

Analysis of discrepancies of core needle biopsy and surgical specimens for accurate evaluation of hormonal receptors and epidermal growth factor receptor 2 status of invasive breast cancer patients

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Abstract

Breast cancer is a serious health problem. It is the most common cancer in women. The aim of this study was to estimate the concordance between ER, PR receptor and HER-2 immunohistochemistry assessment scores in paired CNB (core needle biopsy) and surgical specimens. Histological grade, oestrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor-2 (HER2) status were evaluated in a blinded fashion in CNB and in surgical excision specimens. Absolute concordance rate between core needle biopsies and surgical specimens for histological grade was 50% with κ value (0,15) for ER 92% with κ value (0,79), PR 88% with κ value (0,73) and for HER2 96% with κ value (0,91). CNB can provide reliable information in evaluation of ER, PR and HER2 status in an invasive breast carcinoma.

Ključne reči: breast cancer, core needle biopsy, oestrogen receptors, progesterone receptors, HER-2.

Introduction

Breast cancer poses a serious health problem it is the most common cancer in women with an incidence of 464,000 new cases in Europe in 2012 causes of death 131,000. The mortality has decreased in western countries due to accurate diagnosis and optimal treatment¹. The core needle biopsy (CNB) procedure is now established as the standard method for a diagnostic method for breast cancer and it is almost accurate as a surgical specimens deciding the optimal treatment algorithm². The 2011 European Society of Medical Oncology breast cancer clinical practice guideline required a preoperative disease- related staging

Analiza odstupanja statusa hormonskih receptora i receptora za epidermalni faktor rasta 2 u uzorcima dobijenim iglenom biopsijom i hirurškim uzorkom kod obolelih od invazivnog karcinoma dojke

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Apstrakt

Karcinom dojke je važan zdravstveni problem. To je najčešći karcinom kod žena. Cilj ove studije je da se odredi stepen podudarnosti između imunohistochemijskih skorova estrogenskih, progesteronskih i Her2 receptora kod iglenih biopsija dojke i odgovarajućih operativnih preparata. Evaluirani su histološki gradus, ER, PR i Her2 status u iglenim biopsijama i odgovarajućim operativnim preparatima. Apsolutna podudarnost histološkog gradusa je utvrđena u 50% slučajeva sa κ vrednošću (0,15), za ER 92% sa κ vrednošću (0,79), PR 88% sa κ vrednošću (0,73) i za Her2 96% sa κ vrednošću (0,91). Iglene biopsije mogu obezbediti pouzdane informacije u evaluiranju ER, PR i Her2 statusa kod invazivnih karcinoma dojke.

Key words: karcinom dojke, iglena „core“ biopsija, estrogen receptor, progesteron receptor, HER-2.

including determination of oestrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor-2 (HER2) status by immunohistochemistry (IHC) or in situ hybridization (ISH)³. Optimal determination of both ER, PgR and HER2 gene amplification is a subject of discussion. For breast cancer patients is very important to provide appropriate tissue samples for preoperative pathological analysis. Due to tumour heterogeneity and relatively smaller size of CNB, the biomarker assessment performed on CNB samples may be less reliable than resection specimens. The information provided from CNB may be only information available for determining the patients for preoperative or neoadjuvant therapy. Particularly in settings where neo-adjuvant therapy is used the information obtained from CNB must reasonably reflect that in the whole tissue. Results of previous studies demonstrated that the concordance rate between CNB and surgical specimen were 61.7-99% for ER, 61.5-97.1% for PgR and 80-96% for HER2 respectively⁴.

Our aim was to estimate the concordance between ER, PgR receptor and HER-2 immunohistochemistry assessment scores in paired CNB and surgical specimens.

Materials and Methods

We studied 50 female patients with ductal invasive carcinoma NOS whose ages ranged from 28 to 81 (median ages 58). They underwent CNB and subsequent surgical treatment between January 2013 and 2014 in University Clinical Center of Republic of Srpska. Patients who received neo-adjuvant therapy or radio/chemo therapy between CNB and surgical excision were excluded from study. All the core biopsies were performed under ultrasound guidance using a 16-gauge true-cut needle. Surgical excision was performed in each case. After the CNB 25 (50%) patients underwent quadrantectomy while total mastectomies were carried out in 25 (50%) patients. During both procedures specimens were collected before the initiation of systemic treatment. All the specimens were fixed in 10% buffered formalin, embedded in paraffin, cut in to 4 µm thick slices, staining with haematoxylin-eosin (HE) and immunohistochemically for ER, PgR and HER2, and placed on the glue-coated glass slides.

To determine ER and PgR status of each case, we used ER/PgR pharmDx™ (DAKO, Denmark) with the following antibodies: clones 1D5, ER-2-123 and clone PgR 1294, according to the manufacturer's instructions. All single-receptor positive cases were reevaluated for ER and PgR stains. Repeat ER and PgR stains were reviewed by a pathologist blinded to clinical characteristics and tumour histology. Nuclear staining of any intensity was considered positive in all ER and PgR IHC staining cases. Also, to determine level of HER2 protein expression, we used Dako's pharmDx HercepTest according to the manufacturer's instructions. For epitope retrieval, we used The Epitope Retrieval Solution (HercepTest™ kit). Antibody we used was Rabbit Anti-Human HER2 Protein. Interpretation of the results is carried out by microscoping. To determine number of HER2 gene copies, we used HER2 CISH pharmDx™ Kit (DAKO Corporation, USA). After incubation at 60°C for 60 min, the paraffin sections were deparaffinised in two series of xylol and rehydrated with ethanol series. Pre-treatment of the slides with (20x) solution (Dako, Denmark) in a water bath at 99°C for 10 min was followed by enzymatic digestion with ready-to-use pepsin for 4 min at 37°C on hybridizer (Dako, Denmark). Then, the slides were dehydrated and 10 µl of HER2/cen17 probe was incubated to each tissue sections. Before the hybridization during the night at 37°C, the slides and probe were denatured at 94°C for 5 min. Then, the slides were washed with stringent wash buffer at 63°C for 10 min in a water bath. After dehydration, tissue sections were covered with 10 µl CISH Antibody Mix for 30 min and then incubated with blue and red chromogen for 10 min. Samples were contrasted with haematoxylin. Standard result revision, described as Tanner's paper, was used for the interpretation of HER2 CISH results. Slides from both CNB and the surgery were independently reviewed in a blinded fashion by two pathologists. The following factors were considered: histological grade, ER status, PgR status and HER2 status. In cases of inter-observer disagreement, a conclusion was reached after sufficient discussion. The histological grade was assigned using the Nottingham grading system⁵. The ER and PgR results were assessed semi-quantitatively using Allred's scoring system⁶. The results were categorized as positive when the total score (TS), expressed as the sum of the proportion score (PS) and the intensity score (IS), was more than two. With regard to HER2, membranous

staining was graded as negative (score 0 or 1+), weakly positive (score 2+) and strongly positive (score 3+). Agreement between the results from CNB and those from surgical excision was statistically analysed using the absolute concordance rate and k statistic values.

Results

Histological grade

The histological grade was assessed in 50 cases that were identified as invasive carcinoma by both CNB and subsequent surgical excision. At CNB grade I was present at the 8 (16%) cases grade II at the 38 (76%) cases and grade III at the 4 (8%) cases while at the surgical excision grade I was evaluated at the 3 (6%) cases, grade II at the 24 (48%) cases and grade III at the 23 (46%) patients. There was 50% (25/50 cases) concordance (Table 1.) with a κ statistic value of 0.15. Based on the results of histological grade concordance, in 24 of the 25 discordant cases, the grade at CNB was lower, while in one cases, the grade at CNB was higher than in subsequent surgical excision. However, disagreement case was 1-grade discordant in 24 cases. In one case disagreement was 2-grades. In that case the grade at the CNB was 2- grades lower than in subsequent surgical excision.

Histology grade CNB	Histology grade Surgical excision			
	I	II	III	
I	2	5	1	8 (16%)
II	1	19	18	38 (76%)
III	0	0	4	4 (8%)
	3 (6%)	24 (48%)	23 (46%)	50

Absolute concordance rate, 50% (25/50); kappa statistic value, 0.15.

Table 1. Comparison of the histological grade in CNB and surgical excision

Oestrogen receptor and Progesterone receptor

At the CNB ER 38 (76%) patients had ER and 34 (68%) PgR positive. At the surgical excision, ER was expressed in 72% (36/50) of the cases, while PgR was expressed in 64% (32/50) of the cases (Table 2. and Table 3).

ER CNB	ER Surgical excision		
	Positive	Negative	
Positive	35	3	38 (76%)
Negative	1	11	12 (24%)
	36 (72%)	14 (28%)	50

Absolute concordance rate, 92% (46/50); kappa statistic value, 0.79.

Table 2. Comparison of ER in CNB and surgical excision

ER/PR status of the excisional specimen was regarded as the golden standard. The sensitivity of CNB was 97,3%, specificity 82,4% and positive predictive value 92,2% in ER. The sensitivity of CNB was 94,1%, specificity 81,1% and positive predictive value 88,9% in PgR.

The number of discordant cases in ER was four (8%). Among these, one (2%) case was negative at CNB but positive at excision. Three (6%) cases were positive at CNB but negative at excision. There was 92% concordance with a κ statistic value of 0.79.

The number of discordant cases in PgR was six (12%). Among these, two cases were negative at CNB but positive at excision. Four cases were positive at CNB but negative at excision. There was 88% concordance with a κ statistic value of 0.73.

PgR CNB	PgR Surgical excision		
	Positive	Negative	
Positive	30	4	34 (68%)
Negative	2	14	16 (32%)
	32 (64%)	18 (36%)	50

Absolute concordance rate, 88% (44/50); kappa statistic value, 0.73.

Table 3. Comparison of PgR in CNB and surgical excision

HER2

HER2 was also evaluated in 50 cases. In 16 (32%) of the 50 cases, HER2 was expressed in the excisional specimen. For the CNB HER2 results, 24 (48%) tumours were 0, 1+, 10 (20%) were 2+ and 16 (32%) were 3+. For the resection specimens, 28 (56%) tumours were 0, 1+, 8 (16%) were 2+ and 14 (28%) were 3+. When comparing the results between CNB and resection specimens using the three IHC scores (0 or 1+, 2+, 3+), we observed a significant number of discordant results. A total of 5 tumours were discordant for these three scores and 45 were concordant (90%). These concordances do not all have clinical implications. We also assessed HER2 status as a dichotomous variable (HER2 negative/HER2 positive) according to the current HER2 testing protocols. If the tumour was scored as 0 or 1+, the tumour was HER2 negative. Tumours with 2+ scores were subjected to chromogen in situ hybridization assay (CISH). There was 96% (48/50 cases) agreement with a κ statistic value of 0.91. The sensitivity was 100%, specificity 94,4% and positive predictive value 88,9% of CNB respectively (Table 4).

HER 2 CNB	HER 2 Surgical excision		
	Positive	Negative	
Positive	16	2	18 (36%)
Negative	0	32	32 (64%)
	16 (32%)	34 (68%)	50

Absolute concordance rate, 96% (48/50); kappa statistic value, 0.91.

Table 4. Comparison of HER 2 in CNB and surgical excision

In a two case where the score was 3+ (strongly positive) at CNB at the surgical excision in one case score was 0 (negative) at the surgical excision and in other case score was 2+ (weakly positive) and after CISH testing result was negative. In three cases at the CNB score was 2+ (weakly positive) with negative result after CISH testing, and in the same cases at the surgical excision score was 0 (negative).

Discussion

Accurate determination of histological type, grade, ER, PgR expression and HER2 gene amplification on invasive breast cancers is essential for optimal choice of (neo) adjuvant therapies. Multiple studies have investigated the concordance between CNB and resection specimens, usually with small patient series and with occasionally discrepant results.

In this study, we evaluated the histological grades of specimens taken at CNB and compared these with those of specimens taken at surgical excision. In particular, the histological grade is a powerful prognostic factor of invasive breast carcinoma. There was concordance 50% cases with a κ value (0.15). These result is lower to those reported previously (59–75%)⁷⁻¹¹. In the discordant cases, the grade at CNB tended to be lower than that at surgical excision. Previous studies noted that this tendency of the histological grade was due to underestimation of the mitotic count at CNB.

The hormone receptor status is also a very important and independent prognostic factor in breast cancer. In our study, hormone receptors were accurately evaluated at CNB. An almost perfect κ value (0.79) in ER and a substantial κ value (0.73) in PgR were observed. The concordance of PgR was lower than that of ER. This can be associated with the lower incidence of PgR-expressing cells in the whole tumour because the Allred total score of PgR (mean: 4.0) was lower than that of ER (mean: 5.3). Although the immunohistochemically staining results for ER/PgR at CNB may be reliable for determining therapeutic indications. Four discordant cases (8%) were observed in ER testing. There was one case that was CNB-negative and excision positive and three cases that were of the opposite type for ER assessment. Six discordant cases (12%) were observed in PgR testing. There were two case that were CNB-negative and excision positive and four cases that were of the opposite type for PgR assessment. The characteristics of the former cases are mainly due to intratumoral heterogeneity and some investigators^{12,13} have noted that such situations could exist because of more rapid and constant fixation. Douglas-Jones et al.¹⁴ suggested that ER expression was higher in the CNB than in the excised tumours. ER expression was higher at the periphery of tumours than at the center. The higher ER expression in CNB might reflect the greater possibility of the peripheral part of a tumour being sampled when CNB is performed. CNB ER+/resection specimen ER– tumours were described in 1 patient (2%) and CNB PgR+/resection specimen ER– tumours were described in one patient (2%). Our study provides strong evidence that ER and PgR status can be reliably determined on the CNB. The number of patients misdiagnosed should not to be underestimated. We therefore recommend retesting ER-negative biopsies on the surgical specimen. HER2 positivity on either CNB or surgical specimen is an indication for treatment with the HER2-inhibiting drug such as trastuzumab. Concordance for HER2 IHC testing for the three categories (0 or 1+, 2+, 3+) has revealed that significant discordance exists between CNB and resection specimens^{15,16}. Our study examined the concordance when examining HER2 status as positive or negative (determined with both IHC and in situ hybridization for 2+ cases). The HER2 status, the diagnostic accuracy (particularly the sensitivity) of CNB was high with concordance of 96% and κ value of 0.91. We found one tumour (2%) that was strongly positive 3+ on the CNB, but negative on the resection specimen one tumour (2%) that was strongly positive 3+ on the CNB and weakly positive 2+ on the surgical specimen and negative after in situ hybridisation testing. In this study three tumours (6%) showed weak positivity 2+ on CNB with negative result after in situ hybridisation and on surgical resections they were negative with 0 score. The confirmation of 3+ HER2 results on CNB with in situ hybridization assays could be increase the indication for trastuzumab selection^{17,18}.

Conclusions

Concordance between CNB and surgical specimens was high for ER, PgR and HER2 testing. Core biopsy can provide reliable information on histological grade, hormone receptors and HER2 status of patients.

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