<Study Protocol>

Prediction of pathologic complete response (pCR) by preoperative biopsy in breast cancer with complete clinical response (cCR) after neoadjuvant chemotherapy

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Principal investigator:
Professor Wonshik Han, MD, PhD
Department of Surgery, Seoul National University Hospital
Department of Surgery, Seoul National University College of Medicine
Cancer Research Institute, Seoul National University

Study Overview

(※ Please provide a clear and concise description of each of the following sections in plain terms that can be understood by non-experts.)

Study Title	(Korean) 선행항암요법을 시행한 유방암 환자에서 유방자기공명영상 및 중심침생검 또는 맘모톰조직생검에 의한 병리학적 완전 관해 예측의 정확도에 대한 전향적 연구
	(English) Prediction of pathologic complete response (pCR) by preoperative biopsy in breast cancer with complete clinical response (cCR) after neoadjuvant chemotherapy
Principal Investigator	Prof. Wohshik Han Department of Breast Endocrine Surgery, Seoul National University Hospital
Study Sponsor Institution	Seoul National University Hospital

Study Purpose	● To develop a non-surgical and accurate method to determine the pathological complete response (pCR) in patients with breast cancer after neoadjuvant chemotherapy. ● To develop a basic examination technique that would allow screening of patients based on whether they need local resection or not, when making preoperative determination by pathological response.
Study Design	● This is a prospective clinical trial. ● Patients with breast cancer, who underwent neoadjuvant chemotherapy and whose diagnostic imaging results, including breast magnetic resonance imaging (MRI), showed near complete or complete clinical response (i.e., no residual tumor) will be enrolled. ● On the day before the surgery or on the day of surgery, needle biopsy or vacuum-assisted biopsy will be performed in the eligible patients, focusing on the primary tumor and the peripheral area. The biopsied area will be localized with a wire. Wide excision will be performed subsequently. ● Accuracy of the prediction of pCR by breast MRI and multiple-core needle biopsy or vacuum-assisted breast biopsy will be determined by analysis of final pathological findings of the surgically removed breast tissue.
Study Period	August 12, 2016 ~ August 11, 2018
Study Patients (Investigational product, etc.)	● Patients with breast cancer, who underwent neoadjuvant chemotherapy and was suggested to have near complete or complete clinical response on breast MRI will be included.
Number of Study Patients	40 (20 each for core needle biopsy and vacuum-assisted biopsy)
Vulnerable Patients	● Inclusion of any vulnerable patients (minors/pregnant women, fetuses, newborns/adults with impaired cognition of consent/students, employees/inmates) will be recorded.
Study Method	● Before the surgery, needle biopsy or vacuum-assisted breast biopsy will be performed, focusing on the primary tumor and the peripheral area and will be marked with a clip. Local resection will be performed subsequently. ● Accuracy of the prediction of pCR by breast MRI and multiple-core needle biopsy or vacuum-assisted breast biopsy will be determined by analysis of final pathological findings of the surgically removed breast tissue. ● Clinical pathological factors that may influence the accuracy of the prediction of pCR by breast diagnostic images and multiple-core needle biopsy or vacuum-assisted breast biopsy will be examined. ● The planned number of patients to be enrolled in this study is 40. Of these, 20 patients will receive multiple-core needle biopsy while the remaining 20 will receive vacuum-assisted breast biopsy. A comparative analysis will be done to assess which method has a better prediction rate.
Efficacy	Not applicable.

Evaluation	
Safety Evaluation	Not applicable.
Expected Effects and Results	● Results from this study will help in accurately identifying and screening patients with breast cancer who have achieved pCR after neoadjuvant chemotherapy and hence, do not need surgery. It will allow decreases the economic, psychological, and social burdens on the patient. In addition, issues such as deformation of the breast due to surgery, side-effects arising from various surgical procedures (infection, bleeding, and wounds) and anesthesia can be avoided.

Study Protocol

1. Study Title

Prediction of pathologic complete response (pCR) by preoperative biopsy in breast cancer with complete clinical response (cCR) after neoadjuvant chemotherapy

2. Study Background and Purpose

1) Study Background

- Although a significant number of patients with advanced operable breast cancer achieve pathological complete response (pCR) after treatment with a combination of primary chemotherapy and targeted therapy, not much is known whether surgical resection improves the risk of local recurrence after pCR. pCR is defined as the absence of any residual tumor in the final biopsy results after neoadjuvant chemotherapy.
- Previous studies in patients with breast cancer showed a relatively high local recurrence rate after clinical response was achieved with neoadjuvant radiotherapy.
- Thus, there is a need to develop an accurate prediction method for pCR before the surgery and a method to accurately identify and screen patient groups requiring local resection despite achieving pCR.
- Also, the local recurrence rate was high in patients who did not undergo surgery once they achieved clinical complete response. However, there was no difference in overall survival. Most local recurrences in patients with breast cancer were observed during regular follow-up visits, thus making treatment easier and preventing systemic metastasis of the disease. In addition, the fact that the recurrence rate differed significantly according to the presence of suspected tumor on ultrasound examination, along with clinical observation, also suggests that it is possible to omit surgical treatment when accompanied by accurate preoperative evaluation.
- In other words, it is of critical importance to accurately determine the pCR through the use of advanced breast imaging techniques after neoadjuvant chemotherapy is completed, which is turn can provide important clues for determining future treatment options.
- Advances in anticancer drugs and targeted therapies and a continued development of combination therapies have led to an increase in pathological responses to values as high as 40-67% after neoadjuvant chemotherapy. The results from this study are expected to contribute to making decisions for de-escalation of treatment for an increased number of patients.

2) Study Hypothesis and Purpose

A. To develop a non-surgical and accurate method to determine the pCR after neoadjuvant chemotherapy.

- Accurate prediction of pCR is important for safe omission of surgery in patients deemed to have achieved complete response after neoadjuvant chemotherapy.
- In a previous study, sensitivity to residual tumors after neoadjuvant chemotherapy was measured using breast MRI was at 83%, specificity 47%, positive-predictive value (PPV) 47%, negative-predictive value (NPV) 83%, and accuracy 74%.
- Histological examination of tumors is considered to be an important factor in improving accuracy. The accuracy of pCR prediction is determined to be acceptable when it is 90% or higher, considering that local recurrence rate is about 10% in patients undergoing surgical resection, as observed in previous studies.
- In this study, pCR prediction results of breast magnetic resonance imaging (MRI) and biopsy (multiple-core needle biopsy and vacuum-assisted breast biopsy) will be compared with the prediction results of the breast specimens undergoing quadrantectomy involving primary tumors.
- If residual tumors are observed in the surgical specimens, pathological factors such as the distance from the primary tumor with clipping and the occurrence of necrosis will be analyzed.

B. To develop a basic examination technique that would allow screening of patients based on whether they need local resection or not, when making preoperative determination by pathological response.

- The pCR after neoadjuvant chemotherapy will be determined by non-surgical method.
- Using a RCT design, enrolled patients will be divided into two groups based on whether they received

surgery or not.

- Follow-up with local recurrence rate will be the primary endpoint, while disease-free survival (DFS) and overall survival (OS) will be the secondary endpoints. Based on the results, patients who do not need surgical treatment will be identified.
- In this study, if the pCR can be accurately determined, it is possible to conduct further studies on the extent of surgery (local excisional biopsy with local anesthesia vs. multiple-core needle biopsy vs. segmentectomy)

3. Investigational product and medical device code name (or common name of main ingredient), amount of drug substance, formulation, etc. (including a control drug)

Not applicable.

4. Selection Criteria, Exclusion Criteria, Target Number of Patients and Basis for Calculation

1) Selection Criteria

All patients undergoing neoadjuvant chemotherapy and those who are expected to have near complete or complete clinical response immediately before surgery, as confirmed by MRI will be included. (tumor size ≤0.5 cm or Lesion-to-Background Signal Enhancement Ratio [L-to-B SER] ≤1.6).

2) Exclusion Criteria

- Multifocality (≥3)
- Residual diffuse microcalcification
- Patients who drop out due to complications or cancer progression during neoadjuvant chemotherapy.
- Patients with residual tumors observed immediately before surgery, as observed by MRI and ultrasound.

3) Target number of patients and basis of calculation

The primary endpoint of this study is to perform an additional biopsy after MRI to determine if specificity was greater than 90% (one-sided test). To calculate the number of patients to meet this primary endpoint, the following assumptions were made.

※ Gold standard: biopsy after surgery

(1) Level of significance, $\alpha = 0.05$, one-sided test
(2) Type II error (β) is 0.20, and the test power is maintained at 80%.
(3) The hypothesis of this study is as follows.
Null hypothesis: The specificity of MRI + biopsy is not 64% ($p \neq 0.64$).
Alternative hypothesis: The specificity of MRI + biopsy is greater than 64% ($p > 0.64$). However, a specificity of 90% is expected.
(4) Based on the results from previous studies, MRI data showed that 64% of the patients had no tumors in the actual surgical tissues. Therefore, the specificity of MRI is assumed to be 64%. In case of MRI + biopsy, specificity is expected to be 90%.

Based on this, the number of study patients was calculated using the below formula.

$$n = \frac{(z_\alpha \sqrt{p_0 q_0} + z_\beta \sqrt{p_1 q_1})^2}{\delta^2}$$

Based on the calculation, 17 patients are required to secure 80% probability of rejecting the above null hypothesis at a significance level of 5%.

At this time, 17 patients were counted using the specificity (ratio of complete response in the MRI + needle biopsy results from the patients who would show complete response in the biopsy after surgery [gold standard] results), so it would only represent the number of patients who would have complete response as a result of biopsy after surgery. Therefore, the total number of patients should be calculated by reflecting prevalence (among 100 patients suspected of complete response in our study, the ratio of actual complete response in the postoperative biopsy is 64%) and the results are as follows.

Prevalence (A)	Total number of patients (B)	Number of total study patients considering the dropout rate 10% (C)
64%	27	30

4) Study Patient Recruitment Plan

Based on the above calculations, a total of 30 patients would be sufficient for this study. However, an additional 10 patients were included for further analysis to compare multiple-core needle biopsy and vacuum-assisted breast biopsy, thereby allowing 20 patients in each group.

Among the patients who were admitted for surgery after neoadjuvant chemotherapy (admitted 2 days before surgery), those who were expected to have a complete response immediately before surgery, based on the MRI and ultrasound results, will be given an explanation with regard to study plan, etc.

5. Study Method

1) Detailed study method

A. Breast MRI for assessing response after neoadjuvant chemotherapy

- Breast MRI diagnosis will be interpreted independently of clinical measurements, mammography, and breast ultrasonography results.
- Two breast MRIs will be done for patients who have received neoadjuvant chemotherapy, one before and one after the therapy.
- The images obtained before and after chemotherapy will be examined by a breast diagnostic imaging specialist to assess the exact response of the tumor to neoadjuvant chemotherapy and the presence of residual tumor.
- A complete MRI response means no abnormal contrast enhancement, no mass, or no distortion of the breast tissue in all phases of the MRI and in all compartments of the breast.
- A near complete MRI response means tumor size ≤0.5 cm or Lesion-to-Background Signal Enhancement Ratio [L-to-B SER] ≤1.6.
- The response will be evaluated based on the results of breast ultrasonography and mammography, in addition to breast MRI. In case of residual tumor findings, such as clustered microcalcification on mammography, the patient will be excluded even when an abnormal contrast enhancement is not observed.

B. Biopsy in patients whose complete response is predicted by breast MRI

- In previous studies, attempts to predict the complete response using diagnostic images of breast alone showed about 21% false negative findings. Additional confirmation of pCR using multiple-core needle biopsy or vacuum-assisted breast biopsy can lower such false negative findings.
- A needle biopsy or vacuum-assisted breast biopsy will be performed around the residual tumor and the periphery and a wire will be inserted for localization of the biopsied area. The wire will guided the subsequent wide excision.
- Multiple-core needle biopsy (14-gauge) or vacuum-assisted breast biopsy (10-gauge) will be performed. Obtaining at least five cores is recommended. Multiple-core needle biopsy will be performed at five different locations where the mass is seen before chemotherapy and in areas with the highest blood flow,

as seen in the color Doppler image or the lowest echo on B-mode ultrasound, thus indicating the highest likelihood of residual tumor. The clip inserted during the course of neoadjuvant chemotherapy will aslo be used as reference.

- To minimize variation while preforming biopsy, two different radiologists, with at least five years of experience, will perform the biopsies at sites determined by consensus.
- Amongst the total 40 patients in this study, 20 will undergo multiple-core needle biopsy and 20 will undergo vacuum-assisted breast biopsy. The patients will be assigned alternatively. The prediction rates obtained from the two groups will be compared.
- The rate at which complete responses is achieved by neoadjuvant chemotherapy varies by breast subtype. According to the Seoul National University Hospital data, about 5% of hormone receptor-positive (HR+) breast cancers and 20% or more of Her2-positive (HER2+) or triple-negative breast cancers (TNBC) have shown complete response after neoadjuvant chemotherapy.
- When predicting complete response with breast MRI, PPV also showed high prediction rate for TNBC and non-hormone receptor expression/Her2 positive subtypes and about 45% prediction rate for luminal subtypes.

C. Determining factors that influence the accuracy of pCR prediction after neoadjuvant chemotherapy

- The accuracy of prediction of pCR by breast MRI is likely to be affected by subtypes of breast cancer and the size and location of the tumor prior to neoadjuvant chemotherapy. Thus, the clinical pathological factors that influence the accuracy of the prediction will be analyzed.
- Analysis will be performed by categorizing the tumors according to the patient's age, density of breast tissue (grade), and histological characteristics of the tumor.

2) Comparative group setting and randomization method

The patients will be alternatively assigned in a 1:1 ratio in the multiple-core needle biopsy group and vacuum-assisted breast biopsy group, such that one patient would receive multiple-core needle biopsy and the next will receive vacuum-assisted breast biopsy.

3) Test drug administration and dose, administration/use method, combination therapy, and reason for selection when using a control drug

Not applicable.

4) Observation items, clinical test items, and observation test methods

See attached clinical study report (CSR)

5) Efficacy evaluation criteria and evaluation methods

- Comparison of preoperative MRI and multiple-core needle biopsy or vacuum-assisted breast biopsy with final biopsy results after surgery.
- For the biopsy, patients will be divided into no residual tumor group and residual tumor group (atypical cells, ductal carcinoma *in situ* [DCIS], and infiltrating ductal cancer of the breast [IDCa]) and the degree of consistency will be confirmed by post-surgery biopsy.

6) Differentiation from existing treatments and research

Although complete response is currently predicted by preoperative diagnostic imaging (ultrasound and MRI),

surgery is required for final histological confirmation. In some cases, the final biopsy after surgery may show complete response, but residual tumors may still be present. Hence, confirmation with surgical treatment is required.

This study aims to increase the accuracy of prediction rate of complete response by performing additional multiple-core needle biopsy/vacuum-assisted breast biopsy and preoperative diagnostic imaging. Thus, if complete response can be predicted only by preoperative MRI and biopsy, surgical treatment can be omitted.

7) Benefits and Risks to the Patients

Compared to the previous surgical patients, the patients will undergo an additional multiple-core needle biopsy or vacuum-assisted breast biopsy which would not have been performed before surgery. If a complete response is expected during neoadjuvant chemotherapy, a small clip is inserted into the tumor during the course of neoadjuvant chemotherapy (it is done when the tumor is still present because as the tumor size shrinks, its location cannot be accurately identified in the image). Before surgery, a wire is inserted under ultrasound guidance to mark the location immediately before the surgery, and a wide excision is performed on this site. In order to minimize the discomfort from the additional biopsy, the procedure will be performed at the same time when the wire is inserted just before the surgery. Additional biopsy costs will be funded by the study. The predicted side effects in patients are not expected to be different from the known risks of multiple-core needle biopsy or vacuum-assisted breast biopsy.

8) Data Safety Monitoring Plan (DSMP)

1. Person in charge of data and safety monitoring:

Prof. Wonshik Han

2. Period for the collection and review of data and safety information:

Every 12 months

3. Monitoring procedures for key efficacy endpoints to determine the continuation, change, or discontinuation of the study

: The additional multiple-core needle biopsy and vacuum-assisted breast biopsy that will be used in this study are not new methods but additional one-time procedures that have already been proven and are in use in breast cancer diagnosis.

Consent forms for the study as well as for biopsy will be obtained and related complications will be explained to the patients. As the safety of biopsy procedures have been already demonstrated, it is unlikely to cause any complications. Surgical monitoring will be continuously conducted by the surgeon because preoperative biopsy may lead to problems such as difficulty in the determination of the extent of surgery due to hematoma.

Specimens obtained by biopsy will be duly received by the Department of Pathology and used only for confirmation of pathological results, after which they will be stored and discarded according to the same procedures as followed for other specimens.

4. Reporting procedures to the IRB, sponsor and, where applicable, the regulatory agency (description on the reporting method and period to the IRB, sponsor, or regulatory on adverse events, unexpected problems, non-compliance with the protocol, etc.)

: Problems encountered during the study will be discussed with the principal investigator, Professor Wonshik Han and will also be reported to the IRB office.

9) Data Analysis and Statistical Analysis Method

The results from the preoperative MRI and biopsies (multiple-core needle biopsy and vacuum-assisted breast biopsy) and postoperative pathology will be compared to examine the accuracy of the prediction of complete response or residual tumor.

Primary Endpoint

- Negative-predictive value (NPV)

Secondary Endpoint

- False negative rate (FNR)
- Accuracy
- NPV, FNR, and accuracy analysis for cases meeting all the conditions of MRI size ≤0.5 cm, L-to-B SER ≤1.6, and removal of multiple-core needle biopsy 5 core or more

In addition, the patients will be divided equally into the multiple-core needle biopsy group and the vacuum-assisted breast biopsy group to determine which biopsy method could predict the complete response more accurately.

6. Measures for the Protection of Patients

1) Basic measures for securing research ethics

This study will be initiated after receiving IRB approval and conducted in compliance with the 2013 Helsinki Declaration and ICH-GCP

2) Measures to protect personal information of patients

Patients' information, collected for study purpose, will be stored in the laboratory as password-protected files. Access to these files will be restricted to the principal investigator and co-investigators. Personally identifiable information, acquired during data collection, will not be recorded. Especially, patient's name, national resident number, and hospital registration number will not be documented in the case record. When the results of the study are published, no identification data of the patients will be provided and the case records will be kept separately with the principal investigator, until the final publication of the results after study termination. The data and records will be retained for three years after the termination of the study. Subsequently, these will be discarded under the direction of the principal investigator in accordance with the Article 16 of the Enforcement Decree of the Personal Information Protection Act.

7. Storage and Disposal Methods for Human Biological Materials

Samples from additional biopsies will be received by the Department of Pathology and used only for the purpose of confirming the pathology results. After the confirmation, the samples will be stored and disposed of in accordance with the formal sample procedure of the Department of Pathology and not retained in the Department of Surgery.

8. References

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