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Optimizing breast cancer survival models based on conventional biomarkers and stromal parameters

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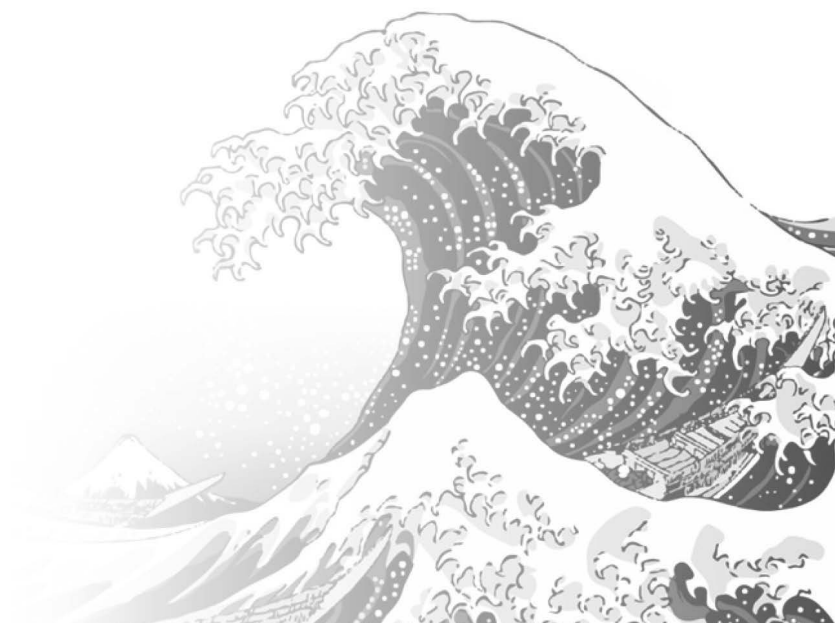
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Reliability of core needle biopsy for determining ER and HER2 status in breast cancer

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Introduction

Breast cancer is the most common cancer in women with an incidence of 421,000 new cases in Europe in 2008 [1]. Due to increasing efficacy of (neo-)adjuvant systemic treatment, positive trends are observed concerning breast cancer patients' survival [2]. Optimal determination of both estrogen receptor (ER) expression and human epidermal growth factor 2 (HER2) gene amplification is a subject of discussion. The American Society of Clinical Oncology and College of Pathology (ASCO/CAP) panel reported on the large number of inaccurate local HER2 testing results, which was estimated to be around 20% of all HER2 tests [3]. The ASCO/CAP panel also estimated that 20% of ER testing might be inaccurate as well, and provided recommendations to increase the reliability including lowering the positivity threshold to 1% ER-positive cells [4]. Despite these discussions, determining ER and HER2 status is still considered as standard care for all invasive breast cancers as these biomarkers are predictive for response of patients to hormonal treatment and/or HER2-inhibiting medication. ER and HER2 status can be tested on both core needle biopsy (CNB) and resection specimens. Using CNB for determining ER and HER2 has the advantage that the final result is available before the surgical procedure. Secondly, for neoadjuvant treatment, CNB is the only material available for molecular testing. Another advantage of CNB is more optimal fixation conditions. For surgical specimens, time from interruption of the blood supply to the initiation of fixation is likely longer, since surgery is a more complicated procedure than CNB. In some centers, breast resection specimens are not immediately sliced and fixed, resulting in poor fixation of the tumor. Fixation protocols are also more standardized for CNB, while many different protocols exist for the fixation of surgical specimens. A disadvantage for using CNB is the possibility of crush artifacts that may lead to false-positive results.

Several series have reported the concordance between preoperative CNB and resection specimens for ER and HER2 determinations [5-27]. These studies generally included small numbers of tumors and have reported some seemingly contradicting results, with concordance percentages ranging from 61.8% to 99.0%, leading to variable recommendations concerning the use of CNB for ER and HER2 testing. Because of these conflicting results, we determined the concordance of CNB and resection specimens for ER and HER2 testing in a series of patients that were treated at our hospital. To compare our results to those published in the literature and to assess the overall concordance of published cases, we combined all published patient series and determined the nature and frequency of discordant results.

Methods

Study population

Determination of ER and HER2 status in the Leiden University Medical Center (LUMC) was routinely carried out on preoperative CNB between 2006 and 2008. Patients that were treated for invasive breast cancer between 2006 and 2008 with ER and HER2 determined on pretreatment CNB were eligible for this study. Biopsies were taken with a 14-gauge needle. For each lesion, at least two cores were required, but more were taken on some occasions (e.g. larger tumors or cores of low quality). Patients who received neoadjuvant chemotherapy were excluded, since this may have substantial influence on the expression of ER and HER2 [28]. For all eligible patients, clinico-pathological parameters including age, tumor size, histological subtype, Bloom-Richardson grade and lymph node stage were retrieved.

TMA construction

A tissue micro-array (TMA) was created from resection materials from patients treated for invasive breast cancer between 2006 and 2008 at the LUMC. These TMAs were originally created as part of a scheme to investigate HER2 testing reliability [29]. To construct the TMA, formalin-fixed paraffin-embedded tissue blocks containing resected invasive breast carcinomas and corresponding hematoxylin and eosin-stained slides were retrieved from our pathology archives. Parts of the tumor that displayed invasive cancer were marked. From each tumor, three 0.6-mm thick cores were collected within the marked area using the Beecher TMA instrument and inserted in a donor block. The use of three cores for the TMA has been shown to correlate strongly to the protein expression as determined on whole sections [30, 31].

Immunohistochemistry and evaluation

After tissues were biopsied or excised, these were kept in neutral buffered formalin overnight in order to ensure a fixation time between 6 and 72 h, which is in accordance to the ASCO/CAP testing guidelines [3, 4]. Immunohistochemistry was carried out with the 1D5 antibody for ER (Dako) and A0485 (rabbit polyclonal) antibody for HER2 on Dako autostainer. Mono color silver in situ hybridization (SISH) was carried out with the Ventana SISH kit on Benchmark XT. To assess the reliability of preoperative biopsies for determining ER and HER2 status, TMA sections were stained for ER and HER2, and results were compared with the final results from the preoperative biopsies. The immunohistochemistry (IHC) results of the biopsies were determined

as part of the routine patient care by multiple pathologists. The TMAs of resected specimens were scored by two observers independently, who were blinded for the biopsy result. The ER and HER2 scores for all three TMA cores were determined. If all three cores were concordant, this was considered the final TMA score. If one of the cores was discordant or if the final score differed from the preoperative CNB result, full-sized slides were stained to assess the final ER and HER2 status. This material was revised by two observers simultaneously for scoring ER status, the threshold of 10% was used for both the CNB and resection specimen. HER2 staining was scored according to the conventional guidelines, with the cut-off for HER2 positivity at 30% of cells. For determining the level of agreement between the CNB and resection score, kappa (κ) values were calculated by using the statistical package SPSS (version 16.0 for Windows, SPPS, Inc., Chicago, IL, USA).

Literature search

A literature search was carried out on 16th September 2011 to identify and to review all studies that have determined the concordance between the CNB and resection specimens for determining ER and/or HER2 status as primary or secondary outcome by using IHC. The search terms used in the three different databases are summarized in supplementary table S1. The Pubmed database was used as the primary search database. Besides Pubmed, the same search strategy was used in Medline and Web of Science. All unique results were identified and added to a reference manager file. The abstracts from all articles were screened for relevance, and full-text articles were obtained for all articles that fitted our inclusion criteria and were available in the English language. The reference list from all articles was searched for other relevant articles. From all articles, the following parameters were noted: the number of patients, the scoring method, the cut-off point for positivity, the number of CNB-/resection specimen+ tumors, the number of CNB+/resection specimen- tumors and number of negative and positive concordant tumors. Parallel to the selection criteria for the LUMC patient series, scientific articles describing patient series that were treated with neoadjuvant chemotherapy between CNB and surgery were excluded from the literature review.

Results

Patient characteristics

The clinico-pathological characteristics of the 122 included patients that were treated at our hospital are summarized in table 1. These patients were all treated for early-stage breast cancer with primary surgery at the LUMC. The mean age was 63 (range 36-91 years). T-stage was pT1 for 87 patients, pT2 for 29 and pT3 for 6. Fifty patients were found to have positive lymph nodes. The most common histological subtype was ductal carcinoma, which was diagnosed in 102 patients (83.6%), followed by lobular carcinoma in 19 (15.6%) and medullary carcinoma in 1 (0.8%). For all tumors, the median number of 14-gauge cores taken was 2 (mean 2.7, range 1-11). For pT1 lesions, the median number of cores was 2 (mean 2.7, range 1-11); for pT2 lesions, the median was 2 (mean 2.5, range 1-6) and for pT3, the median was 4 (mean 3.3, range 1-5). The concordance for the TMA scoring for ER between the two observers was found in 99.1% of cases (κ -value = 0.963). The concordance for the HER2 status was found in 96.4% of cases (κ -value = 0.695). In case of discordance, the final status was resolved by two observers.

ER concordance

Both the CNB and resection specimen ER results were available for 115 patients. Eighteen patients (15.7%) had tumors negative for ER receptor expression on the CNB, 97 (84.3%) were found to have tumors that were positive for ER expression. The final ER result from the resection specimens was negative for 17 tumors (14.8%) and positive for 98 (85.2%) tumors (figure 1A and B). Concordance between the CNB and resection specimen was found for 114 of 115 patients (99.1%) and the κ -value was 0.966, indicating almost perfect agreement (supplementary table S2). Negative CNB result but positive resection specimen was found in 1 tumor. When CNB and resection specimen were compared for this case, marked tumor heterogeneity concerning ER expression was observed in the resection specimen (figure 2A and B). This discordant result was thus likely the result of tumor heterogeneity.

HER2 concordance

HER2 testing was first carried out on all samples with IHC. For the CNB HER2 results, 80 tumors were 0, 1+, 17 were 2+ and 11 were 3+. For the resection specimens, 91 tumors were 0, 1+, 9 were 2+ and 8 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 19 tumors were discordant for these three scores and 89 were concordant (82.4%). This resulted in a κ -value of 0.505, indicating moderate agreement. However, 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 tumor was scored as 0 or 1+, the tumor was HER2 negative. Tumors with 2+ scores were subjected to an in situ hybridization assay. Tumors with 3+ score were considered HER2 positive. For 105 tumors, HER2 status was determined for both CNB and resection specimens. Concordance was found in 96.2% of all tumors (κ -value = 0.813). The discordant cases were four tumors that were found to be positive on the CNB, but negative on the resection specimen (supplementary table S3, figure 3A and B).

Literature review

Our search strategy resulted in 129 results in Pubmed. Additionally, 49 unique results in Embase and 35 in Web of Science were also found and evaluated. From these 213 abstracts, 25 articles were found eligible for our study. An additional eight full-text articles were found as references to these articles. These articles were published between 1996 and 2011.

The CNB-resection concordance for ER status was investigated in 22 studies [5-19, 21-27]. Two studies listed either solely the percentage of concordance or the κ -value for agreement [17, 22], so these studies were left out of our analysis. The remaining 20 studies included a total number of 2507 invasive breast tumors. Concordance was found for 2342 tumors (93.4%). While some studies merely listed the percentage and absolute number of concordant results, we were interested in the reason for the discordant results. This information was available for 16 studies, which investigated a total number of 2244 invasive breast tumors [6-9, 12-16, 19, 21, 23-27]. A number of 51 tumors had negative CNB results, whereas the resection specimen was positive (2.3%). The opposite (positive CNB and negative resection specimen) was true for 77 tumors (3.4%).

The concordance between CNB and resection specimens regarding HER2 status was investigated in 18 studies [6, 8-10, 15, 16, 22-26, 32-35]. Data concerning the concordance concerning three IHC categories (0/1+, 2+, 3+) were available in eight articles [16, 21, 22, 24, 26, 33-35]. Concordant results were observed in 1250 of 1459 tumors (85.7%). The most frequent discordant result was 2+ score on the CNB, and with negative score (0,1+) on the surgical specimen. However, these do not all represent clinically relevant discordant results, because treatment decisions are not solely based

on IHC results. We investigated the number of studies that compared the concordance between CNB and resection specimens when considering tumors as either HER2 negative or HER2 positive [6, 8, 15, 17, 18, 23, 25, 32]. Some of these studies considered all 2+ cases as HER2 positive, which in contrast with current testing guidelines, and these were thus excluded from this analysis [8, 18, 25, 32]. In accordance with HER2 testing guidelines, three studies that determined HER2 status with IHC as initial testing method and *in situ* hybridization assays for all 2+ cases were included [6, 15, 23]. All 543 patients from these studies were pooled. Concordant negative and positive results for CNB and resection specimens were seen in 481 of 543 tumors (88.6%) and 52 of 543 tumors (9.6%), respectively. CNB HER2 positive and resection specimen HER2 negative results were seen in six tumors. Four tumors were HER2 amplified on resection specimens, but negative on the initial CNB.

Combined concordance rates

To evaluate the performance of CNB as a primary method of assessing ER and HER2 status, we combined our results with those described in the literature in order to assess the combined concordance rate of the CNB results with the resection specimen. For determining ER status, the overall concordance was 93.7%, based on our series and 20 published patient series, which included a total number of 2622 patients [5-16, 18, 19, 21, 23-27] (supplementary table S4). The number of CNB+/resection specimen+, CNB-/resection specimen-, CNB+/resection specimen- and CNB-/resection specimen+ tumors was investigated in 2359 patients. This was based on the LUMC series and 16 studies that specified this information in the report [6-9, 12-16, 19, 21, 23-27]. Concordant ER positive and ER negative results were found in 1775 (75.2%) and 455 (19.3%) patients, respectively. The most frequent discordant result was positive ER status determined in CNB, whereas the surgical specimen for the same tumor was found ER negative (77 patients, 3.3%). CNB ER negative with ER positive subsequent surgical specimens was seen in 52 patients (2.2%; table 2).

For determining the HER2 concordance rates, we used the dichotomous categories for HER2 results (HER2 negative or HER2 positive). The number of studies that determined HER2 status according to the currently used HER2 testing protocols were three published reports and our study [6, 15,23] that investigated a total of 646 tumors (supplementary table S5). Overall concordance was found to be 97.8%, 62 tumors (9.6%) were positive on both the CNB and resection specimen, 572 (88.3%) were negative according to both the CNB and resection specimen. Discordant results were seen in 14 patients, 10 of these patients (1.5%) had positive CNB with negative surgical

specimen and 4 (0.6%) were negative on the CNB, whereas the surgical specimen was positive (table 3).

Discussion

Accurate determination of ER 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. We decided to investigate the concordance for ER and HER2 status for a series of patients from our hospital and to pool these data with published patient series in order to more reliably investigate the performance of CNB for determining ER and HER2 status. For ER receptor concordance, we found that this exceeded 99% in our patient series and concordance was 93.7% in the pooled patients from 21 reports including our patient series. Discordance was found for 1 patient in our patient series. This patient had a negative CNB result, whereas the resection specimen was scored positive for ER expression. Our literature search found a similar discordant result in a total number 52 other patients (2.2%) in a pooled series of 2359 patients from 18 studies and our own. These results might be a reflection of the tumor heterogeneity leading to sampling error. This group of patients is currently at risk of being misdiagnosed when solely based on the basis of CNB and might be withheld effective hormonal therapies. Due to the increasing demand for ER testing, the number of patients misdiagnosed should not to be underestimated. We therefore recommend retesting ER-negative biopsies on the surgical specimen.

In the pooled cases from the literature, CNB ER+/resection specimen ER- tumors were described in 77 patients (3.3%). These results might be explained by superior fixation of tissue of the CNB compared with resection specimens or due to over-retrieval. One of the reports that was identified in our literature search recommended testing biopsy material as it was able to identify some tumors with ER expression that were missed in the surgical specimen [16]. Since there is evidence that even patients with low-level ER expression are responsive to hormonal treatment and those with low ER expression are therefore currently considered for hormonal therapy, we feel that it is justified to offer hormonal therapy even to CNB+/resection specimen- tumors.

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. Our study and three others examined the

concordance when examining HER2 status as positive or negative (determined with both IHC and *in situ* hybridization for 2+ cases). We found four tumors that were positive on the CNB, but negative on the resection specimen, and this was found in 10 cases (1.5%) in our combined patient series consisting of the LUMC patient cohort and literature cases. This could be due to artifacts and no real data concerning trastuzumab response for this group of CNB 3+/resection specimen tumors exist. Our center has therefore started testing all 3+ tumors on CNB with *in situ* hybridization in order to assess the final score and to increase the reliability of HER2 positivity ascertained on CNB.

Our study pooled data from several studies that have investigated the concordance between CNB and resection specimens. The weakness of this approach is the heterogeneity of the studies included in our analysis. Some studies have used different methods (fixation, antigen retrieval, antibodies, etc.) and scoring methods (H-score and Allred etc.). However, these studies were included in our analysis as long as both CNB and resection specimens were treated similarly. Although by reviewing all published series in the literature our study is capable of a more reliable evaluation regarding the reliability of CNB for ER and HER2 testing, there is a danger of publication bias. Laboratories with more experience and higher patient volumes are more likely to publish their experience with ER and HER2 testing.

In conclusion, our patient series was combined with other patient series described in the literature in order to assess the frequency and nature of discordant results with more certainty. For ER testing, we have shown that the overall concordance rate described in the literature was 93.7% by pooling data from 20 published reports and our series. Since heterogeneous antigen expression might be a cause for false-negative results on the CNB, we recommend testing tumors with ER-negative biopsy results again on the surgical specimen. Our study provides strong evidence that ER status can be reliably determined on the CNB. Tumors that are ER-positive on the CNB with ER-negative scores on the surgical specimen do exist. Since there is evidence that even patients with low-level ER expression respond to hormonal treatment, we feel that it is justified offering these patients hormonal therapy. For HER2 testing, the overall concordance rate was 97.8% in the pooled series of cases from three previously published reports and our patient series. However, there is a considerable number of tumors that are CNB positive, but negative on surgical specimens. We therefore recommend also confirming 3+ HER2 results on CNB with *in situ* hybridization assays in order to increase the indication for trastuzumab selection.

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Table 1. Patient and tumor characteristics.

	<i>N (%)</i>
Mean age (range)	63 (36–91)
T-stage	
pT1	87 (71.3%)
pT2	29 (23.8%)
pT3	6 (4.9%)
N-stage	
pN0	70 (57.4%)
pN1–3	50 (41.0%)
Unavailable	2 (1.6%)
Tumor grade	
I	20 (16.4%)
II	68 (55.7%)
III	23 (18.9%)
Unavailable	11 (9.0%)
Tumor type	
Ductal	102 (83.6%)
Lobular	19 (15.6%)
Medullary	1 (0.8%)

Table 2. Overall concordance for estrogen receptor in LUMC series combined with literature series.

<i>CNB</i>	<i>Resection specimen</i>		<i>Total</i>
	<i>Positive</i>	<i>Negative</i>	
Positive	1775	77	1852
Negative	52	455	507
Total	1827	532	2359

Table 3. Overall concordance for human epidermal growth factor receptor 2 in LUMC series combined with literature series.

<i>CNB</i>	<i>Resection specimen</i>		<i>Total</i>
	<i>Positive</i>	<i>Negative</i>	
Positive	62	10	72
Negative	4	572	576
Total	66	582	648

Table S1. Search strategy in Pubmed, Embase and Web of Science.

Pubmed:

("Breast neoplasms"[mesh] OR "breast cancer" OR "breast neoplasm" OR "breast neoplasms" OR "breast tumors" OR "breast tumor" OR "breast tumours" OR "breast tumour" OR "mammary carcinoma" OR "mammary carcinomas" OR "breast carcinoma" OR "breast carcinomas" OR "mammary neoplasms" OR "mammary neoplasm" OR "cancer of the breast" OR "cancer of breast") AND ("hormone receptor"[tiab] OR "hormone receptors"[tiab] OR "hormonal receptor"[tiab] OR "hormonal receptors"[tiab] OR "Receptors, Steroid"[mesh] OR "Receptors, Estrogen"[mesh] OR "Receptors, Progesterone"[mesh] OR HER2 OR HER-2 OR erbb2 OR erbB-2 OR "Receptor, erbB-2"[mesh]) AND ("biopsy"[mesh] OR biopsy[tw] OR biopsies[tw]) AND (excision OR excised OR "resection specimen" OR "resection specimens" OR "resected specimen" OR "resected specimens" OR "resected tumor" OR "resected tumour" OR "resected tumors" OR "resected tumours" OR excisional OR excision* OR "tumor resection" OR "tumour resection" OR "tumors resection" OR "tumours resection" OR "final excision") AND ("Comparative Studies" OR "Comparative Study" OR "Comparative Study"[Publication Type] OR comparison OR comparisons OR compared OR compar* OR sensitivity OR specificity OR concordance OR reliably[tw] OR reliability[tw] OR reliable[tw])

Embase:

(exp *Breast tumor/ OR ("breast cancer" OR "breast neoplasm" OR "breast neoplasms" OR "breast tumors" OR "breast tumor" OR "breast tumours" OR "breast tumour" OR "mammary carcinoma" OR "mammary carcinomas" OR "breast carcinoma" OR "breast carcinomas" OR "mammary neoplasms" OR "mammary neoplasm" OR "cancer of the breast" OR "cancer of breast").ti) AND (exp *hormone receptor/ OR ("hormone receptor" OR "hormone receptors" OR "hormonal receptor" OR "hormonal receptors" OR "steroid receptor*" OR "estrogen receptor*" OR "oestrogen receptor*" OR "progesterone receptor*" OR HER2 OR HER-2 OR erbb2 OR erbB-2).ti,ab OR *epidermal growth factor receptor 2/) AND (exp *biopsy/ OR (biopsy OR biopsy OR biops* OR biopt*).ti,ab) AND (exp *excision/ OR (excision OR excised OR "resection specimen" OR "resection specimens" OR "resected specimen" OR "resected specimens" OR "resected tumor" OR "resected tumour" OR "resected tumors" OR "resected tumours" OR excisional OR excision* OR "tumor resection" OR "tumour resection" OR "tumors resection" OR "tumours resection" OR "final excision").ti,ab) AND (exp comparative study/ OR ("Comparative Studies" OR "Comparative Study" OR comparison OR comparisons OR compared OR compar* OR sensitivity OR specificity OR concordance OR reliably OR reliability OR reliable).mp)

Web of science:

TS=("breast cancer" OR "breast neoplasm" OR "breast neoplasms" OR "breast tumors" OR "breast tumor" OR "breast tumours" OR "breast tumour" OR "mammary carcinoma" OR "mammary carcinomas" OR "breast carcinoma" OR "breast carcinomas" OR "mammary neoplasms" OR "mammary neoplasm" OR "cancer of the breast" OR "cancer of breast") AND TS=("hormone receptor" OR "hormone receptors" OR "hormonal receptor" OR "hormonal receptors" OR "steroid receptor*" OR "estrogen receptor*" OR "oestrogen receptor*" OR "progesterone receptor*" OR HER2 OR "HER-2" OR erbb2 OR "erbB-2") AND TI=(biops* OR biopt*) AND TS=(excision* OR excised OR "resection specimen" OR "resection specimens" OR "resected specimen" OR "resected specimens" OR "resected tumor" OR "resected tumour" OR "resected tumors" OR "resected tumours" OR excisional OR excision* OR "tumor resection" OR "tumour resection" OR "tumors resection" OR "tumours resection" OR "final excision") AND TS=("Comparative Studies" OR "Comparative Study" OR comparison OR comparisons OR compared OR compar* OR sensitivity OR specificity OR concordance OR reliably OR reliability OR reliable)

Table S2. Concordance between CNB and resection specimen for ER (LUMC series).

<i>CNB</i>	<i>Resection specimen</i>		<i>Total</i>
	Positive	Negative	
Positive	97	0	97
Negative	1	17	18
Total	98	17	115

Table S3. Concordance between CNB and resection specimen for HER2 (LUMC series).

<i>CNB</i>	<i>Resection specimen</i>		<i>Total</i>
	Positive	Negative	
Positive	10	4	14
Negative	0	91	91
Total	10	95	105

Figure 1. Representative images of tumors negative (A) and positive (B) for nuclear estrogen receptor expression.

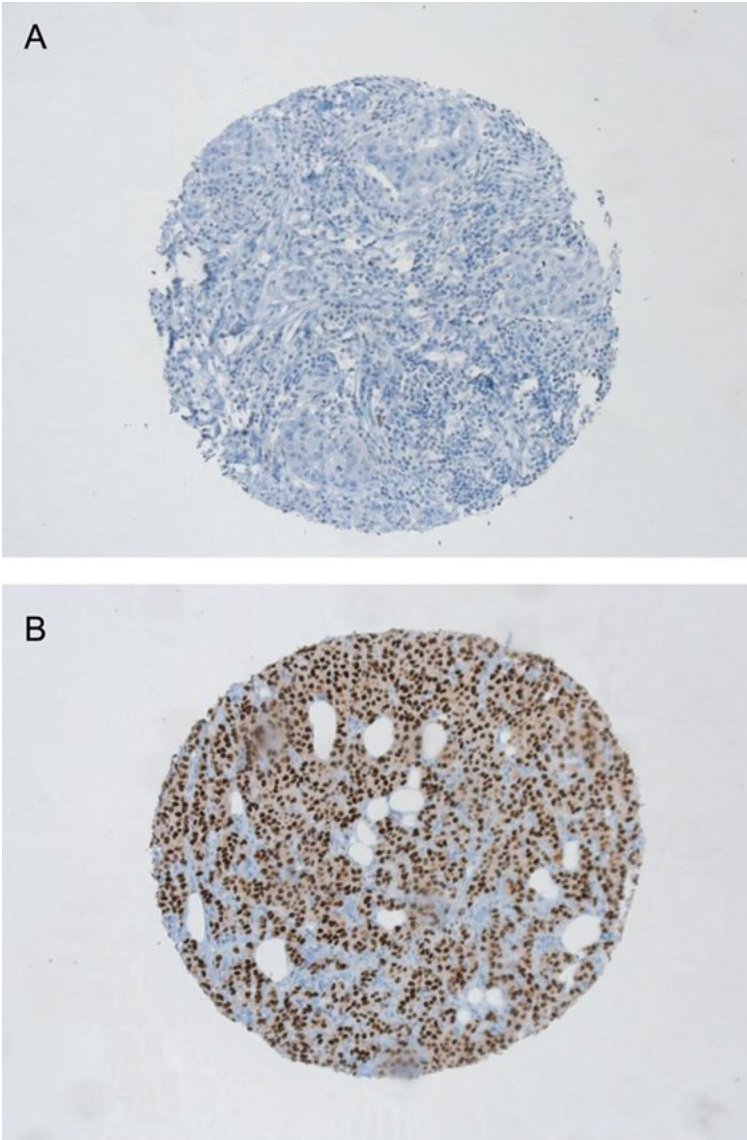
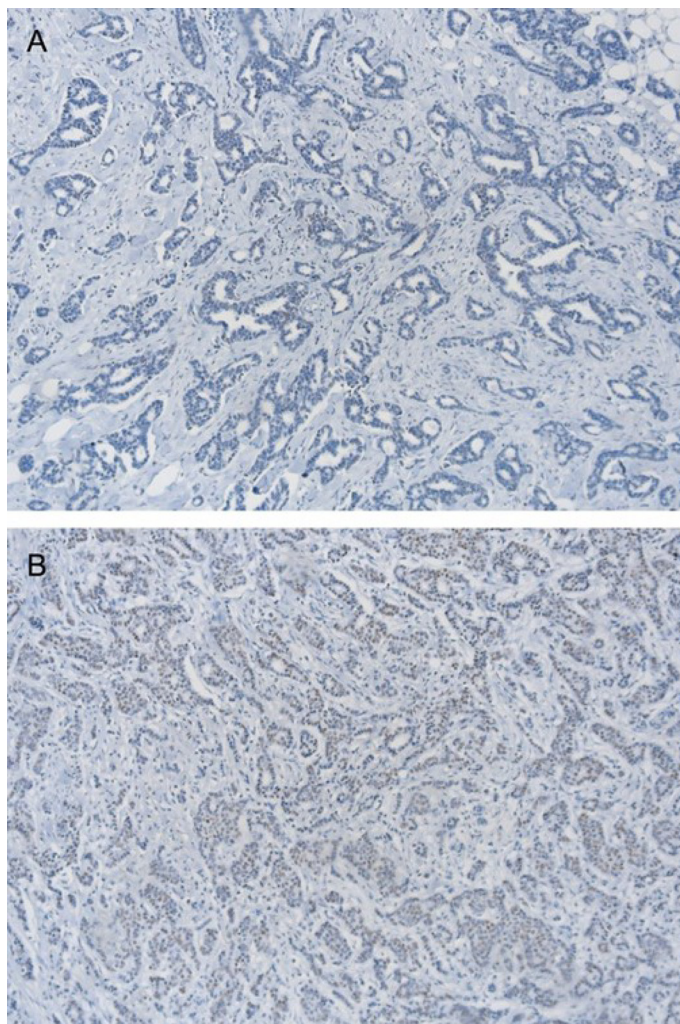


Figure 2. Tumor that displayed tumor heterogeneity for estrogen receptor (ER) expression, while showing both ER-negative (A) and ER-positive (B) tumor fields.



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Figure 3. Tumor that displayed 3+ human epidermal growth factor 2 (HER2) staining on the CNB, while showing 1+ HER2 staining on the resection specimen (and was negative for HER2 gene amplification).

