these biomarkers largely relies on the use of CNB for tissue sampling. However, no consensus exists as to whether CNB or surgical excision leads to more reliable biomarker evaluation.

Wang *et al.* compared the expression profiles of biomarkers in breast adenocarcinoma tissue samples obtained by CNB and by surgical excision, and found no significant differences in PR, HER-2, and Ki-67 expression, whereas ER expression significantly differed between the two groups.[8] Arnedos *et al.* compared the IHC findings of CNB and surgical excision samples from breast adenocarcinoma patients who had not received NAC. The consistency rates of ER, PR, and HER-2 were 98.2%, 85%, and 98.8%, respectively, and the difference in PR expression was statistically significant.[9] Tamaki *et al.* proposed that inadequate sampling was the reason for inaccurate results from CNB samples.[10] Adequate CNB sampling before surgery was suggested to overcome the problem. In our study, pre-surgery CNB samples and samples from surgical excision showed consistency in ER, PR, HER-2, and Ki-67 expression of 97.6%, 95.1%, 97.6%, and 92.7%, respectively, for patients who did not receive NAC. The detection of the four markers was highly consistent across the two groups of samples ($\rho > 0.8$). This might be related to the amount of sampling (2–6 strips per patient). In the NAC group, the inconsistent determination of ER, PR, HER-2, and Ki-67 status might be caused by heterogeneity of the tumors, variation in subjective judgment by the pathologists, or variations in tissue handling and staining, though tissue handling and staining were believed to be a minor contribution to the inconsistency. In the NAC group, the inconsistency rates between CNB and surgical excision samples in ER, PR, HER-2, and Ki-67 were 12.7%, 24.1%, 5.1%, and 38.0%, respectively. Compared with the control group, NAC group samples from CNB and surgical excision showed significant inconsistency in the status of ER, PR, and

Ki-67, while HER-2 status was more consistent. Thus, we believe that CNB and surgical excision can give consistent results in the evaluation of ER, PR, HER-2, and Ki-67 status. CNB samples can reveal the biological characteristics of the whole tumor tissue. Discrepancies in detection of ER, PR, and Ki-67 in the NAC group were because of changes in tumor biological marker by chemotherapy.

Currently, whether NAC could affect ER and PR expression is the focus of considerable attention. Yang *et al*. reported significant changes in ER and PR profiles in breast adenocarcinoma patients after NAC treatment.[11] Zhao *et al*. used the IHC-SP method to measure ER and PR expression in 78 patients with breast adenocarcinoma, before and after NAC, but found no significant difference in the expression of ER and PR.[12] Osako *et al*. reported that changes in ER and PR status were caused by the difference in sampling of tissue before and after chemotherapy. Biopsy samples cannot represent the biological features of the whole tumor.[13] Changes in ER and PR were not caused by NAC.

The current study investigated the ER and PR status of 79 patients with breast adenocarcinoma, before and after receiving NAC. We discovered that NAC could alter ER and PR status. After NAC, the PR^+ rate had significantly decreased. Although the ER^+ rate showed a decreasing trend, the ER^+ rates before and after NAC did not differ significantly. The discrepancies between different investigations might be caused by the variations in sample size, chemotherapy regimen, and numbers of chemotherapy cycles. This awaits proof by a study with a larger sample size.

HER-2 can serve as an independent prognostic marker for breast adenocarcinoma.[14] Faneyte *et al*. reported a non-significant change in HER-2 expression upon NAC treatment.[15] Adams *et al*. suggested the proportion of HER-2 overexpression in breast adenocarcinoma patients increased significantly after NAC.[16] They believed that inadequate CNB sampling might cause pre- and post-NAC differences in HER-2 expression, which, in turn, led to non-representative evaluation of HER-2 status. Research by Yang *et al*. found that, in 113 patients who underwent NAC, 17 cases (15%) exhibited changes in HER-2 status.[11] Although the percentage of cases showing changes was significantly different from that in the control, no trend of change was observed in the NAC group. Mittendorf *et al*. reported that if NAC were combined with trastuzumab, over 43% of patients would exhibit a decrease in HER-2 expression.[17] Our study suggested that HER-2 status did not show a remarkable change before or after NAC. This might be related to the latency in the changes in HER-2 overexpression and gene duplication, which, in turn, explain the constancy of HER-2 status after NAC.[15]

Ki-67 expression status is a reflection on the activity of tumor proliferation. The effect of pre-surgical NAC on Ki-67 expression is still controversial. Faneyte *et al*.[15] showed that the $Ki\text{-}67^+$ rate and expression level declined after NAC treatment. However, a small sample sized study (25 cases) by Arens *et al*. suggested an insignificant change of Ki-67 expression status after NAC treatment.[18] Our study demonstrated a decline of Ki-67 expression level and positive rate after NAC, which was consistent with the results of Faneyte *et al*.

Conclusion

In conclusion, CNB and surgical excision samples provide consistent results in the evaluation of HR, HER-2, and Ki-67, and a CNB sample can reflect the biological characteristics of the whole tumor tissue. NAC can change the status of ER, PR, and Ki-67 expression in patients with breast adenocarcinoma, but it did not exert a significant effect on HER-2 status. After NAC, the PR^+ rate in tumor cells decreased. Although the ER^+ rate showed a decreasing trend after NAC, the change was not statistically significant. Therefore, ER, PR, and Ki-67 expression in tumors should be monitored before and after NAC.

Disclosure

No authors report any conflict of interest.

References

1 Dede DS, Gumuskaya B, Guler G, Onat D, Altundag K, Ozisik Y. Evaluation of changes in biologic markers ER, PR, HER 2 and Ki-67 index in breast cancer with administration of neoadjuvant dose dense doxorubicin, cyclophosphamide followed by paclitaxel chemotherapy. *J BUON* 2013; **18**: 366–71.

2 van de Ven S, Smit V, Dekker TJA, Nortier JWR, Kroep JR. Discordances in ER, PR and HER2 receptors after neoadjuvant chemotherapy in breast cancer. *Cancer Treat Rev* 2011; **37**: 422–30.

3 Ough M, Velasco J, Hieken TJ. A comparative analysis of core needle biopsy and final excision for breast cancer: histology and marker expression. *Am J Surg* 2011; **201**: 692–4.

4 Hammond MEH, Hayes DF, Dowsett M *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med* 2010; **134** (7): e48–72.

5 Wolff AC, Hammond MEH, Schwartz JN *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007; **25**: 118–45.

6 Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes – dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol* 2011; **22**: 1736–47.

7 Ogston KN, Miller ID, Payne S *et al*. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast* 2003; **12**: 320–7.
8 Wang C, Wang J, Mu J *et al*. Clinical study on preoperative core needle biopsy of breast cancer and detection of ER, PR, C-erbB-2, Ki-67. *Chin J Curr Adv Gen Surg* 2011; **14**: 517–20. (In Chinese.)
9 Arnedos M, Nerurkar A, Osin P, A'Hern R, Smith IE, Dowsett M. Discordance between core needle biopsy (CNB) and excisional biopsy (EB) for estrogen receptor (ER), progesterone receptor (PgR) and HER2 status in early breast cancer (EBC). *Ann Oncol* 2009; **20**: 1948–52.
10 Tamaki K, Sasano H, Ishida T *et al*. Comparison of core needle biopsy (CNB) and surgical specimens for accurate preoperative evaluation of ER, PgR and HER2 status of breast cancer patients. *Cancer Sci* 2010; **101**: 2074–9.
11 Yang YF, Liao YY, Li LQ, Xie SR, Xie YF, Peng NF. Changes in ER, PR and HER2 receptors status after neoadjuvant chemotherapy in breast cancer. *Pathol Res Pract* 2013; **209**: 797–802.
12 Zhao Y, Li Y, Zhu Y *et al*. Influence of neoadjuvant chemotherapy on expressions of estrogen receptor, progesterone receptor and prolife rating cell nuclear antigen in patients with breast cancer. *Chin J Gen Pract* 2011; **9**: 1172–3. (In Chinese.)
13 Osako T, Nishimura R, Okumura Y, Toyozumi Y, Arima N. Predictive significance of the proportion of ER-positive or PgR-positive tumor cells in response to neoadjuvant chemotherapy for operable HER2-negative breast cancer. *Exp Ther Med* 2012; **3**: 66–71.
14 Bozzetti C, Musolino A, Camisa R *et al*. Evaluation of HER-2/neu amplification and other biological markers as predictors of response to neoadjuvant anthracycline-based chemotherapy in primary breast cancer: the role of anthracycline dose intensity. *Am J Clin Oncol* 2006; **29**: 171–7.
15 Faneyte IF, Schrama JG, Peterse JL, Remijnse PL, Rodenhuis S, van de Vijver MJ. Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. *Br J Cancer* 2003; **88**: 406–12.
16 Adams AL, Eltoum I, Krontiras H, Wang WO, Chhieng DC. The effect of neoadjuvant chemotherapy on histologic grade, hormone receptor status, and HER2/neu status in breast carcinoma. *Breast J* 2008; **14**: 141–6.
17 Mittendorf EA, Wu Y, Scaltriti M *et al*. Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. *Clin Cancer Res* 2009; **15**: 7381–8.
18 Arens N, Bleyl U, Hildenbrand R. HER2/neu, p53, Ki67, and hormone receptors do not change during neoadjuvant chemotherapy in breast cancer. *Virchows Arch* 2005; **446**: 489–96.

