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Original

Significance of Ki-67 Expression and Risk Category (St. Gallen 2007) in Elderly Breast Cancer Patients, with Emphasis on the Need for Postoperative Adjuvant Therapy

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Abstract: Breast cancer is increasing in the elderly. Although elderly breast cancer patients frequently receive less invasive therapy, its appropriateness is debatable. Ki-67 expression is a controversial prognostic factor and predictor of the efficacy of postoperative adjuvant therapy. This study investigated the value of the Ki-67 labeling index (LI) in elderly breast cancer patients, especially with respect to adjuvant therapy. This retrospective study investigated 82 primary breast cancer patients aged ≥ 70 years who underwent surgery between 1995 and 2005. Their clinicopathological findings were reviewed and their Ki-67 LIs were determined. The patients' mean age was 78 years, the mean observation period was 53.8 months, and 60 patients (73.2%) underwent adjuvant therapy. The St. Gallen (2007) risk category and the Ki-67 LI (mean, 15.3%) were both significantly correlated with relapse and prognosis. In the 31 cases with a low Ki-67 LI (＜10%), 1 patient who underwent adjuvant treatment relapsed, but there were no deaths. Among the intermediate- and high-risk patients, Ki-67 was low in 15; 1 patient who underwent adjuvant treatment relapsed, but there were no deaths. For elderly breast cancer patients aged ≥ 70 years categorized low risk by St. Gallen (2007) or with a low Ki-67 LI, the risk of relapse and death appears to be low regardless of adjuvant therapy. Though further investigation is needed to determine a method of measuring the Ki-67 LI and determining a cut-off value, our findings suggest that the Ki-67 LI helps with the selection of adjuvant therapy in elderly patients.

Key words: adjuvant therapy, breast cancer, elderly, ki-67, risk category

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Introduction

In Japan, primary breast cancer has the highest age-adjusted incidence of all cancers affecting women, and the number of affected individuals is increasing[1]. Breast cancer is also increasing in the elderly because of improved life expectancy. According to a nationwide survey of breast cancer patients by the Japanese Breast Cancer Society, the proportion of breast cancer patients aged 70 years or over was 18.4% in 2004, and had increased to 19.2% by 2007. In many cases, elderly breast cancer patients have underlying diseases[2-4]. Their underlying disease status and degree of activity in daily living need to be taken into account for treatment. Therefore, less invasive treatment is often chosen. However, lack of clear evidence supporting such an approach makes this choice debatable.

At present, adjuvant therapy for primary breast cancer is often performed in Japan with reference to the St. Gallen Consensus Conference. Risk categories were adopted at the 2007 St. Gallen conference and age was included as one of the evaluation factors[5]. However, prognosis standards for elderly breast cancer patients are inconclusive. In contrast, an increase in treatment-related deaths has been reported among the elderly[6], and certain standards have been formulated for adjuvant therapy.

At the 2009 St. Gallen conference, evaluation of the tumor proliferation potential was included as a criterion for chemotherapy[7]. In recent years, the expression of the tumor proliferation potential marker Ki-67 (Ki-67 labeling index [Ki-67 LI]) has been a strong candidate for a prognostic factor and a predictor of the efficacy of adjuvant therapy[8-12]. While broad consensus on its use as a prognostic factor has been obtained, its use as an effective predictor of postoperative adjuvant therapy is still controversial. In addition, there have been recent studies examining the correlation between Ki-67 LI and the need for adjuvant therapy in the elderly.

This retrospective study investigated cases of primary breast cancer surgery in patients aged ≥ 70 years, with each patient being classified into a St. Gallen (2007) risk category. After identifying Ki-67 as a potential marker of tumor proliferation, the correlation between postoperative adjuvant therapy and relapse and prognosis was examined, and patients not requiring therapy were identified.

Materials and Methods

A total of 92 patients aged ≥ 70 years underwent surgery for primary breast cancer at the Showa University Hospital and Toyosu Hospital, Showa University School of Medicine between 1995 and 2005. From these 92 patients, 10 patients were excluded because of ductal carcinoma in situ (6 patients), heterochronous bilateral breast cancer (2 patients), and preoperative chemotherapy (2 patients); the remaining 82 patients were included in this retrospective study. We reviewed the histopathological findings of these 82 patients and examined the relationship with their relapse status and prognosis.

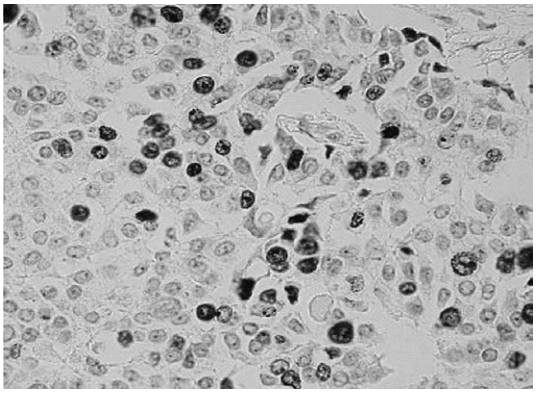

Fig. 1. Example of Ki-67 staining
Tumor cells with positive nuclei were counted, even if the staining was slight.

An automated staining method was used to calculate the Ki-67 LI in each tumor sample (Ventana HX BenchMark System, Ventana Medical Systems, Inc., Tucson, Arizona). The samples were deparaffinized and washed with water, and then heat-activated using EDTA. For primary antibody, a 50×dilution of Ki-67 monoclonal antibody (MIB-1; DAKO, Glostrup, Denmark) was used (reaction time, 32 minutes). The LI was determined using an optical microscope and examination under 400×magnification in three randomly selected fields. Two hundred tumor cells were counted in each, and the proportion of positive nuclei per 600 tumor cells was calculated (Fig. 1).

We also examined the expression of the estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) in each tumor, and assessed the risk category according to the St. Gallen conference (2007). Statistical analyses were performed using the χ^2 test and a *P*-value of < 0.05 was deemed significant.

Results

The mean patient age was 78 years (range, 70–97 years), the mean observation period was 53.8 months (range, 2–124 months), and 60 of the 82 patients (73.2%) underwent adjuvant therapy. There were 14 deaths in the study population, of which 7 were due to other causes: 3 from malignant tumors in other organs, 1 from acute myocardial infarction, 1 from acute subdural hematoma, 1 from pneumonia, and 1 from chronic heart failure. This study only investigated the prognosis for the present illness.

Pathological characteristics are listed in Table 1. Scirrhous carcinoma was the most represented histological type, with 46 cases (56.1%). Special types included 3 cases (3.7%) of mucinous carcinoma, 2 (2.4%) of invasive lobular carcinoma, and 1 (1.2%) of apocrine carcinoma. Immunohistochemistry revealed that 61 cases (74.4%) were ER-positive, 46 cases (56.1%) were PgR-positive, and 12 cases (14.6%) were HER2-positive.

Table 1. Patient histopathology

histological type	scirrhous	46	56(%)
	solid-tubular	14	17(%)
	papillo-tubular	16	20(%)
	mucinous	3	4(%)
	invasive-lobular	2	2(%)
	apocrine	1	1(%)
tumor size	T1	46	56(%)
	T2	29	35(%)
	T3	7	9(%)
nuclear grade	1	47	62(%)
	2	13	17(%)
	3	16	21(%)
vessel invasion	(−)	58	70(%)
	(+)	24	30(%)
lymph node metastasis	0	48	68(%)
	1-3	15	21(%)
	4	8	11(%)
ER	(−)	21	26(%)
	(+)	61	74(%)
PgR	(−)	36	44(%)
	(+)	46	56(%)
HER2	(−)	70	85(%)
	(+)	12	15(%)

Table 2. Correlation between relapse or prognosis and various factors

		total	relapse			prognosis		
			(−)	(+)	p-value	survival	death	p-value
tumor size	T1	46	40	6		43	3	
	T2	29	23	6	$p = 0.15$	26	3	$p = 0.72$
	T3	7	4	3		6	1	
nuclear grade	1	47	45	2		46	1	
	2	13	7	6	$p < 0.001$ (*)	10	3	$p = 0.022$ (*)
	3	16	9	7		13	3	
vessel invasion	(−)	58	52	6	$p = 0.0038$ (*)	57	1	$p < 0.001$ (*)
	(+)	24	15	9		18	6	
lymph node metastasis	0	48	45	3		46	2	
	1-3	15	12	4	$p < 0.001$ (*)	15	0	$p < 0.001$ (*)
	4	8	2	6		4	4	
ER	(−)	21	13	8	$p = 0.006$ (*)	18	3	$p = 0.027$
	(+)	61	54	7		57	4	
PgR	(−)	36	25	11	$p = 0.01$ (*)	32	4	$p = 0.46$
	(+)	46	42	4		43	3	
HER2	(−)	70	59	11	$p = 0.14$	64	6	$p = 0.98$
	(+)	12	8	4		11	1	
St. Gallen risk category (2007)	low	27	27	0		27	0	
	intermediate	42	35	7	$p < 0.001$ (*)	39	3	$p < 0.001$ (*)
	high	13	5	8		9	4	

Based on the St. Gallen risk categories of 2007, 27 patients (39.2%) were classed as low risk, 42 (51.2%) as intermediate risk, and 13 (15.9%) as high risk. The correlations between each of these categories and relapse and prognosis were examined (Table 2). The

Table 3. Relapse and prognosis with and without adjuvant therapy, by risk category

	adjuvant therapy	relapse(+)	relapse(−)	death	survival
low risk	(−)	0/12(0%)	12/12(100%)	0/12(0%)	12/12(100%)
	(+)	0/15(0%)	15/15(100%)	0/15(0%)	15/15(100%)
intermediate risk	(−)	1/9 (11%)	8/9 (89%)	0/9 (0%)	9/9 (100%)
	(+)	6/33(18%)	27/33(82%)	3/33(9%)	30/33(91%)
high risk	(−)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
	(+)	8/13(62%)	5/13(38%)	4/13(31%)	9/13(69%)

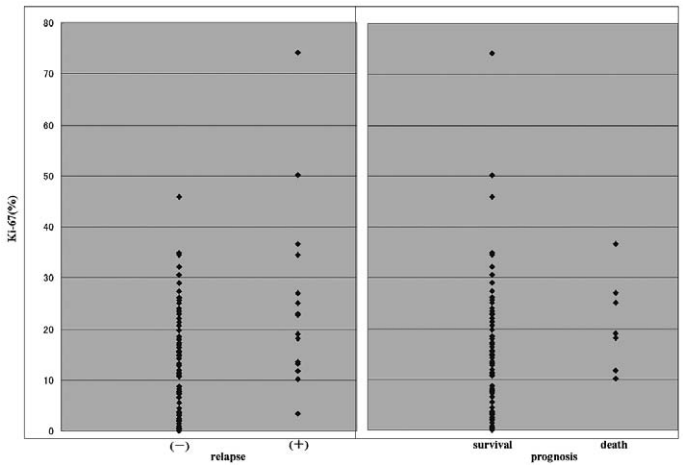

Fig. 2. Ki-67 and relapse, and prognosis
The values of the Ki-67 labeling indexes (Ki-67 LIs) were plotted according to relapse and prognosis. Because no deaths were observed in patients with a Ki-67 LI < 10%, the study was performed by setting the Ki-67 cut-off value at 10%.

risk category was significantly correlated with relapse ($P = 0.00001$). In addition, nuclear grade, the presence of vascular invasion, lymph node metastasis, ER-positive expression, and PgR-positive expression showed significant correlations with relapse. The risk category was also significantly correlated with prognosis ($P = 0.004$). Furthermore, significant correlations were observed between prognosis and nuclear grade and the presence of vascular invasion.

Relapse and prognosis with and without adjuvant therapy are summarized by risk category in Table 3. Of the 27 patients categorized as low risk, 13 did not undergo adjuvant therapy. Regardless of whether they underwent adjuvant therapy, there were no relapses or deaths among the 27 low-risk patients.

The mean Ki-67 LI was 15.3%. The Ki-67 LI of each patient was plotted against relapse and prognosis (Fig. 2). Because no deaths were observed in patients with a Ki-67 LI of less than 10%, the study was performed by setting the Ki-67 cut-off value at 10%. The associations between the Ki-67 LI and various factors are listed in Table 4. The Ki-67 LI

Table 4. Correlation between Ki-67 and various factors

		total	Ki-67 LI < 10%	Ki-67 LI ≥ 10%	p-value
tumor size	T1	46	23	23	p = 0.032 (*)
	T2	29	7	22	
	T3	7	1	6	
nuclear grade	1	47	23	24	p = 0.029 (*)
	2	13	4	9	
	3	16	2	14	
vessel invasion	(−)	58	28	30	p = 0.002 (*)
	(+)	24	3	21	
lymph node metastasis	0	48	19	29	p = 0.72
	1-3	15	6	9	
	4	8	2	6	
ER	(−)	21	2	19	p = 0.002 (*)
	(+)	61	29	32	
PgR	(−)	36	7	29	p = 0.002 (*)
	(+)	46	24	22	
HER2	(−)	70	31	39	p = 0.003 (*)
	(+)	12	0	12	
St. Gallen risk category (2007)	low	27	16	11	p = 0.011 (*)
	intermediate	42	13	29	
	high	13	2	11	
relapse	(−)	67	30	37	p = 0.001 (*)
	(+)	15	1	14	
prognosis	(−)	75	31	44	p = 0.031 (*)
	(+)	7	0	7	

was significantly correlated with tumor size, nuclear grade, the presence of vascular invasion, ER-positive expression, PgR-positive expression, HER2-positive expression, risk category, relapse, and prognosis.

Relapse and prognosis with and without adjuvant therapy are summarized by the Ki-67 LI in Table 5-1. In the 31 patients with a low Ki-67 LI, 1 patient who underwent adjuvant treatment relapsed, but there were no deaths. Of the intermediate- and high-risk patients in this group (Table 5-2), the Ki-67 LI was low in 15 patients. Of these, 1 patient who underwent adjuvant treatment relapsed, but there were no deaths.

Discussion

Characteristics of breast cancer in the elderly

Histopathology

Scattered reports have indicated a high incidence of special-type carcinomas, especially

Table 5-1. Relapse and prognosis with or without adjuvant therapy, by Ki-67 LI

		Ki-67 LI	relapse(+)	death
adjuvant therapy	(−)	< 10%	0/10(0%)	0/10(0%)
		≥ 10%	1/11(9%)	0/11(0%)
	(+)	< 10%	1/21(5%)	0/21(0%)
		≥ 10%	13/50(26%)	7/50(14%)

Table 5-2. Relapse and prognosis with or without adjuvant therapy, by Ki-67 LI (intermediate to high risk)

		Ki-67 LI	relapse(+)	death
adjuvant therapy	(−)	< 10%	0/1 (0%)	0/1 (0%)
		≥ 10%	1/8 (9%)	0/8 (0%)
	(+)	< 10%	1/14(7%)	0/14(0%)
		≥ 10%	13/32(41%)	7/32(22%)

mucinous and apocrine carcinomas, as a pathological feature of breast cancer in the elderly[13]. Furthermore, other reports have demonstrated increases in invasive lobular carcinoma[14]. In this study, mucinous carcinoma was the most commonly occurring special type of carcinoma, at about 4%. In addition, many reports have indicated that ER-positive cases are common in the elderly[2, 3, 13, 14], they accounted for about 74% of the cases in this study, a similar frequency to that reported in the literature.

Prognosis

Past studies of breast cancer in the elderly reported high rates of malignancy and poorer prognoses than in young patients[3]. However, it has been shown that a higher proportion of elderly patients has underlying diseases and they succumb from other causes[2, 3]. Furthermore, elderly patients often present to the doctor later than young patients, and they are given treatment protocols that are less invasive, even in cases with a high risk of relapse[15]. These factors may influence prognosis in the elderly. In a study of 1568 people aged ≥ 55 years at the MD Anderson Cancer Center[4], nonstandard surgical treatments and adjuvant hormonal therapy and chemotherapy for breast cancer patients aged ≥ 75 years made simple comparisons difficult. Lately, a growing number of cases are being diagnosed relatively early among the elderly, and excluding deaths from other causes, reports of prognoses are relatively good[14], though other reports state that there is still no less risk of relapse[16].

Therapy for breast cancer in the elderly

Many opinions on postoperative chemotherapy in the elderly advise a cautious approach. Muss *et al*[6] reported that, while a certain effect can be gained from chemotherapy, there are significantly higher numbers of treatment-related deaths in those aged ≥ 65 years. In contrast, some reports urge chemotherapy for patients with hormone receptor-negative lymph node metastasis[17]. In a 15-year, randomized controlled trial by the Early Breast Cancer

Trialists' Collaborative Group, few patients aged ≥ 70 years received postoperative chemotherapy and so no clear conclusions could be drawn for this age group[15].

The Early Breast Cancer Trialists' Collaborative Group study also reported that tamoxifen was considered an effective hormonal therapy after surgery for all ages[15]. On the other hand, large-scale trials such as the Arimidex, Tamoxifen, Alone or in Combination trial[18] and the Breast International Group trial 1−98[19] reported that an aromatase inhibitor was more useful than tamoxifen even in elderly patients, and they recommended aggressive hormone treatment of hormone receptor-positive patients, even in the elderly.

However, there have also been reports that hormone therapy does not improve the prognosis of patients without lymph node metastases[20], and adjuvant treatment of low-risk patients is still subject to debate. According to the American Society of Clinical Oncology's 2009 consensus[21], aromatase inhibitors are not recommended for low-risk patients of any age.

Ki-67 immunohistochemical staining

Characteristics of Ki-67

Ki-67 is a human nuclear antigen which is associated with all cell nuclei during the proliferating cell cycle except for the G0 phase[22]. In 1983, Gerdes and colleagues[23] developed a monoclonal antibody against Ki-67 from Hodgkin's disease-derived cells, and Ki-67 is now widely used as a biological marker for determining the tumor proliferation potential In recent years it has attracted attention as a prognostic factor and predictor of efficacy of adjuvant therapy in breast cancer. Reports on the connection with prognosis have been particularly numerous[8, 9], and a near-consensus on this has been reached. The Ki-67 LI was also significantly correlated with relapse and prognosis in the present study.

Determination of the Ki-67 labeling index

Ki-67 immunostaining using paraffin staining is widespread at present, but the LI is not determined in any consistent way. Jones *et al*[24] reported that 400×magnification in 10 fields, counting at least 1000 tumor cells, is essential. However, this method is complex and time-consuming in actual clinical practice, so some facilities use automated measurements assisted by analysis software[8], and this technique is also reported to be useful for needle biopsy tissue[25]. However, this automated technique tends to count some nontumor cells, such as lymphocytes, thus affecting its accuracy. In the present study, as a simple, accurate measuring technique, a visual count of 600 tumor cells in three random fields was performed.

Mitotic count and Ki-67

Originally, the mitotic count in the sample was used to determine the nuclear grade with respect to the tumor proliferation potential. As mentioned earlier, because Ki-67 stains the cell nuclei in all growth phases except during the G0 phase, the results do not agree with the mitotic count. However, they should be correlated, and this has been substantiated by

many reports[26, 27]. In contrast, Jalava *et al*[28] examined prognoses classified into subgroups by mitotic count and the Ki-67 LI, and they observed that the prognosis for a high mitotic count was poor even when the Ki-67 LI was low. St. Gallen (2009) observed that either the Ki-67 LI or the mitotic count can be used for evaluation of the tumor proliferation potential, and as an example of a Ki-67 LI cut-off value, enumerated 30% of that used by Jalava *et al*[7].

Ki-67 as a predictor

Several large-scale trials have reported differing results regarding the significance of the Ki-67 LI as a predictor of efficacy of adjuvant therapy. The Breast International Group trial 1-98[8] indicated excellent disease-free survival (DFS) for patients with a high Ki-67 LI (> 11%) in the group treated with letrozole in a tamoxifen and letrozole comparison trial involving 4922 patients. In a Breast Cancer International Research Group trial[10], hormone receptor-positive, HER2-negative breast cancer patients were classified by the Ki-67 LI into luminal A (≤ 11%) and luminal B (> 11%) subtypes. In addition, FAC (fluorouracil, doxorubicin, and cyclophosphamide) and TAC (docetaxel, doxorubicin, and cyclophosphamide) treatment groups were compared. DFS was significantly improved in the luminal B subtype in the TAC treatment group.

The International Breast Cancer Study Group trial looked at both premenopausal and postmenopausal patients[9]. In both cases, hormone therapy alone and hormone therapy with CMF (cyclophosphamide, methotrexate, and fluorouracil) were compared. The Ki-67 LI was included as an item of consideration (cut-off value 19%), but it was not considered a predictor of efficacy of CMF either pre- or post-menopause.

Unfortunately the drugs used were different in each of these investigations, and because of the connection between adjuvant therapy and the Ki-67 LI, the differences in results are likely due to the different treatment regimes. Thus, each individual treatment needs a separate study. In any event, no conclusions have yet been reached on the significance of the Ki-67 LI as a predictor of efficacy of adjuvant therapy after surgery. St. Gallen (2009) adopted evaluation of the tumor proliferation potential as a criterion for chemotherapy after surgery[7], but in the American Society of Clinical Oncology guidelines, Ki-67 is not recognized as a routine biological marker[29], so there is a difference of opinion.

Furthermore, in recent years there has been a focus on the change in Ki-67 before and after preoperative therapy. The Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen study reported that, over the 2 weeks after preoperative hormone therapy, the Ki-67 LI was correlated with DFS[30]. In addition, Jones *et al*[31] reported the Ki-67 LI in samples resected during surgery after preoperative chemotherapy to be a prognostic factor[31]. Thus, it is becoming standard to examine the Ki-67 LI after preoperative treatment.

Problems with Ki-67

Many studies investigating a correlation between Ki-67 and prognosis have been reported,

but their methods of determination and evaluation have not been consistent. Apart from the count method described above, consensus is also needed on whether the determination region of the specimen should be the most-stained region or a random region, and on what degree of staining is considered positive. The cut-off value is also not well established, and reports to date have used a wide range of cut-off values, from 1% to 40%. Ki-67 is likely to have great clinical usefulness, and further investigation is warranted.

Conclusions

This was a retrospective investigation, and we leave decisions pertaining to the details of hormone therapy and chemotherapy to the clinicians. However, at least among elderly breast cancer patients aged ≥ 70 years, categorized as low risk by St. Gallen (2007), or intermediate or high risk with a low Ki-67 LI, the risks of relapse and death regardless of adjuvant therapy are believed to be low, suggesting the possibility of abbreviating adjuvant therapy.

Further investigation is needed to determine a standard method of measuring the Ki-67 LI and a cut-off value. In addition, studies specific to the various adjuvant therapy regimes are also essential.

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