Original Article

A prediction model for early systemic recurrence in breast cancer using a molecular diagnostic analysis of sentinel lymph nodes: A large-scale, multicenter cohort study

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BACKGROUND: The one-step nucleic acid amplification (OSNA) assay can quantify the cytokeratin 19 messenger RNA copy number as a proxy for sentinel lymph node (SN) metastasis in breast cancer. A large-scale, multicenter cohort study was performed to determine the prognostic value of the SN tumor burden based on a molecular readout and to establish a model for the prediction of early systemic recurrence in patients using the OSNA assay. **METHODS:** SN biopsies from 4757 patients with breast cancer were analyzed with the OSNA assay. The patients were randomly assigned to the training or validation cohort at a ratio of 2:1. On the basis of the training cohort, the threshold SN tumor burden value for stratifying distant recurrence was determined with Youden's index; predictors of distant recurrence were investigated via multivariable analyses. Based on the selected predictors, a model for estimating 5-year distant recurrence–free survival was constructed, and predictive performance was measured with the validation cohort. **RESULTS:** The prognostic cutoff value for the SN tumor burden was 1100 copies/μL. The following variables were significantly associated with distant recurrence and were used to construct the prediction model: SN tumor burden, age, pT classification, grade, progesterone receptor, adjuvant cytotoxic chemotherapy, and adjuvant anti–human epidermal growth factor receptor 2 therapy. The values for the area under the curve, sensitivity, specificity, and accuracy of the prediction model were 0.83, 63.4%, 81.7%, and 81.1%, respectively. **CONCLUSIONS:** Using the OSNA assay, the molecular readout–based SN tumor burden is an independent prognostic factor for early breast cancer. This model accurately predicts early systemic recurrence and may facilitate decision-making related to treatment. ***Cancer* 2022;128:1913-1920**.

KEYWORDS: breast cancer, cytokeratin 19, multicenter study, one-step nucleic acid amplification (OSNA) assay, prediction model, sentinel lymph node, total tumor load.

INTRODUCTION

The axillary lymph node status is a key indicator of prognosis in breast cancer.[1] Precise and reproducible pathological node staging (pN) classification is crucial for predicting prognoses and making therapeutic decisions for patients with breast cancer. For almost 30 years, sentinel lymph node (SN) biopsy has remained the standard axillary staging procedure for patients who are clinically node-negative.[2] To reduce the number of false-negative diagnoses, pathologists generally focus their detailed examination on a subset of lymph nodes that are more likely to harbor metastases.[3] The intensive

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examination of SNs increases the detection of low-volume metastases.[4] However, accurate and reproducible quantification of the total metastatic volume of a lymph node is not possible with conventional histopathological examinations.[5] For example, although a node may be step-sectioned and evaluated at each cut surface, potentially critical information can be missed because only a limited area of the node is analyzed.

The one-step nucleic acid amplification (OSNA) assay (Sysmex, Kobe, Japan) offers a solution to the aforementioned issues.[6] This molecular assay allows the whole lymph node to be evaluated and yields the quantitative metastatic tumor burden using the cytokeratin 19 (CK19) messenger RNA (mRNA) copy number as a proxy. Calibration and validation studies[6-8] indicate that the CK19 mRNA copy number provides a good estimate of macrometastasis (>2 mm in size), micrometastasis (>0.2-2 mm), and negative metastasis (≤0.2 mm) as defined by the American Joint Committee on Cancer (AJCC) staging manual.[9] In the clinical setting, more cases of SN metastasis (and, in particular, micrometastasis) are detected with the OSNA assay than via conventional histological examination.[10-12]

The amount of the SN tumor burden as estimated with the OSNA assay is an independent prognostic factor in breast cancer.[13,14] Furthermore, patients with a negative SN status after OSNA analysis have a better prognosis than those whose nodes have undergone conventional histological examination.[15] These observations strongly suggest that the OSNA assay has superior accuracy and reproducibility in comparison with conventional histopathological examinations for prognostication in patients with breast cancer. However, the prognostic cutoff values for the molecular-based tumor burden in SNs have differed by 10-fold between studies.[13,14] Moreover, there has been no validation of prognostic prediction models using the OSNA assay to estimate the probability of recurrence in patients with breast cancer. In this large-scale, multicenter breast cancer cohort study, we evaluated the prognostic impact of a molecular readout–based SN tumor burden and established a model for the prediction of early systemic recurrence using the OSNA assay.

MATERIALS AND METHODS

Patient Selection

This multicenter, retrospective cohort study analyzed a data set registered in a web-based database, Lynolog, operated by the Japanese Association for Theranostics.[16] Each participating hospital registered clinicopathological characteristics, OSNA results, and follow-up information for patients with breast cancer who underwent SN biopsy.

This study included patients who underwent SN biopsy between 2008 and 2012. Patients with any of the following criteria were excluded: 1) male gender, 2) bilateral breast cancer, 3) ductal carcinoma in situ, 4) neoadjuvant drug therapy, 5) recurrence of heterochronous ipsilateral breast cancer, and 6) prior resection of the primary tumor. The review boards of all participating institutions approved this study.

SN Biopsy Using the OSNA Assay

SN mapping and identification were performed with a radioisotope tracer and/or blue dye. Radioactive and/or blue-colored lymph nodes were defined as SNs and removed before evaluation with the OSNA assay with or without a histopathological examination. Complete axillary lymph node resection was performed in SN-positive patients.

Lymph node samples were homogenized in 4 mL of a lysis buffer (Lynorhag; Sysmex) and centrifuged at 10,000*g* at room temperature.[6] The supernatant (2 μL) was analyzed with the RD-100i system (Sysmex), which uses a reverse transcription loop-mediated isothermal amplification method and the LynoampBC kit (Sysmex). The amount of amplification correlated positively with the accumulation of the reaction byproduct, pyrophosphate. Changes in turbidity upon precipitation of magnesium pyrophosphate were then correlated with the CK19 mRNA copy number per microliter of the original lysate. This value was extrapolated from a standard curve that was generated with 3 calibrators containing different amounts of CK19 mRNA. Cutoff values for negative/positive and micro/macrometastasis were set at 250 and 5000 copies/μL, respectively.

In this study, the negative samples were given a CK19 mRNA copy number of 0 copies/μL. The total tumor load (TTL), defined as the aggregate CK19 mRNA copy number of each positive SN sample, was used to quantify the metastatic tumor burden in SNs.[17]

Adjuvant Treatment and Follow-Up

After resection, a combined local and systemic adjuvant treatment course was adopted. Follow-up was based on international standards and national guidelines that took into account both patient and tumor characteristics.

Statistical Analyses

Distant recurrence–free survival (DRFS) was used as the prognostic end point, and it was calculated from the

time of surgery to the first evidence of distant recurrence. Two multivariable logistic models were established: 1) a recurrence-related model for identifying independent variables related to distant recurrence and 2) a recurrence prediction model to estimate the probability of 5-year DRFS rates.

To construct and validate the recurrence-related model, eligible patients were randomly segregated between training and validation cohorts at a ratio of 2:1. The training cohort was used for the cutoff determination of TTL in the SNs, the actual modeling, the model performance measure, and the first internal validation.[18] The validation cohort was used for the second internal validation.

To construct the recurrence-related model with the training cohort, we first set the cutoff value of TTL for stratifying distant recurrence at the maximum value of Youden's index (sensitivity + specificity − 1) for the receiver operating characteristic (ROC) curve.[19] Then, univariable logistic regression analyses were used to screen potential predictors of distant recurrence with P values < .10. Finally, using variables from the univariable analyses, a multivariable logistic regression model for the distant recurrence–related variables was optimized with a stepwise procedure based on the Akaike information criterion.

To measure the model performance, the area under the ROC curve (AUC) of the model was calculated with the training cohort. Next, the first internal validation was performed with the training cohort and a 10-fold cross-validation procedure. At each of the 10 folds, the cutoff value of probability for predicting distant recurrence was set at the maximum value of Youden's index for the ROC curve. Youden's index was based on the individuals' estimated probabilities from the multivariable logistic model and individuals' true values. Finally, the second internal validation was performed with the validation cohort, with the cutoff value calculated with the training cohort.

To construct and validate the recurrence prediction model, the training and validation cohorts were used after the exclusion of patients censored within 5 years. Recurrence 5 years after surgery was defined as no recurrence within the 5-year interval. To construct the model, the regression coefficients of the variables selected in the recurrence-related model were re-estimated with the 5-year prognostic data of the training cohort. The model performance measure and internal validations were performed with the same statistical methods used for the recurrence-related model.

Cumulative survival rates were calculated with the Kaplan-Meier method with censored data. Survival rates between the 2 groups were compared with log-rank tests. Statistical significance was set at $P < .05$, and confidence intervals (CI) were fixed at 95%. All statistical analyses were performed with R software (version 3.6.1).[20]

RESULTS

Patient Characteristics

For this study, 6432 patients were registered in the Lynolog database from 11 participating hospitals in Japan. After the exclusion of 1675 patients according to the exclusion criteria, 4757 eligible patients were randomly assigned to the training cohort (3171 patients) or the validation cohort (1586 patients) at a ratio of 2:1. Demographic characteristics were well balanced between the 2 cohorts (Table 1 and Supporting Tables 1 and 2). A total of 9740 SNs were assessed with the OSNA assay (training cohort, 6496 nodes; validation cohort, 3244 nodes). Of the 4757 patients, 1341 (28.2%) underwent an SN examination combined with the OSNA assay and conventional histopathological examination (Supporting Table 1). The SN tumor burdens assessed between the OSNA assay (50% or 90% of each SN tissue) and the histopathological examination (50% or 10% of each SN tissue) were well correlated (Supporting Tables 3 and 4, respectively).

For the training cohort, 3.5% of the patients (110 of 3171) experienced distant recurrence with a median follow-up of 5.5 years (range, 0.0-9.1 years), and the 5-year DRFS rate was 96.6% (95% CI, 95.9%-97.4%). For the validation cohort, 2.9% of the patients (46 of 1586) experienced distant recurrence with a median follow-up of 5.5 years (range, 0.0-9.2), and the 5-year DRFS rate was 97.4% (95% CI, 96.6%-98.2%).

Determination of the Cutoff Value for TTL in SNs

The discriminative TTL cutoff value in the SNs for calling distant recurrence was set at 1100 copies/μL. With this cutoff value, 2624 patients (82.7%) and 475 patients (15.0%) in the training cohort had TTL values of <1100 and ≥1100 copies/μL, respectively (Table 1). The 5-year DRFS rate was lower for patients with TTL ≥ 1100 copies/μL in the SNs than for patients with TTL < 1100 copies/μL in the SNs (92.3% [95% CI, 89.8%-94.9%] vs 97.5% [95% CI, 96.8%-98.1%]; $P < .01$; Fig. 1).

TABLE 1. Patient Characteristics of the Training and Validation Cohorts (Selected Variables)

Characteristic	Training		Validation	
	No.	%	No.	%
No. of patients	3171	100.0	1586	100.0
Age, y				
20-39	266	8.4	117	7.4
40-49	854	26.9	432	27.2
50-59	752	23.7	388	24.5
60-69	763	24.1	374	23.6
≥70	536	16.9	275	17.3
pT classification				
pT1a-1b	1082	34.1	529	33.4
pT1c	1302	41.1	659	41.6
pT2	715	22.5	356	22.4
pT3	52	1.6	27	1.7
Unknown	20	0.6	15	0.9
Grade				
1	1441	45.4	743	46.8
2 or 3	1716	54.1	837	52.8
Unknown	14	0.4	6	0.4
Estrogen receptor				
≥1%	2695	85.0	1356	85.5
<1%	444	14.0	226	14.2
Unknown	32	1.0	4	0.3
Progesterone receptor				
≥1%	2257	71.2	1150	72.5
<1%	856	27.0	421	26.5
Unknown	58	1.8	15	0.9
HER2				
(–)	2648	83.5	1352	85.2
Equivocal[a]	121	3.8	61	3.8
(+)	391	12.3	170	10.7
Unknown	11	0.3	3	0.2
Subtype				
Luminal	2366	74.6	1221	77.0
Luminal-HER2	231	7.3	95	6.0
HER2	152	4.8	75	4.7
Triple-negative	260	8.2	128	8.1
Unknown	162	5.1	67	4.2
TTL in SNs				
<1100 copies/μL	2624	82.7	1302	82.1
≥1100 copies/μL	475	15.0	236	14.9
Unknown	72	2.3	48	3.0
No. of metastatic SNs				
0	2500	78.8	1249	78.8
1	510	16.1	259	16.3
2	129	4.1	61	3.8
≥3	32	1.0	17	1.1
No. of total metastatic nodes				
0	2500	78.8	1249	78.8
1-3	580	18.3	282	17.8
4-9	78	2.5	44	2.8
≥10	13	0.4	11	0.7
Adjuvant cytotoxic chemotherapy				
No	2307	72.8	1148	72.4
Yes	854	26.9	433	27.3
Unknown	10	0.3	5	0.3
Adjuvant endocrine therapy				
No	952	30.0	460	29.0
Yes	2214	69.8	1120	70.6
Unknown	5	0.2	6	0.4
Adjuvant anti-HER2 therapy				
No	2905	91.6	1465	92.4
Yes	255	8.0	112	7.1
Unknown	11	0.3	9	0.6

Abbreviations: HER2, human epidermal growth factor receptor 2; SN, sentinel lymph node; TTL, total tumor load.
[a]Immunohistochemistry (2+) and *in situ* hybridization not performed.

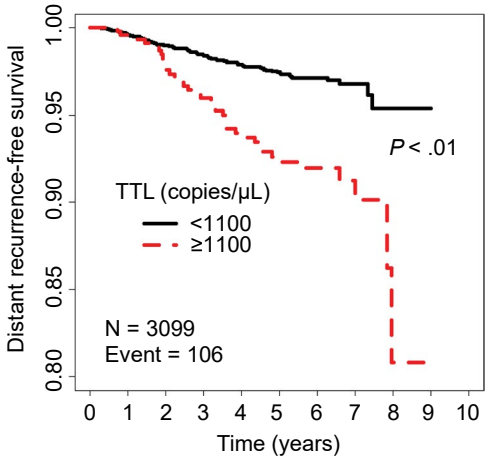

Figure 1. Distant recurrence–free survival according to the total tumor load (TTL) in sentinel lymph nodes in the training cohort.

Construction and Validation of the Recurrence-Related Model

Upon univariable analysis using the training cohort, we observed 17 variables that were associated with distant recurrence ($P < .10$): age, breast surgery procedure, axillary surgery procedure, pT classification, grade, lymphovascular invasion, estrogen receptor status, progesterone receptor status, subtype, SN macro/micrometastasis, TTL in the SNs, number of metastatic SNs, number of metastatic non-SNs, number of total metastatic nodes, positive SN ratio, adjuvant cytotoxic chemotherapy, and adjuvant anti–human epidermal growth factor receptor 2 (HER2) therapy (Table 2 and Supporting Table 5). In the multivariable analysis using the training cohort, the following 7 variables were selected for the optimal model: TTL in the SNs, age, pT classification, grade, progesterone receptor status, adjuvant cytotoxic chemotherapy, and adjuvant anti-HER2 therapy (Table 3). TTL in the SNs remained statistically significant ($P < .01$).

The AUC of the model was 0.82 (95% CI, 0.77-0.87; Supporting Fig. 1). For the first internal validation using the training cohort with the 10-fold cross-validation procedure, the average sensitivity, specificity, and accuracy of the model were 73.5%, 77.0%, and 76.9%, respectively (Supporting Table 6). For the second internal validation using the validation cohort, the sensitivity, specificity, and accuracy of the model were 61.9%, 80.0%, and 79.5%, respectively, with a cutoff value of 4.8% (Table 4 and Supporting Fig. 1).

We performed the same analysis on the luminal breast cancer subtype in the cohort. However, the

TABLE 2. Univariable Analysis of Predictive Factors for Distant Recurrence in the Training Cohort (Selected Variables)

Variable	Estimate	SE	Z	*P*	Odds Ratio (95% CI)
Age, y					(n = 3171)
Intercept	–2.89	0.27	–10.53	<.01[a]	0.06 (0.03-0.10)
20-39					1.00
40-49	–0.53	0.34	–1.58	.11	0.59 (0.30-1.14)
50-59	–0.13	0.32	–0.40	.69	0.88 (0.47-1.66)
60-69	–0.89	0.37	–2.42	.02[b]	0.41 (0.20-0.84)
≥70	–0.53	0.37	–1.43	.15	0.59 (0.29-1.22)
pT classification					(n = 3151)
Intercept	–5.37	0.45	–11.99	<.01[a]	0.00 (0.002-0.01)
pT1a-1b					1.00
pT1c	2.07	0.47	4.37	<.01[a]	7.89 (3.12-19.93)
pT2	2.83	0.47	6.00	<.01[a]	16.89 (6.71-42.51)
pT3	3.34	0.62	5.35	<.01[a]	28.10 (8.27-95.45)
Grade					(n = 3157)
Intercept	–4.12	0.21	–19.61	<.01[a]	0.02 (0.01-0.02)
1					1.00
2 or 3	1.19	0.24	5.02	<.01[a]	3.29 (2.07-5.24)
Estrogen receptor					(n = 3139)
Intercept	–3.62	0.12	–29.93	<.01[a]	0.03 (0.02-0.03)
≥1%					1.00
<1%	1.26	0.21	6.02	<.01[a]	3.51 (2.33-5.28)
Progesterone receptor					(n = 3113)
Intercept	–3.92	0.15	–25.74	<.01[a]	0.02 (0.01-0.03)
≥1%					1.00
<1%	1.39	0.20	6.90	<.01[a]	4.00 (2.70-5.92)
HER2					(n = 3160)
Intercept	–3.38	0.11	–31.02	<.01[a]	0.03 (0.03-0.04)
(–)					1.00
Equivocal[c]	0.01	0.52	0.01	.99	1.01 (0.36-2.79)
(+)	0.41	0.26	1.57	.12	1.50 (0.90-2.50)
Subtype					(n = 3009)
Intercept	–3.68	0.13	–27.71	<.01[a]	0.03 (0.02-0.03)
Luminal					1.00
Luminal-HER2	0.59	0.35	1.68	.09	1.80 (0.91-3.57)
HER2	0.79	0.39	2.05	.04[b]	2.21 (1.04-4.72)
Triple-negative	1.57	0.24	6.53	<.01[a]	4.80 (3.00-7.69)
TTL in SNs					(n = 3099)
Intercept	–3.61	0.12	–29.60	<.01[a]	0.03 (0.02-0.03)
<1100 copies/μL					1.00
≥1100 copies/μL	1.14	0.21	5.42	<.01[a]	3.13 (2.07-4.72)
No. of metastatic SNs					(n = 3171)
Intercept	–3.62	0.13	–28.83	<.01[a]	0.03 (0.02-0.03)
0					1.00
1	0.78	0.23	3.36	<.01[a]	2.18 (1.38-3.43)
2	1.43	0.32	4.51	<.01[a]	4.20 (2.25-7.84)
≥3	1.68	0.55	3.05	<.01[a]	5.35 (1.82-15.70)
No. of total metastatic nodes					(n = 3171)
Intercept	–3.62	0.13	–28.83	<.01[a]	0.03 (0.02-0.03)
0					1.00
1-3	0.91	0.21	4.26	<.01[a]	2.48 (1.63-3.76)
4-9	1.31	0.42	3.14	<.01[a]	3.69 (1.64-8.34)
≥10	1.92	0.78	2.46	.01[b]	6.81 (1.48-31.35)
Adjuvant cytotoxic chemotherapy					(n = 3161)
Intercept	–3.87	0.15	–26.28	<.01[a]	0.02 (0.02-0.03)
No					1.00
Yes	1.34	0.20	6.81	<.01[a]	3.83 (2.60-5.64)
Adjuvant endocrine therapy					(n = 3166)
Intercept	–3.13	0.16	–19.36	<.01[a]	0.04 (0.03-0.06)
No					1.00
Yes	–0.30	0.20	–1.46	.14	0.74 (0.50-1.11)
Adjuvant anti-HER2 therapy					(n = 3160)
Intercept	–3.39	0.10	–32.47	<.01[a]	0.03 (0.03-0.04)
No					1.00
Yes	0.61	0.29	2.15	.03[b]	1.85 (1.06-3.24)

Abbreviations: CI, confidence intervals; HER2, human epidermal growth factor receptor 2; SE, standard error; SN, sentinel lymph node; TTL, total tumor load.
[a]*P* < .01.
[b]*P* < .05.
[c]Immunohistochemistry (2+) and *in situ* hybridization not performed.

validation procedure could not be completed because of the small number of recurrences (data not shown).

Construction and Validations of the Recurrence Prediction Model

After the exclusion of patients censored within 5 years, 2085 and 1059 patients remained in the training and validation cohorts, respectively. The regression coefficients of the 7 variables selected from the recurrence-related model were re-estimated, and the TTL in the SNs was statistically significant ($P < .01$; Table 3). The AUC of the model was 0.83 (95% CI, 0.78-0.88; Supporting Fig. 1). For the first internal validation using the training cohort with the 10-fold cross-validation procedure, the average sensitivity, specificity, and accuracy of the model were 81.5%, 70.0%, and 70.5%, respectively (Supporting Table 7). For the second internal validation using the validation cohort, the sensitivity, specificity, and accuracy of the model were 63.4%, 81.7%, and 81.1%, respectively, with a cutoff value of 5.7% (Table 4 and Supporting Fig. 1). The freely accessible online tool for estimating the probability of 5-year DRFS rates is available at https://www.theranostics.jp/sln/show.

DISCUSSION

The current study is the largest study of molecular diagnostic analysis for lymph node metastasis. Data from approximately 4800 patients with breast cancer whose SNs were evaluated with the OSNA assay were collected from 11 institutions through a web-based patient registration system in Japan. We confirmed herein that the SN tumor burden, estimated with the CK19 mRNA copy number as a proxy, is an independent prognostic factor in early-stage breast cancer. Moreover, as far as we

TABLE 3. Two Multivariable Logistic Regression Models Using the Training Cohort: 1) Recurrence-Related Model for Identifying Independent Variables Related to Distant Recurrence and 2) Recurrence Prediction Model for Estimating the Probability of 5-Year Distant Recurrence–Free Survival

	Recurrence-Related Model (n = 2831)					Recurrence Prediction Model (n = 2085)				
Variable	Estimate	SE	Z	*P*	Odds Ratio (95% CI)	Estimate	SE	Z	*P*	Odds Ratio (95% CI)
Intercept	–5.78	0.57	–10.05	<.01[a]	0.00 (0.001-0.01)	–5.49	0.59	–9.29	<.01[a]	0.00 (0.001-0.01)
TTL in SNs ≥1100 (vs <1100)	0.71	0.25	2.91	<.01[a]	2.04 (1.26-3.30)	0.80	0.27	2.94	<.01[a]	2.23 (1.31-3.81)
Age, 40-49 y (vs 20-39 y)	–0.40	0.38	–1.05	.30	0.67 (0.32-1.42)	–0.47	0.39	–1.21	.23	0.62 (0.29-1.34)
Age, 50-59 y	–0.30	0.37	–0.81	.42	0.74 (0.36-1.53)	–0.62	0.39	–1.62	.11	0.54 (0.25-1.14)
Age, 60-69 y	–1.04	0.42	–2.51	.01[b]	0.35 (0.16-0.80)	–1.56	0.46	–3.41	<.01[a]	0.21 (0.09-0.52)
Age, ≥70 y	–0.47	0.42	–1.11	.27	0.63 (0.27-1.43)	–0.81	0.46	–1.75	.08	0.44 (0.18-1.10)
pT1c (vs pT1a-1b)	1.69	0.49	3.48	<.01[a]	5.40 (2.09-13.99)	1.73	0.49	3.52	<.01[a]	5.66 (2.16-14.86)
pT2	2.21	0.50	4.46	<.01[a]	9.13 (3.46-24.14)	2.41	0.51	4.77	<.01[a]	11.15 (4.14-30.02)
pT3	2.72	0.66	4.13	<.01[a]	15.18 (4.17-55.23)	3.16	0.70	4.55	<.01[a]	23.67 (6.05-92.58)
Grade 2 or 3 (vs 1)	0.58	0.28	2.08	.04[b]	1.79 (1.03-3.10)	0.76	0.31	2.46	.01[b]	2.14 (1.17-3.91)
Progesterone receptor <1% (vs ≥1%)	1.41	0.24	5.82	<.01[a]	4.08 (2.54-6.56)	1.68	0.26	6.47	<.01[a]	5.39 (3.23-8.98)
Adjuvant cytotoxic chemotherapy, yes (vs no)	0.40	0.26	1.57	.12	1.50 (0.90-2.49)	–0.14	0.29	–0.49	.62	0.87 (0.49-1.54)
Adjuvant anti-HER2 therapy, yes (vs no)	–0.48	0.34	–1.42	.16	0.62 (0.32-1.20)	–0.40	0.36	–1.11	.27	0.67 (0.33-1.36)

Abbreviations: CI, confidence intervals; HER2, human epidermal growth factor receptor 2; SE, standard error; SN, sentinel lymph node; TTL, total tumor load.
[a]*P* < .01.
[b]*P* < .05.

TABLE 4. Predictive Performance of the Recurrence-Related Model and the Recurrence Prediction Model Using the Validation Cohort

	Recurrence-Related		Recurrence Prediction	
Cutoff[a]	4.8%		5.7%	
Predicted/actual	Yes	No	Yes	No
Yes	26	292	21	188
No	16	1165	12	838
Sensitivity	61.9%		63.4%	
Specificity	80.0%		81.7%	
Accuracy	79.5%		81.1%	

[a]Maximum values of Youden's index for the receiver operating characteristic curves based on the individuals' estimated probabilities from the multivariable logistic model and individuals' true values.

are aware, this study is the first to use the OSNA assay to construct and validate a risk prediction model to estimate the probability of recurrence in patients with breast cancer.

Two multivariable logistic models were established for this study. The recurrence-related model aimed to elucidate the prognostic impact of the molecular-based tumor burden in SNs. The recurrence prediction model was designed to estimate the probability of 5-year DRFS rates. These 2 models had moderately accurate discriminative ability (AUC, 0.82-0.83) and moderately high performance (validation accuracy, 70%-80%).

Herein, we set the prognostic cutoff value of the SN tumor burden at 1100 copies/μL of CK19 mRNA. With this cutoff value, the SN tumor burden was an independent prognostic factor in the 2 multivariable models. This cutoff value is similar to that of a previous single-institution study (2810 copies/μL)[13] because both cutoff values are within the range of the tumor burden equivalent to AJCC micrometastasis (250-5000 copies/μL).[6] Therefore, patients with AJCC micrometastasis in the SNs were divided into good and poor prognosis groups based on the metastatic volume. The OSNA assay accurately and consistently estimates small metastatic volumes and, therefore, could be used to determine the patients' prognoses with precision.

The prediction model developed in the current study can accurately predict early systemic recurrence after standard adjuvant therapies for early-stage breast cancer. Although distant metastasis occurred in <5% of the study population and this low prevalence of events may negatively influence the predictive performance, the predictive accuracy of the model was as high as 70% to 80% by the 2 procedures of the internal validations. Using the prediction model, we developed a freely accessible online tool for estimating 5-year DRFS, which is available on the website of the Japanese Association for Theranostics. This online tool can help clinicians to predict prognosis accurately and reproducibly with the OSNA assay and to guide more precise therapeutic strategies for patients who undergo SN biopsy.

For patients with hormone receptor–positive and HER2-negative (luminal) breast cancer, the indication

of adjuvant chemotherapy has recently been guided by a multigene assay.[21,22] In the current study, 75.4% of the patients (3587 of 4757) had luminal breast cancer, and 80.7% of them (2896 of 3587) were treated without adjuvant cytotoxic chemotherapy. Thus, our prediction model might be useful for the selection of patients with luminal breast cancer who have an excellent prognosis without chemotherapy, even in the absence of the multigene assay. When patients are estimated to have a poor prognosis by our model, the addition of the multigene assay would help to assess potential benefits from adjuvant chemotherapy.

There are 3 potential limitations to the construction, validation, and clinical utility of our prognostic prediction model. First, the median follow-up of 5.5 years to assess the prognosis of early-stage breast cancer is relatively short. Thus, distant metastasis was detected in <5% of the patients. Further follow-up of patients and improvement of the model to predict the 10- or 15-year prognosis are desirable. Second, external validation (temporal or geographic validation) could not be performed for our model.[18] In this study, however, the patient selection bias appears negligible because this is a large-scale, multicenter study, and the predictive performance was evaluated in 2 ways: 10-fold cross-validation using the training cohort (>2000 patients) and validation using the validation cohort (>1000 patients). Finally, our model cannot be used to identify patients who can benefit from adjuvant cytotoxic chemotherapy. We suggest that such a model could be developed with data from the patients with luminal breast cancer in the cohort after a 10-year or longer follow-up.

In conclusion, the molecular readout–based SN tumor burden using the OSNA assay can serve as an independent prognostic indicator in early-stage breast cancer. The prognostic cutoff value in the SNs was within the tumor burden range equivalent to AJCC micrometastasis. Furthermore, the prediction model that we developed during the course of this study can accurately predict early systemic recurrence after standard adjuvant therapies. It could, therefore, facilitate therapeutic decision-making for clinicians who care for patients with breast cancer.

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CONFLICT OF INTEREST DISCLOSURES

Tomo Osako received honoraria from Diaceutics outside the submitted work. Kenzo Shimazu received honoraria from AstraZeneca, Pfizer, Sysmex, Novartis, Chugai, Daiichi-Sankyo, Taiho, Eisai, and Eli Lilly outside the submitted work. Takashi Ishikawa received honoraria from AstraZeneca, Eisai, Daiichi Sankyo, Pfizer, Taiho, Lilly, Nippon Kayaku, Kyowa Kirin, and Takeda outside the submitted work. Nobuaki Sato received honoraria from Chugai, Kyowa Kirin, Taiho, Eli Lilly, and Nippon Kayaku outside the submitted work. Seigo Nakamura reported payments to his institution from Eisai, Konica Minolta Japan, Shimadzu, Sysmex, Taiho, and Chugai and payments to him from AstraZeneca, Daiichi Sankyo, Chugai, and Becton Dickinson outside the submitted work. Hitoshi Tsuda received research funding from Taiho, Goryo Chemical, and Roche Diagnostics and scholarship donations from Chugai, Takeda, and Eli Lilly outside the submitted work. Shinzaburo Noguchi is an advisor for Sysmex and has received consulting fees and research funding from Sysmex; he is an advisor for AstraZeneca and Nittobo and has received honoraria and/or consulting fees from AstraZeneca, Eli Lilly, Chugai, Nittobo, and Sysmex outside the submitted work; and he holds joint patents not related to this study with Sysmex. The other authors made no disclosures.

AUTHOR CONTRIBUTIONS

Tomo Osako: Design of work, acquisition of data, analysis of data, interpretation of data, draft, editing, and revision. **Masaaki Matsuura:** Design of work, analysis of data, interpretation of data, writing-review and revision. **Daisuke Yotsumoto:** Acquisition of data and writing-review. **Shin Takayama:** Acquisition of data and writing-review. **Koji Kaneko:** Acquisition of data and writing-review. **Mina Takahashi:** Acquisition of data and writing-review. **Kenzo Shimazu:** Acquisition of data and writing-review. **Katsuhide Yoshidome:** Acquisition of data and writing-review. **Kazuya Kuraoka:** Acquisition of data and writing-review. **Masayuki Itakura:** Acquisition of data and writing-review. **Mayumi Tani:** Acquisition of data and writing-review. **Takashi Ishikawa:** Acquisition of data and writing-review. **Yasuyo Ohi:** Conception of work, design of work, and writing-review. **Takayuki Kinoshita:** Conception of work, design of work, and writing-review. **Nobuaki Sato:** Conception of work, design of work, and writing-review. **Masahiko Tsujimoto:** Conception of work, design of work, and writing-review. **Seigo Nakamura:** Conception of work, design of work, and writing-review. **Hitoshi Tsuda:** Conception of work, design of work, interpretation of data, writing-review, and revision. **Shinzaburo Noguchi:** Conception of work, design of work, interpretation of data, writing-review, and revision. **Futoshi Akiyama:** Conception of work, design of work, interpretation of data, and writing-review. The final version was approved by all authors.

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