# Prospective Study of the Effect of the 21-Gene Assay on Adjuvant Clinical Decision-Making in Japanese Women With Estrogen Receptor-Positive, Node-Negative, and Node-Positive Breast Cancer☆

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## Abstract

**In a prospective study in 124 Japanese women with estrogen receptor-positive (ER+) invasive early breast cancer (EBC), the effect of the 21-gene assay on adjuvant decision-making was examined. Overall, treatment recommendations changed in 33% (95% confidence interval [CI], 24%-43%) of node-negative (N0) and 65% (95% CI, 41%-85%) of node-positive (N+) patients, predominantly from chemohormonal to hormonal therapy. Results from this Japanese population confirm US and European experiences.**

**Background:** In this study we investigated if the 21-gene assay result affects adjuvant decision-making in Japanese women with ER+ invasive EBC. **Patients and Methods:** A total of 124 consecutive eligible patients with ER+, HER2-negative EBC and 0 to 3 positive lymph nodes were enrolled. Treatment recommendations, physicians' confidence and patients' decisional conflict before and after knowledge of the Recurrence Score results of the 21-gene assay were recorded. **Results:** One-hundred four patients (84%) had N0 disease, including micrometastases, and 20 (16%) had N+ disease. Overall, recommendations changed in 33% (95% CI, 24%-43%) of N0 and 65% (95% CI, 41%-85%) of N+ patients. In 27 of 48 (56%) of N0 and 13 of 15 (87%) of N+ patients an initial recommendation for chemohormonal therapy was revised to only hormonal therapy after assay results, and in 7 of 56 (13%) of N0 and 0 of 5 N+ patients from only hormonal to combined chemohormonal therapy. Decisions appeared to follow the Recurrence Score results for low and high values. For patients with intermediate Recurrence Score values, overall recommendations for chemohormonal treatment tended to decrease after assay results. Physicians' confidence increased in 106 of 124 (85.5%; 95% CI, 78%-91%) cases. Patients' decisional conflict significantly improved as indicated by changes in the total score and the 5 defined subscores ($P = .014$ for Informed Subscore; $P < .001$ for all others). **Conclusion:** Results from this prospective study in a Japanese population confirm an effect of the 21-gene assay results on adjuvant treatment decision-making, consistent with reported experiences from the United States and Europe.

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## Introduction

The incidence of breast cancer is still increasing in Japan.[1] Although breast cancer mortality rates in Western countries are decreasing, they are still increasing in Japan.[2] Preventing future distant recurrences is the crucial primary objective of adjuvant therapy. Hormone receptor positive disease accounts for roughly



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75% of Japanese breast cancer cases.[3] Routinely, such patients receive adjuvant hormonal treatment. Many of these patients are also treated with adjuvant chemotherapy although a substantial proportion will not derive any clinical benefit in terms of a further reduction of their risk of recurrence.[4] Recently, the traditional instrumentarium of clinical and histopathological prognostic markers has been complemented by genomic markers such as the multigene 21 gene Recurrence Score assay.

The 21 gene assay measures the mRNA expression of 16 cancer related and 5 reference genes selected based on correlation of gene expression and risk of distant recurrence in 3 development studies.[5-7] The assay is based on reverse transcription polymerase chain reaction, which was specifically optimized to be used in archival formalin fixed, paraffin embedded tumor tissue,[8,9] and can thus be performed on routinely processed and archived tumor blocks or slides. Using an algorithm based on the results of clinical studies, the Recurrence Score result a numeric score between 0 and 100 is calculated.[10] The score is a continuous variable quantifying the risk of distant recurrence at 10 years for the individual patient[10] with estrogen receptor positive (ER+) early breast cancer treated with adjuvant hormonal therapy. A lower Recurrence Score value corresponds to a lower risk of recurrence, and a higher value corresponds to a higher risk of recurrence. Three risk categories have been defined: low, intermediate, and high risk groups for Recurrence Score values $< 18$, 18 to 30, and $\geq 31$, respectively.[10] The prognostic significance of the 21 gene assay for node negative (N0) and node positive (N+) disease has been validated using tumor specimens from patients with ER+ early breast cancer enrolled prospectively in large randomized phase III studies.[4,10-12] Furthermore, the assay was shown to be predictive of the benefit of chemotherapy in N0 and N+ ER+ patients.[4,12] Patients with tumors that had a high Recurrence Score result had the largest proportional benefit of chemotherapy, and those presenting with a tumor with a score $< 18$, did not appear to benefit from chemotherapy.

The 21 gene assay has been included in guidelines of scientific societies such as American Society of Clinical Oncology,[13] National Comprehensive Cancer Network (NCCN),[14] and European Society for Medical Oncology.[15] The updated 2011 St Gallen Consensus Panel acknowledges the test as the only multiparameter gene assay considered useful, not only as a prognostic test, but also as a marker predictive of chemotherapy responsiveness in hormone receptor positive early breast cancer where uncertainty remains after consideration of other tests.[16]

Several clinical utility studies have demonstrated that knowledge of Recurrence Score results affects management of patients. Results of these retrospective and prospective studies are very consistent for N0 ER+ disease and show a revision of treatment recommendations in approximately 35% of cases as reported in a recent metaanalysis.[17] Recommendations shift predominantly from adjuvant chemohormonal treatment to hormonal treatment alone. The database for N+ disease is still evolving.[18,19] Results suggest a similar effect for patients with 1 to 3 positive lymph nodes.

It was also shown that the 21 gene assay was applicable to adjuvant therapy decision making beyond the largely Caucasian populations in which it was originally validated. A recently published confirmatory study demonstrated that the assay provided prognostic information in a population of Japanese women with ER+ N0 early breast cancer treated with adjuvant tamoxifen.[2] Notably, the authors reported that the expression profiles of individual genes and gene groups for the Japanese patients were very similar to those for the patients from the validation study National Surgical Adjuvant Breast and Bowel Project B 14: A Clinical trial to assess Tamorifen in patients with primary breast cancer and negative axillary nodes whose tumors are positive for estrogen receptors with confidence intervals for the hazard ratios for distant recurrence for the 2 studies overlapping for all genes and gene groups. Physicians in Japan have started to use the assay as a tool in routine adjuvant decision making. Japanese guidelines describe the assay as an option for consideration to aid decisions on whether chemotherapy should be used for hormone receptor positive breast cancer in the adjuvant setting.[20] However, thus far, no prospective clinical utility data of the 21 gene assay have been generated in a population of women in Japan. Thus, we conducted a clinical study to analyze the influence of Recurrence Score information on the adjuvant decision making process in Japanese patients with ER+ N0 or N+ early stage breast cancer.

## Patients and Methods

This was a prospective, multicenter study performed in 2 Japanese centers. The study was approved by the respective institutional ethics committees. All patients provided written informed consent.

### *Study Objectives*

The primary study objective was to characterize the degree to which Recurrence Score results affect physician recommendations for adjuvant therapy and physicians' expressed level of confidence in the recommended treatment plan in a cohort of consecutive patients with ER+, HER2 negative breast cancer with up to 3 positive lymph nodes.

A secondary study objective was to assess the effect of assay results on patients' level of decisional conflict. An additional secondary objective was to provide a basis for indirect estimates of net cost effects and savings from a Japanese societal perspective that might result from using the assay. This health economic assessment is beyond the scope of the current report.

### *Patients*

Enrollment was offered consecutively to eligible women who had operable ER+, HER2 negative breast cancer, either with N0 (pre and postmenopausal patients) or micrometastatic disease (postmenopausal patients) or with histologically verified lymph node metastases in 1 to 3 lymph nodes (postmenopausal patients only). Patients had to be 18 years of age or older with adequate performance status to be candidates for systemic chemotherapy, and to be able to give consent and answer written questions in Japanese. To participate in the study, patients were required to incur the costs of the assay as an out of pocket expense.

### *Physicians*

Seventeen physicians participated in the study. They had to be either medical oncologists or surgeons making adjuvant treatment recommendations to breast cancer patients. At least 1 physician of a participating center needed to have previously ordered the 21 gene assay.

### *Physician Questionnaires*

A baseline questionnaire developed for use in this study on the basis of a published questionnaire[21] captured physicians' initial treatment recommendations, largely based on effective Japanese[20] and NCCN guidelines,[14] and answers to queries regarding their confidence in their treatment recommendations before the assay was performed. A follow up questionnaire recorded physicians' treat ment recommendations and confidence in their recommendations after knowledge of the assay results. For the latter, physicians responded to the statement "I am more confident in my treatment recommendation after ordering the assay" according to a Likert scale with the options: "strongly disagree," "disagree," "neither disagree nor agree," "agree," "strongly agree," and "do not know."

### *Patient Questionnaires and Decisional Conflict Scale*

At baseline and after results of the assay were discussed, patients completed the 16 item Decisional Conflict Scale (DCS). This scale has been validated to assess patient perceptions of uncertainty in making decisions about health care treatment options and satisfac tion with treatment decision making.[22,23] Regarding the DCS, the test retest (2 weeks later) reliability coefficient was 0.81. Internal consistency coefficients ranged from 0.78 to 0.92.

The DCS has a Total Score and 5 subscores: the Informed, Values Clarity, Support, and Uncertainty Subscores are based on 3 items each and the Effective Decision Subscore is based on the remaining 4 items.

### *Statistical Methods*

The proportion of patients whose treatment recommendations changed from baseline to follow up was calculated along with the respective 95% confidence interval (CI) using the Clopper Pearson method. McNemar's test was used to assess whether the proportion of patients who were initially recommended chemotherapy was changed after the 21 gene assay. These analyses were conducted separately according to nodal status (N0, including micrometastases [N1mic], vs. N+), and combined. The proportion of cases in which the physician either agreed or strongly agreed that they were more confident in their treatment recommendation after the assay was calculated along with the respective 95% CI.

The DCS data from the baseline and follow up questionnaires were analyzed similarly. Each of the 5 subscores was calculated as the sum of the component items only if there were responses to each of the defined items, and transformed to a range from 0 to 100 with smaller scores reflecting less decisional conflict. If any subscore was missing, the Total Score was set to missing. If all 5 subscores were not missing, then the Total Score was calculated as:

Total Score = (3 × [Informed Subscore] + 3 × [Values Clarity Subscore] + 3 × [Support Subscore] + 3 × [Uncertainty Subscore] + 4 × [Effective Decision Subscore])/16. The changes from baseline to follow up in the DCS Total Score and each of the subscores were analyzed using paired sample *t* tests.

The study was designed to enroll 200 patients, with the original intent to estimate a decision change rate of 20% with a precision of ±5% to 6%. However, it was decided to halt enrollment after 124 patients were enrolled because the accumulating data indicated that there were statistically significant reductions in treatment recommendations for chemotherapy in N0 and N+ patient subgroups.

## Results

### *Patient and Tumor Characteristics*

One hundred twenty four patients were enrolled between July 2009 and June 2011. Complete patient and tumor characteristics and the distribution of Recurrence Score values are listed in Table 1. In the N0 subset, 50 (48%) patients had a low score < 18, 37 (36%) had an intermediate score of 18 to 30, and 17 (16%) had a high

**Table 1 Patient Characteristics**

| Characteristic | All (n = 124) | N0 (n = 104) | N+ (1-3 Positive Nodes) (n = 20) |
|---|---|---|---|
| **Mean Age** | | | |
| Years | 51.4 | 49.8 | 59.9 |
| **Tumor Size** | | | |
| ≤2 cm | 76 (61.3) | 63 (60.6) | 13 (65.0) |
| >2 cm | 48 (38.7) | 41 (39.4) | 7 (35.0) |
| **Tumor Grade** | | | |
| Well | 44 (35.5) | 31 (29.8) | 13 (65.0) |
| Moderate | 35 (28.2) | 30 (28.8) | 5 (25.0) |
| Poor | 45 (36.3) | 43 (41.3) | 2 (10.0) |
| **Menopausal Status** | | | |
| Premenopausal | 62 (50.0) | 62 (59.6) | 0 (0.0) |
| Postmenopausal | 62 (50.0) | 42 (40.4) | 20 (100.0) |
| **Recurrence Score Values** | | | |
| Low | 62 (50.0) | 50 (48.1) | 12 (60.0) |
| Intermediate | 44 (35.5) | 37 (35.6) | 7 (35.0) |
| High | 18 (14.5) | 17 (16.3) | 1 (5.0) |

Data are reported as n (%) excepte where otherwise noted.
Abbreviations: N0 = node negative; N+ = node positive.

**Table 2** Chemotherapy Recommendations Before and After Assay Overall and According to Nodal Status

| | After Assay | | |
|---|---|---|---|
| | HT | CHT | All |
| **All Evaluable Patients** | | | |
| **Before Assay** | | | |
| HT | 54 (44%) | 7 (6%) | 61 (49%) |
| CHT | 40 (32%) | 23 (19%) | 63 (51%) |
| All | 94 (76%) | 30 (24%) | 124 |
| **Node-Negative Patients** | | | |
| **Before Assay** | | | |
| HT | 49 (47%) | 7 (7%) | 56 (54%) |
| CHT | 27 (26%) | 21 (20%) | 48 (46%) |
| All | 76 (73%) | 28 (27%) | 104 |
| **Node-Positive Patients** | | | |
| **Before Assay** | | | |
| HT | 5 (25%) | 0 (0%) | 5 (25%) |
| CHT | 13 (65%) | 2 (10%) | 15 (75%) |
| All | 18 (90%) | 2 (10%) | 20 |

McNemar's test exact $P < .001$.
Abbreviations: CHT = chemohormonal therapy; HT = hormonal therapy.

score of ≥ 31. Distribution of patients in the smaller N+ subset according to risk group was 12 (60%) patients with low, 7 (35%) with intermediate, and 1 (5%) with high Recurrence Score values.

### *Treatment Recommendations Before and After Knowledge of Recurrence Score Result*

Treatment recommendations before and after the 21 gene assay are listed in Table 2. Initial treatment recommendations were revised in 47 of 124 (38%; 95% CI, 29% 47%) of all patients, 34 of 104 (33%; 95% CI, 24% 43%) of patients with N0 and 13 of 20 (65%; 95% CI, 41% 85%) of patients with N+ disease after knowledge of the Recurrence Score results.

For all patients recommended chemohormonal therapy (CHT) before the assay, treatment recommendations were revised to hormomal therapy (HT) only in 40 of 63 (63%; 95% CI, 50% 75%) total patients, including 27 of 48 (56%; 95% CI, 41% 71%) with N0 disease, and 13 of 15 (87%; 95% CI, 60% 98%) with N+ disease. For all patients initially recommended HT alone, the recommendations after assay changed to CHT in 7 of 61 (11%; 95% CI, 5% 22%) total patients, all 7 of whom were from those 56 patients with N0 disease (13%; 95% CI, 5% 24%).

Overall, the shift in treatment recommendations was predominantly from CHT to HT ($P < .001$ for N0 patients and $P < .001$ for N+ patients by McNemar's test), ultimately resulting in a net reduction of adjuvant chemotherapy (Table 2 and Fig. 1). All patients in the low Recurrence Score group were recommended HT and, similarly, 100% of patients in the high Recurrence Score group were recommended CHT, indicating that for N0 and N+ patients, treatment recommendations after assay appeared to directly follow the low and high Recurrence Score categorizations (Fig. 1). For patients with intermediate Recurrence Score values, in N0 patients recommendations for CHT decreased by an absolute 19%, and in N+ patients by an absolute of 86% after the assay (Table 3).

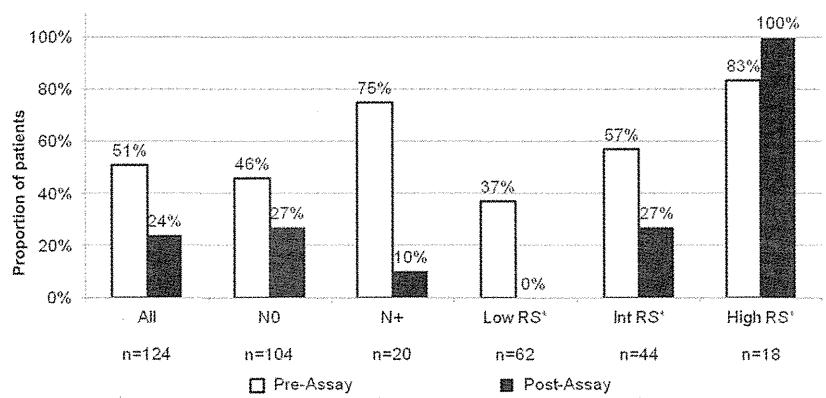
### *Physicians' Confidence in Treatment Recommendation*

Physicians either agreed or strongly agreed that they were more confident in their treatment recommendations after the assay in 106 of 124 (85%; 95% CI, 78% 91%) cases. Physicians disagreed in 7% of cases and neither agreed nor disagreed in 8% of cases (Fig. 2).

### *Patients' Decisional Conflict Before and After the 21-Gene Assay*

The Total Score of the Decisional Conflict Scale before and after assay was available for 116 patients. The mean values of the 5 subscores and the Total Score are listed in Table 4. Each of the 5 subscores and the Total Score decreased significantly ($P = .014$

**Figure 1** Recommendation for Adjuvant Chemotherapy Pre- and Post-Assay




Abbreviation: RS = Recurrence Score result.
*Regardless of nodal status.

**Table 3** Changes in Treatment Recommendations

| Patients | n | Overall Change Rate, Before to After Assay | CHT to HT | HT to CHT | No Change | CHT to CHT | HT to HT |
|---|---|---|---|---|---|---|---|
| **All Evaluable** | 124 | 47 (38%; 95% CI, 29% 47%) | 40 (32%) | 7 (6%) | 77 (62%) | 23 (19%) | 54 (44%) |
| Low RS | 62 | 23 (37%) | 23 (37%) | 0 (0%) | 39 (63%) | 0 (0%) | 39 (63%) |
| Intermediate RS | 44 | 21 (48%) | 17 (39%) | 4 (9%) | 23 (52%) | 8 (18%) | 15 (34%) |
| High RS | 18 | 3 (17%) | 0 (0%) | 3 (17%) | 15 (83%) | 15 (83%) | 0 (0%) |
| **Node-Negative** | 104 | 34 (33%; 95% CI, 24% 43%) | 27 (26%) | 7 (7%) | 70 (67%) | 21 (20%) | 49 (47%) |
| Low RS | 50 | 16 (32%) | 16 (32%) | 0 (0%) | 34 (68%) | 0 (0%) | 34 (68%) |
| Intermediate RS | 37 | 15 (41%) | 11 (30%) | 4 (11%) | 22 (59%) | 7 (19%) | 15 (41%) |
| High RS | 17 | 3 (18%) | 0 (0%) | 3 (18%) | 14 (82%) | 14 (82%) | 0 (0%) |
| **Node-Positive** | 20 | 13 (65%; 95% CI, 41% 85%) | 13 (65%) | 0 (0%) | 7 (35%) | 2 (10%) | 5 (25%) |
| Low RS | 12 | 7 (58%) | 7 (58%) | 0 (0%) | 5 (42%) | 0 (0%) | 5 (42%) |
| Intermediate RS | 7 | 6 (86%) | 6 (86%) | 0 (0%) | 1 (14%) | 1 (14%) | 0 (0%) |
| High RS | 1 | 0 (0%) | 0 (0%) | 0 (0%) | 1 (100%) | 1 (100%) | 0 (0%) |

95% Confidence intervals calculated using the Clopper Pearson method.
Abbreviations: CHT = chemohormonal therapy; HT = hormonal therapy; RS = Recurrence Score result.

for Informed Subscore; $P < .001$ for all others), indicating an overall reduction in patients' decisional conflict after knowledge of the Recurrence Score result. The mean Total Score improved by 26% after patients received the assay results.

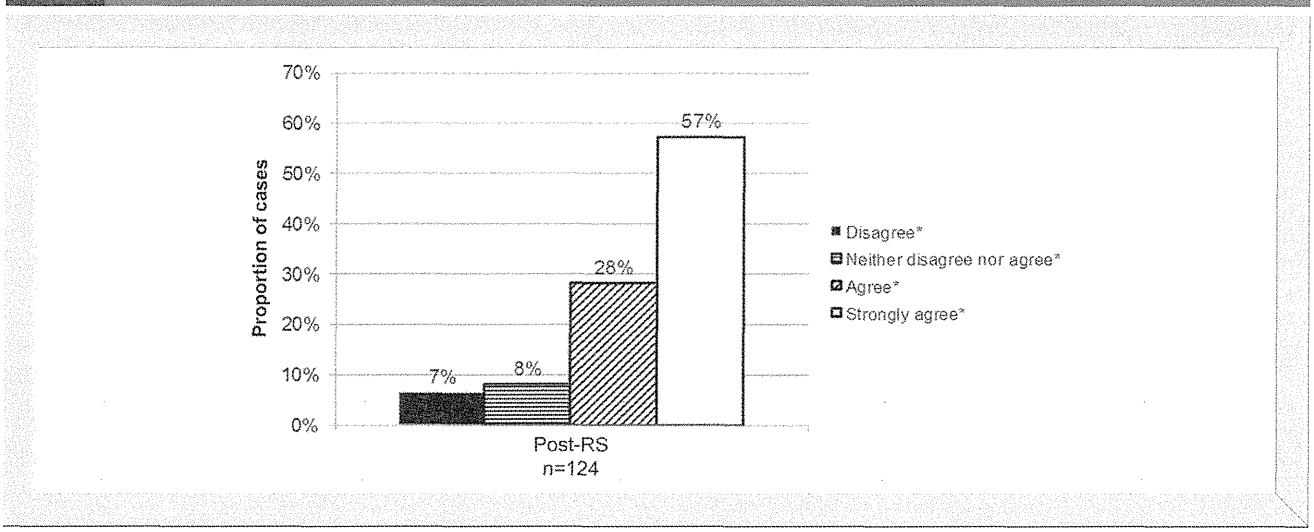
## Discussion

This is the first study of the effect of the 21 gene assay on clinical decision making in early invasive breast cancer in an Asian patient population. Moreover, our study is one of the first decision impact studies for the assay that includes N0 and N+ patients.

Regarding N0 disease, the results of our study are consistent with those reported from other prospective decision impact studies from the United States,[21] Spain,[24] and Germany.[19] Overall change rates in these prospective studies ranged from 30% to 32%. The metaanalysis of 9 studies and 1154 patients reported a change rate of 35%.[17] We found an overall change rate of 33%. Change rates in the United Kingdom[25] and Australia[18] were somewhat lower with 27% and 24%, perhaps in part because the proportion of patients with an initial recommendation for chemotherapy in these studies was much lower (40% and 24%, respectively), than in our study (51%) and the other 3 cited. However, regardless of baseline tendencies to use either more conservative or aggressive treatment approaches across all studies to date, decision changes attributable to the 21 gene assay appear to occur in both directions foregoing chemotherapy in many patients, and adding it in others.

Regarding N+ disease, results vary among other studies of the effect of Recurrence Score results in N+ early breast cancer pati ents. A retrospective study in 135 patients with ER+ disease including 9 patients with N1mic and 11 patients with N+ disease found an overall change rate in treatment recommendations of 25%. The authors found no correlation of therapy change and

**Figure 2** Change in Physicians' Confidence After The 21-Gene Assay




Abbreviation: RS = Recurrence Score result.
*Answers to the question (post RS): "I am more confident in my treatment recommendation."

**Table 4** Changes From Before Assay to After Assay in Decisional Conflict

| Score | n[a] | Pre-RS Mean | Post-RS Mean | Mean Change (95% CI) | P[b] |
|---|---|---|---|---|---|
| **Informed Subscore** | 121 | 26.2 | 22.1 | 4.1 (0.9 7.4) | .014 |
| **Values Clarity Subscore** | 122 | 28.6 | 22.3 | 6.2 (2.9 9.5) | <.001 |
| **Support Subscore** | 120 | 22.6 | 17.6 | 5.0 (2.2 7.8) | <.001 |
| **Uncertainty Subscore** | 121 | 44.6 | 30.5 | 14.0 (9.0 19.1) | <.001 |
| **Effective Decision Subscore** | 122 | 24.3 | 17.7 | 6.6 (3.9 9.2) | <.001 |
| **Total Score** | 116 | 28.8 | 21.4 | 7.4 (4.7 10.0) | <.001 |

Abbreviation: RS = Recurrence Score result.
[a]Number of patients for whom all items were not missing for the pre assay and the post assay questionnaires.
[b]*P* value from paired *t* test.

nodal stage.[26] A US Web based retrospective physician survey reported a change rate of 51% in 138 N+ ER+ patients with a change from CHT to HT in 33%.[27] In the Australian study,[18] the Recurrence Score result led to a 26% change in treatment recommendations in 50 patients with 1 to 3 positive lymph nodes: 12 patients changed to HT and 1 to CHT. In the German study there was a 39% change rate in 122 patients, with a predominant change from CHT to HT in 28% of all N+ cases and a 37% change among the 92 N+ patients with an initial recommendation for CHT.[19] In this study, we saw a 65% (95% CI, 41% 85%) shift in treatment recommendations in the 20 N+ patients, with all changes made from CHT to HT. These patients all had low and intermediate Recurrence Score values. It should be noted that we only offered the test to N+ patients who were postmenopausal, in accordance with the validation study in N+ disease.[12] This was not a prerequisite in the other studies cited. Thus, physicians in our study might more readily have omitted chemotherapy. Furthermore, because all patients were required to pay out of pocket for the cost of the assay, the study might have preselected patients who were more inclined and generally more confident to forego chemotherapy from the outset. The small number of patients with N+ disease in our study is a major limitation to drawing more general conclusions, and further studies might be warranted to better define the effect of the assay when offered to N+ patients.

Generally, for patients in the low and in the high Recurrence Score groups, treatment recommendations after assay corresponded completely with the Recurrence Score results in our study. The US, Spanish, and German studies have similarly observed that the shifts in treatment recommendations followed the Recurrence Score values. However, although all patients with high Recurrence Score results were recommended chemotherapy in these studies, a small minority of patients in the low Recurrence Score groups remained with recommendations for chemotherapy despite Recurrence Score values < 18. For physicians in our study, the assay appeared to be the final decisive parameter after consideration of all other factors. One explanation might be that patients might have been more motivated to avoid chemotherapy, particularly if their scores were low, because they paid out of pocket for the assay in this study.

For patients in this study with intermediate Recurrence Score results, the physicians appeared to have taken the continuous nature of the score into account, because the tendency to change from CHT to HT was greater for patients with low intermediate scores between 18 and 25 compared with those with high intermediate scores from 26 to 30. It should also be noted that the assay was not offered to patients in whom a clear decision for the type of adjuvant therapy had already been made.

Similar to other studies, we found that physicians' confidence in their treatment recommendation increased in 85% of cases. In comparison, changes in physician confidence levels were 76% in the US study,[21] 60% in the Spanish study,[24] 46% in the Australian study,[28] and 45% in the German study.[19] Although all decision impact studies report sizable increases in physician confidence after receipt of Recurrence Score information, the wide range of improvements in physician confidence might reflect differences in baseline experience with use of the 21 gene assay among physician investigators in each study.

In our assessment of patients' decisional conflict, we found each of the 5 subscores and the Total Score to improve significantly, indicating overall reduction in patients' decisional conflict with knowledge of the Recurrence Score results. The mean total Decisional Conflict Score improved by 26% after knowledge of the Recurrence Score results. The analysis of the Decisional Conflict Scale in the US study[21] was conducted on the raw Total Scores. Applying the scaling rules used in our study to enable comparison, the mean Total Score decreased from 24.8 to 17.3, a reduction of 7.5 units, which is comparable with the mean reduction of 7.4 units seen in our study.

## Conclusion

The results from this Japanese population confirm an effect of the 21 gene assay on adjuvant treatment decision making, consistent with studies in predominantly Caucasian populations in North America and Europe. Moreover, results indicate that the Recurrence Score values were adopted as a critical tool in adjuvant decision making in ER+ early breast cancer in centers with previous experience with the assay. The use of the assay ultimately resulted in a net reduction in treatment recommendations for adjuvant chemotherapy. The effect on the Japanese health care system should be assessed systematically. In another article we report on health economic analyses assessing the cost effectiveness of an adjuvant decision making process guided by the 21 gene assay for the Japanese health care system.

### *Clinical Practice Points*

- The 21 gene assay was shown to be of prognostic significance and to be predictive of the benefit of chemotherapy in patients with estrogen receptor positive early breast cancer in both node negative and node positive disease.

- A confirmatory study in a population of Japanese women with ER+ node negative early breast cancer treated with adjuvant tamoxifen demonstrated that it also provided prognostic infor mation beyond the largely Caucasian populations it was origi nally validated in.
- The 21 gene assay has been included in guidelines of major scientific societies.
- Several clinical utility studies have demonstrated that knowledge of Recurrence Score results affects management of patients.
- In node negative ER positive disease results consistently show a revision of treatment recommendations in approximately 35% of cases and a predominant shift of recommendations from adju vant chemohormonal treatment to hormonal treatment alone.
- Similar effects have been described for patients with 1 to 3 positive lymph nodes.
- The results of this prospective study in a Japanese population confirm an impact of the 21 gene assay on adjuvant treatment decision making, consistent with studies in predominantly Caucasian populations in North America and Europe.
- The use of the assay ultimately resulted in a net reduction in treatment recommendations for adjuvant chemotherapy as well as an increase in physicians' confidence and an improvement in patients' decisional conflict.
- The data may contribute to a wider adoption of the 21 gene assay as a critical tool in adjuvant decision making in ER+ early breast cancer in Japanese clinical practice.

## Disclosure

Dr Yamauchi has received honoraria from SRL Inc. Ms Naka gawa, Dr Hell, and Dr Nakamura serve as consultants for Genomic Health, Inc. Dr Chao and Dr Yoshizawa are employees of Genomic Health, Inc. All other authors state that they have no conflicts of interest.

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# Sentinel Node Biopsy After Neoadjuvant Chemotherapy in Cytologically Proven Node-Positive Breast Cancer

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## Abstract

**We evaluated the accuracy of sentinel node biopsy after neoadjuvant chemotherapy (NAC) in 95 patients with cytologically proven positive nodes before chemotherapy. The identification rate was 85.3% and the false negative rate was 15.7%. Sentinel node biopsy in the patients was not feasible but the appropriate selection of the subgroup might enable minimization of false negative results.**

**Introduction:** Several studies have assessed the feasibility of sentinel lymph node biopsy (SLNB) after NAC in patients with breast cancer, but diagnostic accuracy has varied. We prospectively evaluated the diagnostic accuracy of SLNB in detecting axillary lymph node (ALN) metastases after NAC in patients with cytologically proven positive nodes before chemotherapy. **Patients and Methods:** We studied 95 breast cancer patients with cytologically proven positive nodes and a partial or complete clinical response to NAC in the breast lesions confirmed using magnetic resonance imaging. Patients then underwent SLNB followed by ALN dissection. The identification rate of sentinel lymph nodes (SLNs) and the false negative rate of nodal metastases were assessed. Subgroup analysis was conducted according to several clinical factors. **Results:** SLNs were successfully identified in 81 (85.3%) of the 95 patients. Among these 81 patients, 51 (63.0%) had ALN metastases on final pathologic examination after NAC. Eight of the 51 patients with ALN metastases had negative results on SLNB (false negative rate, 15.7%). Univariate analysis indicated that the false negative rate was significantly lower only in the HER2-negative group ($P = .003$). **Conclusion:** SLNB after NAC did not correctly predict the presence or absence of axillary node metastases in patients with breast cancer who had cytologically proven positive nodes before NAC. However, the diagnostic accuracy might be different in cancer subtypes, therapeutic effect of chemotherapy, or sentinel lymph node status after chemotherapy. Well-powered studies are needed to confirm diagnostic accuracy of SLNB after NAC according to subgroup in patients with breast cancer.

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**Keywords:** False negative rate, Fine needle aspiration cytology, Identification rate, Neoadjuvant chemotherapy, Sentinel lymph node biopsy

## Introduction

Sentinel lymph node biopsy (SLNB) is a standard procedure in patients with clinically node negative, early breast cancer. Several comparative trials have shown that axillary lymph node dissection (ALND) is associated with a higher incidence of lymphedema than SLNB.[1-4] Avoidance of ALND might thus improve patients' quality of life. However, clinically positive axillary lymph nodes (ALNs) are a contraindication to SLNB, and current treatment guidelines recommended ALND as a standard procedure.[5]

Neoadjuvant chemotherapy (NAC) is comparable to adjuvant chemotherapy in terms of safety and efficacy in operable breast cancer. NAC is widely used because a good response to NAC enhances the rate of breast conserving surgery, and a pathologic complete response (pCR) is considered a predictor of better survival.[6] Moreover, the NSABP B 27 (National Surgical Adjuvant Breast and Bowel Project Protocol B 27) trial showed that adding a taxane to an anthracycline based regimen increases the likelihood

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of pCR.[7] However, complete ALND is performed after NAC in patients who have clinically node positive disease before NAC because reliable methods for predicting the disappearance of nodal metastasis in response to NAC are currently unavailable. If a way to accurately predict pCR in ALNs were available, ALND would be omitted in selected patients, avoiding its potential complications. Several groups have studied whether SLNB can accurately predict ALN status after NAC in patients with node positive breast cancer. A metaanalysis of 21 published studies of SLNB after NAC reported an overall false negative rate of 12%, with rates varying widely from 0% to 33% in individual studies.[8] The wide variability in false negative rates most likely reflects differences among studies in the numbers of patients and the indications for SLNB, and in surgical technique, response to chemotherapy, characteristics of breast cancer, and extent of lymph node involvement.

Fine needle aspiration cytological analysis (FNAC) is widely used to diagnose clinically suspicious ALN metastases and has a specificity of nearly 100%.[9] In patients with ALN metastasis on FNAC, ALND can be performed without SLNB, which is considered unnecessary in such patients. Positive lymph node status is an important indication for chemotherapy, and information available before surgery serves as the basis for evaluating the need for NAC. ALN metastases identified on FNAC are generally macrometastases consisting of bulky tumor nests, often associated with multiple metastases.[10] The possibility of omitting ALND by assessing sentinel lymph nodes (SLNs) after NAC in patients with clinical evidence of ALN metastasis is of great interest.

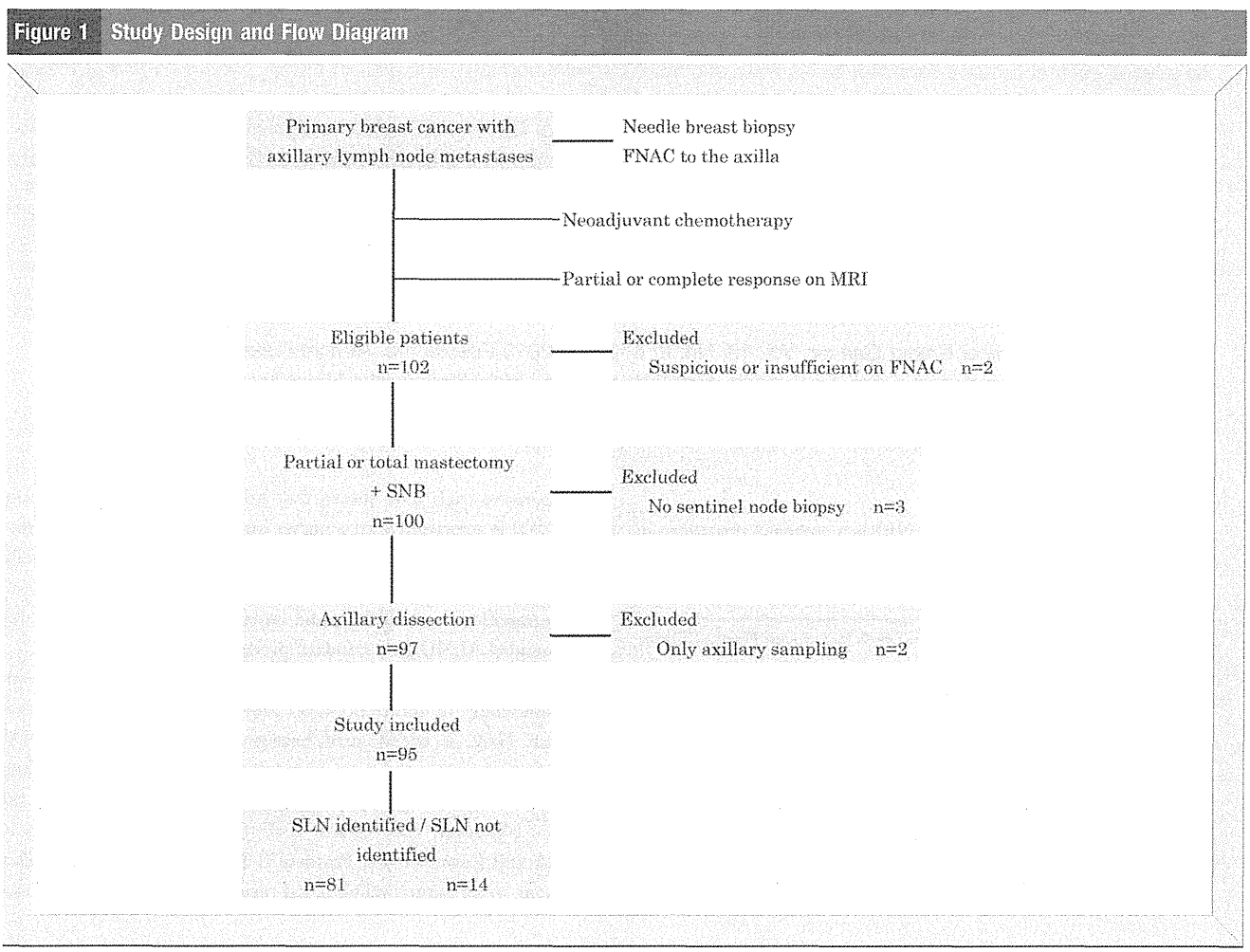
We conducted a prospective study of SLNB followed by ALND after NAC in patients with cytologically proven positive nodes to assess the identification rate of the SLN and the false negative rate of nodal metastases. We also attempted to identify subgroups of patients with a minimal risk of false negative results on SLNB after NAC.

## Patients and Methods

### *Eligibility*

Eligible patients for this single center prospective study underwent surgery by 3 well trained breast surgical oncologists at St. Luke's International Hospital between February 2007 and April 2009. The inclusion criteria were untreated, primary invasive breast cancer confirmed histologically using percutaneous needle biopsy of the breast; ALN metastasis confirmed using FNAC of suspicious ALNs; and a clinical partial or complete response to NAC, evaluated using dynamic contrast enhanced magnetic resonance imaging (MRI). Clinical complete response (cCR) was defined as the complete or probable disappearance of all target breast lesions, and

**Figure 1** Study Design and Flow Diagram




Abbreviations: FNAC = fine needle aspiration cytology; MRI = magnetic resonance imaging; SLN = sentinal lymph node; SNB = sentinel node biopsy.

clinical partial response was defined as a 30% decrease in the sum of diameters of the target lesions. Changes in the axillary nodes were not taken into account while defining tumor response. Patients were excluded if they had undergone ALND without SLNB, if they did not undergo ALND after SLNB, or if they had distinct clinical evidence of ALN metastasis after NAC. An institutional review board approved the study protocols, and informed consent was obtained from all patients.

### *Lymphatic Mapping and Surgical Procedure*

On the evening of the day before or in the morning before surgery, 2.0 or 1.0 mCi of technetium 99 phytate colloid was injected into the subareolar region. Lymphoscintigraphy was per formed approximately 2 hours after the injection. With the patient under general anesthesia, 1 mL of 2% patent blue dye was injected into the subareolar region approximately 15 minutes before starting surgery. SLNB was done via an ordinary skin incision used to perform total mastectomy or via a longer incision in the axillary region, similar to that used to perform ALND, apart from the incision for partial mastectomy. Hot or blue nodes were excised with hard palpable nodes, followed by ALND, regardless of the presence or absence of SLN metastasis. Breast surgery was per formed before or after ALND.

### *Pathological Assessment*

Estrogen receptor (ER), progesterone receptor (PgR), and human epithelial growth factor 2 (HER2) status of the tumor were evalu ated using immunohistochemical (IHC) staining of breast speci mens obtained using needle biopsy. For ER and PgR, an Allred score of 0 to 3 was defined as negative, and a score of 4 to 8 was defined as positive. HER2 positivity was defined as 3+ staining on immunohistochemistry, or 2+ staining and a ratio of 2 or more on fluorescence in situ hybridization (FISH). Each SLN was sent to the pathology department and completely cut into 2 mm thick slices and subjected to frozen section analysis with hematoxylin and eosin staining. The remainder of the node was then embedded in paraffin and reexamined with hematoxylin and eosin staining and IHC staining for cytokeratin AE1/AE3, if necessary. Lymph nodes that contained isolated tumor cells were categorized as positive nodes. pCR was evaluated based on the characteristics of the primary tu mor, axillary metastasis, or both.

### *Outcomes*

The main outcomes were the identification rate of SLNs in pa tients for whom an SLNB was attempted and the false negative rate of SLNB in patients who were found to have no positive SLN, but had 1 or more positive non SLN(s). Univariate analysis was per formed using the $\chi^2$ test for finding the subgroup that minimized the false negative rate.

## Results

### *Patient and Tumor Characteristics*

A total of 102 patients were enrolled (Fig. 1). Two patients with suspicious or insufficient cytologic results of ALNs, 3 who under went only ALN sampling during surgery, and 2 who did not undergo SLNB were excluded. Finally, 95 patients were included for further study. The median age of the patients at the time of surgery was 49 years (range, 28 76 years) (Table 1). The histological diagnosis of the primary tumor on needle biopsy of the breast was invasive ductal carcinoma in 94 patients and invasive, mixed ductal and lobular carcinoma in 1 patient. ER was positive in 73 patients (77%), PgR was positive in 53 (53%), and HER2 was positive in 22 (23%). As NAC, 90 patients sequentially received taxanes and anthracyclines (Table 2). Trastuzumab was administered during cycles of taxane therapy in all patients with HER2 positive tumors.

**Table 1 Clinicopathologic Characteristics of Patients (n = 95) Before Chemotherapy**

| Characteristic | n | % |
|---|---|---|
| **Age, years** | | |
| Median | 49 | |
| Range | 28 76 | |
| **Body Mass Index** | | |
| Median | 21.6 | |
| Range | 16.9 36.1 | |
| **Tumor Location, Quadrant** | | |
| Upper outer | 54 | 57 |
| Upper inner | 16 | 17 |
| Lower outer | 9 | 9 |
| Lower inner | 4 | 4 |
| Central | 12 | 13 |
| **Clinical Tumor Classification, AJCC** | | |
| cT0 | 2 | 2 |
| cT1 | 21 | 22 |
| cT2 | 56 | 59 |
| cT3 | 15 | 16 |
| cT4 | 1 | 1 |
| **Primary Tumor Histology** | | |
| Invasive ductal | 94 | 99 |
| Mixed ductal and lobular | 1 | 1 |
| **Nuclear Grade** | | |
| 1 | 50 | 53 |
| 2 | 28 | 29 |
| 3 | 17 | 18 |
| **Estrogen Receptor Status** | | |
| Positive | 73 | 77 |
| Negative | 22 | 23 |
| **Progesterone Receptor Status** | | |
| Positive | 50 | 53 |
| Negative | 45 | 47 |
| **HER2 Status** | | |
| HER2 positive, 3+, 2+, and FISH+ | 22 | 23 |
| HER2 negative, 0, 1+, 2+, and FISH | 73 | 77 |

Abbreviations: AJCC = American Joint Committee on Cancer; FISH = fluorescence in situ hybridization.

### *Accuracy of SLN Status*

Sentinel lymph nodes were identified in 81 patients (85.3%) and not detected in 14 patients. ALND was performed in all patients. In response to NAC, 21 patients (22%) had a pCR in breast tumor,

**Table 2** Treatment Methods and Pathological Results in 95 Patients

| Characteristic | n | % |
|---|---|---|
| **Regimens of Chemotherapy** | | |
| FEC DTX | 56 | 59 |
| AC DTX | 1 | 1 |
| AC wPTX | 5 | 5 |
| FEC | 2 | 2 |
| FEC DTX + H | 10 | 11 |
| FEC wPTX + H | 1 | 1 |
| DTX FEC | 16 | 17 |
| DTX + H FEC | 1 | 1 |
| TC | 3 | 3 |
| **Breast Surgery** | | |
| Partial mastectomy | 68 | 72 |
| Total mastectomy | 28 | 29 |
| **Clinical Response on MRI** | | |
| Partial response | 61 | 64 |
| Complete response | 32 | 36 |
| **Lymphoscintigraphy** | | |
| Not detectable | 30 | 32 |
| Detectable | 65 | 68 |
| **Sentinel node biopsy** | | |
| Not detectable | 14 | 15 |
| Detectable | 81 | 85 |
| **Level of Axillary Dissection** | | |
| 1 | 3 | 3 |
| 1 and 2 | 92 | 97 |
| **Number of Excised Axillary Nodes** | | |
| Median | 10 | |
| Range | 1 28 | |
| **Axillary Node Metastasis** | | |
| Negative (complete pathologic LN response) | 31 | 33 |
| Positive | 64 | 67 |
| **Pathologic Response, Primary Tumor** | | |
| Partial | 74 | 78 |
| Complete | 21 | 22 |

Abbreviations: AC = doxorubicin, cyclophosphamide; DTX = docetaxel; FEC = fluorouracil, epirubicin, cyclophosphamide; H = trastuzumab; LN = lymph node; MRI = magnetic resonance imaging; TC = docetaxel, cyclophosphamide; wPTX = weekly paclitaxel.

and 31 (32.6%) had a pCR in ALN. The median number of SLNs was 2 (range, 1 7) in the 81 patients with identified SLNs (Table 3). On frozen section analysis, 42 patients were assessed to have negative SLNs. On assessment of permanent sections, however, 4 of these 42 patients had positive SLNs. Overall, 43 of the 81 patients had positive SLNs, and 51 (43 SLN positive and 8 false negative) had positive ALNs. The identification rate was 85.3% (81 of 95 patients), and the false negative rate was 15.7% (8 of 51 patients). Thirteen of the 14 patients in whom SLNs were not identified had positive ALNs (Table 4).

In univariate analysis, the false negative rate was significantly lower in the HER2 negative group than in the HER2 positive group ($P = .001$) (Table 4). False negative cases are shown in Table 5.

**Table 3** Proportion of Patients in Whom Sentinel Nodes Were (n = 81) and Were Not (n = 14) Detected

| Characteristic | Detected, n | Not Detected, n |
|---|---|---|
| **Clinical Response on MRI** | | |
| Partial response | 66 | 14 |
| Complete response | 15 | 0 |
| **Lymphoscintigraphy** | | |
| Detectable | 62 | 3 |
| Not detectable | 19 | 11 |
| **Diagnosis on Frozen Section** | | |
| Negative | 42 | — |
| Positive | 39 | — |
| **Diagnosis on Permanent Section** | | |
| Negative | 38 | — |
| Positive on H & E and IHC staining | 43 | — |
| **Sentinel Node Metastasis on Frozen Section** | | |
| Negative | 42 | — |
| Positive | 39 | — |
| **Sentinel Node Metastasis on Permanent Specimen** | | |
| Negative | 38 | — |
| Positive | 43 | — |
| **Axillary Node Metastasis** | | |
| Negative | 30 | 1 |
| Positive (range, 1 15; median 2) | 51 | 13 |
| **Pathological Response, Primary Tumor** | | |
| Partial | 60 | 14 |
| Complete | 21 | 0 |

Abbreviations: H & E = hematoxylin and eosin; IHC = immunohistochemical; MRI = magnetic resonance imaging.

## Discussion

In this prospective study, the overall identification rate of SLNs on SLNB after NAC was low, and the false negative rate was high in patients with cytologically proven positive nodes. The identification rate and false negative rate were considered unacceptable clinically. In the presence of bulky metastasis, the flow of radioactive tracer or blue dye in lymphatic vessels might be unsatisfactory because of modification by chemotherapy. Our results showed that 13 of 14 patients in whom SLNs were not been detected had residual node metastasis, and such patients should undergo ALND. The high false negative rate might have been caused by NAC induced changes in lymphatic flow routes. Eight patients with false negative results had a median number of 3 (range, 1 6) residual nodal metastases as shown in Table 5. At present, SLNB can thus not be routinely recommended after NAC for patients with cytologically proven positive lymph nodes before NAC.

In our study, minimal false negative results were obtained only in patients with HER2 negative tumors. A characteristic biological feature of HER2 positive tumors is that they might influence lymphatic flow despite chemotherapeutic effects. It is completely unclear whether trastuzumab has an influence on the high rate of false negative results. In a subgroup of ER negative tumors, with

**Table 4** False Negative Rates of Sentinel Node Biopsy According to Clinicopathological Factors

| Characteristic | Number of Node-Positive (n = 51) | Number of False Negative (N=8) | P |
|---|---|---|---|
| **Body Mass Index** | | | .866 |
| <20 | 14 | 2 | |
| ≥20 | 37 | 6 | |
| **Clinical Tumor Classification** | | | .297 |
| cT0 1 | 11 | 3 | |
| cT2 | 33 | 5 | |
| cT3 4 | 7 | 0 | |
| **Clinical Response on MRI** | | | .310 |
| Partial response | 46 | 8 | |
| Complete response | 5 | 0 | |
| **Lymphoscintigraphy** | | | .106 |
| Detectable | 40 | 8 | |
| Not detectable | 11 | 0 | |
| **Estrogen Receptor** | | | .369 |
| Positive | 47 | 8 | |
| Negative | 4 | 0 | |
| **Progesterone Receptor** | | | .684 |
| Positive | 35 | 5 | |
| Negative | 16 | 3 | |
| **HER2 Status** | | | .001 |
| HER2 positive, 3+, 2+, and FISH+ | 7 | 4 | |
| HER2 negative, 0, 1+, 2+, and FISH | 44 | 4 | |
| **Number of Sentinel Nodes** | | | .141 |
| 1 | 25 | 6 | |
| 2 | 12 | 2 | |
| ≥3 | 14 | 0 | |

Abbreviations: FISH = fluorescence in situ hybridization; MRI = magnetic resonance imaging.

complete response on MRI, or with multiple SLNs (3 or more), false negative results were seen, however, this rate was not signifi cant. Therefore, a larger patient groups is needed to clearly under stand the significance. cCR in breast tumor on MRI does not always indicate a pCR, but MRI is more sensitive than physical examina tion or mammography for estimating chemotherapeutic effective ness, and cCR in the breast on MRI correlates with tumor response.[11 14] Moreover, the effect of NAC has been shown to differ according to breast cancer subtype.[15 17] ER positive tumors often show a poor response to chemotherapy, which might be related to the increased false negative rate. In contrast, ER negative tumors often respond well to chemotherapy. Our findings suggest that the specific subtype of breast cancer, the evaluation of tumor response on imaging studies, and a high number of sentinel nodes can potentially affect the accuracy of SLNB after NAC in patients with positive ALNs.

**Table 5** Cases of False Negative Results

| Pt | Age, years | T | NG | ER | PR | HER2 | BMI | LS | SLN | Hot | Blue | No. | MRI | pR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 56 | 1 | 1 | 8 | 2 | | 18.5 | 1 | 2 | + | + | 2 | PRe | PRe |
| 2 | 50 | 2 | 2 | 7 | 0 | + | 26.1 | 1 | 1 | + | + | 1 | PRe | PRe |
| 3 | 55 | 2 | 2 | 4 | 3 | | 36.1 | 1 | 1 | + | + | 3 | PRe | PRe |
| 4 | 67 | 2 | 1 | 8 | 7 | | 23.8 | 1 | 1 | + | + | 1 | PRe | PRe |
| 5 | 45 | 2 | 2 | 7 | 6 | | 18.4 | 1 | 1 | + | + | 1 | PRe | PRe |
| 6 | 43 | 1 | 1 | 8 | 5 | | 22.5 | 2 | 1 | + | + | 6 | PRe | PRe |
| 7 | 48 | 2 | 3 | 6 | 6 | | 22.5 | 1 | 2 | + | + | 1 | PRe | PRe |
| 8 | 42 | 1 | 2 | 8 | 4 | | 20.1 | 1 | 2 | + | + | 2 | PRe | PRe |

Abbreviations: Blue = blue node; BMI = body mass index; ER = estrogen receptor; Hot = hot node; LS = number of positive nodes using lymphoscintigraphy; MRI = clinical response on magnetic resonance imaging; NG = nuclear grade; No. = number of positive nodes; pR = pathological response; PR = progesterone receptor; PRe = partial response; Pt = patient; SLN = sentinel lymph node; T = clinical tumor classification.

**Table 6** Studies of Sentinel Node Biopsy After Neoadjuvant Chemotherapy in Patients With Node-Positive Breast Cancer

| Study | Patients, n | Method Used to Assess Axillary Metastasis | Identification Rate | False Negative Rate | Overall Accuracy |
|---|---|---|---|---|---|
| Shen et al[19] | 69 | FNAC | 64/69 (92.8%) | 10/40 (25%) | 38/56 (67.9%) |
| Lee et al[20] | 219 | Palpation and FNAC or US and 18F FDG PET | 170/219 (77.6%) | 7/124 (5.6%) | 163/170 (95.9%) |
| Canavese et al[21] | 64 | Palpation or US | 60/64 (93.8%) | 2/39 (5.1%) | 58/60 (96.7%) |
| Present study | 95 | FNAC | 81/95 (85.3%) | 8/51 (15.7%) | 73/81 (90.1%) |

Abbreviations: 18F FDG PET = 2 deoxy 2[F 18]fluoro D glucose positron emission tomography; FNAC = fine needle aspiration cytology; US = ultrasound.

Mamounas et al evaluated the success rate of sentinel node identification and false negative rate in the neoadjuvant setting of NSABP B 27.[18] The success rate was 87.6% using radioisotope and blue dye, and false negative rate was 10.7%. In this study, confirmation of lymph node status was not done either cytologically or pathologically. So, the patient selection was largely different from our indication and these cannot be simply compared.

Four studies, including ours, have evaluated the results of SLNB after NAC in patients with clinically positive ALNs (Table 6).[19,21] Shen et al reported a high SLN identification rate (92.8%) with a high false negative rate (25%) after NAC in 64 patients with cytologically proven, node positive breast cancer.[19] They concluded that the SLNB does not provide a reliable indication of the presence or absence of residual disease in the axilla. Lee et al compared the accuracy of SLNB in patients with clinically node positive breast cancer who received (219 patients) or did not receive (363 patients) chemotherapy.[20] The SLN identification rate was significantly lower in the patients who received chemotherapy (77.6%) than in those who did not (97.0%), and the false negative rates were similar (5.6% and 7.4%, respectively). Canavese et al evaluated the results of SLNB in 64 patients with clinically positive nodes.[21] The SLN identification rate was 93.8%, and the false negative rate was 5.1%. The variability in the false negative rate might be attributed to heterogeneity among patients and tumor characteristics in these studies.

The status of SLNs is usually diagnosed on frozen section analysis during surgery, followed by permanent section analysis after surgery. In our series, 4 (9.5%) of 42 patients who were evaluated to be SLN negative on intraoperative frozen section analysis were found to be SLN positive on permanent section analysis (including immunohistochemistry) after surgery, shown in Table 3. Pathological diagnosis of specimens obtained using SLNB after NAC is often difficult because of chemotherapy induced changes. Biopsy specimens stained with hematoxylin and eosin that are evaluated to be node negative on frozen section analysis during surgery should be assessed using immunohistochemistry,[22,23] or IHC staining of permanent sections should be performed, if necessary, for the accurate detection of residual tumor cells.

## Conclusion

The strengths of our study include the fact that we assessed breast cancer subtypes using needle biopsy before treatment and confirmed the clinical response of the tumors to chemotherapy using preoperative MRI imaging. In addition, SLNB was performed by 3 well experienced breast surgical oncologists according to a standardized procedure. We also examined differences between the results of frozen and permanent section analyses of SLNs. Our study had a limitation in that it was a small study performed at a single center.

The results of this prospective study do not support the routine use of SLNB for evaluating the presence or absence of residual axillary metastasis after NAC in patients with cytologically proven, node positive breast cancer. Our findings suggest that we might identify patients at minimal risk for false negative results by evaluating the HER2 status of tumors. Moreover, the response to NAC on MRI, the ER status, and the number of sentinel nodes could also have a potential role. The validity of our results should be confirmed in large prospective clinical trials.

### *Clinical Practice Points*

- The utility of sentinel node biopsy after NAC in breast cancer patients with clinically positive nodes has not been established.
- Our data showed that the identification rate was low and the false negative rate was high, which did not support clinical use of the sentinel node biopsy.
- HER2 negative tumors can be a good indication for sentinel node biopsy.

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## Disclosure

The authors have stated that they have no conflicts of interest.

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ORIGINAL ARTICLE

# Prevalence and differentiation of hereditary breast and ovarian cancers in Japan

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**Abstract**
*Background* We assembled needed data on the prevalence and characteristics of *BRCA1/2* in Japan.
*Materials and methods* Our study of *BRCA1/2* collected data at eight institutions in Japan on 320 individuals with a strong family history of breast cancer, according to the NCCN guidelines, by the end of March 2012.
*Results* Among 260 proband cases, 46 (17.7 %) were positive for *BRCA1,* and 35 (13.5 %) were *BRCA2*-positive. Therefore, the total pathological mutation rate was 30.7 %. Pathology data after breast surgery were obtained from 37 cases of *BRCA1* mutation, 23 (62.2 %) of which were triple negative (TN). On the other hand, 29 cases (82.9 %) of *BRCA2* mutations were Luminal type. The most prevalent *BRCA1* mutation site was L63X, found in 10 families. L63X was reported previously by studies in Japan, and it may be a founder mutation. We found two cases of large deletion detected by multiplex ligation-dependent probe amplification. One was an entire deletion of exon 20 and the lacked exons 1 9. TN with a family history of ovarian cancer was 11/20 (55 %). TN under 40-year-old (y.o.) 15/23 (65.2 %) and TN with one or more breast cancers in family history 17/32 (53.1 %) showed higher incidences of *BRCA1* mutation.
*Conclusion* Hereditary breast and ovarian cancer (HBOC) may have nearly the same prevalence in Japan as

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in the US or Europe. If TN cases are taken into account, the ratio of *BRCA1* is higher. L63X may be one of the founder mutations in Japan. A nationwide database of HBOC is important to develop risk models for *BRCA1/2* carriers in Japan.

**Keywords** HBOC · *BRCA1* · *BRCA2* · Triple negative · L63X

## Introduction

Breast cancer ranks among the most common of female cancers, according to the Center for Cancer Control and Information Services of the National Cancer Center in Japan. According to a database maintained by the Center for Cancer Control, the breast cancer incidence has reached 60000 patients. However, the lifetime risk of breast cancer is markedly lower (1 in 16) than in the United States (1 in 7).

In the US, about 5 10 % of breast cancers are thought to be hereditary. Most inherited cases of breast cancer are associated with two susceptibility genes, *BRCA1* and *BRCA2* [1 5]. Germ line mutations of *BRCA1* and *BRCA2* (*BRCA1/2*) can cause very high rates of breast and ovarian cancer, the so-called hereditary breast and ovarian cancers (HBOC). This study collected data to estimate the prevalence and characteristics of *BRCA1/2*, which was not accurately assessed with a larger cohort in the Japanese population.

## Materials and methods

Data on *BRCA1/2* were collected at eight institutions in Japan from 1996 until the end of March 2012. Candidates were recruited from breast or genetic counseling clinics in each institution. We evaluated 320 subjects with a strong family history according to NCCN guidelines [6].

The definition of high risk was:

Personal history of breast cancer plus one or more of the following:

1. Diagnosed under 40-years-old
2. Diagnosed at age 50-years-old with at least one close blood relative with breast cancer under 50 y.o. and/or at least one blood relative with epithelial ovarian/fallopian tube and/or primary peritoneal cancer at any age
3. Two primary breast lesions when the first cancer diagnosis occurred prior to age 50
4. Diagnosed at any age, with two close blood relatives with breast and/or epithelial ovarian/fallopian tube/primary peritoneal cancer at any age
5. Close male blood relative with breast cancer
6. Personal history of epithelial ovarian/fallopian tube/primary peritoneal cancer
7. For an individual of ethnicity associated with a higher mutation frequency (Ashkenazi Jewish), no additional family history may be required
8. Personal history of epithelial ovarian/fallopian tube/primary peritoneal cancer
9. Personal history of male breast cancer

Family history only first-degree, second-degree or third-degree relatives (Third must have at least two close blood relatives with breast cancer under 50 years and/or ovarian at any age) meeting any of the above criteria.

The criteria for HBOC testing revised in 2011 added important new points for affected individuals and a slight clarification to the recommendation for individuals with family history only:

- Affected: triple-negative (ER−, PR−, HER2−) breast cancer diagnosed <60 y.o.
- Affected: diagnosed <50 y.o. with a limited family history (see clarification below).
- Affected: personal history of breast and/or ovarian cancer at any age with at least two close blood relatives with pancreatic cancer at any age.
- Affected: personal history of pancreatic adenocarcinoma at any age with at least two close blood relatives with breast and/or ovarian and/or pancreatic cancer at any age.
- Family history only: third-blood relative with breast cancer and/or ovarian/fallopian tube/primary peritoneal cancer and two close blood relatives with breast cancer (at least one with breast cancer under 50 y.o.) and/or ovarian cancer.

Genetic testing for *BRCA1/2* was performed by direct sequencing, as described previously [7]. In addition, multiplex ligation-dependent probe amplification (MLPA) analysis was performed for 145 subjects by the their wish to search for large genomic rearrangements (LGR) [8 10].

Data on *BRCA1/2* and clinical background of subjects were registered to the group study conducted by JBCS (2011 2012). The database was developed with FileMaker Pro ver.11. All data are anonymized and can be reidentified by linking the coded information with the identity of the participants based on their comprehensive agreement. This

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study protocol was approved by the local review boards of each institution.

## Results

Subject background and genetic testing results are shown in Table 1. Among the 260 proband cases, 46 (17.7 %) were positive for *BRCA1*, and 35 (13.5 %) for *BRCA2*. Therefore, the total pathological mutation rate was 30.7 % (Fig. 1).

**Table 1** Subject background and genetic testing results in *BRCA1/2* data collected by the HBOC study group

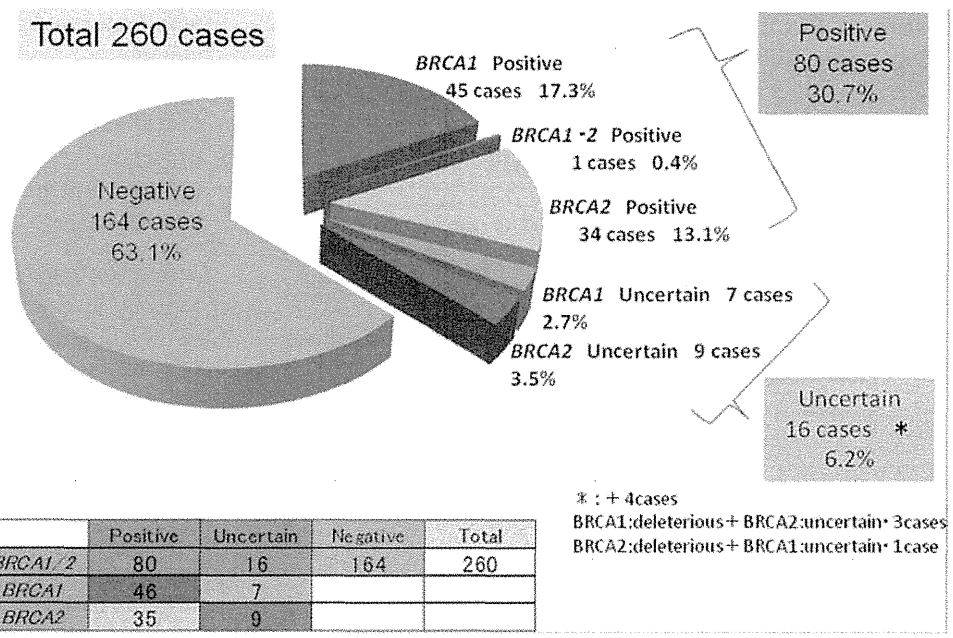
| Subject background | Breast or ovarian cancer diagnosed | Result of *BRCA1/2* genetic testing |
|---|---|---|
| Proband: 260 | Yes: 244 | Positive |
| | | *BRCA1*: 46 |
| | | *BRCA2*: 32[a] |
| | | Negative: 167 |
| | No: 16 | Positive |
| | | *BRCA1*: 0 |
| | | *BRCA2*: 3 |
| | | Negative: 13 |
| HBOC family member: 60 | Yes: 14 | Positive |
| | | *BRCA1*: 6 |
| | | *BRCA2*: 9[a] |
| | | Negative: 0 |
| | No: 46 | Positive |
| | | *BRCA1*: 11 |
| | | *BRCA2*: 10 |
| | | Negative: 25 |
| Total: 320 | | |

[a] Included one case for *BRCA1* and *BRCA2* double mutations

Table 2 shows deleterious mutation types and family numbers detected in this study. The most prevalent relevant site of mutation on *BRCA1* was L63X; it was found among 10 families. There were two cases of LGR that could not be detected by the current PCR-based method. However, they were diagnosed by an MLPA procedure, becoming the first and second cases reported in Japan. One was a deletion of the entire exon 20 and the other delete exons 1 9. There was only one case of double mutation: a combination of L63X in *BRCA1* and 5804del4 in *BRCA2*.

Uncertain variants were detected in 16 cases. Seven cases of *BRCA1* (2.7 %) and nine *BRCA2* (3.5 %) were uncertain variants. In addition, there were four detected cases of uncertain variants with deleterious mutations in the other *BRCA* gene (Table 3). 60 cases of HBOC family data were also included in breast cancer subtype analyses. Figure 2 is the prevalence of breast cancer subtypes in our *BRCA1/2* data of probands and family members. 23 cases (62.2 %) among the *BRCA1* mutations were triple-negative (TN), and 11 (29.7 %) were Luminal type. On the other hand, 29 cases (82.9 %) among *BRCA2* mutations were Luminal type.

Figure 3 presents TN cases classified through patient/family history and *BRCA1* mutations. TN with a history of ovarian cancer in their families (11/20, 55 %), TN under 40 y.o. (15/23, 65.2 %) and TN with a family history of one or more breast cancers (17/32, 53.1 %) had a higher incidence of *BRCA1*.




| | Positive | Uncertain | Negative | Total |
|---|---|---|---|---|
| *BRCA1/2* | 80 | 16 | 164 | 260 |
| *BRCA1* | 46 | 7 | | |
| *BRCA2* | 35 | 9 | | |

**Fig. 1** Result of *BRCA1/2* genetic testing of 260 proband cases

**Table 2** *BRCA1/2* mutation types and family numbers

| Mutation type | *BRCA1* | No. | *BRCA2* | No. |
|---|---|---|---|---|
| Frame shift | 575delCA | 1 | 1506delA | 1 |
| | 589delCT | 1 | 2041insA | 1 |
| | 1231delC | 1 | 3036del4 | 1 |
| | 1239delA | 1 | 3423del4 | 1 |
| | 1343delA | 1 | 3827delGT | 1 |
| | 1406insA | 1 | 5358del4 | 1 |
| | 2508delGA | 1 | 5804del4 | 4 |
| | 2632dellA | 1 | 5903del1 | 1 |
| | 2730delCC | 1 | 6491del5 | 1 |
| | 2798del4 | 1 | 6674del5 | 1 |
| | 2805delA | 1 | 6696delTC | 1 |
| | 3127delTT | 1 | 6854delTA | 1 |
| | 3561delG | 2 | 8663ins19 | 1 |
| | 3699ins4 | 1 | 8817insA | 1 |
| | | | 8896delC | 1 |
| Large rearrangement | exon1a 9del | 1 | | |
| | exon20del | 1 | | |
| Nonsense mutation | Q60X | 1 | Q1089X | 1 |
| | L63X | 10 | S1882X | 2 |
| | Y130X | 1 | R2318X | 4 |
| | E352X | 1 | R2520X | 1 |
| | K503X | 1 | S2835X | 1 |
| | Q934X | 2 | Q2893X | 1 |
| | E1214X | 2 | R3128X | 1 |
| | Q1721X | 1 | | |
| | R1835X | 1 | | |
| Missense mutation | C24Y | 1 | S2670L | 1 |
| | C61G | 1 | I2675V | 2 |
| | C64R | 1 | | |
| | S1655F | 1 | | |
| Splicing | IVS14 2A > G | 1 | IVS5 + 1G > A | 1 |
| | IVS17 + 3A > G | 1 | P3039P | 2 |
| | IVS19 + 2insT | 1 | | |
| | IVS20 1G > A | 1 | | |
| | IVS20 1G > C | 1 | | |
| Total | 35 types | 46 | 26 types | 35 |

We used a simple checklist-based status/family history according to the NCCN guidelines to detect suspected HBOC patients. Patient conditions and detection rates in cases that met only one condition are shown in Table 4.

Figure 4 shows *BRCA1/2* mutation and breast cancer incidence by age. 260 proband cases were classified into three groups of *BRCA1*-positive, *BRCA2*-positive and *BRCA*-negative (the double mutation was included in the *BRCA1* group). The age at which breast cancers were diagnosed among patients and family member (excluding males) were binned in 5-year spans from 25 to 85 y.o. Japanese Breast Cancer data from the National Cancer Center for 2007 was used as the general reference breast cancer statistics, and the incidence in each age group is overlaid in Fig. 4. The age of diagnosis for ovarian cancers in patient or family member was evaluated in the same way. The incidence in each age group is presented as a proportion of all ovarian cancer patients in Fig. 5.

**Table 3** Uncertain variants of *BRCA1/2* mutation detected in 260 proband cases

| *BRCA1* | *BRCA2* |
|---|---|
| L52F | V208G |
| P209L | M764V[a] |
| E259V | I770V |
| G401E | F1273Y[a] |
| Q855P[a] | D1728N |
| R1645S | D1990A[a] |
| Y1853C | D1990A |
| IVS17 9A > G | V2010G |
| | P2802L |
| | D2900G |
| | H3056Y |
| | L3274W |

[a] The other *BRCA* gene has deleterious mutation

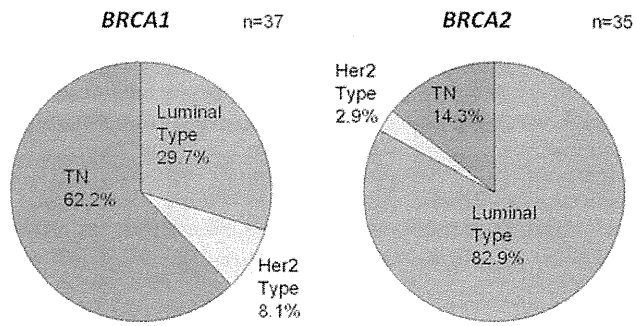



**Fig. 2** Type of breast cancer with *BRCA1/2* mutation in probands and family members

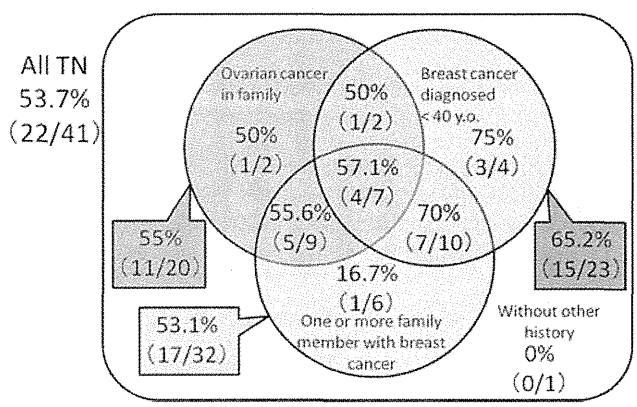



**Fig. 3** Prevalence of *BRCA1* mutation of probands with TN classified with patient/family history

**Table 4** Detection rate for one condition only in simple checklist

| | Number of subjects | *BRCA1*(+) | *BRCA2*(+) | Positive rate |
|---|---|---|---|---|
| Breast cancer diagnosed <40 years in family | 34 | 2 | 4 | 17.6 |
| Ovarian cancer (including fallopian tube and/or peritoneal) diagnosed at any age in family | 28 | 3 | 1 | 14.3 |
| Two or more primary breast cancers diagnosed at any age for one family member | 17 | 0 | 1 | 5.9 |
| Male breast cancer patient in family | 0 | | | |
| 3 or more breast cancer patients in family | 22 | 0 | 2 | 9.1 |
| Triple negative breast cancer patients in family | 4 | 0 | 0 | 0.0 |

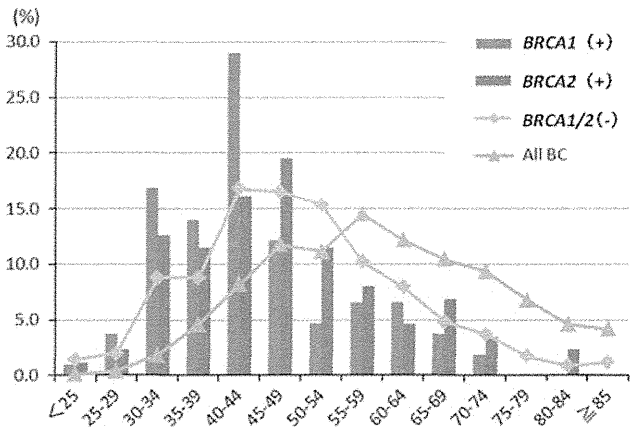



**Fig. 4** *BRCA1/2* mutation and breast cancer incidence by age

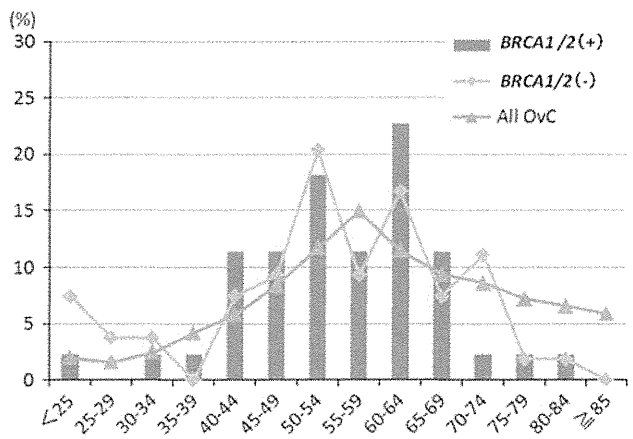



**Fig. 5** *BRCA1/2* mutation and incidence of ovarian cancer by age

**Table 5** Genetic testing for a choice of breast cancer surgery option

| Test results | Number of total subjects | Breast cancer surgery | |
|---|---|---|---|
| | | Conservative operation | Mastectomy |
| Positive | 16 | 2 (12.5 %) | 14 (87.5 %) |
| Negative | 39 | 28 (71.8 %) | 11 (28.2 %) |

After receiving the results of *BRCA1/2* testing, the number of surgical options actually selected, such as conservative operation or mastectomy, is shown in Table 5.

## Discussion

The prevalence of *BRCA1/2* germ line mutations in Japanese patients was initially reported in 2008 by Sugano et al. [11]. They examined 135 cases by full sequence analysis of the *BRCA1/2* gene and found 28 types of deleterious mutations in 36 cases (26.7 %), including 13 types of *BRCA1* mutation in 17 cases (12.6 %) and 15 types of *BRCA2* mutation in 19 cases (14.1 %). In our study, 46 cases (17.7 %) were positive for *BRCA1* mutations and 35 (13.5 %) were *BRCA2*-positive among 260 proband cases. The total pathological mutation rate was 30.7 %. One of the reasons for the greater number of *BRCA1* deleterious mutations was our inclusion of TN breast cancer as a risk factor [12]. Fostira et al. [13] identified 65 deleterious *BRCA1* mutations among the 403 TN breast cancer patients (16 %). TN breast cancers with a *BRCA1* mutation were more common among those younger than 40 years: 38 (36 %) of 106 women. Moreover, mutations were found in 48 % (50/105) of the TN breast cancer patients with a family history of breast or ovarian cancer. These results indicate that women with early-onset, TN breast cancer, and possibly all TN breast cancer patients, are candidates for genetic testing for *BRCA1*, even in the absence of a family history of breast or ovarian cancer. The NCCN HBOC guidelines of 2011 include TN breast cancer under 60 y.o. as a test criterion.

Breast cancers occurring in carriers of *BRCA1* mutations are more likely to be estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, HER2/neu receptor-negative, and have a basal phenotype. Ovarian cancers associated with *BRCA1* are more likely to be a higher grade and of serous histopathology.

Sekine et al. [14], analyzed genetic alterations in *BRCA1* and *BRCA2* genes among 82 families in Japan with ovarian cancer victims. Their criterion for an ovarian cancer family was: two or more members with well documented

epithelial ovarian cancer among second-degree relatives and no breast cancer cases in third-degree relatives. When the family had at least one breast cancer case in a third degree-relative, it was classified as a breast ovarian cancer family. Using a direct sequencing method, 45 of the 82 ovarian cancer families had deleterious mutations of *BRCA1* or *BRCA2*. The breakdown of mutations was 40 with *BRCA1* and five with *BRCA2*. L63X and Q934X mutations were detected in seven and eight independent families, respectively. In our study, the most prevalent mutation site of *BRCA1* also was L63X, and it was found among 10 families. This mutation had been pointed out by Sekine et al., in the series of Familiar Ovarian Cancer Study Group in Japan [10]. Therefore, it may be a founder mutation unique to the Japanese population.

We detected two cases of large deletion by MLPA; the first cases reported in Japan. One was an entire deletion of exon 20 and the other lost exons 1 9. Both cases were positive for *BRCA1*. Previously, the available techniques only allowed identification of small genomic alterations, but new technology allows LGR to be detected rapidly. LGR in *BRCA1* are responsible for between 0 and 27 % of the disease-causing mutations identified in numerous populations [15, 16]. Such alterations are far less common in the *BRCA2* gene [17, 18]. One large German study showed that all rearrangements were detected in families with: (1) at least two breast cancer cases prior to the age of 51 y.o.; (2) breast and ovarian cancer cases; (3) families with at least two ovarian cancer cases; or (4) a single breast cancer case prior to the age of 36 years [19]. No mutations were detected in families with no or only one breast cancer case prior to 51 y.o. *BRCA2* MLPA screening is recommended only for families who present with co-occurrence of female and male breast cancers.

Models are available in the US to predict an individual's lifetime risk of developing breast cancer [20, 21]. Additional models exist to predict an individual's likelihood of having a *BRCA1* or *BRCA2* mutation [22 24]. Each model is appropriate only when the patient's characteristics and family history are similar to the study population on which the model was based. Therefore, we need a Japanese risk calculation model that can be applied to individual Japanese.

Breast cancer is also a common feature of Li Fraumeni syndrome caused by *TP53* mutations and a feature of Cowden syndrome from *PTEN* mutations [25]. Women with an abnormal *BRCA1* or *BRCA2* gene, who had lumpectomy plus radiation are between 4 and 5 times more likely to develop another cancer (either the same cancer recurring or a new cancer) in the same breast compared to women with an abnormal *BRCA1* or *BRCA2* gene who had a mastectomy. However, when the women who had lumpectomy plus radiation also had chemotherapy after surgery, their risk of developing another breast cancer was about the same as women who have mastectomy. Based on these results, the researchers suggested that lumpectomy plus radiation therapy would be a good choice for women with an abnormal breast cancer gene, but only if chemotherapy is included in the treatment plan.

When a result positive for *BRCA1/2* was obtained, our data suggested that mastectomy was preferred over breast-conserving surgery (BCS). IBTR risk after BCS in carriers of *BRCA1/2* mutations once was considered greater than for patients who had sporadic breast cancer. Likewise, the risk of CBC seems to be higher in this group. These risks, and the likelihood of developing new primary tumors, should be discussed with carriers interested in breast conservation, as well as when choosing risk-reducing strategies. However, the rate of ipsilateral tumors was no higher in mutation carriers than in non-carriers or controls. BCT is a treatment option for tumors in *BRCA1/2* mutation carriers because they may be more sensitive to radiation [26, 27].

## Conclusion

HBOC in Japan may have the same trends as in the US or Europe. If TN cases are taken into consideration as a risk factor, the ratio of *BRCA1* will be increased in our data. L63X may be one of the founder mutations of *BRCA1* in Japan. A national database of HBOC is warranted to clarify these unsolved questions among Japanese:

1. To develop risk models to estimate the prevalence of *BRCA1/2* carriers in Japan
2. To identify the best means to detect early cancer occurrence among Japanese *BRCA1/2* carriers
3. To differentiate whether uncertain mutation variants found in Japan are deleterious.

To accomplish these goals, efforts must continue toward maintaining a mandatory national database.

**Conflict of interest** Seigo Nakamura has no conflict of interest. Masato Takahashi has no conflict of interest. Mitsuhiro Tozaki has no conflict of interest. Takahiro Nakayama has no conflict of interest. Tadashi Nomizu has no conflict of interest. Yoshio Miki received $30,000/year as a royalty for Breast Cancer Gene U 2090 from the University of Utah. Yoshie Murakami has no conflict of interest. Daisuke Aoki has no conflict of interest. Takuji Iwase has no conflict of interest. Seiichiro Nishimura has no conflict of interest. Hideko Yamauchi has no conflict of interest. Shozo Ohsumi has no conflict of interest. Shinichi Baba has no conflict of interest. Tadao Shimizu has no conflict of interest.

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