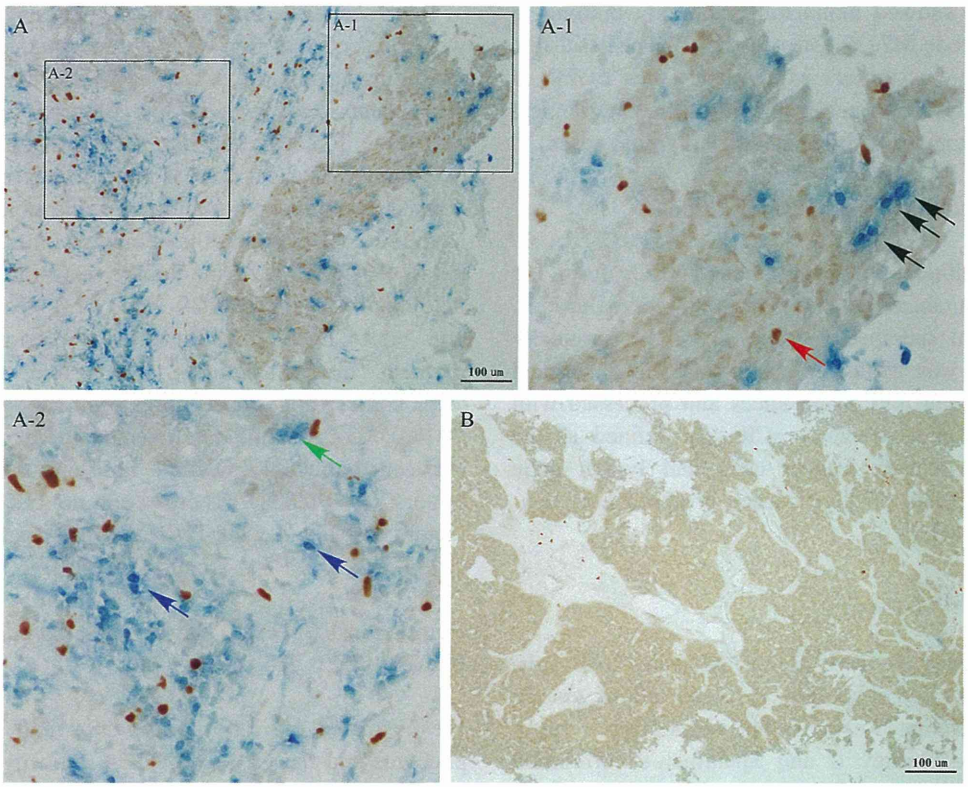

Fig. 1 Immunohistochemical double staining of tumor-infiltrating CD8 (*blue*) and FOXP3 (*brown*). The representative tumor tissue of high (**a**) and low infiltration (**b**) of CD8 and FOXP3. Tumor-infiltrating lymphocytes were counted in the three compartments of each tumor: the intra-tumoral compartment (*black arrow* points to CD8+ lymphocytes and *red arrow* points to CD8+ lymphocytes), adjacent stromal compartment (*green arrow* points to CD8+ lymphocytes) and distant stromal compartment (*blue arrow* points to CD8+ lymphocytes)

counted, and the ratio of CD8+ to FOXP3+ was calculated [17, 25]. These procedures were performed in the following three compartments of each tumor: the intra-tumoral compartment (within the tumor nests), the adjacent stromal compartment (the distance between the lymphocytes and tumor nest is less than or equal to the size of one tumor cell), and the distant stromal compartment (the distance between the lymphocytes and tumor nest is greater than the size of a single tumor cell) [17] (Fig. 1).

Immunohistochemistry for vasohibin-1 and CD31

We performed immunohistochemical staining for vasohibin-1 and CD31 on the biopsy specimens. Microvessels were identified as the lumen lined by endothelial cells stained with anti-CD31 antibody (code M0823, Dako). We counted the microvessels in one high power field (×200) after the areas with the greatest number of microvessels had been selected from low magnifications (×40 and ×100) [20, 21]. Vasohibin-1 signals were counted in the same hot spot and in the same field in which the highest number of anti-CD31+ vessels was identified. We defined the vasohibin-1-positive ratio (VPR) as the number of vasohibin-1-positive signals divided by the number of CD31-positive signals [26, 27].

Evaluation of ER, PgR, HER2, Ki-67, EGFR, and CK5/6

The ER and PgR statuses were evaluated by immunostaining using monoclonal antibodies (code 107925 and 102333, Roche Diagnostics), and nuclear staining of more than 1 % was considered positive. The HER2 status was evaluated by immunohistochemistry (HercepTest, code K5204, Dako) or by fluorescence in situ hybridization (the PathVysion Kit; Abbott). HER2 positivity was defined in accordance with the American Society of Clinical Oncology/College of American Pathologists Guideline [28]. The Ki-67 was determined with an anti-MIB-1 monoclonal antibody (code M7240, Dako) by counting 1,000 tumor cells in the hot spots [29, 30]. EGFR was interpreted as positive if the membranes of 10 % or more carcinoma cells were stained using monoclonal antibodies (code K1492, Dako). CK5/6 was interpreted as positive if 10 % or more

carcinoma cells showed monoclonal antibody binding in the cytoplasm (code M7237, Dako) [31, 32]. The basal-like type was defined as tumors expressing either EGFR or CK5/6. Two pathologists performed all of the pathological diagnoses and staining assessments of individual cases.

Clinical information and pathological response

We collected clinical information on TNBCs from the individual breast cancer databases of the three institutions. The pathological therapeutic response of the surgically resected tumor was evaluated after NAC. The surgical specimens were cut into 5 mm slices and processed with hematoxylin–eosin staining. A pCR was defined as the absence of all invasive cancer cells and lymph node metastasis, regardless of the presence or absence of non-invasive cancer cells.

Statistical analyses

All statistical analyses were performed using SAS software, JMP Pro 11 (Tokyo, Japan). Associations among variables were evaluated using Fisher's exact test or the χ^2 test. The Mann–Whitney U and Spearman's correlation tests were used to compare non-continuous parameters. Logistic regression analyses were performed for univariate and multivariate analyses to determine the independent variables for the prediction of pCR. All tests were two sided, and a P value of less than 0.05 was considered statistically significant.

Results

Clinicopathological factors and their association with CD8 and FOXP3 and the ratio of CD8+/FOXP3+

After immunohistochemical re-examination of ER, PgR and HER2 status, eight patients who presented with low ER status of between 1 and 10 % were excluded in this study. An evaluation could not be performed in fourteen patients because of little tumor specimens in the biopsy materials. Immunohistochemical analyses of CD8, FOXP3, vasohibin-1, CD31, EGFR, CK5/6, and Ki-67 and the CD8+/FOXP3+ ratio by the double-staining method were subsequently performed in the tumors from 88 TNBCs treated with NAC. Seventy-eight patients (89 %) were treated with NAC regimens containing both anthracyclines and taxanes, and almost all of the other patients were treated with anthracyclines. Twenty-six patients were diagnosed as having a pCR according to the detailed pathological examination, and the pCR rate was 29.5 % (26/88).

Representative images of the immunohistochemistry were presented in Fig. 1. The clinicopathological factors, including vasohibin-1, CD31 and VPR, were examined for the patients with high or low infiltration of CD8+ and FOXP3+ T cells, and the CD8+/FOXP3+ ratio was determined. The cut-offs of high or low infiltration were defined as the median number of infiltrating cells per field as follows: CD8, 86.75 (the numbers of infiltrating cells per field); FOXP3, 73.25 (the numbers of infiltrating cells per field) and CD8+/FOXP3+ ratio, 1.0 (Table 1). TNBCs with high CD8+/FOXP3+ ratios were significantly more frequent in the patients with premenopausal status ($P = 0.002$) and those diagnosed at an earlier age ($P = 0.003$). A significant inverse correlation was detected between the CD8+/FOXP3+ ratio and the VPR in these tumors ($P = 0.021$). There were no clinicopathological factors associated with TNBCs with a high infiltration of CD8+ and FOXP3+ T cells (Table 1).

Correlations between CD8, FOXP3, the CD8+/FOXP3+ ratio and pCR

We evaluated the infiltrating CD8+ and FOXP3+ lymphocytes and the CD8+/FOXP3+ ratio by double-immunostaining in the three compartments of each tumor. In this TNBC cohort, positive correlations were detected between CD8+ lymphocyte infiltration and FOXP3+ lymphocyte infiltration in the intra-tumoral ($r = 0.5583$, $P < 0.0001$), adjacent stromal ($r = 0.5834$, $P < 0.0001$) and distant stromal compartment ($r = 0.4803$, $P < 0.0001$; Fig. 2a–c). The statistically positive correlation was detected between the total CD8 and FOXP3 levels in the entire field, which represents the summation of CD8 and FOXP3 levels in these three compartments ($r = 0.4798$, $P < 0.0001$; Fig. 2d).

The TNBCs were classified into high and low groups for CD8, FOXP3 and the CD8+/FOXP3+ ratio using the cut-off values defined as the median number of infiltrating cells per field as described in Fig. 3. In the intra-tumoral compartment, the patients whose tumors had a high CD8 level or high CD8+/FOXP3+ ratio had a significantly higher pCR rate than those with a low CD8 level or low CD8+/FOXP3+ ratio (46 vs. 15 %, $P = 0.002$ and 52 vs. 19 %, $P = 0.003$, respectively; Fig. 3a, c). In the adjacent stromal compartment, the patients whose tumors had a high CD8 or high CD8+/FOXP3+ ratio also had a significantly higher pCR rate than those with weakly infiltrated tumors (49 vs. 13 %, $P < 0.001$ and 45 vs. 17 %, $P = 0.005$, respectively; Fig. 3a, c). The pCR rate in high CD8 tumors was not significantly different from that in low CD8 tumors (40 vs. 22 %, $P = 0.097$) but the patients whose tumors had a high CD8+/FOXP3+ ratio were associated with a significantly higher pCR rate than did those with weakly

Table 1 Clinicopathological factors and association with CD8, FOXP3, the ratio of CD8/FOXP3 in TNBC

	CD8[a]			FOXP3[b]			CD8/FOXP3[c]		
	High	Low	*P* value	High	Low	*P* value	High	Low	*P* value
Age (years)			0.198			0.668			0.003*
≤50	23	16		21	18		27	12	
>50	21	28		23	26		18	31	
Menopausal status			0.081			1.000			0.002*
Premenopausal	22	13		18	17		25	10	
Postmenopausal	22	31		26	27		20	33	
Tumor size			0.051			1.000			0.469
≤5.0 cm	37	28		32	33		35	30	
>5.0 cm	7	16		12	11		10	13	
Nodal status			1.000			0.783			0.784
Negative	8	8		7	9		9	7	
Positive	36	36		37	35		36	36	
Histological grade			0.087			0.087			1.000
I, II	16	25		16	25		21	20	
III	28	19		28	19		24	23	
Basal-like type			0.372			0.372			0.824
Basal-like	26	31		26	31		30	27	
Non basal-like	18	13		18	13		15	16	
Ki-67 LI (cut-off 57.5)			1.000			0.831			0.394
Low	22	22		21	23		25	19	
High	22	22		23	21		20	24	
Vasohibin-1 (cut-off 20)			0.199			1.000			0.134
Low	27	20		23	24		28	19	
High	17	24		21	20		17	24	
CD31 (cut-off 40)			0.670			0.201			0.831
Low	24	21		26	19		24	21	
High	20	23		18	25		21	22	
VPR (vasohibin-1/CD31; cut-off 0.6)			1.000			0.831			0.021*
Low	21	21		20	22		27	15	
High	23	23		24	22		18	28	

LI labeling index, *VPR* vasohibin-1 positive ratio

* The *P* value is significant

[a] The cut-off of CD8 infiltration is 86.75

[b] The cut-off of FOXP3 infiltration is 73.25

[c] The cut-off of CD8/FOXP3 ratio is 1.0

infiltrated tumors in the distant stromal compartment (45 vs. 12 %, $P = 0.001$; Fig. 3a, c). No differences were detected between tumors infiltrated with high levels of FOXP3+ lymphocytes and those infiltrated with low levels, in the intra-tumoral, adjacent stromal and distant stromal compartments (38 vs. 23 %, $P = 0.163$; 37 vs. 23 %, $P = 0.242$; and 30 vs. 29 %, $P = 0.816$, respectively; Fig. 3b). In the entire carcinoma areas, the patients whose TNBC tumors had a high total CD8 level or total CD8+/FOXP3+ ratio achieved significantly higher pCR rates than those with a low total CD8 level or total CD8+/FOXP3+ ratio (41 vs. 18 %, $P = 0.034$ and 44 vs. 14 %, $P = 0.002$, respectively; Fig. 3a, c).

Correlation between vasohibin-1, CD31 and pCR

The microvessel density was counted at the hot spot of CD31 staining, and the median was 39 (range 10–90) per field. Vasohibin-1 immunoreactivity was only detected in endothelial cells, and the median number of vasohibin-1-positive microvessels in the hot spot was 20 (range 6–60) per field. The median of VPR calculated was 0.618 (range

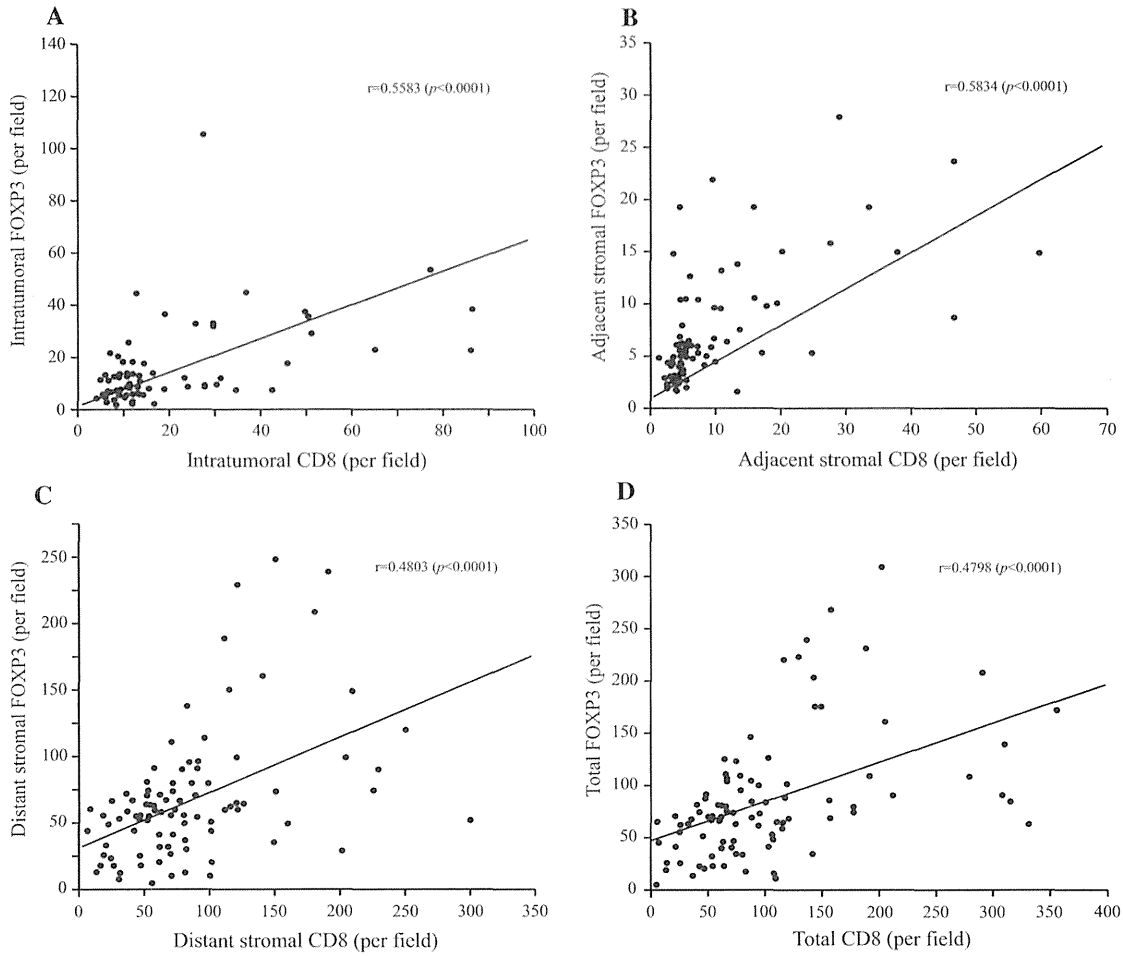

Fig. 2 The correlation diagrams of the infiltrating CD8+ and FOXP3+ lymphocytes in the compartments of each tumor: **a** the intra-tumoral compartment, **b** adjacent stromal compartment, **c** distant stromal compartment and **d** entire field. In this TNBC cohort, moderate positive correlations were observed between CD8+ lymphocyte infiltration and FOXP3+ lymphocyte infiltration in all of the compartments

0.152–0.917). Breast tumors were classified into high and low groups for vasohibin-1, CD31 and VPR, with the median. No significant differences in the vasohibin-1 or CD31 levels were detected between the pCR and non-pCR groups. However, low VPR tumors were significantly correlated with a higher pCR rate than high VPR tumors in the univariate analysis (odds ratio = 0.36, 95 % CI 0.14–0.92, $P = 0.031$; Table 2; Supplementary Figure).

Predictive value of the ratio of CD8+/FOXP3+ and the Ki-67 for pCR in multivariate analysis

The variables, including clinicopathological factors, CD8, FOXP3, vasohibin-1, CD31 and Ki-67, were investigated for their association with pCR after NAC, using multivariate analyses. Among these variables, earlier age at diagnosis ($P = 0.009$), pre-menopausal status ($P = 0.027$), smaller tumor size ($P = 0.004$) and a high Ki-67 ($P = 0.003$) were all significantly associated with pCR, as were a high CD8 level ($P = 0.013$), a high CD8+/FOXP3+ ratio ($P = 0.001$) and a low VPR level ($P = 0.031$; Table 2; Supplementary Figure). These significant variables and the suggestive variable "basal-like type" ($P = 0.075$) were all assessed in together to verify their predictive value for pCR in multivariate analysis. Results indicated that a high CD8+/FOXP3+ ratio was a markedly powerful predictor of pCR, with an odds ratio (OR) of 5.32 (95 % CI 1.62–19.98, $P = 0.005$). A high Ki-67 LI was also significantly associated with pCR (OR = 5.69, 95 % CI 1.83–20.24, $P = 0.002$; Table 2).

In addition, ROC curve analysis was performed for two independent factors: the CD8+/FOXP3+ ratio and the Ki-67. The area under the curve (AUC) was 0.708 for the CD8+/FOXP3+ ratio ($P = 0.012$) and 0.702 for Ki-67 ($P = 0.006$; Fig. 4). The sensitivity and specificity of the CD8+/FOXP3+ ratio and Ki-67 for pCR were summarized in Fig. 4. We also investigated the pCR rates of

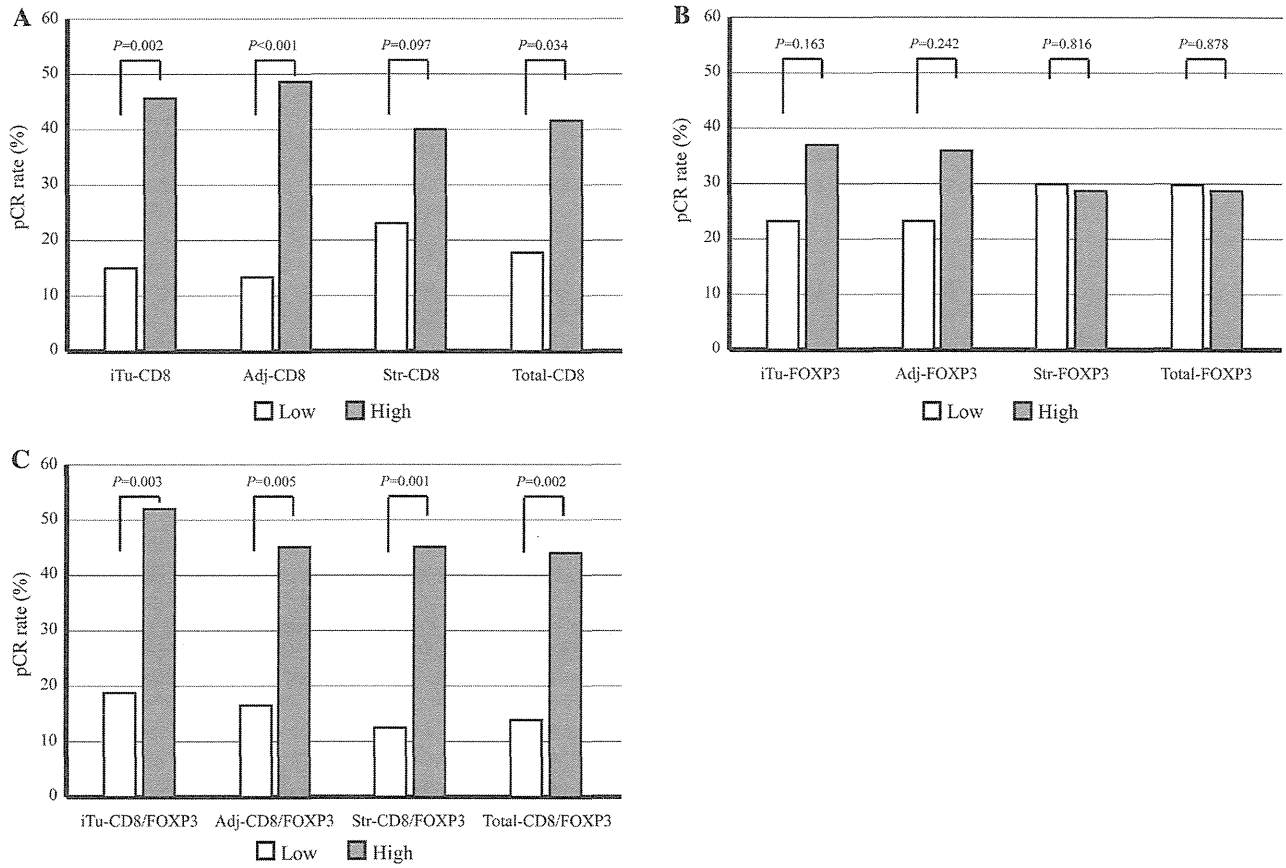

Fig. 3 Pathological complete response (pCR) rates between high and low groups for **a** CD8, **b** FOXP3 and the **c** CD8+/FOXP3+ ratio using the cut-offs of the each compartment was defined as the median number of infiltrating cells per field as follows: CD8, 4; FOXP3, 7 and CD8+/FOXP3+ ratio, 1 in the intra-tumoral (iTu) compartment: CD8, 4; FOXP3, 5 and CD8+/FOXP3+ ratio, 1 in the adjacent stromal (Adj) compartment: CD8, 70; FOXP3, 60 and CD8+/FOXP3+ ratio, 1 in the distant stromal (Str) compartment: CD8, 86.75; FOXP3, 73.25 and CD8+/FOXP3+ ratio, 1 in the entire field

TNBCs using the combination of the CD8+/FOXP3+ ratio and the Ki-67. Results demonstrated that TNBCs could be classified into several types that had extremely different pCR rates. The patients with breast tumors with a high CD8+/FOXP3+ ratio and a high Ki-67 achieved the highest pCR rate (70 %) following NAC. In contrast, the pCR rate for patients whose tumors had a low CD8+/FOXP3+ ratio and a low Ki-67 was only 5 % (Fig. 5). The pCR rate for patients whose tumors had a high CD8+/FOXP3+ ratio and a low Ki-67 and that had a low CD8+/FOXP3+ ratio and a high Ki-67 tumor were 24 and 21 %, respectively (Fig. 5).

Discussion

Results of this study indicated that a high CD8+/FOXP3+ ratio in biopsy specimens is an accurate predictor of pCR following NAC in TNBCs. Breast tumors with relatively high numbers of intra-tumoral CD8+ lymphocytes were reported to have favorable clinical outcomes [12–14] but the presence of FOXP3+ lymphocytes in breast tumors has been reported to be associated with paradoxically both reduced survival [12, 17] and improved survival [19]. Liu et al. reported that the density of intra-tumoral FOXP3+ infiltrates and the peritumoral CD8+/FOXP3+ ratio could represent independent prognostic factors correlated with the prognosis or clinical outcome of ER-positive or -negative breast cancer patients [12]. However, West et al. reported that intra-tumoral FOXP3+ lymphocytes were associated with robust anti-tumor immunity and a favorable prognosis in ER-negative breast cancer patients [19]. It is also important to note that the status of FOXP3+ was also by no means associated with breast cancer-specific survival after adjusting for known prognostic factors [14]. These discrepancies could be derived from the heterogeneity of the studied breast cancer patients. These studies were conducted for several breast cancer subtypes with different biological characteristics and treated with different therapeutic drugs; for example, endocrine therapy for

Table 2 Univariate and multivariate analyses of variables in the prediction for pCR in TNBC

	Univariate analysis			Multivariate analysis		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Age (≤50 vs. 50<)	3.43	1.34–9.31	0.009*	1.66	0.11–8.75	0.706
Menopausal status (pre vs. post)	2.86	1.13–7.52	0.027*	1.42	0.09–11.48	0.797
Tumor size (≤5.0 vs. 5.0 cm<)	4.01	1.75–12.01	0.004*	2.91	0.47–12.1	0.137
Nodal status (pos vs. neg)	1.78	0.51–8.36	0.383			
Grade (III vs. I, II)	1.32	0.52–3.47	0.571			
Basal-like versus non-basal-like	2.60	0.92–8.64	0.075	3.37	0.91–13.54	0.062
Ki-67 LI (high vs. low)	4.46	1.62–13.58	0.003*	5.69	1.83–20.24	0.002*
CD8 (high vs low)	3.52	1.29–10.42	0.013*	1.94	0.51–7.73	0.329
FOXP3 (high vs. low)	0.79	0.31–2.04	0.632			
CD8/FOXP3 (high vs. low)	4.93	1.82–15.09	0.001*	5.32	1.62–19.98	0.005*
Vasohibin-1 (high vs. low)	0.78	0.31–1.97	0.601			
CD31 (high vs. low)	0.91	0.09–9.06	0.939			
VPR (vasohibin-1/CD31; high vs. low)	0.36	0.14–0.92	0.031*	0.72	0.22–2.36	0.582

A logistic regression analyses were performed for univariate and multivariate analyses

OR odds ratio, *95 % CI* 95 % confidence interval, *LI* labeling index, *VPR* vasohibin-1 positive ratio

* The *P* value is significant

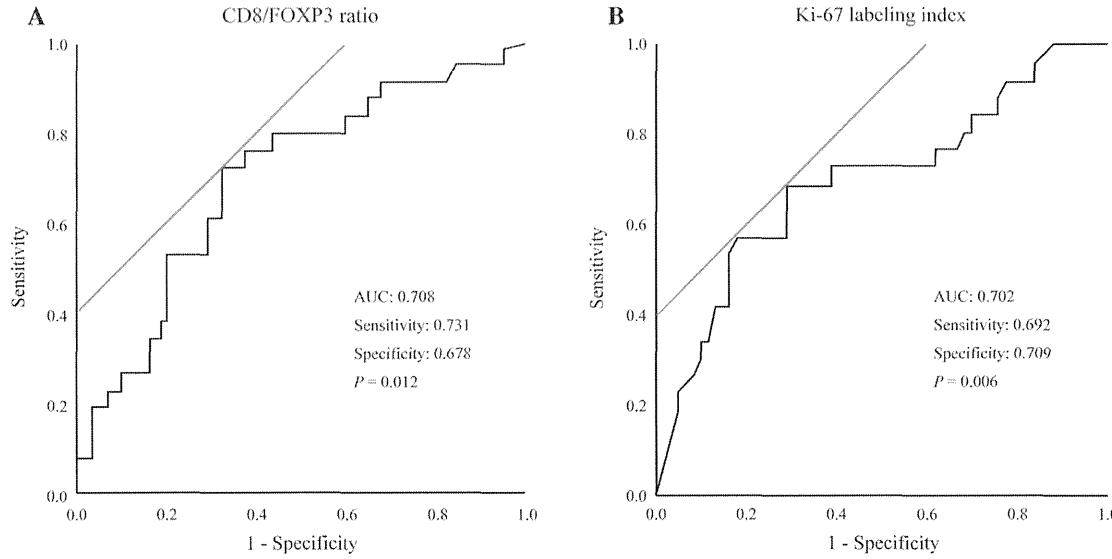

Fig. 4 ROC curve analysis for two independent factors: the CD8+/FOXP3+ ratio and the Ki-67 labeling index. *AUC* area under the curve

ER-positive breast cancers and anti-HER2 therapy for HER2-positive breast cancers, and the sensitivities to the drugs and the prognosis could markedly vary among different subtypes. The possible association between the prognostic significance of infiltrating lymphocytes and the breast cancer subtype have been explored by several investigators [33, 34]. Significant differences in the status of FOXP3+ infiltration and the CD8+/FOXP3+ ratio have been reported in the five molecular subtypes of breast cancer [12]. Different types of immune responses in different subtypes of breast cancer could, therefore, explain the contradictory results. Therefore, we conducted an immunohistochemical study of CD8 and FOXP3 only in the TNBC subtype, generally treated with current standard regimens containing anthracyclines and taxanes.

Biopsy specimens in the neoadjuvant setting that were not affected by treatment can be used to estimate the efficacy of drugs. This design is possible for TNBC, for which pCR after NAC has been validated as a reliable surrogate marker for survival. To the best of our knowledge, two studies evaluated the roles of CD8 and FOXP3 in biopsy specimens to predict pCR following NAC [18, 35]. Ladoire et al. reported that significantly decreased levels of FOXP3+ lymphocytes after NAC were more likely to generate strong anti-tumor immunity and achieved a high pCR rate in breast tumors harboring high levels of CD8+ lymphocytes remaining unchanged [35]. Oda et al. reported that FOXP3+ lymphocyte and the Ki-67 were both independent predictors of pCR [18]. However, these studies evaluated all breast cancer subtypes, including

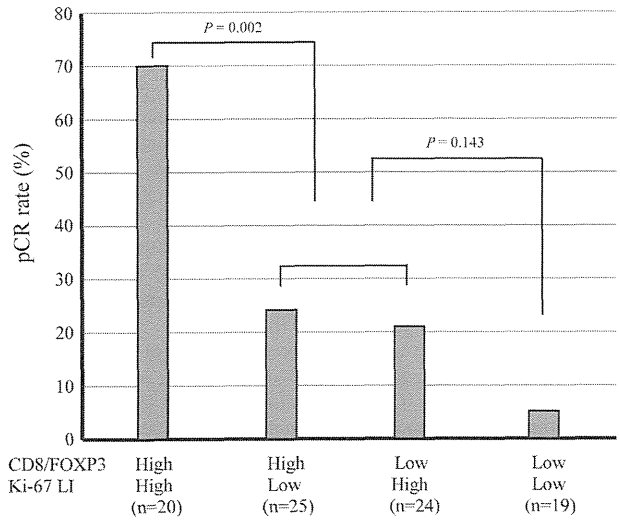

Fig. 5 Pathological complete response (pCR) rates of TNBC tumors using the combination of the CD8+/FOXP3+ ratio and the Ki-67 labeling index (LI)

approximately 20–30 TNBCs. In our present study, the predictive roles of status of intra-tumoral CD8 and FOXP3, together with neoangiogenesis, basal and proliferation markers, in biopsy specimens was investigated in a relatively large number of TNBC samples for NAC cohorts (slightly fewer than 100 patients). We set the cut-off value of high or low Ki-67 as the median number. For the reason, the statistical analyses could not be performed using the cut-off of 20 % that is recommended at the 13th St Gallen International Breast Cancer Conference 2013 because there were only eight cases (9 %) classified into low Ki-67 group in our cohort of aggressive triple-negative breast cancer [36]. Results of our present study did indicate that a high CD8+/FOXP3+ ratio and a high Ki-67 were significantly associated with pCR in a multivariate analysis. In addition, the patients whose breast tumors had a high CD8+/FOXP3+ ratio and a high Ki-67 achieved a 70 % pCR rate; therefore, these markers could be clinically valuable predictors of the response to NAC.

A few studies have recently examined the localization of CD8+ and FOXP3+ infiltrating lymphocytes to evaluate the microenvironment and expansion of lymphocytes in breast tumors [17, 19]. Mahmoud et al. reported that an association with adverse clinical outcome was detected in intra-tumoral and tumor-adjacent stromal FOXP3+ lymphocytes [17]. West et al. reported that the number of FOXP3+ lymphocytes was correlated with the number of CD8+ lymphocytes regardless of whether CD8+ cells were present in the tumor or stroma and that these levels were associated with prolonged survival [19]. In the present study, we performed the assessments of the localization using the immunohistochemical double-staining method for CD8 and FOXP3, allowing evaluation of the balance between CD8+ and FOXP3+ infiltrating lymphocytes in the same field of the same section. Statistically positive correlations were detected between CD8+ and FOXP3+ in each of the three compartments. Therefore, regardless of the localization in breast tumors, a high CD8 level or a high CD8+/FOXP3+ ratio was associated with a significantly higher pCR rate, and there was no difference between tumors that were infiltrated with high numbers and low numbers of FOXP3+ T cells. Based on these data, the same degree of anti-tumor immune response could be induced in each region.

We also investigated the VPR which was reported to be associated with neovascularization and breast cancer survival [26, 27]. The inverse correlation between the CD8+/FOXP3+ ratio and the VPR was detected in our TNBCs. Neoangiogenesis factors, including vasohibin-1, which is a negative feedback suppressor induced by vascular endothelial growth factor (VEGF)-A, were previously reported to suppress tumor-infiltrating lymphocytes and anti-tumor immune responses [37, 38]. Results of our study demonstrating the inverse correlation between the CD8+/FOXP3+ ratio and the VPR were of interest. The tumors with a low VPR were significantly correlated with a higher pCR than those with a high VPR in the univariate analysis and no significant association of VPR with pCR was detected in the multivariate analysis.

In summary, results of our present study demonstrated that both CD8+/FOXP3+ ratio and the Ki-67 were independent predictors of pCR in TNBC patients treated with the current standard regimens of NAC. By examining both the CD8+/FOXP3+ ratio and the Ki-67, which are conveniently available in the great majority of diagnostic laboratories, we could predict both the subgroup expected to have a high pCR rate and subsequently favorable prognosis and the subgroup that should be treated with new investigational drugs. However, results in our present retrospective study need to be validated in a prospective study.

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Conflict of interest None.

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ORIGINAL ARTICLE

Prognostic significance of the progesterone receptor status in Ki67-high and -low Luminal B-like HER2-negative breast cancers

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Abstract
Background Breast cancer is a heterogeneous disease, and immunohistochemical evaluation is a surrogate marker that is widely used in clinical settings to identify the intrinsic subtypes. The definition of the Luminal B-like breast cancer was changed at the 2013 St. Gallen meeting; therefore, we investigated the clinicopathological features of the new Luminal B-like breast cancer categorized in the latest definition. We also compared the conventional PgR-high Luminal B-like breast cancer with the conventional PgR-low or -negative Luminal B-like breast cancer.
Patients We investigated 118 Luminal HER2-negative breast cancer patients who were operated in 2005–2008 at a single institution. Data on each patient's medical history were retrieved.
Results A subset of patients (14.4 %) was categorized as the new Luminal B-like due to low or negative PgR: 58.8 % were histological grade I, 65 % were T1 in tumor size, and half had node involvement. Chemotherapy was performed in half of the cases. Breast cancer-related events were more frequent for the new Luminal B-like breast cancer than for the Luminal A-like breast cancer and were less frequent than for the conventional Luminal B-like breast cancer. Based on multivariate analysis, low or negative expression of PgR and the absence of hormonal therapy were worse prognostic factors. When categorized into two groups by the PgR status, 48.1 % of the conventional Luminal B-like breast cancer was PgR-high; tumor size was smaller, and nodal involvement was less in this group. The rate of adjuvant chemotherapy of the conventional PgR-high Luminal B-like breast cancer was less than that of the conventional PgR-low or -negative Luminal B-like breast cancer. Breast cancer-related events were significantly lower in the conventional PgR-high Luminal B-like breast cancer.
Conclusions Our results show the possibility that PgR status has some influence on the prognosis for Luminal HER2-negative breast cancers. Therefore, attention should be paid to the PgR status as well as Ki-67.

Keywords Luminal B-like · PgR · Ki-67 · Prognosis · Adjuvant chemotherapy

Introduction

Breast cancer is a heterogeneous disease, and gene expression studies have revealed different intrinsic molecular subtypes with different prognosis [1–5]. However, gene expression-based assays are not readily available worldwide because of their cost and assay times [6]. Thus, substitutional immunohistochemical (IHC) assays are still being widely used to analyze surrogate markers for identifying intrinsic molecular subtypes in a clinical setting.

The definition of the Luminal B-like HER2-negative breast cancer was changed at the 2013 St. Gallen

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International Breast Cancer Conference meeting. In short, ER-positive/PgR-low or -negative/Ki-67-low breast cancers are newly categorized into the "Luminal B-like" breast cancer [2]. This recommendation is based on only one study which concluded that IHC expression of PgR was useful to add prognostic value within the Luminal A-like or B-like breast cancer definition by improving the identification of outcome of breast cancers [7]. A PgR cut-point of ≥20 % is recommended; however, whether the PgR cut-point is suitable for all laboratory examinations is still controversial [2, 8, 9]. Thus, assurance programs should be essential for discussing this matter and necessary to verify the status of PgR in Asian patients.

Here, we investigated the clinicopathological features of the "new Luminal B-like" breast cancer and compared them with other Luminal HER2-negative breast cancer. At the same time, focusing on the PgR status, we compared the conventional PgR-high/Ki67-high Luminal B-like breast cancer with the conventional PgR-low or -negative/Ki67-high Luminal B-like breast cancer.

Patients and methods

We investigated 118 female Japanese patients who had Luminal HER2-negative primary breast cancers and underwent surgery in January 2005 to December 2008 at the Department of Surgical Oncology, Tohoku University Hospital. Comprehensive agreement was obtained to use specimen for clinical research in every cases and institutional review board approval was obtained for the present study. The data on each patient's medical history were retrieved. Surgically removed breast cancer specimens were used, and pathological examination was performed. All of the cancers were invasive ductal carcinoma, and other histological types were excluded. We performed staining with hematoxylin–eosin (H&E) and IHC for ER, PgR, HER2 and Ki67. Surgical specimens were fixed in 10 % formalin, embedded in paraffin, cut into 4 μm-thick sections, and placed on glue-coated glass slides. To determine the hormone receptor status, we employed the avidin–streptavidin immunoperoxidase method using the clone 6F11 antibody (Ventana, Tucson, AZ, USA) for ER, the clone 6 antibody (Ventana) for PgR, and the MIB-1 mouse monoclonal antibody (code M7240: Dako, Copenhagen, Denmark) for Ki-67 immunohistochemistry. A standardized immunohistochemistry kit (HercepTest for Immunoenzymatic Staining: Dako, Copenhagen, Denmark) was used for HER2 staining.

We examined the surgical specimens for the following parameters: histological type, histological grade, ER, PgR, HER2, Ki-67 and nodal involvement. Histopathological evaluations were based on the World Health Organization histological classification of tumors of the breast and Rosen's Breast Pathology. ER status was determined by nuclear staining, graded from 0 to 8 using the Allred score [10] and was categorized as positive when the score was 3 or more. The PgR status was determined as the percentage of nuclear staining and was considered high when 10 % or more. Additionally, we set another cut-point of PgR as 20 % recommended at the 2013 St. Gallen meeting. With regard to HER2 evaluation, membranous staining was graded as follows: score 0, 1+, 2+ and 3+. A score of 0 was defined as no staining or membrane staining in <10 % of tumor cells, and 1+ was defined as faint/barely perceptible membrane staining detected in ≥10 % of the tumor cells. A score of 2+ was assigned when there was weak to moderate complete membrane staining in >10 % tumor cells, whereas a score of 3+ consisted of uniform, intense membrane staining in ≥10 % tumor cells. Tumors scored 2+ were further subjected to FISH assays to assess gene amplification. A HER2 score of 3+ or successful gene amplification was determined HER2-positive cancer [11]. Ki67 was determined by nuclear staining at the hot spots and was considered high when 20 % or more cells were stained [12].

Definition of each subtype

We defined each Luminal HER2-negative breast cancer as follows. (1) Luminal A-like: PgR-high/Ki67-low, (2) new Luminal B-like: PgR-low or -negative/Ki67-low and (3) conventional Luminal B-like: any PgR/Ki67-high. And we divided the conventional Luminal B-like subtype into two group for discussion. (4) conventional PgR-high Luminal B-like: PgR-high/Ki67-high and (5) conventional PgR-low or -negative Luminal B-like: PgR-low or -negative/Ki67-high.

Statistics

Statistical analyses were performed using JMP Pro 11.0.0 software (SAS, North Carolina, USA). The Fisher's exact test and the Chi square test for trends were used to assess the association between categorical and ordinal variables, respectively. The primary end-points were the incidence of locoregional relapse, contralateral breast cancer, distant metastases and breast cancer-related events. Locoregional relapse included breast cancer in the chest wall and skin of the ipsilateral breast. Distant metastasis included all sites of recurrence, except locoregional relapses and contralateral breast cancer. When locoregional relapse and distant metastasis occurred simultaneously, it was included as a distant metastasis case. Breast cancer-related events included locoregional relapses, distant metastases and contralateral breast cancer. Cumulative incidence was

drawn using the Kaplan–Meier method, and the Cox proportional hazards model was used to estimate the hazard ratio.

Results

Characteristics of each Luminal breast cancers

Patient characteristics are shown in Table 1. The median age of the 118 patients was 55.0 years (range 27–87 years) and was not significantly different among any subtypes. A subset of patients (14.4 %) was categorized into the new Luminal B-like breast cancer with the cut-point of PgR as 10 %. 62.7 % were placed into the Luminal A-like breast cancer (Table 1); 22.9 % of patients were categorized into the conventional Luminal B-like breast cancer. Nearly all cases placed into the new Luminal B-like and the Luminal A-like breast cancer were histological grade I or II, whereas approximately one-thirds of the conventional Luminal B-like breast cancer were grade III. Sixty-five percent of tumors in the new Luminal B-like breast cancer were smaller than 2 cm (T1); 82 % of the Luminal A-like breast cancer and 56 % of the conventional Luminal B-like breast cancer were T1.

About half of cases in the new Luminal B-like breast cancer had node involvement. Eighteen percent of the Luminal A-like breast cancer and half of the conventional Luminal B-like breast cancer were node positive.

Approximately half of the new Luminal B-like breast cancer, 23 % of the Luminal A-like breast cancer and over half of the conventional Luminal B-like breast cancer had received chemotherapy.

Significant difference was observed in Histological grade ($p = 0.0001$), nodal status ($p = 0.0022$) and chemotherapy status ($p = 0.0043$). The Luminal A-like and

Table 1 Patient characteristics

	Total	Luminal A-like	New Luminal B-like	Conventional Luminal B-like	*p* value
Number	118	74	17	27	
Age					0.3641*
<35	4 (3.4)	1 (1.4)	0 (0.0)	3 (11.1)	
35–49	34 (28.8)	24 (32.4)	5 (29.4)	5 (18.5)	
50–69	57 (48.3)	35 (47.3)	8 (47.1)	14 (51.9)	
≥70	23 (19.5)	14 (18.9)	4 (23.5)	5 (18.5)	
PgR					
<10 %	31 (26.3)	0 (0.0)	17 (100)	14 (51.9)	
≥10 %	87 (73.7)	74 (100)	0 (0.0)	13 (48.1)	
Histological Grade					0.0001*
I	56 (47.5)	44 (59.5)	10 (58.8)	2 (7.4)	
II	51 (43.2)	28 (37.8)	7 (41.2)	16 (59.3)	
III	11 (9.3)	2 (2.7)	0 (0.0)	9 (33.3)	
Ki-67 labeling index					
<20 %	91 (77.1)	74 (100)	17 (100)	0 (0.0)	
≥20 %	27 (22.9)	0 (0.0)	0 (0.0)	27 (100)	
T					0.0502*
1	87 (73.7)	61 (82.4)	11 (64.7)	15 (55.6)	
2	21 (17.8)	9 (12.2)	4 (23.5)	8 (29.6)	
3	1 (0.8)	1 (1.4)	0 (0.0)	0 (0.0)	
4	9 (7.6)	3 (4.1)	2 (11.8)	4 (14.8)	
Nodal status					0.0022**
Negative	84 (71.2)	61 (82.4)	9 (52.9)	14 (51.9)	
Positive	34 (28.8)	13 (17.6)	8 (47.1)	13 (48.1)	
Chemotherapy					0.0043**
Yes	40 (33.9)	17 (23.0)	8 (47.1)	15 (55.6)	
No	78 (66.1)	57 (77.0)	9 (52.9)	12 (44.4)	
Hormonal therapy					1.0000*
Yes	115 (97.5)	72 (97.3)	17 (100)	26 (96.3)	
No	3 (2.5)	2 (2.7)	0 (0.0)	1 (3.7)	

Values in parentheses are percentages of the number in each subtype

T Primary tumor size, *T1* tumor ≤20 mm in greatest dimension, *T2* >20 mm but ≤50 mm, *T3* > 50 mm, *T4* tumor of any size with direct extension to the chest wall and/or to the skin

* Fisher's exact test was used to estimate *p* values

** χ^2 test was used to estimate *p* values

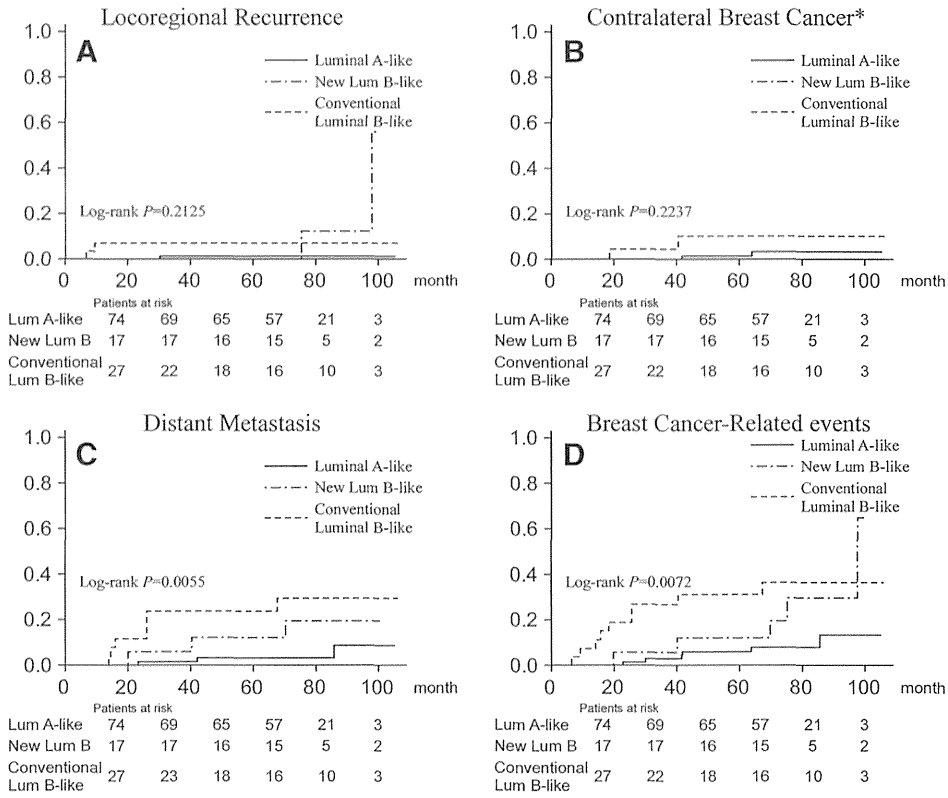

Fig. 1 Clinical outcomes of 118 women with Luminal subtype breast cancer. The incidence of locoregional relapse (**a**), contralateral breast cancer (**b**), distant metastases (**c**) and breast cancer-related events (**d**). When locoregional relapse and distant metastasis occurred at the same time, it was included as a distant metastasis case. * We experienced no contralateral breast cancer in the new Luminal B-like breast cancers

the new Luminal B-like breast cancers were low histological grade than the conventional Luminal B-like (Luminal A-like vs. conventional Luminal B-like: $p < 0.0001$, new Luminal B-like vs. conventional Luminal B-like: $p = 0.0002$). The Luminal A-like breast cancer had less nodal involvement than the other breast cancer (Luminal A-like vs. new Luminal B-like: $p = 0.0141$, Luminal A-like vs. conventional Luminal B-like: $p = 0.0027$). Chemotherapy was less applied to the Luminal A-like breast cancer than to the conventional Luminal B-like breast cancer ($p = 0.0023$).

Breast cancer-related events were more frequent for the new Luminal B-like breast cancer than for the Luminal A-like breast cancer and were less frequent for the conventional Luminal B-like subtype (Fig. 1). Significant differences in distant metastatic-free survival and breast cancer-related events among the three luminal groups were observed.

In detail, the new Luminal B-like and the Luminal A-like breast cancer experienced locoregional recurrence or contralateral breast cancer that were considered non-life threatening and could be completely resected by re-operation. However, distant metastasis occurred more often for the conventional Luminal B-like breast cancer than in the other groups.

When 20 % was used for the cut-point of PgR, same tendency was observed compared with when 10 % was used (Suppl. Figure 1).

PgR status as a prognostic factor for Breast cancer-related events

Based on the univariate analysis (Table 2), low or negative expression of PgR (PgR-high vs. -low or -negative, HR 4.79, 1.98–12.25, 95 % CI), high histological grade (grade I/II vs. III, HR 5.08, 1.64–13.34, 95 % CI), high Ki-67 expression (Ki-67 < 20 % vs. ≥ 20 %, HR 3.12, 1.25–7.56, 95 % CI), large tumor size ($T \leq 2$ cm vs. > 2 cm, HR 3.03, 1.21–7.35, 95 % CI) and not receiving hormonal therapy (hormonal therapy yes vs. no, HR 17.56, 2.71–65.68, 95 % CI) were worse prognostic factors in this study.

Based on the multivariate analysis (Fig. 2), low or negative expression of PgR (PgR-high vs. -low or -negative, HR 3.80, 1.40–10.88, 95 % CI) and not receiving hormonal therapy (hormonal therapy yes vs. no, HR 37.32, 4.71–194.32, 95 % CI) were worse prognostic factors.

When 20 % was used for the cut-point of PgR, we obtained the results as follows (Suppl. Figure 2); Based on the multivariate analysis, not receiving hormonal therapy (hormonal therapy yes vs. no, HR 34.52, 4.48–175.32, 95 % CI) were worse prognostic factors. Expression of PgR was not so powerful prognostic factor (PgR-high vs. -low or -negative, HR 2.27, 0.81–6.63, 95 % CI).

Table 2 Univariate analysis of 118 women with Luminal subtype breast cancer

	Univariate analysis		
	HR	(95 % CI)	*p* value
PgR			0.0006
≥10 %	1.00		
<10 %	4.79	(1.98–12.25)	
Histological Grade			0.0073
I/II	1.00		
III	5.08	(1.64–13.34)	
Ki-67 labeling index			0.0158
<20 %	1.00		
≥20 %	3.12	(1.25–7.56)	
Tumor size			0.0187
≤2 cm	1.00		
>2 cm	3.03	(1.21–7.35)	
Hormonal therapy			0.0066
Yes	1.00		
No	17.56	(2.71–65.68)	
Nodal status			0.0817
Negative	1.00		
Positive	2.24	(0.90–5.44)	
Chemotherapy			0.2403
No	1.00		
Yes	1.71	(0.69–4.13)	

Univariate analysis of clinicopathological factors for Breast cancer-related events

HR hazard ratio, *CI* confidence intervale

The conventional PgR-high Luminal B-like breast cancer had better prognosis compared with the conventional PgR-low or -negative Luminal B-like breast cancer

Furthermore, we categorized the conventional Luminal B-like breast cancer into two groups based on the PgR status (Table 3). Nearly half (48.1 %) of the conventional Luminal B-like breast cancer were PgR-positive, and 51.9 % were PgR-low or -negative. The histological grade was II in nearly all of the conventional PgR-high Luminal B-like breast cancer, whereas 51.7 % of the conventional PgR-low or -negative Luminal B-like breast cancer was grade III. The tumor size was smaller for the conventional PgR-high Luminal B-like breast cancer compared with the conventional PgR-low or -negative Luminal B-like breast cancer. Slightly more than a third (38.5 %) of the conventional PgR-high Luminal B-like breast cancer and 57.1 % of the conventional PgR-low or -negative Luminal B-like breast cancer were node positive. Thus, the percentage of patients in the conventional PgR-high Luminal B-like breast cancer who received adjuvant chemotherapy was less than that of the conventional PgR-low or -negative Luminal B-like breast cancer (PgR-high, 38.5 % vs. -low or -negative, 71.4 %). In both groups, nearly all patients received adjuvant hormonal therapy.

The number of breast cancer-related events in the conventional PgR-high Luminal B-like breast cancer was significantly lower than that of the conventional PgR-low or -negative Luminal B-like breast cancer. The conventional PgR-high Luminal B-like breast cancer experienced only contralateral breast cancer and then could be resected properly (Fig. 3).

In addition, same result was observed when 20 % was used for the cut-point of PgR (Suppl. Figure 3).

Discussion

In this study, we investigated the more suitable IHC-based surrogate definition of Luminal breast cancers focusing on the expression level of PgR and Ki-67. First, we investigated the features of the new Luminal B-like breast cancer. Their clinicopathologic features and event-free survival were between those of the Luminal A-like and the

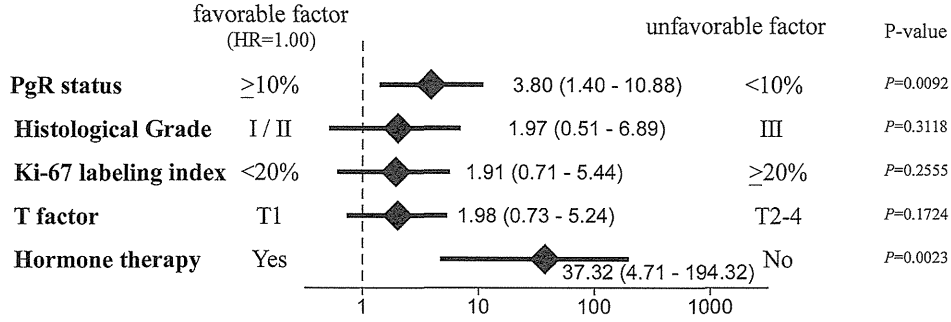

Fig. 2 Multivariate analysis in 118 women with Luminal subtype breast cancer. Multivariate analysis of clinicopathological factors for Breast cancer-related events. Hazards ratio (HR) and 95 % confidence intervals (CI) obtained from a multivariable COX proportional hazards model that was adjusted for PgR status, histological grade, Ki-67, tumor size, hormonal therapy

Table 3 Characteristics of conventional Luminal B-like breast cancer according to the PgR status

	Conventional PgR-high Luminal B like	Conventional PgR-low or negative Luminal B-like	*p* value
Number	13	14	
Age			0.5851*
<35	1 (7.7)	2 (14.3)	
35–49	1 (7.7)	4 (28.6)	
50–69	8 (61.5)	6 (42.9)	
≥70	3 (23.1)	2 (14.3)	
PgR			
<10 %	0 (0.0)	14 (100)	
≥10 %	13 (100)	0 (0.0)	
Histological Grade			0.0160*
I	1 (7.7)	1 (7.1)	
II	11 (84.6)	5 (35.7)	
III	1 (7.7)	8 (57.1)	
Ki-67 labeling index			
<20 %	0 (0.0)	0 (0.0)	
≥20 %	13 (100)	14 (100)	
T			0.3702*
1	9 (69.2)	6 (42.9)	
2	3 (23.1)	5 (35.7)	
3	0 (0.0)	0 (0.0)	
4	1 (7.7)	3 (21.4)	
Nodal status			0.3317**
Negative	8 (61.5)	6 (42.9)	
Positive	5 (38.5)	8 (57.1)	
Chemotherapy			0.0850**
Yes	5 (38.5)	10 (71.4)	
No	8 (61.5)	4 (28.6)	
Hormonal therapy			0.3261**
Yes	13 (100)	13 (92.9)	
No	0 (0.0)	1 (7.1)	

Values in parentheses are percentages of the number in each subtype

* Fisher's exact test was used to estimate *p* values

** χ^2 test was used to estimate *p* values

conventional Luminal B-like breast cancer. However, the frequency of node involvement in the new Luminal B-like subtype showed the same tendency as the conventional Luminal B-like breast cancer; therefore, the new luminal B-like patients received adjuvant chemotherapy as frequently as the conventional Luminal B-like patients.

A higher rate of relapse in the new Luminal B-like subtype patients might be due to the PgR status, which is influenced by the efficacy of hormonal therapy. Previous analyses investigated whether the PgR status provided additional value to the ER status and improved the prediction of benefit from endocrine treatment among patients with primary breast cancer who received adjuvant endocrine therapy [13–15]. The authors concluded that PgR-negative patients benefit less from adjuvant endocrine therapy than PgR-positive patients and that they may want to consider chemotherapy [8, 13]. Another study that investigated the Ki-67 status during endocrine therapy revealed that Ki-67 was reduced to a greater extent in PgR-positive cancers when compared with PgR-negative cancers [16], suggesting that treating PgR-negative cancers with hormonal therapy could not completely prevent cancer cell activation. Other researchers showed that a PgR-negative status was an important contributor to the relapse risk in early [17] and late periods [18]. The same result was published for metastatic breast cancer. In women with ER-positive metastatic breast cancer, PgR-positive patients had significantly longer overall survival than PgR-negative patients. Therefore, they also concluded that the PgR status is an important prognostic factor [19].

Next, we investigated the influences of PgR status on the conventional Luminal B-like breast cancer. Interestingly, although the Ki-67 expression was high, the conventional PgR-high Luminal B-like breast cancer experienced less breast cancer-related events than the conventional PgR-low or -negative Luminal B-like breast cancer, and the rate of adjuvant chemotherapy showed a similar trend. In our study, the difference in the histological grade of the groups was significant ($p = 0.0160$). A previous study reported that the histological grade (I, II vs. III) was a more powerful prognostic factor for chemotherapy responsiveness and recurrent-free survival than Ki-67 expression in Luminal (HER2-negative) breast cancer [20]. Our results support this result; therefore, different strategies may be necessary for distinguishing the conventional PgR-high Luminal B-like breast cancer and the conventional PgR-low or -negative Luminal B-like breast cancer. This emphasizes the questions regarding whether chemotherapy regimen is better for treating the conventional PgR-high Luminal B-like breast cancer, or whether adjuvant chemotherapy is really needed. At the same time, the rate of node involvement was also lower in these breast cancers. The reason for higher node involvement in the conventional PgR-low or -negative Luminal B-like might be explained by the following. A previous study indicated that PgR expression decreased due to recurrence [21], and the PgR status changing from positive to negative was a poor prognostic factor [22]. In the same way, the PgR status of breast cancer changing from positive to negative might be a trigger, and as a result, breast cancer could obtain the worse property of metastasizing to axillary lymph nodes.

Third, we examined which value is more suitable for a PgR cut-point (Suppl. Figure 1–3). Based on the multivariate analysis (Fig. 2, Suppl. Figure 2), the value of 10 % for PgR cut-point was stronger prognostic factor than the

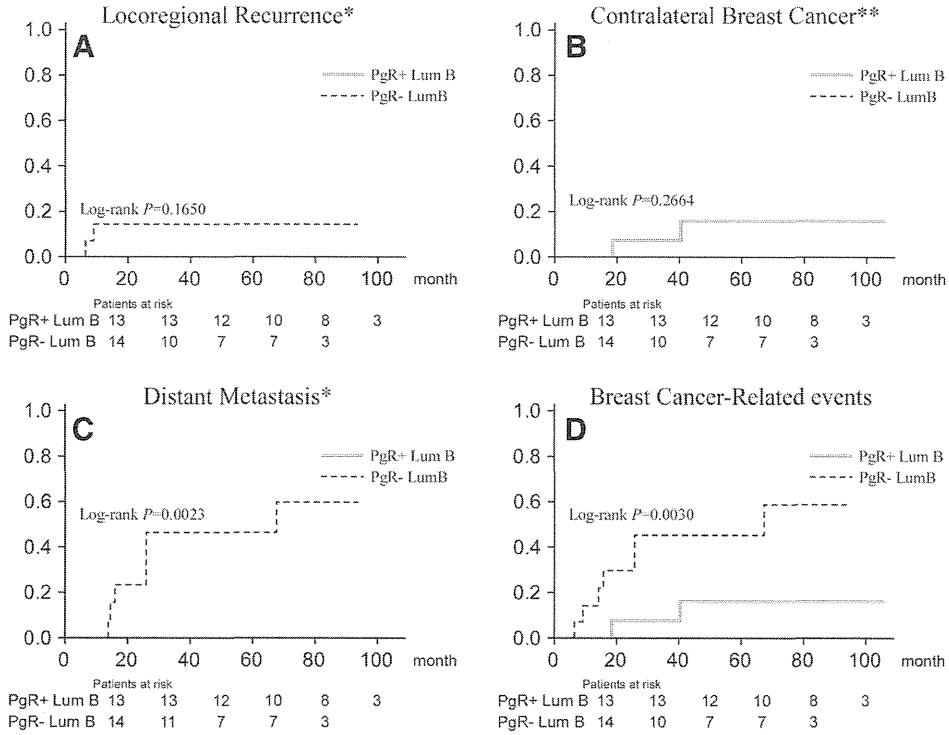

Fig. 3 Clinical outcomes of conventional Luminal B-like subtype breast cancer. PgR + LumB: conventional PgR-high Luminal B-like subtype breast cancer, PgR-LumB: conventional PgR-low or -negative Luminal B-like subtype breast cancer. The incidence of locoregional relapse (**a**), contralateral breast cancer (**b**), distant metastases (**c**) and breast cancer-related events (**d**). * We experienced no locoregional recurrence or distant metastasis in the conventional PgR-high Luminal B-like breast cancers. ** We experienced no contralateral breast cancer in the conventional PgR-low or -negative Luminal B-like breast cancers

value of 20 % (10 %: HR 3.80, $p = 0.0092$, 20 %: HR 2.27, $p = 0.1170$). However, the number of breast cancer-related events was not so different between when the value of 10 % was used for a PgR cut-point and when the value of 20 % was used (Suppl. Figure 1, 3). It is ture that the number of the patients was relatively small but the results still provided sufficient evidence to support the value of the PgR cut-point. Further investigations employing larger numbers of patients with longer periods of clinical follow-up may be required for determining the most clinically relevant PgR cut-point.

The new Luminal B-like and the conventional Luminal B-like breast cancers are still considered a heterogeneous group of breast cancers [8]. As mentioned above, we demonstrated using multivariate analysis that the PgR status was one of the most important prognostic factors for the Luminal-like HER2 negative breast cancer; new Luminal B-like breast cancer had better prognosis than conventional Luminal B-like breast cancer. In conventional Luminal B-like breast cancer alone, the PgR status was also important prognostic factor. This result suggests the possibility of different therapeutic approach whether chemotherapy is really needed or not for the conventional PgR-high Luminal B-like breast cancer. Although the present study had some limitations in that it was retrospective and included a relatively small number of patients and a short period, it was the first time that the clinicopathological feature of the Luminal B-like breast cancer categorized in the latest definition was investigated and the importance of the PgR status was reported from Asian single institution. And this results might provide a clue for designing better treatments for Luminal HER2-negative breast cancers.

Conclusion

Our results showed the possibility that PgR status has some influence on the prognosis of the Luminal HER2-negative breast cancers. To improve the outcome of Luminal HER2-negative breast cancers, the PgR status as well as the Ki67-labeling index should be considered.

Conflict of interest The authors declare that they have no conflict of interest.

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ABSTRACT

Role of ultrasonography in breast cancer screening

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The purpose of cancer screening is to reduce cancer mortality. While mammography is the only method of breast cancer screening for which there is established evidence of a reduction in mortality, low sensitivity of the technique in dense breasts is a serious issue to be resolved. Although ultrasonography is a useful candidate for overcoming this problem, the recent trend toward introducing ultrasonography for population-based screening is still too early. Ultrasonography may offer higher sensitivity because it can detect breast cancer at an early stage based on mass shape, even in the dense parenchyma of premenopausal women. However, there is no manual on a standardized method of examination, no diagnostic criteria, and no evidence that ultrasonography screening reduces mortality. The Japan Strategic Anticancer Randomized Trial (J-START) was the first large-scale RCT to verify the quality and effectiveness of ultrasonography for breast cancer screening in women aged 40–49 years. The primary endpoints of this trial were inter-group comparisons of sensitivity and specificity, and the secondary endpoint was the inter-group comparison of the accumulated incidence rate of advanced breast cancer during the follow-up period. The primary and secondary endpoints are expected to be published at the end of 2014. Endorsement of the new modality is expected to increase the detection rate; however, it might also increase the recall rate, which would be an adverse effect of screening. It is necessary to make ultrasonography screening available, but it should not be introduced too hastily until evidence has been established.

(HEP. 2014;41:315-321.)

KEY WORDS **breast cancer screening, mammography, ultrasonography, J-START**

特集 総合健診とがん

乳がん検診における超音波診断の役割

鈴木 昭彦[1)] 石田 孝宣[2)] 大内 憲明[2)]

抄 録

がん検診の目的は、受診者の当該がんの死亡率の低減にある。この目的を達成するために、科学的に有効性の証明された検診方法で、正しく検診が行われることが重要で、さらに多くの対象者が受診することで検診対象集団の中での効果が明らかとなる。乳がん対策の分野で、現時点で科学的な死亡率低減の根拠が示された検診方法はマンモグラフィをベースとした検診のみであるが、近年、任意型検診や一部の対策型検診において超音波検査を導入する動きがある。超音波検診は標準的な検査方法や診断基準が確立されておらず、乳がん死亡率の低減効果も証明されていない。検診には検査の偽陽性や追加の画像検査、身体的侵襲をともなう生検、精神的および経済的負担の増加、過剰診断などの多くの不利益もともなうため、検診の利益が不利益を確実に上回ることが証明されていない検診は、安易に取り入れられるべきではない。我が国では世界に先駆けて2006年から「乳がん検診における超音波検査の有効性を検証するための比較試験（J-START）」が進行中であり、その解析結果によって超音波検査を乳がん検診の中で有効に運用していくシステムが構築されてゆくであろう。検診が真に有用であることとは、発見率のみならず、精度管理や、多くの不利益に対する検証も行われなければならず、エビデンスの確立していない現時点での拙速な超音波検査の導入は、慎重に判断しなければならない。

（総合健診，2014；41：315-321.）

キーワード 乳がん検診、マンモグラフィ、超音波検診、J-START

▶▶▶ はじめに

乳がんに限らずがん検診の目的は、受診者の当該がんの死亡率の低減にある。この目的を達成するために、科学的に有効性の証明された検診方法で、正しく検診が行われることが重要で、さらに多くの対象者が受診することで検診対象集団の中での効果が明らかとなる。乳がん対策の分野で、現時点で科学的な死亡率低減の根拠が示された検診方法はマンモグラフィをベースとした検診のみであるが、画像診断技術の向上に伴い、若年者・高濃度乳房への対策としての超音波検査の追加によって検診精度を高めようとする動きがある。本稿では、我が国の乳がん検診の現況と、精度向上を目的とした超音波診断の役割に関して解説する。

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▶▶▶ 乳がん検診の現況

（1）乳がん検診の変遷

我が国では1981年に「がん（悪性新生物）」が死亡率の第一位となり、がん対策の重要性が認識されるようになった。国策としての検診は1982年のからの第一次老人保健事業の中で胃がんと子宮頸がんの検診が開始された（表1）。乳がんは1987年の第二次老人保健事業において導入された。しかしながら、導入された検診方法は視触診単独による検診であり、その有効性に関する科学的根拠や検証の無いままの導入であった。その後、生存率の比較による研究および症例対照研究が行われたが、明らかな死亡率減少効果は証明されず、1998年3月のがん検診の有効性評価に関する研究班報告書[1)]では「視触診による乳がん検診は、無症状の場合は死亡リスク低減効果が認められるが、有効性を示す根拠は必ずしも十分でない」とされ、有効性の証明された検診への転換が急務となった。一方、欧米では1960年代から既にマンモグラフィ検診の有効性の検証が行われ

表1　日本の乳がん検診の歴史

1969年	厚生省がん予防対策要綱
1983年	老人保健法胃がん・子宮頸がん検診が対象
1987年	肺がん、乳がん、子宮体がん検診追加
1999年	がん検診の有効性に関する研究報告書
1999年	がん検診の一般財源化
2000年	老健第65号によりマンモグラフィ導入 50歳以上・2年に一度
2001年	新たながん検診手法の評価報告書
2004年	がん検診に関する検討会中間報告書
2004年	老老発第0427001号通達 マンモグラフィを原則とする 40歳代・2方向・2年に一度
2005年	厚生労働省がん検診検討会「老人保健事業に基づく乳がん検診及び子宮がん検診における事業評価の手法について」

ており、科学的根拠が証明された検診方法として定着していた。我が国においてマンモグラフィ検診は1989年から検証が行われ[2,3]、その結果は欧米における結果と比較しても遜色のないものであることが報告され、我が国においても画像診断を基礎とした検診方法へと転換することとなった。現行のマンモグラフィによる乳がん検診は2000年3月に‘がん予防重点健康教育及びがん検診実施のための指針’（老健第65号）を改訂し、50歳以上の女性に対して導入された。さらに2003年12月に厚生労働省に設置された‘がん検診に関する検討会’において、乳がん検診のありかた、特に40歳代へのマンモグラフィ導入の是非とその具体的方法が審議され、2004年3月表1に示す中間報告がまとめられた。これを受けて厚生労働省は2004年4月27日、‘乳がんについては、マンモグラフィ（乳房エックス線検査）を原則として実施すること’（老老発第0427001号）と指針を一部改訂し、検診対象年齢を40歳以上、受診間隔を2年に1度とする現行方式を推奨することになった。

（2）検診実施状況

乳がん検診の実施主体である市区町村単位の実施状況は、厚生労働省の平成25年度の 調査[4]によれば、調査対象となった全国1,738市区町村のうち、1,735市区町村から返答があり、未回答の3市区町村を除く1,735の市区町村で乳がん検診が行われている（表2）。検診方法は国の基準に従ったマンモグラフィを原則とする方法が99％（1,718市区町村）で採用されていたが、検診に超音波検査を追加する方法を行っている市区町村が547件（31.5％）、検診の対象者を国の指針より拡大（30歳代への検診か）している市区町村が595件（34.3％）あった。一方で国の指針より対象者を制限している市区町村も721件（44.9％）みられ、定員を設けて先着順や抽選で対象者を絞り込む方法がとられている。

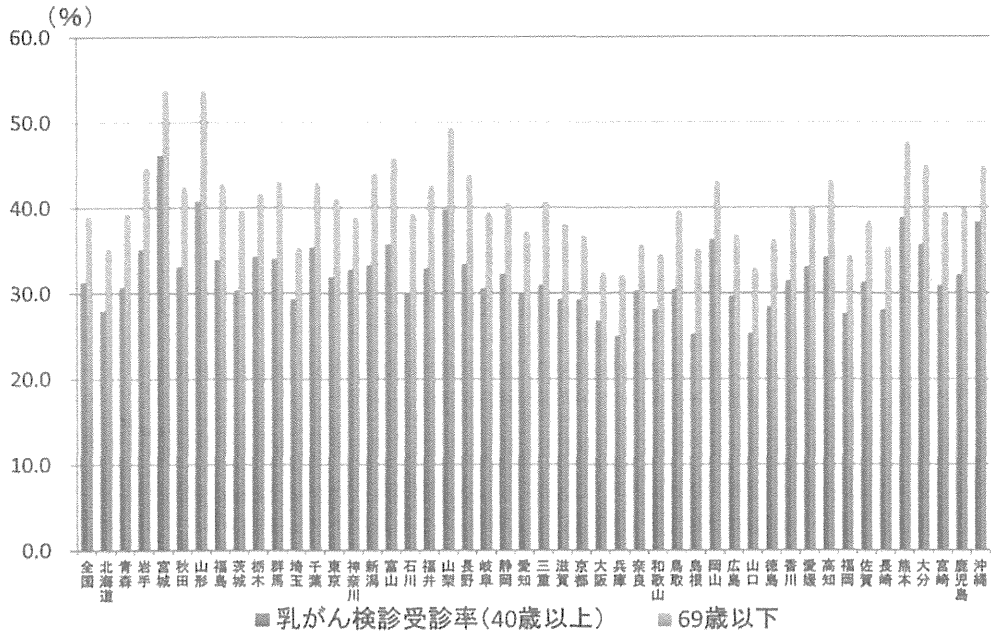
表2　乳がん検診実施状況

乳がん検診	集団検診、個別検診のいずれかでも実施している市区町村	
	市区町村数	（％）
問診	1,411	81.30％
視触診	1,502	86.60％
マンモグラフィ	1,718	99.00％
超音波検査	547	31.50％
乳がん検診実施市区町村数	1,735	

文献4より抜粋

乳がん検診は上記の住民検診の他に、労働安全衛生法に基づく職域検診のオプションとして行われる検診や、個人が主体となって受診する任意型検診などがある。住民検診レベルでの受診率は、2010年の統計（2009年、2010年の受診合計）で全国平均22.86％と推計されているが、職域や任意型の検診も含めて計上する国民生活基礎調査のデータでは2010年の受診率（2009年、2010年の受診合計）は40歳以上の全ての人口を対象とすると31.4％（図1）、さらに対象をがん対策推進基本計画で求めている40歳以上69歳以下に限定して算出した場合には推定受診率は39.1％と報告されている[5]。がん対策推進基本計画は、がん対策基本法（平成18年法律第98号）に基づき政府が策定するものであり、はじめは平成19年6月に策定、5年経過した平成24年に新たな基本計画が策定された[6]。乳がん検診の受診率に関しては5年以内に50％を達成するよう求めており、がん検診推進事業によるクーポン券の配布などを通して、受診率の向上に努めている。

図1　国民生活基礎調査に基づく乳がん検診受診率（2010年、過去2年合計）



▶▶▶ 乳がん検診の方法に関する諸問題

（1）超音波による乳がん検診

超音波検査はマンモグラフィと比較して高濃度乳房での小腫瘤の診断力が高く、マンモグラフィの弱点を補う手段として期待されるが、現在のところ超音波単独、またはマンモグラフィと超音波を併用した乳がん検診の有効性（死亡率低減効果）を報告したランダム化比較試験はない。超音波検診は精度管理が難しいく、マンモグラフィと比較して偽陽性、偽陰性、生検率が高いことなどから超音波単独による検診を推奨する根拠は存在しない。ただし、検診としてマンモグラフィに超音波を併用した研究では、いずれの研究も癌発見率の向上に関しては肯定的である[7-9]。ACRIN6666 は高リスク女性を対象として超音波検診の診断能力の検証と、診断技術の標準化を主な目的とした多施設共同試験である[10]。マンモグラフィに超音波検査を加えることにより乳癌発見率は上昇するが、先行研究と同様に偽陽性は増加し、特異度は低下した。統制のとれた精度管理の元に行われた試験であることは多いに評価できるが、高リスク女性に限定されたトライアルであることや、参加者数が少ないこと（2,809例）などから、超音波検査を対策型検診に導入する根拠としてこの結果のみでは不十分であると言わざるを得ない。

我が国における超音波検診の実態として、厚生労働省の市区町村アンケート調査がある[4]。この調査によれば、乳がん検診に超音波検査を追加する方法を行っている市区町村が547件（31.5%）あったと報告されている。対象集団の全体としての利益が不利益を確実に超えることが条件となる対策型検診で、これだけ多くの超音波検診がエビデンスの裏付けが無いままに実施されていることは憂慮される事態である。

（2）精度管理とエビデンスの確立

超音波検診が乳がん検診の方法として一般化されるためには、その検診方法が精度管理可能な検診方として標準化されることと、標準化された検診法で乳がんの死亡率を減少させる効果があることを科学的に証明することが不可欠である。ここで言う精度管理とは超音波検査機器の仕様や検査方法、及び読影技術、診断基準、更には教育方法までを含むシステム全体を検証可能なかたちに確立することを指す。我が国では世界に先駆けて2006年から「乳がん検診における超音波検査の有効性を検証するための比較試験（J-START）」を行っている[11]。2010年度までにおよそ76,000名の検診受診者を登録しており、結果の集積と検討が続いている（図2）。J-START では、40歳代女性を対象とする乳がん検診の方法として、超音波による検診の標準化と精度管理基準の統一を図った上で、マンモグラフィに超音波検査を併用する（介入）群と併用しない（非介入）群との間でランダム化比較試験を行い、2群間で検診精度と有効性を検証することを目的とした。 プライマ