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Original Article

Blood vessel invasion and other variables as predictors of long-term survival in Japanese and British patients with primary invasive breast cancer

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Abstract: This study was undertaken to investigate the associations of blood vessel invasion (BVI), lymphatic vessel invasion (LVI) or other variables and long-term survival in 173 Japanese and 184 British patients with primary invasive breast cancer, and whether they are associated with survival differences between Japanese and British patients. BVI was detected by objective methods, using both factor VIII-related antigen (F-VIII) staining and elastica van Gieson (E v G) staining. BVI was classified into three subtypes. 1) BVI e, BVI detected by E v G staining alone, 2) BVI f, BVI detected by F-VIII staining alone, 3) BVIef, BVI evaluated by combining BVIf and BVIe. LVI was also detected by objective methods, using lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) staining alone. There was a borderline significance between the frequencies for BVIef of British patients and those of Japanese patients (8.2% vs 3.5%; $P = 0.06$) but not for LVI ($P = 0.36$). British patients had a significantly worse relapse-free survival (RFS) and overall survival (OS) than Japanese patients ($P < 0.01$, $P < 0.01$, respectively) even though their tumors were smaller and more ER-positive with a similar prevalence of lymph-node involvement. LVI was not significantly associated with RFS and OS, however, BVIef positive tumors had a significantly worse RFS and OS compared with BVIef negative patients, after statistical adjustment for the other variables ($P = 0.02$, $P = 0.01$, respectively). The present study shows that BVIef variability might contribute to the Japanese and British disparities in breast cancer outcomes.

Keywords: Breast cancer, ethnicity, blood vessel invasion, lymphatic vessel invasion, LYVE-1 staining, *KDR/Flk-1*

Introduction

Previous studies have shown that Asian-American and Japanese patients tend to have a lower incidence of breast cancer and have a better prognosis than Caucasians [1-4]. Racial differences in age-adjusted mortality rates are likely to be due to many factors, such as genetics, diet, reproductive patterns, socioeconomic status, geographic and environmental exposures and other unidentified cultural or biological factors [5-11]. Although there have been numerous studies about racial disparities in breast cancer incidence and outcomes, the reason for such disparities has not been identified.

As green tea, consumed many times daily by the average Japanese, inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells [12, 13], the authors have hypothesized that differences in microvessel density (MVD) might contribute to the Japanese and British disparities in breast cancer outcomes. The study showed that although a difference in MVD exists between Japanese and British patients, it did not explain the survival [4]. The authors speculated that other surro-

gate markers of angiogenesis or hematogenous dissemination contributed to the poorer survival of British patients.

The purpose of this study is to investigate the associations of blood vessel invasion (BVI) and other variables such as lymphatic vessel invasion (LVI), *p53*, *Bcl-2* and vascular endothelial growth factor receptor -2 (*KDR/Flk-1*), with long-term survival, and whether they accounted for survival differences between Japanese and British patients.

Materials and methods

Patients and specimens

The original study consisted of 217 Japanese and 219 British patients. Patients with non-invasive, Stage IV, bilateral, male, or inflammatory cancers, have been excluded [4]. Patients underwent surgery at the Tokyo Women's Medical University Hospital or the John Radcliffe Hospital, Oxford between 1991 and 1993. Cases where insufficient material remained in the tissue blocks for immunohistochemical evaluation of factor VIII related antigen were excluded. Eleven Japanese and 14 British samples for study were identified retrospectively as having no carcinoma and paraffin-embedded tissue blocks of 33 Japanese and 21 British cases were insufficient because they have been used for other research. These left 173 cases in the Japanese and 184 cases in British group for which recent follow up data and tissue were available. The date of the last note in the medical record in Tokyo was 2009, whilst in Oxford it was 2012. Only death from breast cancer alone was taken as an endpoint and cases that died from other diseases were excluded. The results of the study of clinical, biochemical and histopathological features carried out on these patients have been described previously [4] and the data of PT-stage, ER status, lymph-node status and grade were used for multivariate analysis between those conventional factors and new prognostic factors. Estrogen receptor (ER) content was determined biochemically using the dextran-coated charcoal (DCC) method in Tokyo and Oxford. Tumors were classified as ER-positive if the content exceeded 5 fmol/μg protein.

The pathological specimens from both hospitals were reviewed without any knowledge of the clinical outcome by the same investigator (T. K.). Conventional pathological features were observed and recorded, including lymph-node status. Operative and pathological sizes were used for the pT-stage in Japanese patients but only pathological size for British patients. Grade was determined as previously described [14].

Immunohistochemistry

Serial sections were prepared from representative formalin-fixed and paraffin-embedded tissue blocks from this series of breast cancer. 5 μm tissue sections were stained with H&E and were used to select the area with the largest invasive components. Immunostaining for factor VIII-related antigen was performed on paraffin sections using the streptavidin-biotin-immunoperoxidase method as previously described [15, 16]. Briefly, formalin-fixed, paraffin-embedded sections were de-waxed in 100% Citroclear, re-hydrated through graded industrial methylated spirit (IMS) series, and immunostaining was performed using a polyclonal antibody (von Willebrand factor, Dako, Copenhagen, Denmark) applied at 1:200 for 1 hour at room temperature. Technical details of the polyclonal and monoclonal lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) staining are outlined in a previous study [17]. A normal human tonsil served as a positive control. Rabbit polyclonal antibody and mouse monoclonal antibody to LYVE-1 (LYVE-1/PCAB and LYVE-1/MCAB) were generated as described previously [18, 19].

The primary antibodies used were against *p53* (DO7 MoAb, DAKO, Denmark), *Bcl2* (clone 100 MoAb, DAKO, Denmark) and *KDR* (SC6251, Santa Cruz, CA, USA). The secondary antibody (DAKO anti-mouse envision HRP polymer) was applied at room temperature, followed by DAB chromogen (DAKO, Denmark) for 5 minutes and counterstained with Meyer's hematoxylin and mounted. Positive controls were sections of tonsil.

Nuclear labeling by *p53* protein staining was evaluated by counting 500 consecutive cells along the border between cancer nests and the stroma at high power (20×). *p53* scoring (cancer cells (CCs)) was described as follows: low, less than mean of labeling index (LI); and high, more than or equal to mean of LI (**Figure 1A**).

The cytoplasm of the cancer cells was stained by *Bcl-2* and was scored as a percentage of the

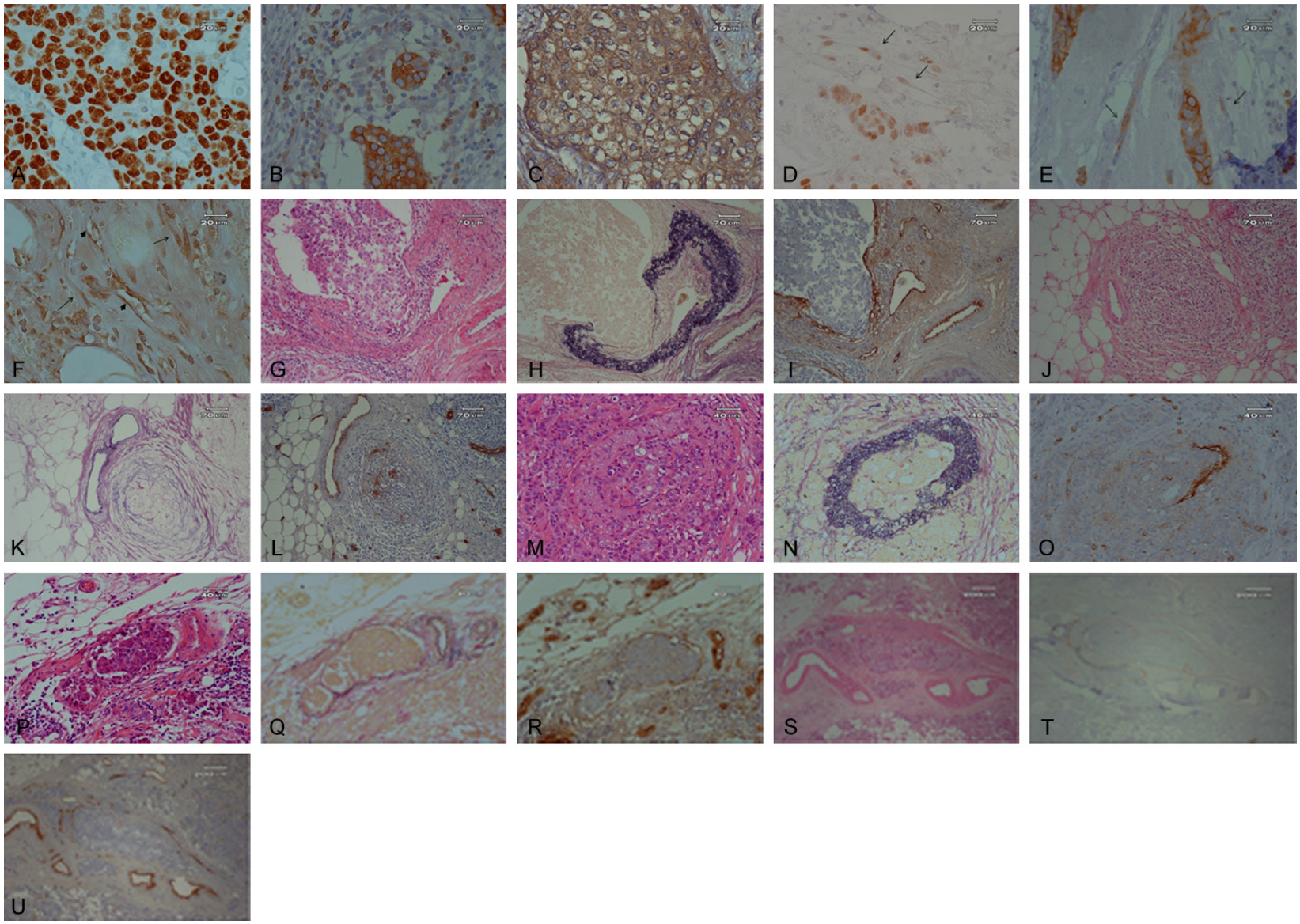
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Figure 1. (1) Immunohistochemical staining of tumor cells, endothelial cells and stromal fibroblasts with antibodies to *p53, Bcl-2 or KDR*. Tumor cells are immunostained with the three antibodies (A: *p53* staining, B: *Bcl-2* staining, C: *KDR* staining). Lymphocytes always stained intensely (B). The arrows indicate stromal fibroblasts stained by *p53*, *Bcl-2* and *KDR* staining (D: *p53* staining, E: *Bcl-2* staining, F: *KDR* staining). The arrowheads indicate endothelial cells stained by *KDR* staining (F). (2) Representative examples of blood vessel invasion. Blood vessels directly infiltrated by cancer cells (Type I, G: H&E staining; H: elastica van Gieson staining; I: factor VIII-related antigen staining, hematoxylin counter stain). (3) Blood vessels filled with tumor cell emboli (Type II, J: H&E staining; K: elastica van Gieson staining; L: factor VIII-related antigen staining, hematoxylin counter stain). (4) Blood vessels with growing of cancer cells between endothelium and lamina elastica interna (Type III, M: H&E staining; N: elastica van Gieson staining; O: factor VIII-related antigen staining, hematoxylin counter stain). Endothelial cells collapsed in expanding tumors are stained by factor VIII-related antigen staining. (5) Blood vessels with floating tumor cells (Type IV, P: H&E staining; Q: elastica van Gieson staining; R: factor VIII-related antigen staining, hematoxylin counter stain). (6) It is difficult to distinguish LVI from ductal carcinoma in situ (DCIS) or Type II BVI by H&E staining alone (S: H&E staining). The monoclonal anti LYVE-1 antibodies yielded specific and consistent staining of endothelial cells in the lymphatic vessels (T: LYVE-1 staining, hematoxylin counter stain). The pattern of staining in the lymphatic vessel by factor VIII-related antigen staining was very faint, discontinuous and inconsistent in contrast to the intense and continuous staining observed in the vascular endothelium (U: factor VIII -related antigen staining, hematoxylin counter stain).

total number of cancer cells as follows: score 0, < 25%; score 1, 25% to 50%; score 2, > 50%. *Bcl-2* scoring (CCs) was described as follows: Score 0 was considered as negative and score 1 or score 2 as positive (**Figure 1B**).

The cytoplasm of the cancer cells was stained by *KDR* and was scored as a percentage of the total number of cancer cells as follows: score 0, < 10%; score 1, 11% to 30%; score 2, 31% to 50%; score 3, > 51%. *KDR* scoring (CCs) was described as follows: Score 0 or score 1 was considered as negative and score 2 or score 3 as positive (**Figure 1C**).

To evaluate endothelial cells and stromal fibroblasts, one maximal area of all the cut surfaces exhibiting invasive components in each tumor was scanned at low power (4×). The number of endothelial cells and stromal fibroblasts stained by *p53*, *Bcl-2* and *KDR* in the 3 areas scanned at high power (20×) along the border between cancer nests and the stroma was recorded. The average number of each in the 3 fields was calculated and the number per square millimeter was employed for *p53* (endothelial cells (ECs)), *Bcl2* (ECs) and *KDR* (ECs) (**Figure 1D-F**). They were described as follows: low, less than the mean of all the numbers; and high, more than or equal to those.

Assessment of BVI and LVI

BVI was defined as the presence of tumor cell emboli within a blood vessel space or between the endothelium and the lamina elastica interna, which were identified by endothelial lining stained by factor VIII-related antigen and elastica van Gieson staining. BVI was evaluated on one section stained with HE, factor VIII-related antigen staining and elastica van Gieson staining as follows: 1) BVI e, BVI detected by elastica van Gieson staining alone, 2) BVI f, BVI detected by factor VIII-related antigen staining alone, 3) BVI ef, BVI evaluated by combining BVIf and BVIe. BVI-positive tumors were defined as lesions showing BVI f or BVI e or BVI ef. BVI-negative tumors showed no invasion by any staining method.

The author morphologically classified the BVI into 4 types according to patterns of these blood vessels invaded by cancer cells as previously described [15, 16]. Briefly, type I was a blood vessel directly infiltrated by tumor cells (**Figure 1G-I**), type II was a blood vessel filled with tumor cell emboli (**Figure 1J-L**), type III was a blood vessel with cancer cell growth between the endothelium and the lamina elastica interna (**Figure 1M-O**), and type IV was a blood vessel with floating tumor cells (**Figure 1P-R**).

LVI was defined as the presence of tumor cell emboli within a lymphatic vessel space, which were identified by associated fibrin clot and/or endothelial lining stained by LYVE-1 staining. As the distinction between lymphatic vessels and blood vessels stained with HE staining alone is difficult and sometimes arbitrarily determined, LYVE-1 staining was used to distinguish between them (**Figure 1S-U**). All doubtful cases were considered to be negative.

Statistical analysis

Statistical analysis of the data was performed with the Survival Tools for Statview-J 5.0 package (Abacus Concepts, Berkeley, CA). For asso-

Table 1. Clinicopathological characteristics of Japanese and British patients

Characteristics	Japanese (%)	British (%)	*P*-value
Patients	173	184	
Age, y			
Median	51	56	
Range	24-86	27-83	
Survival follow-up, Yrs			
Median	8.1	12.2	
Range	0.1-17.3	0.5-21.6	
Recurrence	40	80	
Deaths	21	64	
BVIf			0.19
Negative	138 (79.8)	136 (73.9)	
Positive	35 (20.2)	48 (26.1)	
BVIe			0.31
Negative	152 (89.0)	157 (85.3)	
Positive	19 (11.0)	27 (14.7)	
Unknown	2	0	
BVIef			0.06
Negative	167 (96.5)	169 (91.8)	
Positive	6 (3.5)	15 (8.2)	
LVI			0.36
Negative	117 (67.6)	116 (63.0)	
Positive	56 (32.4)	68 (37.0)	
p53 scoring (CCs)[a]			0.07
Low	97 (62.6)	93 (63.0)	
High	58 (37.4)	83 (47.2)	
Unknown	18	8	
p53 (ECs)[b]			0.01
Low	130 (83.9)	91 (51.7)	
High	25 (16.1)	85 (48.3)	
Unknown	18	8	
Bcl-2 scoring (CCs)[c]			0.01
Negative	95 (55.2)	70 (38.5)	
Positive	77 (44.8)	112 (61.5)	
Unknown	1	2	
Bcl-2 (ECs)[d]			0.01
Low	105 (61.0)	45 (24.7)	
High	67 (39.0)	137 (75.3)	
Unknown	1	2	
KDR scoring (CCs)[e]			0.01
Negative	19 (11.8)	49 (26.8)	
Positive	142 (88.2)	134 (73.2)	
Unknown	12	1	
KDR (ECs)[f]			0.01
Low	116 (72.0)	102 (55.7)	
High	45 (28.0)	81 (44.3)	
Unknown	2	1	

ciation with clinicopathological factors and the two groups (Japanese and British patients), chi-square test or Fisher exact tests were used. The authors examined the univariate relationships between prognostic indicators and relapse-free survival (RFS) and overall survival (OS) by fitting Kaplan-Meier survival curves [20] and then looking for differences among the curves using the log-rank test [21]. The Cox proportional hazards regression model was also used for the multivariate analysis [22].

Results

Pathological analysis of the 357 patient samples

Tumor tissue for the study was available from 357 patients. The median follow-up duration for the Japanese patients was 8.1 years (range, 0.1 to 17.3) and for the British 12.2 years (range, 0.5 to 21.6). Clinical and pathological data are listed in **Table 1**. Seven patients were lost to follow up and one patient was dead within one year. Median ages were 51 years for Japanese and 56 years for the British patients. Japanese patients had proportionately more pT2 and pT3 cases than did the British patients (46.8%, 8.1% vs 34.8%, 2.2%, respectively, $P < 0.01$), while the number of ER-positive patients was significantly higher in British patients (57.2% vs 78.8%, $P < 0.01$). No differences in the prevalence of lymph-node metastases were found (37.5% vs 39.1%, $P = 0.75$). There was a borderline significance between the frequencies for the BVIef of British patients and those of Japanese patients (8.2% vs 3.5%; $P = 0.06$), but not for the frequencies of BVIf, BVIe and LVI. A large proportion of the British patients had a higher percentage of stained cancer cells for *Bcl-2* scoring (CCs) but not for *p53* scoring (CCs). Moreover the proportion of British patients with a higher staining percentage for *Bcl-2* (ECs) and/or *p53* (ECs) was also bigger. A larger proportion of Japanese patients had a positive staining for *KDR* in cancer cells but the British had the highest proportion of patients with stromal and endothelial staining.

RFS and OS stratified by population

British patients had a significantly worse RFS and OS compared with Japanese patients ($P < 0.01$, $P < 0.01$, respectively; **Figure 2A** and **2B**).

BVIf: blood vessel invasion stained by factor VIII related antigen; BVIe: blood vessel invasion stained by elastica van Gieson staining; BVIef: both blood vessel invasion including BVIf and BVIe; LVI: lymphatic vessel invasion; KDR: vascular endothelial grpwth factor receptor-2; [a]p53 scoring (CCs): labeling index of cancer cells stained by p53 antigen staining; [b]p53 (ECs): No of endothelial cells or stromal fibroblasts stained by p53 antigen staining; [c]Bcl-2 scoring (CCs): scoring of cancer cells stained by Bcl-2 antigen staining; [d]Bcl-2 (ECs): No of cancer cells and endothelial cells or stromal fibroblasts stained by Bcl-2 antigen staining; [e]KDR scoring (CCs): scoring of cancer cells stained by KDR antigen staining; [f]KDR (ECs): No of endothelial cells or stromal fibroblasts stained by KDR antigen staining.

Univariate analysis of RFS and OS

The prognostic factors found to be significantly associated with RFS were; population, lymph-node status, pT-stage, grade, BVIf, BVIef and *KDR* positivity. Moreover, population, lymph-node status, pT-stage, grade, BVIf, BVIe and BVIef were also associated with OS, while *KDR* scoring (CCs) was of borderline significance (**Table 2**).

Multivariate analyses of RFS and OS

Model 1 in **Table 3** indicates that British patients had a significantly worse RFS and OS compared with Japanese patients and patients with node-positive carcinoma or pT2 or pT3-stage had a significantly worse RFS and OS compared with the patients with node-negative carcinoma or pT1-stage. Model 2 in **Table 3** indicates that British patients also had a significantly worse RFS and OS compared with Japanese patients and patients with node-positive carcinoma or pT2 or pT3-stage had a significantly worse RFS and OS compared with the patients with node-negative carcinoma or pT1-stage. Patients with BVIef-positive carcinoma had a significantly worse RFS and OS compared with the patients with BVIef-negative carcinoma.

Discussion

Some investigators have reported that Japanese patients with breast cancer have a better survival than British patients [4, 7, 8]. However, the reason for such disparities has not been identified. To our knowledge, this is the first study to compare the prognostic value of BVI and LVI detected by objective methods in all Japanese and British patients with invasive breast cancer.

The clinico-pathological difference of hematogenous versus lymphatic metastatic spread remains unclear. Though several investigators think it is impractical to distinguish between the blood and lymphatic vessel systems as independent routes of tumor dissemination because they are so interrelated [23], the authors have examined the difference between BVI and LVI to see if they are different pathways to metastasis [15, 16]. In the current study, the authors used the objective methods to examine the associations of BVI and LV and investigated whether they are associated with survival differences between Japanese and British patients.

The rate of BVI observed by Lauria et al was 4.2% [24]. They detected BVI using H&E staining alone and the prevalence of BVI was particularly low in their study. In a previous study Kato et al also evaluated BVI by H&E staining alone and found a rate of 6.5% similar to Lauria et al [15, 24]. By H&E staining alone, it was difficult to detect blood vessels filled with tumor cell emboli, to distinguish between small blood vessel invasion and lymphatic vessel invasion, to find the blood vessels with cancer cell growing between the endothelium and the lamina elastica interna and to distinguish BVI from ductal carcinoma in situ (DCIS) covered with elastic fibers [15]. Therefore, there was a possibility of underestimating the degree of blood vessel invasion in these studies.

The authors previously used both factor VIII-related antigen and elastica van Gieson staining in two studies of Japanese patients with breast cancer where the rate of BVI was 27.4% and 29.3% respectively [15, 16]. The current study using elastica van Gieson staining, factor VIII-related antigen and LYVE-1 staining showed that the rate of BVI was 34.6% in Japanese and 48.9% in British breast cancer patients. Whereas the rate of BVI in Japanese patients was similar to the previous studies, the prevalence of BVI in British patients was particularly high.

Previous reports have suggested that vascular invasion (blood vessel invasion and lymphatic vessel invasion) [25-28] or LVI [24, 29] are significant prognostic factors. However a few recent studies show that BVI has been associated with overall survival [15, 30]. A wide range of frequencies has been reported for BVI among patients with breast cancer and the prognostic significance has not yet been made clear [15, 24, 30, 31].

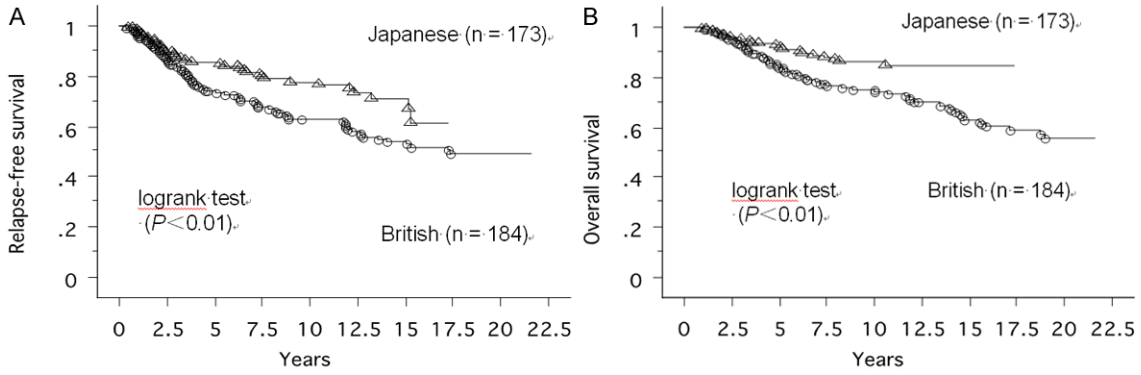

Figure 2. Kaplan-Meier survival curves for all patients with breast cancer. A. Relapse-free survival stratified by population. B. Overall survival related to population.

Table 2. Unvariate analysis of the value of prognostic factors for relapse-free survival and overall survival among Japanese and British patients

	Relapse-free survival			Overall survival		
Variables	HR[a]	95% CI	*P*-value	HR[a]	95% CI	*P*-value
Population						
British vs. Japanese	1.6	1.1-2.4	0.01	2.2	1.3-3.7	0.01
ER status						
Negative vs Positive	0.7	0.4-1.1	0.16	0.7	0.4-1.3	0.35
Lymph-node status						
Positive vs Negative	2.5	1.8-3.7	0.01	2.7	1.7-4.2	0.01
pT-stage						
pT2 vs pT1	2.1	1.4-3.0	0.01	2.3	1.4-3.6	0.01
pT3 vs pT1	3.2	1.5-6.8	0.01	4.3	1.8-9.8	0.01
Grade						
II vs I	1.9	1.1-3.1	0.01	2.1	1.1-3.9	0.01
III vs I	2.1	1.3-3.6	0.01	2.5	1.3-4.7	0.01
BVIf						
Positive vs Negative	1.7	1.1-2.5	0.01	1.9	1.2-2.9	0.01
BVIe						
Positive vs Negative	1.3	0.8-2.2	0.23	1.7	1.0-2.9	0.04
BVIef						
Positive vs Negative	2.1	1.1-3.8	0.01	2.5	1.3-4.9	0.01
LVI						
Positive vs Negative	1.1	0.8-1.6	0.41	0.8	0.5-1.3	0.51
p53 scoring (CCs)[b]						
High vs. Low	0.9	0.6-1.3	0.82	1.0	0.6-1.6	0.81
p53 (ECs)[c]						
High vs. Low	1.0	0.7-1.5	0.85	0.8	0.5-1.4	0.61
Bcl-2 scoring (CCs)[d]						
Negative vs Positive	1.2	0.9-1.8	0.16	1.4	0.9-2.1	0.11
Bcl-2 (ECs)[e]						
Low vs. High	1.2	0.8-1.8	0.17	1.0	0.6-1.6	0.73
KDR scoring (CCs)[f]						
Negative vs. Positive	1.6	1.0-2.5	0.01	1.6	0.9-2.6	0.05
KDR (ECs)[g]						
High vs. Low	1.0	0.7-1.5	0.70	1.0	0.6-1.6	0.87

It has been suggested that a high rate of neovascularization could facilitate cancer cells easy invasion into blood vessels and thereby facilitate the metastatic process [32, 33]. Nagy et al demonstrated that the newly formed blood vessels were of a leaky nature, increasing their permeability and making the vascular invasion easier to accomplish [34]. This suggests a very strong relationship between neovascularization and small blood vessel invasion or capillaries invasion [35]. A few cancer cells that invade capillaries or small blood vessels at the points of microcirculation, such as BVIf, did not affect the prognosis of breast cancer patients, however, a heavy infiltration of large blood vessels, as indicated by BVIe or BVIef at the relatively proximal vessels, strongly affected the prognosis of them, as we reported in the previous paper [15]. The current study shows similar results.

Population, pT-stage, lymph-node status and BVIef were significantly independent prognostic factors in all Japanese and British patients, after statistical adjustment for the other variables. British patients had a significantly worse RFS and OS than Japanese patients even though their tumors were smaller with a similar prevalence of lymph-node involvement. Although there was a borderline significance

[a]HR: hazards ratio; 95% CI: 95% confidence interval; ER: estrogen receptor; Grade: histological grade; BVIf: blood vessel invasion stained by factor VIII related antigen; BVIe: blood vessel invasion stained by elastica van Gieson staining; BVIef: both blood vessel invasion including BVIf and BVIe; LVI: lymphatic vessel invasion; *KDR*: vascular endothelial grpwth factor receptor-2; Hazard ratio from Cox regeression analysis; [b]*p53* scoring (CCs): labeling index of cancer cells stained by *p53* antigen staining; [c]*p53* (ECs): No of endothelial cells or stromal fibroblasts stained by *p53* antigen staining; [d]*Bcl-2* scoring (CCs): scoring of cancer cells stained by *Bcl-2* antigen staining; [e]*Bcl-2* (ECs): No of cancer cells and endothelial cells or stromal fibroblasts stained by *Bcl-2* antigen staining; [f]*KDR* scoring (CCs): scoring of cancer cells stained by *KDR* antigen staining; [g]*KDR* (ECs): No of endothelial cells or stromal fibroblasts stained by *KDR* antigen staining.

between the frequencies for the BVIef of British patients and those of Japanese patients in the present study, BVIef variability might contribute to the Japanese and British disparities in breast cancer outcomes.

Quantitation of tumor lymphatic vessels has for decades been problematic. Although morphology can sometimes distinguish lymphatic vessels from blood vessels by the frequent absence of a basement membrane and lack of erythrocytes in the latter, neither is a reliable method for routine use. More recently however, the development of specific markers such as LYVE-1 and D2-40 have allowed many new experimental studies of tumor lymphatics to be initiated [17, 36, 37]. Our previous work confirms that both LYVE-1 polyclonal and monoclonal antibodies distinguish efficiently between lymphatic and blood vessels in pathological specimens [17].

The current study has used LYVE-1 staining to increase the accuracy and rate of detection of LVI since using this method makes it easy to distinguish from BVI. In recent human studies the rate of LVI fell to within the range of 13.3% and 53.3% [38, 39], while in this study the rate of LVI detected by LYVE-I staining was seen in 32.4% in Japanese patients and 37.0% in British patients and was similar to a previous study [17]. Schoppmann *et al* demonstrated that LVI assessed by anti-podoplanin immunostaining has been strongly associated with the presence of lymph-node metastases and unfavorable overall survival in human breast cancer [40]. However, the present study showed that LVI did not contribute to the Japanese-British disparity in breast cancer and LVI variability did not explain the survival differences between Japanese and British patients.

p53 accumulation has been associated with a poor prognosis in other studies of patients with breast cancer [41] and the expression of *Bcl-2* in breast cancer is associated with favorable prognostic factors such as low-grade histology, ER positivity and slow proliferation [42]. In this study, *p53* accumulation and *Bcl2* expression in all breast cancer patients was not statistically associated with clinical outcome and did not explain the survival differences between Japanese and British patients.

KDR is a transmembrane receptor that plays an important role in endothelial cell development and *KDR* actions are related to angiogenesis [43, 44]. Some investigators reported that *KDR* positivity was observed in 91of 141 cases (64.5%) in invasive breast cancer [45] and *KDR* expression was associated with the nuclear grade. In the present study, *KDR* (CCs) positivity was observed in 142 of 161 cases (88.2%) in Japanese patients and in 134 of 183 cases (73.2%) in British patients. The *KDR* (CCs) positivity rate in both Japanese and British patients was higher than that of the previous study. Moreover, the ratio of *KDR* (CCs)-positive Japanese patients was significantly higher than that of British patients. Nakopoulou et al and Ryden et al demonstrated that *KDR* positivity in breast cancer was not significantly associated with survival [45, 46], although in this study it was associated with a favorable prognosis.

These results suggest that a hematogenous pattern of dissemination may be more important than a lymphatic one for ethnic disparities in breast cancer outcomes. The authors acknowledge that a large number of patients will be required to better assess these relationships but we conclude that BVIef is an important surrogate marker of hematogenous dissemination and might contribute to the Japanese and British disparities in breast cancer outcomes.

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Table 3. Multivariate analysis of the value of prognostic factors for relapse-free survival and overall survival among all Japanese and British patients

model	model 1[b]						model 2[c]					
	relapse-free survival			overall survival			relapse-free survival			overall survival		
Variable	HR[a]	95% CI	*P*-value	HR[a]	95% CI	*P*-value	HR[a]	95% CI	*P*-value	HR[a]	95% CI	*P*-value
Population												
British vs. Japanese	2.1	1.3-3.2	0.01	3.1	1.7-5.5	0.01	2.1	1.3-3.3	0.01	3.0	1.7-5.3	0.01
pT-stage												
pT2 *vs* pT1	2.2	1.5-3.4	0.01	2.6	1.6-4.3	0.01	2.3	1.5-3.5	0.01	2.6	1.6-4.3	0.01
pT3 *vs* pT1	4.3	1.9-9.8	0.01	6.3	2.6-15.2	0.01	4.8	2.1-10.9	0.01	6.6	2.7-16.0	0.01
Lymph-node status												
Positive vs. Negative	2.5	1.7-3.6	0.01	2.6	1.6-4.0	0.01	2.6	1.8-3.8	0.01	2.7	1.7-4.2	0.01
Grade												
II *vs* I	1.7	1.0-2.9	0.03	2.6	1.6-4.0	0.08	1.8	1.0-3.1	0.02	1.8	0.9-3.5	0.06
III *vs* I	1.6	0.9-2.8	0.08	1.6	0.8-3.2	0.16	1.7	1.0-3.0	0.04	1.8	0.9-3.6	0.08
BVIf												
Positive vs Negative	1.2	0.8-1.9	0.22	1.4	0.9-2.2	0.12						
BVIef												
Positive vs Negative							2.0	1.1-3.7	0.02	2.2	1.1-4.4	0.01
KDR (CCs)												
Negative vs Positive	1.2	0.8-1.9	0.22	1.1	0.7-1.9	0.56	1.2	0.7-1.8	0.36	1.0	0.6-1.7	0.78

[a]HR: hazards ratio; 95% CI: 95% confidence interval; Grade: histological grade; BVIf: blood vessel invasion stained by factor VIII related antigen staining; BVIef: blood vessel invasion including BVIf and BVIe; KDR: vascular endothelial grpwth factor receptor-2; *KDR* scoring (CCs): labeling index of cancer cells stained by *KDR* antigen staining Hazards ratio from Cox regression analysis; [b]Multivariate model 1 adjusted for population, pT-stage, lymph-node status, grade, BVIf and *KDR* scoring (CCs); [c]Multivariate model 2 adjusted for population, pT-stage, lymph-node status, grade, BVIef and *KDR* scoring (CCs).

Disclosure of conflict of interest

None.

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