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ERRATUM

Erratum to: Thoracic and cardiovascular surgery in Japan during 2013

Annual report by The Japanese Association for Thoracic Surgery

Committee for Scientific Affairs, The Japanese Association for Thoracic Surgery[1] · Munetaka Masuda[2] · Hiroyuki Kuwano[3] · Meinoshin Okumura[4] · Hirokuni Arai[5] · Shunsuke Endo[6] · Yuichiro Doki[7] · Junjiro Kobayashi[8] · Noboru Motomura[9] · Hiroshi Nishida[10] · Yoshikatsu Saiki[11] · Fumihiro Tanaka[12] · Kazuo Tanemoto[13] · Yasushi Toh[14] · Hiroyasu Yokomise[15]

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Erratum to: Gen Thorac Cardiovasc Surg (2015) 63:670–701
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The following errors appeared in the above-cited article.

(A) Cardiovascular surgery section: in the 3rd paragraph, on the 4th line, ''9168 cases in 2003'' should read ''9165 cases in 2003''; on the 6th line, "15,757 cases in thoracic aortic aneurysm" should read "15,758 cases in thoracic aortic aneurysm"; on the 8th line, "4.0, 4.6 and 14.6 %, respectively" should read "4.0, 4.7 and 14.6 %, respectively"; on the 10th line, "16,752 cases" should read "16,560 cases"; and on the 13th line, "83.4 % increase" should read "83.8 % increase". In the 5th paragraph, on the 14th line, "6.7 % in 2003" should read "6.9 % in 2003". In the 6th paragraph, on the 2nd line, "83.4 %" should read "83.8 %"; on the 4th line, "placement" should read "replacement", and "2.2 and 3.7 %" should read "2.9 and 5.4 %"; on the 6th line, "0.8 %" should read "1.1 %"; on the 25th line, "38.2 %" should read "38.3 %"; and on the 26th line, "41.9 %" should read "59.4 %". In the 7th paragraph, on the 11th line, "23.4 %" should read "22.4 %". In the 8th paragraph, on the 3rd line, "1.0 and 1.7 %" should read "0.8 and 1.4 %"; on the 10th line, "5.5 %" should read "7.3 %"; and on the 14th line, "8.5–6.4 %" should read "8.0–6.4 %". In the 9th paragraph, on the 2nd line, "414 operations" should read "840 operations"; and on the 4th line, "298 operations" should read "371 operations". In the 11th paragraph, on the 6th

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Table 5 Thoracic aortic aneurysm (total; 15,758)
(1) Dissection (total; 6787)

Replaced site	Stanford type																								
	Acute								Chronic								Concomitant operation					Redo			
	A				B				A				B												
	Cases	30-day mortality		Hospital mortality	Cases	30-day mortality		Hospital mortality	Cases	30-day mortality		Hospital mortality	Cases	30-day mortality		Hospital mortality	AVP	AVR	MVP	MVR	CABG	Cases	30-day mortality		Hospital mortality
		Hospital	After discharge			Hospital	After discharge			Hospital	After discharge			Hospital	After discharge								Hospital	After discharge	
1. Ascending Ao.	2608	186 (7.1)	2 (0.1)	217 (8.3)	4	0 (0.0)	0	1 (25.0)	186	3 (1.6)	0	6 (3.2)	8	0 (0.0)	0	0 (0.0)	216	111	17	8	132	51	2 (3.9)	0	4 (7.8)
2. Aortic Root	197	34 (17.3)	0	35 (17.8)	0	0	0	0	65	6 (9.2)	0	7 (10.8)	1	0	0	0	37	185	6	1	42	38	5 (13.2)	0	5 (13.2)
3. Ascending Ao. + Arch	1393	108 (7.8)	0	124 (8.9)	32	1 (3.1)	0	2 (6.3)	282	6 (2.1)	0	11 (3.9)	112	3 (2.7)	0	4 (3.6)	106	62	3	4	95	91	4 (4.4)	0	8 (8.8)
4. Arch + descending Ao.	25	1 (4.0)	0	2 (8.0)	6	1 (16.7)	0	2 (33.3)	31	4 (12.9)	0	4 (12.9)	56	4 (7.1)	0	4 (7.1)	0	0	0	0	2	17	6 (35.3)	0	5 (29.4)
5. Aortic Root + Asc. Ao. + Arch	86	12 (14.0)	0	14 (16.3)	2	0	0	0	33	1 (3.0)	0	1 (3.0)	13	0	0	0	22	85	1	0	15	18	0	0	0
6. Descending Ao.	13	3 (23.1)	0	3 (23.1)	37	6 (16.2)	0	5 (13.5)	84	2 (2.4)	0	3 (3.6)	270	12 (4.4)	0	17 (6.3)	0	0	0	0	4	41	1 (2.4)	0	3 (7.3)
7. Thoracoabdominal Ao.	1	0	0	0	11	1 (9.1)	0	3 (27.3)	29	4 (13.8)	0	4 (13.8)	145	9 (6.2)	0	12 (8.3)	0	0	0	0	1	47	5 (10.6)	0	6 (12.8)
8. Extra-anatomical bypass	14	2	0	2 (14.3)	24	2 (8.3)	0	3 (12.5)	2	0	0	0 (0.0)	3	0	0	0	0	0	0	0	0	3	1 (33.3)	0	1 (33.3)
9. Stent graft[*a]	107	9 (8.4)	0	7 (6.5)	181	17 (9.4)	0	21 (11.6)	139	0	0	1 (0.7)	587	6 (1.0)	1 (0.2)	12 (2.0)	6	2	0	0	3	70	2 (2.9)	0	3 (4.3)
1) TEVAR[*b]	48	7 (14.6)	0	7 (14.6)	179	16 (8.9)	0	20 (11.2)	119	0	0	1 (0.8)	556	6 (1.1)	1 (0.2)	12 (2.2)	5	1	0	0	1	68	2 (2.9)	0	3 (4.3)
2) Open stent	59	2 (3.4)	0	3 (5.1)	2	1 (50.0)	0	1 (50.0)	20	0	0	0	31	0	0	0	1	1	0	0	2	2	0	0	0
a) With total arch[*c]	59	2 (3.4)	0	3 (5.1)	2	1 (50.0)	0	1 (50.0)	20	0	0	0	16	0	0	0	1	1	0	0	2	1	0	0	0
b) Without total arch[*d]	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	1	0	0	0
3) Unspecified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	4444	355 (8.0)	2 (0.05)	404 (9.1)	297	28 (9.4)	0	37 (12.5)	851	26 (3.1)	0	37 (4.3)	1195	34 (2.8)	1 (0.1)	49 (4.1)	387	445	27	13	294	376	26 (6.9)	0	35 (9.3)

Values in parenthesis represent mortality %

Ao aorta, *AVP* aortic valve repair, *AVR* aortic valve replacement, *MVP* mitral valve repair, *MVR* mitral valve replacement, *CABG* coronary artery bypass grafting, *TEVAR* thoracic endovascular aortic (aneurysm) repair

Acute, within 2 weeks from the onset

*a = *b + *c + *d + unspecified

Table 5 continued
(2) Non-dissection (total; 8971)

Replaced site	Unruptured				Ruptured				Concomitant operation					Redo				CPB (−)			
	Cases	30-day mortality		Hospital mortality	Cases	30-day mortality		Hospital mortality	AVP	AVR	MVP	MVR	CABG	Cases	30-day mortality		Hospital mortality	Cases	30-day mortality		Hospital mortality
		Hospital	After discharge			Hospital	After discharge								Hospital	After discharge			Hospital	After discharge	
1. Ascending Ao.	1201	25 (2.1)	0	32 (2.7)	62	7 (11.3)	0	9 (14.5)	40	820	67	39	147	95	7 (7.4)	0	10 (10.5)	2	0	0	0
2. Aortic Root	928	16 (1.7)	1 (0.1)	24 (2.6)	31	8 (25.8)	0	8 (25.8)	232	631	54	24	95	145	13 (9.0)	0	14 (9.7)	1	0	0	0
3. Ascending Ao. + Arch	2151	44 (2.0)	0	72 (3.3)	173	25 (14.5)	0	37 (21.4)	33	201	28	8	327	105	8 (7.6)	0	10 (9.5)	6	0	0	0
4. Arch + descending Ao.	104	6 (5.8)	0	9 (8.7)	23	3 (13.0)	0	5 (21.7)	0	2	0	1	7	11	3 (27.3)	0	4 (36.4)	8	0	0	0
5. Aortic root + Asc. Ao. + Arch	109	1 (0.9)	0	2 (1.8)	5	2 (40.0)	0	2 (40.0)	24	80	5	1	6	20	3 (15.0)	0	3 (15.0)	2	0	0	0
6. Descending Ao.	343	12 (3.5)	0	18 (5.2)	84	16 (19.0)	0	18 (21.4)	0	0	0	0	4	33	3 (9.1)	0	6 (18.2)	26	2 (7.7)	0	2 (7.7)
7. Thoracoabdominal Ao.	372	17 (4.6)	1 (0.3)	28 (7.5)	52	9 (17.3)	0	15 (28.8)	0	0	0	0	1	42	1 (2.4)	0	3 (7.1)	11	0	0	0
8. Extra-anatomical bypass	35	0	0	1 (2.9)	2	0	0	0	1	0	0	0	1	1	0	0	0	2	0 (0.0)	0	0
9. Stent graft[*a]	2928	43 (1.5)	1 (0.03)	73 (2.5)	368	39 (10.6)	3 (0.8)	55 (14.9)	7	7	1	0	25	122	6 (4.9)	1 (0.8)	7 (5.7)	1079	19 (1.8)	3 (0.3)	28 (2.6)
1) TEVAR[*b]	2774	37 (1.3)	1 (0.04)	66 (2.4)	358	38 (10.6)	3 (0.8)	53 (14.8)	3	1	0	0	5	118	5 (4.2)	1 (0.8)	6 (5.1)	1059	19 (1.8)	3 (0.3)	28 (2.6)
2) Open stent	154	6 (3.9)	0	7 (4.5)	10	1 (10.0)	0	2 (20.0)	4	6	1	0	20	4	1 (25.0)	0	1 (25.0)	20	0	0	0
a) With total arch[*c]	112	6 (5.4)	0	7 (6.3)	10	1 (10.0)	0	2 (20.0)	4	5	1	0	16	2	1 (50.0)	0	1 (50.0)	0	0	0	0
b) Without total arch[*d]	42	0	0	0	0	0	0	0	0	1	0	0	4	2	0	0	0	20	0	0	0
3) Unspecified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	8171	164 (2.0)	3 (0.04)	259 (3.2)	800	109 (13.6)	3 (0.4)	149 (18.6)	337	1741	155	73	613	574	44 (7.7)	1 (0.2)	57 (9.9)	1137	21 (1.8)	3 (0.3)	30 (2.6)

Values in parenthesis represent mortality %

Ao aorta, *AVP* aortic valve repair, *AVR* aortic valve replacement, *MVP* mitral valve repair, *MVR* mitral valve replacement, *CABG* coronary artery bypass grafting, *TEVAR* thoracic endovascular aortic (aneurysm) repair

*a = *b + *c + *d + unspecified

Table 7 Assisted circulation (total; 1713)

Sites	VAD									Heart–lung assist					
	Device			Results						Method		Results			
	Centrifugal	VAS	Others	Not weaned			Weaned			PCPS	Others	Not weaned		Weaned	
				On going	Death	Transplant	Alive	Deaths	Transplant			Deaths	Transplant	Deaths	Alive
Post cardiotomy															
Left	38	4	7	8	30 (61.2)	0	3	8 (16.3)	0						
Right	0	0	0	0	0	0	0	0	0						
Biventricle															
Left	1	3	0	0	3 (75.0)	0	1	0	0	499	69	274 (48.2)	0	85 (15.0)	209
Right	4	0	0												
Congestive heart failure															
Left	50	41	92	112	38 (20.8)	7	16	9 (4.9)	0						
Right	2	1	0	1	0	0	2	0	0						
Biventricle															
Left	5	22	3	5	14 (46.7)	0	8	3 (10.0)	0	685	29	360 (50.4)	0	105 (14.7)	249
Right	18	11	1												
Respiratory failure										106	22	46 (35.9)	0	16 (12.5)	66
Total	118	82	103	126	85 (28.1)	7	30	20 (6.6)	0	1290	120	680 (48.2)	2	206 (14.6)	524

Values in parenthesis represent mortality %

VAD ventricular assist devise, *VAS* ventricular assist system, *PCPS* percutaneous cardiopulmonary support

Table 33
14. Combined resection of neighboring organ(s)

	Cases	30 day mortality		Hospital mortality
		Hospital	After discharge	
14. Combined resection of neighboring organ(s)	1581	7 (0.4)	3 (0.2)	19 (1.2)
(A) Primary lung cancer (organ resected)				
Aorta	16	0	0	0
Superior vena cava	40	0	0	0
Brachiocephalic vein	12	0	0	0
Pericardium	177	2 (1.1)	0	3 (1.7)
Pulmonary artery	227	1 (0.4)	0	1 (0.4)
Left atrium	45	0	0	1 (2.2)
Diaphragm	98	1 (1.0)	0	1 (1.0)
Chest wall (including ribs)	500	1 (0.2)	0	9 (1.8)
Vertebra	31	0	0	3 (9.7)
Esophagus	12	0	0	0
Total	1158	5 (0.4)	0	18 (1.6)
(B) Mediastinal tumor (organ resected)				
Aorta	3	0	0	0
Superior vena cava	69	0	0	1 (1.4)
Brachiocephalic vein	93	1 (1.1)	0	1 (1.1)
Pericardium	267	1 (0.4)	0	2 (0.7)
Pulmonary artery	9	0	0	0
Left atrium	2	0	0	0
Diaphragm	16	0	0	0
Chest wall (including ribs)	20	0	0	0
Vertebra	7	0	0	0
Esophagus	1	0	0	0
Lung	277	1 (0.4)	0	1 (0.4)
Total	764	3 (0.4)	0	5 (0.7)

Values in parenthesis represent mortality %

line, “8171 cases” should read “8971 cases”; and on the 9th line, “2.2 %” should read “3.2 %”. In the 13th paragraph, on the 4th line, “42 % increase” should read “4.2 % increase”.

Additionally, there are some errors in the following tables. Table 3 (3), on the 4th line (4 Damus–Kaye–Stansel operation), values of the columns “Neonate; Hospital mortality”, “Infant; 30-day mortality; Hospital”, and “Infant; Hospital mortality” should be “1 (33.3)”, “4 (8.0)”, and “6 (12.0)”, not “1”, “4”, and “6”, respectively. The title for Table 9 should read “Pacemaker + ICD (total; 4660)”. In (B) General thoracic surgery section, Table 33, the first line of the column “30-day mortality; Hospital” should read “7 (0.4)”, not “7 (1.4)”. Other tables with errors and their corrections are given below.

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MIB-1 labeling index, Ki-67, is an indicator of invasive intraductal papillary mucinous neoplasm

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Abstract. Despite strict criteria for the observation of intraductal papillary mucinous neoplasm (IPMN), it remains difficult to distinguish invasive IPMN from non-invasive IPMN. The aim of the present study was to identify an indicator of invasive IPMN. The present study retrospectively evaluated 53 patients (28 with non-invasive and 25 with invasive IPMN) who underwent resection of IPMN, and examined the usefulness of the MIB-1 labeling index as an indicator of invasive IPMN. The MIB-1 labeling indexes in patients with invasive IPMN were significantly higher compared with those with non-invasive IPMN (P<0.001). A receiver operating characteristic curve revealed that the area under the curve was 0.822. These results suggested that a cut-off level for the MIB-1 labeling index should be set to 15.5% to distinguish invasive from non-invasive IPMN. A multivariate analysis using a logistic regression model revealed the MIB-1 labeling index (hazard ratio, 18.692; 95% confidential interval, 4.171-83.760; P<0.001) and the existence of mural nodules (hazard ratio, 6.187, 95% confidential interval, 1.039-36.861; P=0.045) were predictive factors for invasive IPMN. However, no statistically significant differences were observed between patients with a lower MIB-1 labeling index and patients with a higher MIB-1 labeling index (P=0.798). The MIB-1 labeling index must be considered as a candidate for the classification of IPMN.

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Abbreviations: IPMN, intraductal papillary mucinous neoplasm; PBS, phosphate-buffered saline

Key words: IPMN, MIB-1, Ki-67, invasive IPMN, mural nodule

Introduction

Since Ohashi *et al* (1) first described intraductal papillary mucinous neoplasm (IPMN) in 1982, IPMNs have become recognized as the most common of all cystic tumors of the pancreas, accounting for up to 70% (2). On the basis of the location of ductal involvement, IPMNs are divided into three groups: Main duct IPMN, branch duct IPMN and mixed type IPMN (3). The first International Consensus Guidelines for IPMN management were published in 2006 (3) and were later updated in 2012 (4). According to the guidelines, surgical resection is recommended for all main duct IPMNs due to the high risk of malignancy (61.6%) and invasive carcinoma (43.1%) (4,5). By contrast, the frequency of malignant and invasive IPMNs in branch duct IPMN were reported to be 25.5 and 17.7%, respectively (4). The latest International Consensus Guidelines, however, described worrisome features of malignancy, including a cyst >3 cm, thickened and enhanced cyst walls, main pancreatic duct size 5-9 mm, non-enhancing mural nodule, abrupt change in caliber of duct with distal pancreatic atrophy and lymphadenopathy (4). No criterion has been proven accurate in predicting an invasive progression in main duct IPMN (6). Several previous studies described predictors of malignancy of main duct IPMN: Older age, more frequent incidence of jaundice and/or worsening of diabetes, >15 mm dilatation of the main pancreatic duct and a mural nodule (5,7). However, 29% of the patients with malignant main duct IPMN were asymptomatic (5), and those with smaller main duct dilatation and no mural nodule had invasive carcinomas (7). Previously, a number of additional predictors of malignancy in branch duct IPMNs were reported: Elevated tumor markers, an increase of cyst size over time, family history, multifocal IPMN or obesity (8-12).

An unsettled definition of IPMN malignancy makes comparison of the described data difficult. Certain reports included cases with carcinoma *in situ* into those of malignant IPMNs, while other studies enrolled patients with invasive IPMN only into those of malignant disease. The new International Consensus Guidelines described carcinoma *in situ* as high-grade dysplasia (4).

By contrast, the MIB-1 index has been used for diagnosing malignancy in other diseases. In neuroendocrine tumors, those

with an MIB-1 labeling index of <2% are classified as G1, and those with an index between 2 and 20% as G2. Tumors with an index of >20% are classified as neuroendocrine carcinoma (13). In early breast carcinoma, patients with a high MIB-1 labeling index have a poor prognosis (14). As for IPMNs, several reports have presented data of the MIB-1 labeling index (15-22). However, confusing criteria for the definition of malignant IPMNs prevent us from comparing these results.

The aim of the present study was to identify clinical and pathologic features of invasive IPMN using our cohort approach that simply classifies patients into two groups: Non-invasive and invasive IPMN. The present study also aimed to identify the role of the MIB-1 labeling index as an indicator of invasive IPMNs.

Materials and methods

Patients. A total of 53 patients with IPMNs who underwent resection of tumors between 2000 and 2010 were enrolled, in accordance with the guidelines for informed consent and approval from the Ethics Committee of our institute. Of these patients, 28 patients exhibited non-invasive IPMN, including three patients with carcinoma *in situ* of IPMN, and 25 patients with invasive IPMN. The neoplasms were classified into non-invasive IPMNs and invasive IPMNs. Minimally invasive IPMNs were classified into invasive IPMNs. The neoplasms in the head, neck or uncinate process of the pancreas were treated with pancreaticoduodenectomy, and neoplasms in the pancreatic body or tail were treated with open or laparoscopic distal pancreatectomy accordingly.

Analysis on factors for invasive IPMN. As for the clinical features in determining predictive factors for invasive IPMN, age, gender, tumor size, type of involved duct (main or mixed type vs. branch duct), with or without symptoms, dilatation of the main duct and a mural nodule in pre-operative imaging modalities, and the MIB-1 labeling index were investigated.

Immunohistochemical analysis. The MIB-1 labeling index was assessed by immunohistochemistry using an avidin-biotin-peroxidase complex method. Formalin-fixed, paraffin-embedded tissue samples were cut into 4 μm-thick sections. The sections were deparaffinized in xylene and rehydrated through a series of decreasing alcohol concentrations. Following this, they were rinsed three times in phosphate-buffered saline (PBS), and the sections were immersed in an absolute methanol solution containing 0.3% H_2O_2 for 30 min at room temperature to inhibit endogenous peroxidase. Antigens were retrieved by autoclaving sections on slides in 0.01 M (pH 6.0) citrate buffer for 10 min. After rinsing in PBS, the sections were incubated with monoclonal mouse anti-human antibody against Ki-67 (Clone, MIB-1; cat. no. M724001; Dako, Tokyo, Japan; 1:50) overnight at 4˚C. A further wash in PBS was followed by treatment with peroxidase-labeled anti-mouse antibody (Histofine Simple Stain Max-PO (M); Nichirei, Tokyo, Japan) as the secondary antibody for 30 min at room temperature. The staining was visualized with diaminobenzidine. Immunohistochemical evaluations were performed with a microscope (magnification, x100). A total of 1,000 tumor cells were counted to assess positive staining, and the percentages of positively stained cells were determined as the MIB-1 labeling index.

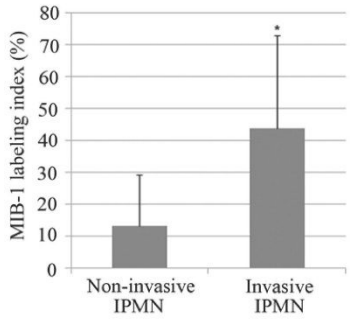

Figure 1. MIB-1 labeling index in patients with non-invasive and invasive IPMN. The MIB-1 labeling index was 13.4±15.8 and 42.4±30.3 in patients with non-invasive and invasive IPMN, respectively. The data are expressed as the mean ± standard deviation (*P<0.001). IPMN, intraductal papillary mucinous neoplasm.

Statistical analysis. Categorical variables were evaluated by either the χ^2 or Fisher's exact test. Predictors of invasive IPMNs were determined with univariate and multivariate analyses using a logistic regression model. To assess the performance characteristics of the MIB-1 labeling index, receiver operating characteristic curves were generated and the area under the curve was calculated. The survival time was observed between the date of surgery and date of the last follow-up. Overall survival was calculated using the Kaplan-Meier method and differences between the groups were assessed by the log-rank test. The data are presented as the mean ± standard deviation. All statistical calculations were performed using SPSS® version 22 (IBM SPSS, Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

MIB-1 labeling index. The MIB-1 labeling index was 13.4±15.8 in patients with non-invasive IPMN and 42.4±30.3 in patients with invasive IPMN (Fig. 1). A statistically significant difference was observed between the groups (P<0.001). A histogram of the MIB labeling index was generated according to the pathological grade (Fig. 2). The labeling index of four patients with invasive IPMN was under 5% (1 patient, 1% and 3 patients, 5%), while that of three patients with non-invasive IPMN (2 with carcinoma *in situ* component and 1 with high grade dysplasia) was over the mean labeling index of patients with invasive IPMN.

Performance of MIB-1 labeling index. The receiver operating characteristic curve is shown in Fig. 3. The calculated area under the curve was 0.822. At a cut-off level set to an index of 15.5%, sensitivity was 0.84 and specificity was 0.79. This resulted in an accuracy of 81% and four patients with invasive IPMN would be misdiagnosed. As shown in Fig. 2, the four patients with invasive IPMN exhibited 1, 5, 5 and 5% in

Table I. Comparison of various characteristics between patients with non-invasive and invasive intraductal papillary mucinous neoplasm.

Characteristic	Non-invasive (n=28)	Invasive (n=25)	P-value
Age (mean ± SD)	66.0±8.7	69.4±9.9	0.190
Gender			0.184
Male	14	17	
Female	14	8	
Involved duct			0.059
Branch	10	3	
Main or Mixed	18	22	
Size			0.743
<3.0	19	18	
≥3.0	9	7	
Symptom			0.694
No	25	21	
Yes	3	4	
Main Duct Dilatation			0.509
No	7	4	
Yes	21	21	
Mural Nodule			**0.011**
No	25	14	
Yes	3	11	
MIB-1 labeling index			**<0.001**
<15.5%	25	4	
≥15.5%	3	21	

Bold print denotes statistical significance. SD, standard deviation.

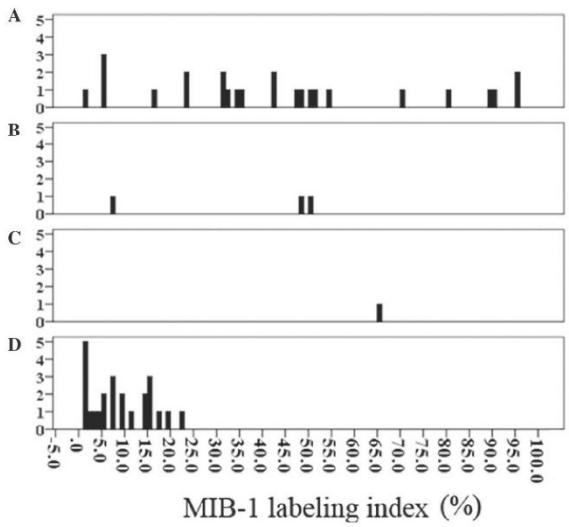

Figure 2. Histogram of the MIB-1 labeling index. (A) Invasive IPMN, and the non-invasive groups, (B) IPMN with carcinoma *in situ* component, (C) IPMN with high grade dysplasia and (D) IPMN with low grade dysplasia were plotted. IPMN, intraductal papillary mucinous neoplasm.

Table II. Univariate and multivariate analysis of potential predictive factors for invasive invasive intraductal papillary mucinous neoplasm.

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
Tumor size (>3 cm)	0.821	0.252-2.670	0.743			
Main duct or mixed type	4.074	0.972-17.071	0.055			
Symptoms	1.587	0.319-7.905	0.573			
Main duct dilatation	1.750	0.445-6.882	0.423			
Mural nodule	6.548	1.560-27.484	**0.010**	6.187	1.039-36.861	**0.045**
MIB-1 labeling index (≥15.5%)	19.250	4.750-78.011	**<0.001**	18.692	4.171-83.760	**<0.001**

Bold print denotes statistical significance. HR, hazard ratio; CI, confidential interval.

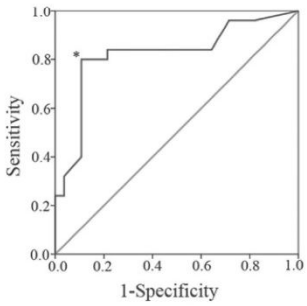

Figure 3. Receiver operating characteristics curve. The calculated area under the curve was 0.822. *The coordinate point when the cut-off threshold of the index was set to 15.5%.

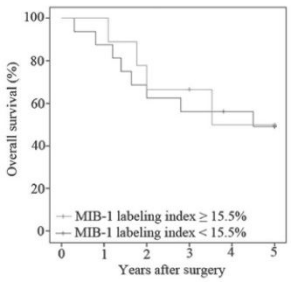

Figure 4. Survival of patients with invasive IPMN according to the level of the MIB-1 labeling index. No statistically significant difference was observed between the groups (P=0.798). IPMN, intraductal papillary mucinous neoplasm.

the MIB labeling index. Therefore, it was estimated that the cut-off level of the MIB-1 labeling index be set to 15.5% as a lower cut-off level was more likely to be inaccurate.

Comparison of characteristics of patients between non-invasive and invasive IPMN. Table I shows the comparison of characteristics of patients between non-invasive and invasive IPMN. The mean ages of patients with non-invasive IPMNs and invasive IPMNs were 66.0±8.7 and 69.4±9.9, respectively. As for the MIB-1 labeling index, patients with ≥15.5% were classified into the higher group and those with <15.5% into the lower group. The existence of a mural nodule and a higher MIB-1 labeling index were significantly more frequent in the patients with invasive IPMN compared with those with non-invasive IPMN (P=0.011 and P<0.001, respectively). No statistically significant difference was observed between non-invasive and invasive IPMN in the other examined characteristics.

Univariate and multivariate analyses of patients with non-invasive or invasive IPMN. To determine which factors are predictors of invasive IPMN, each one was measured using a logistic regression model. The results are shown in Table II. In the univariate analysis, the existence of a mural nodule and the MIB-1 labeling index achieved statistically significant differences (P=0.01 and P<0.001, respectively). In the multivariate analysis, the existence of a mural nodule (hazard ratio, 6.187; 95% confidential interval, 1.039-36.861; P=0.045) and the MIB-1 labeling (hazard ratio, 18.692; 95% confidential interval, 4.171-83.760; P<0.001) were independent predictors of invasive IPMN.

Survival of patients. The MIB-1 labeling index of the patients with invasive IPMN was 43.8±29.1. The present study decided to classify the patients into two groups to evaluate prognosis of those with invasive IPMN: Patients with a labeling index ≥50% and the patients with a labeling index <50%. Fig. 4 shows the overall survival of the patients with invasive IPMN according to the level of the labeling index. The median survival of patients with a lower MIB-1 labeling index was 4.50 years, whereas that of those with a higher index was 3.53 years. No statistically significant differences were observed between the groups (P=0.798).

Discussion

The present results revealed that the MIB-1 labeling index and the existence of a mural nodule were predictive factors for

invasive IPMN. Takeshita *et al* (22) reported the MIB-1 labeling index as a prognostic factor; however, to the best of our knowledge, this is the first report describing the MIB-1 labeling index as a predictor of invasive IPMNs. The report by Takeshita *et al* (22) revealed statistically significant differences of the MIB-1 labeling index between low- or intermediate-grade dysplasia and high-grade dysplasia, or carcinoma *in situ* component, or an invasive component of IPMN (22). Other previous reports have described the MIB-1 labeling index in the context of cell proliferation (15-21). Abe *et al* (18) reported that the MIB-1 labeling index increased in accordance with adenoma, borderline lesion and carcinoma *in situ*. However, as a result of the confusing criteria for defining malignant IPMN, it is difficult to compare these results (18). Therefore, the present study classified the patients into two groups: Non-invasive, including IPMNs with carcinoma *in situ* component, and invasive IPMN. By contrast, the existence of a mural nodule has been reported to be a predictive factor for invasive IPMN (6,7).

As for the performance of the MIB-1 index as a predictor of invasive IPMNs, the receiver operating characteristic curve proved that an index threshold of 15.5% was the best to distinguish between non-invasive and invasive IPMN. However, 4/25 patients with invasive IPMN exhibited 1, 5, 5 and 5% in the MIB labeling index. These patients could therefore not be detected by a lowered cut-off level. Among these patients, three patients exhibited a cyst size >3.0 cm, main duct dilation, or an abnormal tumor marker. Additionally, two of those exhibtied a mural nodule. They could be detected as high risk for malignancy by the worrisome features and/or high risk stigmata. Therefore, a combination of clinical features, including worrisome features and high risk stigmata with the MIB-1 labeling index would be useful.

In terms of the prognosis of invasive IPMNs, the analysis of the present study revealed no statistical significance between patients with a lower or higher MIB-1 labeling index. Takeshita *et al* (22) reported that IPMN with low- or intermediate-grade dysplasia exhibited a significantly improved prognosis compared with IPMN with an associated invasive carcinoma, while no statistically significant difference was observed between the prognosis of IPMN with high grade dysplasia and with an associated invasive carcinoma (22). It was also reported that the MIB-1 labeling index significantly increased from 1.8% in IPMN with low- or intermediate-grade dysplasia to 14-23% in carcinoma, concluding that a sudden change in proliferative activity occurred between the two. A difference in classification of IPMN existed between the previous report and the present study, which classified high grade dysplasia into non-invasive IPMN.

To use the MIB-1 labeling index as a predictor, pre-operative assessment is required. Fine-needle aspiration biopsy using endoscopic ultrasonography can provide a preoperative opportunity to assess the labeling index. However, due to concerns over intra-abdominal dissemination, fine-needle aspiration is not always performed prior to operation (23,24). The feasibility of assessing the MIB-1 labeling index using fine needle aspiration biopsy under endoscopic ultrasonography must be determined by a clinical research in the future since tumors have heterogeneity in the MIB-1 labeling index.

In conclusion, the MIB-1 labeling index and the existence of mural nodules were proven to be useful as an indicator of invasive IPMN. Although malignancy of certain patients failed to be detected by the MIB-1 labeling index, a combination of worrisome features and high risk stigmata, including the existence of mural nodule) may assist in accurately diagnosing patients with invasive IPMN. The MIB-1 index must be considered as a candidate for the classification of IPMNs.

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ORIGINAL ARTICLE

Tumor-infiltrating CD45RO$^+$ memory cells are associated with a favorable prognosis breast cancer

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Abstract

Background CD45RO is a marker for memory lymphocytes. Whether CD45RO$^+$ tumor-infiltrating lymphocytes (TILs) prevent breast cancer recurrence is unclear.

Methods In the present study, we evaluated CD45RO expression in TILs as a predictor of prognosis in 98 patients with breast cancer who underwent radical surgery without neoadjuvant chemotherapy. Patients were classified as CD45RO$^+$/TILs^{High} or CD45RO$^+$/TILs^{Low} based on median immunohistochemistry levels.

Results CD45RO$^+$/TILs^{High} were associated with smaller tumor size. The CD45RO$^+$/TILs^{High} group also had significantly fewer metastatic lymph nodes ($P = 0.0082$) and fewer peritumoral lymphatic invasions ($P = 0.0284$). The CD45RO$^+$/TILs^{High} group enjoyed longer recurrence-free survival ($P = 0.0453$) but not cancer-specific survival ($P = 0.0640$) in univariate analysis.

Conclusions These results suggested that CD45RO$^+$ effector cells may both help eradicate local tumors and prevent metastases to the lymphatic systems in breast cancer patients. High ratio of CD45RO expressing TILs was associated with recurrence-free survival improvement and a trend toward cancer-specific survival improvement in breast cancer patients.

Keywords Tumor-infiltrating lymphocytes · CD45RO · Breast cancer · Prognosis

Introduction

On encountering pathogenic microbes, naïve Ag-specific T cells proliferate and differentiate into effector T cells. Effector T cells eliminate pathogens; some of them becomes memory T cells, which resist subsequent exposure to antigens [1]. Effector T cells also help eradicate tumor cells by detecting tumor-specific antigens [2]. Although memory T cells are known to protect against microbe re-exposure, whether they can prevent cancer recurrence after tumor resection is unclear.

CD45 is known as the leukocyte common antigen. It functions as a tyrosine phosphatase in leukocyte signaling [3]. CD45RO$^+$ is a marker for memory cells—expressed when naive CD45 leukocyte antigen (CD45RA) changes into an activated isoform. As memory cells may prevent recurrence in cancer patients, CD45RO expression in tumor-infiltrating lymphocytes (TILs) might predict immune response to recurrence after tumor resection. Several studies have associated CD45RO$^+$ lymphocytes and cancer prognosis, but their results were controversial [3–15]. In the present study, we evaluated CD45RO$^+$ TIL levels as predictors of breast cancer outcomes. We retrospectively investigated whether CD45RO$^+$ TILs were generated in breast cancer specimens, and how their expression related to recurrence, and other clinicopathological factors.

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Patients and methods

Patients and specimens

Between January and December 2006, 102 women underwent radical breast surgery at the Gunma Prefectural Cancer Center. All of them were diagnosed with invasive ductal carcinoma (scirrhous/papillotubular/solid tubular types), and all had clear resected margins. None of the patients received neoadjuvant systemic chemotherapy. All patients gave informed consent. Of the 102 patients, 1 had incomplete clinical data and 3 had samples that were not appropriate for staining, leaving us with 98 subjects for whom formalin-fixed, paraffin-embedded (FFPE) tissue samples were obtained. Outcomes were recurrence-free survival (RFS) and breast cancer-specific survival (CSS), calculated from the date of surgery. Follow-up was completed in July 2013; 11 patients were lost to follow-up. Details extracted from the database were age, histological types, primary tumor size, nuclear grade, number of involved lymph nodes, lymphatic or vascular invasion, estrogen receptor (ER) or progesterone receptor (PgR) status, and HER2 score of the primary tumor. Tumors were considered as ER or PgR positive if the Allred score was $\geq$3. Lymphatic and venous invasion were categorized according to the Japanese Classification of Carcinoma: no invasion (ly0, v0), minimal invasion (ly1, v1), moderate invasion (ly2, v2), and severe invasion (ly3, v3) [16]. The recorded treatment details were type of surgery, radiotherapy, chemotherapy, and hormonal therapy given for ER^+ and/or PgR^+ cancers. Disease staging was based on the TNM classification of the International Union for Cancer Control (UICC), 7th ed.

About surgical and radiation therapies, 32 patients were received breast-conserving surgery only. Total mastectomy was performed to 61 patients, and 14 of them received radiation therapy, too. Four of 70 patients had not received hormonal therapy though they were ER+. Chemotherapy treatments were given in 50 patients, divided into 3 regimens: 6 courses of cyclophosphamide, epirubicin, and fluorouracil (CEF), and 4 courses of CEF followed by 4 courses of paclitaxel or docetaxel (taxane). Six lymph nodes positive patients refused adjuvant chemotherapy.

Immunohistochemistry

CD45RO expression was evaluated by immunohistochemistry. FFPE tissue samples were cut into 4-μm sections each, deparaffinized in xylene, and rehydrated through an ethanol gradient. No antigen retrieval was performed. Slides were peroxidase-blocked in 0.3 % H_2O_2 in methanol for 30 min. After blocking for 20 min with 10 % normal rabbit serum at room temperature in moisture boxes, these sections were then incubated for 30 min in room temperature, and overnight at 4 °C with anti-human CD45RO antibody (UCHL1, monoclonal mouse; Dako, Tokyo, Japan). The primary antibody used at the dilution of 1:800. Then these slides were washed with 1 % TBST and PBS, incubated with secondary antibody (Histofine, Nichirei Biosciences, Tokyo, Japan) at room temperature for 30 min. Sections were then washed with TBST and PBS, and visualized with DAB for about 2 min. These sections were counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene, and coverslipped. We evaluated TILs as lymphocytes located in cancer nest and intratumoral stroma except those within lymphatic vessels as previously reported [17–19]. For each slide, 3 different areas of the most highly-stained cells in the primary tumor (containing stroma near the tumor nest) were selected under ×400 magnification. We counted the total number of $CD45RO^+$ cells and TILs, and assessed the percentage of CD45RO-expressing cells among the TILs. And Ki67 expression was evaluated using anti-human Ki67 antibody (Clone SP6, monoclonal rabbit; Thermo SCIENTIFIC Inc.). Samples were considered to be high expression when the proportion of stained cells was $\geq$14 %.

Statistical analysis

The software program, JMP 5.0 (SAS Institute, Cary, NC, USA), was used to perform statistical analysis. Student's *t* test was used to analyze statistically significant differences between continuous variables, and the Chi-square test was used for categorical variables. Fisher's exact test or the Chi-square test with or without Yates' correction was used to perform univariate statistical analyses. The Kaplan–Meier method was used to develop survival curves. The log-rank test was used to assess differences between these curves. To evaluate independent prognostic significance and relative risk, we performed univariate analysis of clinicopathological factors. Any variables that were significant in the univariate analyses were included in multivariate analyses. Cox's logistic regression was used to perform the multivariate analyses. $P < 0.05$ was considered significant.

Results

CD45RO expression in TILs from breast cancer samples

To show the role of $CD45RO^+$ memory cells in breast cancer, we immunohistochemically stained $CD45RO^+$

cells from resected-breast cancers. CD45RO expression was detected in both cancer nests and stromal areas near the cancer cells. $CD45RO^+$ cells had brown-stained membrane (Fig. 1). The $CD45RO^+$ cells were in the range of 12–555, TILs were in the range of 95–928, and $CD45RO^+$/TILs was in the range of 7.4–72.9 %. The median numbers of the $CD45RO^+$, TILs, and $CD45RO^+$/TILs were 209.5, 478.5, and 49.5 %, respectively. The $CD45RO^+$/TILs had a significant relation for RFS in univariate analysis, whereas the $CD45RO^+$ number had not (Table 2). So we divided the 98 patients into 2 groups of 49 subjects each—the $CD45RO^+/TILs^{High}$ group and the $CD45RO^+/TILs^{Low}$ group–on the basis of the median calculated number of $CD45RO^+$/TILs.

Correlation between tumor-infiltrating $CD45RO^+$ cells and clinicopathological features in breast cancer patients

We performed a univariate analysis to determine the relationship between CD45RO expression and clinicopathological variables in breast cancer patients (Table 1), which

Table 1 Relations between CD45RO expression and clinicopathological characteristics

	$CD45RO^+/TILs^{High}$ ($n = 49$)		$CD45RO^+/TILs^{Low}$ ($n = 49$)		Univariate *P* value	Multivariate *P* value
	n	%	*n*	%		
Age (years)	58.4 ± 9.0		60.8 ± 10.1		0.2203	
Histopathology						
Scirrhous	29	59.2	29	59.2	0.6311	
Papillotubular	13	26.5	10	20.4		
Solidtubular	7	14.3	10	20.4		
Tumor size (mm)	19.9 ± 12.6		25.0 ± 11.8		0.0419	0.9916
Nuclear grade						
1	17	34.7	17	34.7	0.4402	
2	15	30.6	10	20.4		
3	17	34.7	22	44.9		
Positive LNs number	0.29 ± 1.24		1.55 ± 3.00		0.0082	0.1475
pStage						
I	32	65.3	15	30.6	0.0080	0.1826
IIA	12	24.5	17	34.7		
IIB	3	6.1	9	18.4		
IIIA	2	4.1	7	14.3		
IIIC	0	0.0	1	2.0		
Lymphovascular invasion						
v0	37	75.5	31	63.3	0.3213	
v1	11	22.5	13	26.5		
v2	1	2.0	4	8.2		
v3	0	0.0	1	2.0		
ly0	10	20.4	5	10.2	0.0284	0.9971
lyl	30	61.2	22	44.9		
ly2	9	18.4	20	40.8		
ly3	0	0.0	2	4.1		
ER status positive	34	63.4	36	73.5	0.6547	
PgR status positive	27	55.1	32	65.3	0.3021	
HER2 score						
0	12	24.5	7	14.4	0.2054	
1	12	24.5	9	18.4		
2	18	36.7	19	38.8		
3	7	14.3	14	28.6		
Ki67 index high	30	61.2	28	57.1	0.6810	

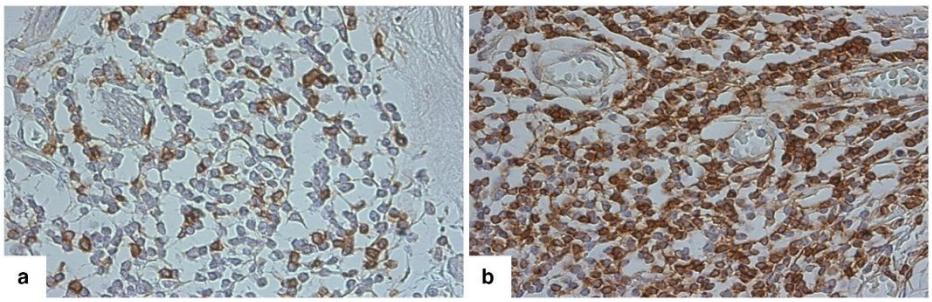

Fig. 1 Immunohistochemical expression of CD45RO. Positive cells have brown-stained membranes. **a** Low expression, **b** high expression (magnification ×400)

showed that the $CD45RO^{+}/TILs^{High}$ group tended to have smaller tumors ($P = 0.0419$), fewer metastatic lymph nodes ($P = 0.0082$), lower grade of peritumoral lymphatic invasion ($P = 0.0284$), and lower pathological stage ($P = 0.0080$) than the $CD45RO^{+}/TILs^{Low}$ group. However, these factors were not significant in multivariate analysis. These results imply that $CD45RO^{+}$ effector cells in TILs might both help eradicate local tumors and prevent metastases to the lymphatic systems in breast cancer patients.

CD45RO expression was associated with disease recurrence

As memory T cell numbers depend on the degree of effector T cells expansion, we examined the relationship between $CD45RO^{+}$ TILs and recurrence after breast cancer resection.

Recurrences were observed in 13 patients (13.3 %) over a median follow-up duration of 84.0 months; 10 of these patients died from their breast cancer recurrences; 2 had distant metastases after locoregional recurrences, 1 had axillary lymph nodes recurrence only, and 10 patients had distant metastases only. In the $CD45RO^{+}/TILs^{High}$ group, 3 patients (6.1 %) had recurrences, and 2 of the 3 had distant metastases and they died. However, in the $CD45RO^{+}/TILs^{Low}$ group, 10 patients (20.4 %) had distant metastases, and only two of them had been alive. The RFS rate of the $CD45RO^{+}/TILs^{High}$ group was significantly lower than that of the $CD45RO^{+}/TILs^{Low}$ group ($P = 0.0453$; Fig. 2). The CSS rate appeared to show lower survival with the $CD45RO^{+}/TILs^{Low}$ group, but not significantly so ($P = 0.0640$). The hazard ratio of $CD45RO^{+}/TILs^{High}$ for RFS was 0.539 in univariate analysis. As same as the $CD45RO^{+}$/TILs, the numbers of CD45RO expressing cells and the TILs were divided using these median numbers. The hazard ratio of high CD45RO expressing cells group and the high TILs group had not significant relations for RFS. Clinicopathological factors that were significantly associated with RFS in univariate

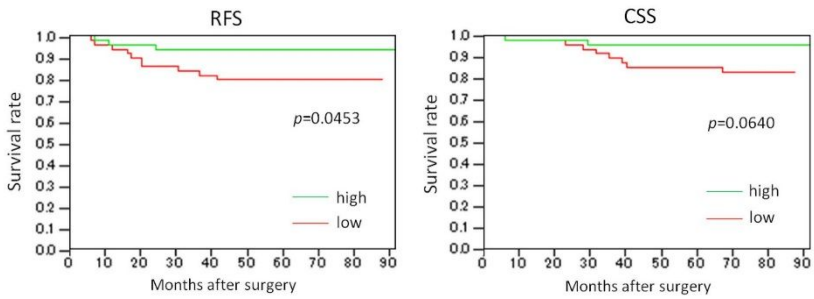

Fig. 2 Time to tumor recurrence according to Kaplan–Meier curves varies among patients depending on $CD45RO^{+}$/TILs. Over 84.0-month median follow-up period, RFS curves show significantly lower survival for patients with low $CD45RO^{+}$/TILs

Table 2 Predictors of recurrence-free survival

RFS	Univariate analysis			Multivariate analysis	
	HR	95 % CI	*p* value	HR	95 % CI
$CD45RO^+$/TILs High	0.539	0.255–0.974	0.0405	0.476	0.105–1.293
TILs High	1.552	0.885–2.981	0.1271		
$CD45RO^+$ High	0.781	0.429–1.352	0.3799		
Age <60 years	0.895	0.480–1.631	0.7129		
Tumor size >20 mm	1.529	0.840–2.988	0.1650		
Nuclear grade 3	2.056	1.105–4.393	0.0221	1.324	0.422–4.101
Positive lymph nodes ≥4	2.697	1.446–4.919	0.0029	1.140	0.372–3.193
Lymphovascular invasion					
ly ≥2	2.043	1.122–3.993	0.0196	0.997	0.358–3.126
v ≥1	2.569	1.381–5.492	0.0026	2.976	0.839–15.195
ER negative	1.482	0.796–2.702	0.2045		
PgR negative	2.090	1.124–4.468	0.0192	1.358	0.545–3.684
HER2 score ≥2	1.366	0.735–2.919	0.3369		
Surgical/radiation therapy: breast-conserving only	0.445	0.104–1.017	0.0557		
Adjuvant chemotherapy not performed	0.924	0.496–1.684	0.7950		
Hormonal therapy not performed	2.973	1.124–6.417	0.0317	2.099	0.515–7.377

HR hazard ratio, *CI* confidence interval

analysis are shown in Table 2, but these factors, including $CD45RO^+$/TILs, lost their significance in multivariate analysis. Among therapeutic influences, hormonal therapy with cancelation was a negative factor in univariate analyses but was not significant in multivariate analysis. Taken together, $CD45RO^+$ memory cells may be important in preventing tumor recurrence after resection in breast cancer patients.

Discussion

Several studies have shown relationships between TILs (especially $CD8^+$ T cells) and cancer prognosis [20, 21]. TILs (especially $CD8^+$ T cells) are reportedly correlated with better prognosis in breast cancer patients [17, 22–24]. Some reports attempted to present TILs as a predictive factor of neoadjuvant chemotherapy response in breast cancer [18, 19, 25–27]. Therefore, TILs both eradicated local tumors and prevented recurrence in breast cancer patients.

The presence of different cell-surface CD45 isoforms seems to distinguish naive and memory T cells. Cell-surface CD45RA was thought to indicate naive cells, whereas CD45RO indicated memory cells. After antigen exposure, effector cells and memory cells gain expression of CD45RO and lose expression of CD45RA. However, distinguishing between effector and memory cells can be difficult because both effector and memory cells express CD45RO. In the present study, we examined expression of CD45RO in TILs from resected breast cancers. Almost all $CD45RO^+$ TILs were probably effector cells because effector cells migrate mainly to tumors, whereas memory cells migrate to lymphoid tissue. In the present study, we found that the $CD45RO^+/TILs^{High}$ group tended to have smaller tumors than the $CD45RO^+/TILs^{Low}$ group. Furthermore, the $CD45RO^+/TILs^{High}$ group had significantly less frequent axillary lymph node metastasis than the $CD45RO^+/TILs^{Low}$ group. These results suggest that $CD45RO^+$ effector cells among TILs may both help eradicate local tumors but also prevent metastasis to the lymphatic systems in breast cancer patients.

Although memory cells are critical for protection against secondary infection after microbe re-exposure, whether they prevent recurrence after cancer resection is unclear. Reportedly, $CD45RO^-$ TILs are associated with better overall survival (OS) and/or disease-free survival (DFS) in esophageal squamous carcinoma [4], gastric cancer [5, 6], colorectal cancer [7, 8, 11, 12], and so on. However, Hotta et al. revealed paradoxical OS in renal cell carcinoma [3]. Wakatsuki et al. showed that $CD45RO^+$ associated with better prognosis only in advanced cases in gastric cancer [5], but in colon cancer, it is an independent prognostic variable in early-stage disease [8, 13]. In breast cancer, we knew of no studies that correlated CD45RO and prognosis, although one report on medullary carcinoma of the breast described a significantly higher proportion of $CD45RO^+$ TILs in medullary carcinoma compared with atypical medullary carcinoma [28, 29]. In this study, we found that the $CD45RO^+/TILs^{High}$ group had a significantly lower

recurrence rate than in the $CD45RO^{+}/TILs^{Low}$ group, indicating that memory cells may help prevent recurrence. Because lymph node metastasis is an important prognostic factor in patients with breast cancer, the $CD45RO^{+}/TILs^{High}$ group may have a better prognosis because of the low frequency of the presence of lymph node metastasis. However, almost all patients who suffered recurrence had distant recurrences but not locoregional lymph node recurrences. Among patients with no lymph node metastasis, the $CD45RO^{+}/TILs^{High}$ group was comparable to the $CD45RO^{+}/TILs^{Low}$ group for RFS (data not shown). Among patients with lymph node metastases, no $CD45RO^{High}$ patients had relapsed, whereas almost 32 % of the $CD45RO^{Low}$ patients had relapsed, implying that $CD45RO^{+}$ cells prevent recurrence in node-positive patients. Larger cohorts of patients are needed to clarify this possibility.

CD45RO is a surface marker for mature leukocytes, because effector/memory T cells are largely of the $UCHL1^{+}$ phenotype. But UCHL1 can label activated mature memory T cells, as well as a subpopulation of resting T cells within both the CD4 and CD8 subsets. Some monocytes/macrophages, granulocytes and dendritic cells are also labeled, whereas normal B cells and NK cells are negative [30]. Moreover, $UCHL1^{+}$ cells are activated by some antigen but not by tumor-specific effector/memory T cells, strictly. Therefore, additional research for detailed differential leukocyte markers using flow cytometry is warranted to explore the significance of $CD45RO^{+}$ TILs for breast cancer prognosis. This study has several other potential limitations. The major limitation is that it used retrospective methods of data collection. Additionally, the number of patients in our study was relatively small.

In conclusion, our results suggest that the ratio of the $CD45RO^{+}$/TILs are higher in earlier-stage breast cancer, and are associated with better RFS. The $CD45RO^{+}$/TILs could be a useful marker for better RFS in breast cancer.

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Conflict of interest The authors declare that they have no competing financial interests.

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