Table 3. Frequencies of audits in each deficiency ascribed to each responsible participant in 42 approvals judged as conformation with proviso

Responsible participants	Deficiencies	*n* (%)
Institution	Qualification requirements of hospitals were not met	6 (14.3)
	Lack of appropriate SOP	0 (0.0)
	All investigators were not identified in the contract	0 (0.0)
	Inappropriate contract	6 (14.3)
	Inappropriate informed consent	11 (26.2)
	CRFs filled incorrectly/and or insufficiently	8 (19.1)
	Problems related to archives	19 (45.2)
	Delay in communication of safety information	3 (7.1)
	Others	6 (14.3)
Investigator	Eligibility criteria were not met	13 (31.0)
	Prohibited concomitant therapies	7 (16.7)
	Laboratory tests were not performed according to the defined protocol	9 (21.4)
	Nonobservance of dose and/or schedule provided by the protocol	8 (19.1)
	Others	8 (19.1)
Sponsor	Inappropriate monitoring	24 (57.1)
	Delay in communication of safety information to institution	4 (9.5)
	Others	2 (4.8)
IRB	Qualification requirements of IRB were not met	2 (4.8)
	Lack of appropriate SOP	1 (2.4)
	Insufficient review	4 (9.5)
	Insufficient minutes of meetings	2 (4.8)
	Others	7 (16.7)

IRB, institutional review board; SOP, standard operational procedure; CRFs, case report forms.

Table 4. Frequencies of audits in which one or more deficiencies ascribed to the responsible participants were found by GCP inspection in oncology and other registered trials [*n* (%)]

Responsible participants	Medicinal type		Total	*P* value[a]
	Oncology	Others		
Institution				0.184
Yes	15 (37.5)	168 (49.0)	183 (47.8)	
No	25 (62.5)	175 (51.0)	200 (52.2)	
Investigator				0.309
Yes	13 (32.5)	145 (42.3)	158 (41.3)	
No	27 (67.5)	198 (57.7)	225 (58.8)	
IRB				0.696
Yes	10 (25.0)	78 (22.7)	88 (23.0)	
No	30 (75.0)	265 (77.3)	295 (77.0)	
Sponsor				0.740
Yes	21 (52.5)	169 (49.3)	190 (49.6)	
No	19 (47.5)	174 (50.7)	193 (50.4)	

[a]Fisher's exact test for contingency table of the presence of deficiencies ascribed to each responsible participant and medicinal types.
GCP, Good Clinical Practice; IRB, institutional review board.

Drug development generally takes considerably long due to the on-site GCP audit in response to a trial application. However, problems related to archives would essentially relate to the reliability of the registration trial regarding the existing subjects, ethics, and science. We noted no problems related to archives in the oncology drug registration trials; the frequency of this deficiency was clearly lower for oncology drugs as compared with other drugs. Thus, the compliance with GCP regarding archives was satisfactory in oncology drug registration trials.

The frequency of protocol deviation in oncology fields is lower than that for other medicinal classifications; however, protocol deviations for eligibility criteria or use of prohibited concomitant therapies would influence subject safety in registration trials. Therefore, investigators, clinical research coordinators (CRC), and other health care professionals who support registration trials should make an effort to have sufficient knowledge regarding the target disease and treatment and keep track of details regarding the protocol and GCP. The incidence of deficiencies at domestic investigational sites with CRC was 21% (N = 270/1260), which was lower than that of deficiencies at domestic investigational sites without CRC, i.e. 58% (N = 188/325) [7, 18]. Therefore, an effective approach for reducing deficiencies associated with protocol deviation would entail the careful selection of trial institutions with sufficient numbers of well-trained CRCs and suitable conditions for carrying out monitoring.

In the present study, deficiencies in monitoring were most frequent both overall and in sponsor deviations. Monitoring of the medical institution by the sponsor is enforced by GCP in order to ensure appropriate operation of the registration trial according to trial protocol and GCP. A previous study indicated that typical monitoring issues associated with sponsors in the fiscal year 2005 were as follows: operation of monitoring associated with standard operation procedure and source document verification (41%), timing of monitoring (9.5%), taking appropriate precautions to prevent deviation by monitoring report (8.5%), submission of monitoring report (5.5%), and other (35.5%) [18]. Appropriate monitoring for registration trial by a monitor who has been specifically trained and possesses scientific and clinical knowledge is important for ensuring quality control and quality assurance of registration trials. For further improvement in reducing deficiencies in monitoring, the monitor in the sponsor organization or contract research organization (CRO) should be sufficiently familiar with the protocol and GCP. Improved performance of various parties in the registration trial would not only facilitate operation of the registration trial by the sponsor but also the operation of investigator-initiated registration-directed clinical trials by the investigator, according to the revised GCP enforced from July 2003 [19].

Another major item of deficiency related to the sponsor is a delay in communicating information regarding adverse drug reactions; this is related to subject safety, ethics, and operation of the registration trial. A seamless communication system for delivering critical information is important for ensuring subject safety and appropriate operation of the registration trial. In

Table 5. Frequencies of audits in which each deficiency was found by GCP inspection in oncology drug and other drug applications [*n* (%)]

Responsible participants	Deficiencies	Oncology	Others	Total	*P* value[a]
Institution	Qualification requirements of hospitals were not met	1 (2.5)	6 (1.8)	7 (1.8)	0.541
	Lack of appropriate SOP	0 (0.0)	0 (0.0)	0 (0.0)	–
	All investigators were not identified in the contract	0 (0.0)	3 (0.9)	3 (0.8)	1.000
	Inappropriate contract	2 (5.0)	17 (5.0)	19 (5.0)	1.000
	Inappropriate informed consent	3 (7.5)	26 (7.6)	29 (7.6)	1.000
	CRFs filled incorrectly/and or insufficiently	8 (20.0)	81 (23.6)	89 (23.2)	0.696
	Problems related to archives	1 (2.5)	47 (13.7)	48 (12.5)	0.043
	Delay in communication of safety information	2 (5.0)	21 (6.1)	23 (6.0)	1.000
	Others	4 (10.0)	36 (10.5)	40 (10.4)	1.000
Investigator	Eligibility criteria were not met	2 (5.0)	43 (12.5)	45 (11.8)	0.201
	Prohibited concomitant therapies	0 (0.0)	28 (8.2)	28 (7.3)	0.099
	Laboratory tests were not carried out according to the defined protocol	6 (15.0)	59 (17.2)	65 (17.0)	0.823
	Nonobservance of dose and/or schedule provided by the protocol	5 (12.5)	23 (6.7)	28 (7.3)	0.195
	Others	5 (12.5)	48 (14.0)	53 (13.8)	1.000
IRB	Qualification requirements of IRB were not met	1 (2.5)	5 (1.5)	6 (1.6)	0.487
	Lack of appropriate SOP	0 (0.0)	2 (0.6)	2 (0.5)	1.000
	Insufficient review	7 (17.5)	19 (5.5)	26 (6.8)	0.012
	Insufficient minutes of meetings	0 (0.0)	12 (3.5)	12 (3.1)	0.623
	Others	2 (5.0)	49 (14.3)	51 (13.3)	0.138
Sponsor	Inappropriate monitoring	19 (47.5)	136 (39.7)	155 (40.5)	0.395
	Delay in communication of safety information to institution	5 (12.5)	50 (14.6)	55 (14.4)	1.000
	Others	1 (2.5)	13 (3.8)	14 (3.7)	1.000

[a]Fisher's exact test for contingency table of the presence of each deficiency and medicinal types.
GCP, Good Clinical Practice; IRB, institutional review board; SOP, standard operational procedure.

recent drug development protocols, registration trials such as randomized clinical trials are carried out globally in various trial institutions; in such a scenario, worldwide regional offices of the sponsor would be ideal for improving communication systems and ensuring smooth and timely communication.

There have been various approaches for improving social and scientific infrastructure for clinical research in Japan by academia, industry, and the government. In 2003, the MHLW drew up and published the nationwide 3-year clinical trial activation plan, under which it promoted various measures, including the creation of clinical trial networks and fostering of CRC. Subsequently, the MHLW created the office of clinical trial promotion, research, and development and launched the new 5 yearly clinical trial activation plan in 2007, which was expected to reinforce clinical research infrastructure to ensure patient safety and to secure access to new drugs and devices [20]. Furthermore, the MHLW science research grants 'research on clinical trials infrastructure development' were inaugurated to support framework development for promoting clinical trials (comprising grants to 10 leading academic medical centers). Thus, a study on 'the development of individual health care institution infrastructure models aimed at equally sharing cancer research infrastructure development' was started, and it became possible to pursue favorable institutional infrastructure development and human resources training concerning the ethical aspects of clinical research and methods of new drug development in the National Cancer Center Hospital [21, 22]. Furthermore, the Japanese Ministry of Education, Culture, Sports, Science and Technology provided grants to five universities and a clinical research organization named 'Coordination, Support and Training Program for Translational Research' in 2007 and onward [22, 23]. These various approaches promoted the establishment of a clinical trial infrastructure; we believe that an adequate infrastructure would be the optimal influence for ensuring compliance with GCP in registration trials.

Our study had certain limitations. We were not able to use the full data of on-site GCP audits for a number of trial institutions—such as the trial institution background, i.e. scale (university hospital, national hospital, private hospital, and clinic), region (Japan or other countries), number of subjects under on-site GCP audit, presence of supporting system for registered trial (CRC, site management organization, CRO, etc.)—because the PMDA review reports for on-site GCP audit are the only available data source and these do not have detailed data. Therefore, it is difficult to directly compare the results of the present study with those of previous studies. Because there

are few reports of on-site GCP audits by regulatory agencies, the present study described differences in deficiencies from on-site GCP audits between Japan and other countries. For further improving global compliance with GCP, we consider that each regulatory agency should disclose detailed results of on-site GCP audits on a regular basis.

GCP inspections have indicated certain deficiencies in the data of registration trials and the operation systems of registration trials; these were evaluated in the regulatory reviews of NDA or sNDA. However, the most important purpose of GCP inspection is to prevent a recurrence of GCP deficiencies for establishing higher quality in drug development. In 2009, the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA), USA, initiated the EMEA–FDA GCP initiative that focuses upon enhanced and systematic GCP-related information exchanges between the EMEA and FDA combined with collaboration in the conduct of GCP inspections of registration trials [24]. The results of the present study suggest that the principle of compliance with GCP for registration trials has reached Japanese investigators and trial institutions, and high-quality GCP inspections are thereby being carried out by the PMDA. The clinical development of medicines is a global undertaking. Therefore, in the future, we consider it important that all regulatory agencies work in a collaborative and synergistic manner in order to achieve a system for the optimal use of GCP inspection resources and results and implement information exchanges.

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disclosure

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original article

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Participation of elderly patients in registration trials for oncology drug applications in Japan†

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Background: The objective of this study was to evaluate the age-based enrolment of cancer patients into registration trials of new drug applications or expanding the indications for use.

Materials and methods: The data from 234 registration trials in Japan and overseas of 43 drugs, which were reviewed by the Pharmaceuticals and Medical Devices Agency and approved by the Ministry of Health, Labour and Welfare in Japan between 1999 and 2008, were retrospectively analyzed according to the age distribution of enrolled patients. The age distribution of the Japanese cancer population was derived from Cancer Statistics in Japan 2003 and Annual Report on Health, Labour and Welfare 2003–2004.

Results: In the Japanese cancer population, the estimated median age of cancer patients is 70 years, and 66% of cancer patients are aged 65 years or more. The estimated median age of cancer patients in all registration trials conducted in Japan was 59 years, whereas it was 55 years in the registration trials conducted overseas. The proportion of patients aged 65 years or more enrolled in registration trials conducted in Japan was 35%; this number was 28% in registration trials conducted overseas.

Conclusion: Elderly patients are underrepresented in oncology registration trials in Japan.

Key words: cancer, clinical trial, elderly patient, new drug application

original article

introduction

Cancer is a disease of the elderly, with 60% of patients aged 65 years or more [1]. The elderly population in Japan has considerably increased when compared with the numbers in the other countries [2]. Twenty-two percent of the Japanese population was aged 65 years or more in 2008, and approximately 41% of the Japanese population will be aged 65 years or more by 2055.

Many studies reported that elderly cancer patients are generally underrepresented in cancer clinical trials [3, 4]. Previous studies by clinical trial groups (i.e. SWOG, NCI, NCIC CTG) reported the overall population of patients aged 65 years or more in clinical trials were 25%, 32%, 22%, whereas corresponding numbers in the cancer population of these countries were 63%, 61%, 58%, respectively [5–7]. In oncology registration trial, the United States (US) Food and Drug Administration (FDA) reported that the overall proportion of patients aged 65 years or more enrolled in the registration trials was 36%, whereas the corresponding number for the US cancer population was 60% [8], which is slightly higher than that reported in studies by clinical trial groups.

The Pharmaceuticals and Medical Devices Agency (PMDA) is a Japanese regulatory agency working in conjunction with the Ministry of Health, Labour and Welfare (MHLW) [9]. The major roles of the PMDA are to conduct drug and medical device reviews, evaluate postmarketing safety, and provide relief services with regard to adverse effects of drugs. The PMDA conducts scientific reviews of marketing authorization applications for pharmaceuticals and medical devices and also clinical trial consultations. On the basis of these reviews, the MHLW approves new drug applications (NDAs) or extension of indications (EI) for Japan. The PMDA has also analyzed the median age of patients enrolled in 68 registration trials of NDA or EI approved in Japan from 1999 to 2005 [10]. This study also reported that the median age of patients enrolled in the registration trial conducted in Japan was lower than that of the Japanese cancer population, but the proportion of patients aged 65 years or more was not investigated. In this study, we evaluated elderly cancer patient enrollment of registration trials for the application of NDA or EI approved in Japan between 1999 and 2008. For each type of cancer, drug, and application, the estimated median age of the

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patients enrolled in registration trials conducted in Japan were respectively compared with those conducted in countries other than Japan as well as the Japanese cancer population overall. Similarly, the rate of enrollment of patients aged 65 years or more was compared with the corresponding rates in overseas trials and the Japanese cancer population.

materials and methods

registration trial data sources

Data from patients enrolled in registration trials, which included trials conducted in Japan and overseas, for NDA or EI of oncology drugs, or drugs for supportive care of cancer, reviewed by the PMDA between 1999 and 2008, were extracted from the review reports of the PMDA and the documents submitted by the application sponsors, which have been publicly released on the websites of the PMDA and the Japan Pharmacists Education Center [11, 12]. This study excluded data from phase I oncology registration trials because these trials generally included patients with any type of cancer. Global registration trials including patients in Japan and overseas were also excluded. For each registration trial, data with respect to the type of cancer, drug, treatment, application, region, median age of patients enrolled in the registration trial, and percentage of patients aged 65 years or more were collected.

age distributions of Japanese and US cancer populations

For each type of cancer and all cancers combined, the median age and the proportion of patients aged 65 years or more in the Japanese cancer population were estimated as follows. We obtained the age-specific incidence from Cancer Statistics in Japan 2003 [13], and the population by age group from the Annual Report on MHLW 2003–2004 [14], and multiplied them to estimate the number of age-specific, newly diagnosed cancer patients. The median age and the proportion of patients aged 65 years or more were calculated on the basis of the number and ages of newly diagnosed cancer patients. For each type of cancer and all cancers combined, the median age and the proportion of patients aged 65 years or more in the US cancer population, from the Surveillance, Epidemiology, and End Results Program for the period 2002–2006 and released by the National Cancer Institute, were used as a reference for the age distribution of patients enrolled in overseas registration trials [15].

comparisons of age distribution between registration trials and general cancer populations

For each type of cancer and all cancers combined, we calculated the average median age and the average proportion of patients aged 65 years or more enrolled in registration trials conducted in Japan, and we compared that data with the data from trials conducted overseas and Japanese and US cancer populations. Comparisons were also made for each type of drug, treatment, or application.

results

registration trials

An overview of the 234 registration trials of 43 drugs (32 NDA and 11 EI) analyzed in this study is given in Table 1. The trials were conducted on 19 cytotoxic drugs, 6 small molecular target drugs, 5 hormonal drugs, 4 antibody drugs, 3 supportive care drugs, and 6 miscellaneous drugs.

comparison of median age

Table 2 shows the estimated median age of patients in Japanese and US cancer populations and the distribution of the median age of patients in registration trials for each type of cancer and all cancers combined. The average median age of cancer patients in the 234 registration trials was 59 years, whereas it was 55 years in the registration trials conducted overseas and 70 years in the Japanese cancer population. For each type of cancer except for uterine cancer, the average median age of patients in the registration trials was lower than the estimated median age of the Japanese cancer population but was similar to that in the case of the overseas registration trials. It should be noted that the difference in the median age between the registration trials and the Japanese cancer population was smaller in patients with breast and prostate cancer than in those with other solid cancers. On the other hand, the average median age of patients enrolled in registration trials for hematologic malignancies was more than 10 years lower than the estimated median age of the Japanese cancer population. Additionally, for each cancer and all cancers combined, with the exception of liver and prostate cancer, the average median age of patients in registration trials conducted overseas was lower than the estimated median age of the US cancer population.

Table 3 shows the distribution of median age for each type of drug and application in Japan and overseas. In Japan and overseas, the average median age was approximately 60 years for all types of drugs except for supportive care drugs. The average median age in NDA trials was higher than that in those studying EI by approximately 5 years.

Table 1. Background data of 234 registration trials

Variables	Number of trials (%)
Region	
Japan	78 (33.1)
Overseas	156 (66.1)
Phase	
I/II	15 (6.4)
II	165 (70.5)
III	54 (23.1)
Type of drug	
Cytotoxic	99 (42.3)
Molecular target	36 (15.4)
Antibody	43 (18.4)
Hormonal	32 (13.7)
Supportive care	10 (4.3)
Miscellaneous	14 (5.9)
Type of cancer	43 (18.4)
Solid cancer	186 (79.5)
Hematologic malignancy	48 (20.5)
Type of application	
New drug application	170 (72.7)
Extension of indications	64 (27.4)

comparison of the proportion of patients aged 65 years or more

Of the 234 registration trials, the 66 registration trials were available to obtain the data pertaining to the proportion of patients aged 65 years or more from the data sources. Table 4 shows the estimated proportion of patients aged 65 years or more in Japanese and US cancer populations and the

Table 2. Estimated median age of the cancer population and distribution of median age in the registration trials in Japan and overseas

Type of cancer	Region	Estimated median age[a]	Number of trials	Average median age	30–34 years	35–39 years	40–44 years	45–49 years	50–54 years	55–59 years	60–64 years	65–69 years	70–74 years
CNS	Japan	63	1	53	0	0	0	0	1	0	0	0	0
	Overseas	56	4	51	0	0	1	0	2	1	0	0	0
Oral/pharynx	Japan	67	—	—	—	—	—	—	—	—	—	—	—
	Overseas	62	11	56	0	0	0	1	0	8	2	0	0
Lung	Japan	73	10	66	0	0	0	0	0	0	2	7	1
	Overseas	71	12	61	0	0	0	0	0	3	8	1	0
Gastric	Japan	70	2	56	0	0	0	0	0	2	0	0	0
	Overseas	71	4	58	0	0	0	0	1	3	0	0	0
Liver	Japan	70	2	64	0	0	0	0	0	0	1	1	0
	Overseas	64	1	69	0	0	0	0	0	0	0	1	0
Gall-bladder	Japan	76	1	61	0	0	0	0	0	0	1	0	0
	Overseas	64	—	—	—	—	—	—	—	—	—	—	—
Colon	Japan	70	13	62	0	0	0	0	0	3	9	1	0
	Overseas	71	27	62	0	0	0	0	0	6	17	3	1
Kidney	Japan	70	2	61	0	0	0	0	0	1	1	0	0
	Overseas	64	6	59	0	0	0	0	0	4	2	0	0
Bladder	Japan	73	5	66	0	0	0	0	0	0	1	2	2
	Overseas	73	5	66	0	0	0	0	0	0	3	0	2
Pancreas	Japan	73	—	—	—	—	—	—	—	—	—	—	—
	Overseas	72	4	62	0	0	0	0	0	0	4	0	0
Skin	Japan	75	—	—	—	—	—	—	—	—	—	—	—
	Overseas	60	1	59	0	0	0	0	0	1	0	0	0
Breast	Japan	57	23	55	0	0	1	2	8	7	4	1	0
	Overseas	61	36	57	0	0	1	4	10	5	5	11	0
Uterus	Japan	53	3	63	0	0	0	0	0	1	1	0	1
	Overseas	62	—	—	—	—	—	—	—	—	—	—	—
Ovary	Japan	59	—	—	—	—	—	—	—	—	—	—	—
	Overseas	63	1	62	0	0	0	0	0	0	1	0	0
Prostate	Japan	74	5	69	0	0	0	0	0	0	1	1	3
	Overseas	68	9	71	0	0	0	0	0	0	0	3	6
Lymphoma	Japan	70	4	42	1	1	0	1	0	1	0	0	0
	Overseas	64	2	35	1	1	0	0	0	0	0	0	0
Myeloma	Japan	74	2	60	0	0	0	0	0	1	1	0	0
	Overseas	70	4	62	0	0	0	0	0	0	4	0	0
Leukemia	Japan	67	5	52	0	1	0	1	0	2	1	0	0
	Overseas	66	29	54	3	0	3	2	3	5	12	1	0
Total	Japan	70	78	59	1	2	1	4	9	18	23	13	7
	Overseas	66	156	55	4	1	5	7	16	36	58	20	9

[a]Estimated median ages for the overseas group are values of the US cancer population.
CNS, central nervous system.

Table 3. Distribution of median age for each type of drug and application in Japan and overseas

	Region	Number of trials	Average median age	30–34 years	35–39 years	40–44 years	45–49 years	50–54 years	55–59 years	60–64 years	65–69 years	70–74 years
Type of drugs												
Cytotoxic	Japan	44	60	1	2	0	0	8	6	13	11	3
	Overseas	55	60	1	0	1	1	8	10	25	7	2
Molecular target	Japan	7	59	0	0	0	0	0	4	3	0	0
	Overseas	29	58	0	0	2	2	2	8	13	2	0
Antibody	Japan	4	58	0	0	0	0	0	3	1	0	0
	Overseas	39	58	0	0	0	3	5	17	10	2	2
Hormonal	Japan	14	60	0	0	1	1	0	4	5	1	2
	Overseas	18	65	0	0	1	0	0	0	5	8	4
Supportive care	Japan	3	48	0	0	0	2	1	0	0	0	0
	Overseas	7	42	3	1	1	1	0	0	0	0	1
Miscellaneous	Japan	6	62	0	0	0	1	0	1	1	1	2
	Overseas	8	61	0	0	0	0	1	1	5	1	0
Type of application												
NDA	Japan	48	62	0	1	0	1	3	12	15	10	6
	Overseas	122	60	1	0	2	4	12	33	47	17	6
EI	Japan	30	56	1	1	1	3	6	6	8	3	1
	Overseas	34	55	3	1	3	3	4	3	11	3	3

NDA, new drug application; EI, extension of indications.

distribution of the proportion of patients aged 65 years or more in the registration trials for each type of cancer and all cancers combined. The average proportion of patients aged 65 years or more in 26 registration trials conducted in Japan was 35%; the corresponding number was 28% in the 40 registration trials conducted overseas and 66% in the Japanese cancer population. For each type of cancer except for uterine cancer, the average proportion of patients aged 65 years or more in registration trials was lower than the estimated proportion of patients aged 65 years or more in the Japanese cancer population, although the number of trials was small. In Japan and overseas, the average proportion of patients aged 65 years or more in registration trials for prostate cancer was more than 80%. The average proportion of patients aged 65 years or more in registration trials conducted in Japan was similar to that of the trails conducted overseas for lung cancer, gastric cancer, kidney cancer, breast cancer, prostate cancer, and lymphoma.

Table 5 shows the distribution of the proportion of patients aged 65 years or more for each type of drug and application in Japan and overseas. The average proportions of patients aged 65 years or more in registration trials conducted in Japan for cytotoxic, molecular target, and hormonal drugs were the same. Additionally, the average proportion of patients aged 65 years or more in registration trials conducted in Japan for NDA was higher than that in the trials conducted to study EI. These trends were also observed in registration trials conducted overseas.

discussion

The present study indicated that the estimated median age of patients and the proportion of patients aged 65 years or more in the registration trials conducted in Japan were almost the same as those in overseas trials for each type of cancer and all cancers combined, but was lower than the estimated values of the Japanese cancer population. The proportion of patients aged 65 years or more in the registration trials conducted in Japan was similar to that reported in studies by clinical trial groups and FDA (35% versus 25%, 32%, 22%, and 36%) [5–8]. Our study confirmed that elderly patients were underrepresented in the registration trials and that this is a significant problem observed worldwide.

Registration trials for NDA or EI are generally conducted in patients of all ages, and their results are reviewed by the relevant regulatory agency in order to evaluate the generalizability of efficacy and safety for the general cancer population. Evidence based on the results of registration trials with lower enrollment of elderly patients may not be entirely suitable for elderly cancer patients. Effective strategies are needed to include an adequate number of elderly patients in registration trials and to conduct specific clinical trials in order to evaluate the risks and benefits of cancer therapies in them [7, 16]. The following approaches may be useful in motivating pharmaceutical companies and investigators to conduct registration trials or postmarketing studies for elderly patients: (i) prioritizing a review if the application includes data from a registration trial conducted specifically for elderly patients, (ii) making it mandatory for pharmaceutical companies to conduct postmarketing studies if subgroup analyses reveal differential toxicity profiles or safety risks for elderly patients, and (iii) establishing a research grant to encourage clinical trial groups to conduct postmarketing studies that include elderly patients.

Similarly, the underrepresentation of elderly patients in Japan and other countries may be attributed to the following barriers [3, 4, 17]: (i) trial-design barriers whereby elderly patients do not meet the eligibility requirements because of comorbidities or deteriorating organ or psychological function;

Table 4. Estimated proportion of cancer patients aged 65 years or more and distribution of the proportion of patients aged 65 years or more in registration trials in Japan and overseas

	Region	Estimated proportion[a], %	Number of trials	Average of proportion, %	0–19%	20–39%	40–59%	60–79%	80–100%
Type of cancer									
CNS	Japan	47	1	22	0	1	0	0	0
	Overseas	35	4	8	4	0	0	0	0
Oral/pharynx	Japan	57	—	—	0	0	0	0	0
	Overseas	43	2	0	2	0	0	0	0
Lung	Japan	77	2	47	0	1	0	1	0
	Overseas	68	2	38	0	2	0	0	0
Gastric	Japan	68	1	17	1	0	0	0	0
	Overseas	64	2	23	1	1	0	0	0
Liver	Japan	71	—	—	0	0	0	0	0
	Overseas	49	1	61	0	0	0	1	0
Gall-bladder	Japan	83	—	—	0	0	0	0	0
	Overseas	49	—	—	0	0	0	0	0
Colon	Japan	66	2	26	1	1	0	0	0
	Overseas	65	3	48	0	1	2	0	0
Kidney	Japan	66	2	39	0	1	1	0	0
	Overseas	50	4	31	1	2	1	0	0
Bladder	Japan	76	3	48	0	0	0	0	0
	Overseas	72	—	—	0	1	1	1	0
Pancreas	Japan	74	—	—	0	0	0	0	0
	Overseas	68	1	46	0	0	1	0	0
Skin	Japan	77	—	—	0	0	0	0	0
	Overseas	41	1	33	0	1	0	0	0
Breast	Japan	33	10	22	4	5	1	0	0
	Overseas	41	8	36	2	1	5	0	0
Uterus	Japan	27	1	65	0	0	0	1	0
	Overseas	43	—	—	0	0	0	0	0
Ovary	Japan	38	—	—	0	0	0	0	0
	Overseas	47	—	—	0	0	0	0	0
Prostate	Japan	87	2	83	0	0	0	1	1
	Overseas	62	3	84	0	0	0	1	2
Lymphoma	Japan	63	1	0	1	0	0	0	0
	Overseas	50	2	0	2	0	0	0	0
Myeloma	Japan	78	1	46	0	0	1	0	0
	Overseas	64	—	—	0	0	0	0	0
Leukemia	Japan	57	—	—	0	0	0	0	0
	Overseas	54	7	6	6	1	0	0	0
Total	Japan	66	26	35	7	10	4	4	1
	Overseas	55	40	28	18	9	9	2	2

[a]Estimated proportion of patients aged ≥65 years mentioned for the overseas group are values of the US cancer population.
CNS, central nervous system.

(ii) physicians tending to have a negative bias against the enrollment of elderly patients; and (iii) patient factors including a desire to choose their own treatment, distance from the treatment institution, and socioeconomic factors. Most elderly patients are willing to consider participation in cancer clinical trials, but they do not appear to actively seek out clinical trials and few seem to be informed of the availability of clinical trials [18].

In addition to the abovementioned barriers, the present study showed that both Japanese and overseas researchers may tend to enroll a higher number of elderly patients in trials for treatments with lower toxicity than in those for treatments with higher toxicity. For breast and prostate cancers, the differences in the estimated median age of patients and the proportion of patients aged 65 years or more between the registration trials and general cancer populations in Japan and overseas were relatively small, probably because the registration trials for evaluating hormonal drugs for these cancers, which are comparatively less toxic, included more elderly patients than the registration trials for the other cancers [7]. In Japan and overseas, the estimated median age of patients and the proportion of patients aged 65 years or more in registration trials for hematologic malignancies were lower than those in the registration trials for solid cancers. Investigators may hesitate to enroll elderly patient with hematologic malignancies since these

Table 5. Distribution of the proportion of patients aged 65 years or more for each type of drug and application in Japan and overseas

	Region	Number of trials	Average of proportion	0–19%	20–39%	40–59%	60–79%	80–100%
Type of drugs								
Cytotoxic	Japan	6	34	1	3	1	1	0
	Overseas	7	18	5	1	1	0	0
Molecular target	Japan	5	34	1	2	2	0	0
	Overseas	12	29	4	5	2	1	0
Antibody	Japan	1	18	1	0	0	0	0
	Overseas	2	0	2	0	0	0	0
Hormonal	Japan	8	34	2	4	1	0	1
	Overseas	10	53	1	1	5	1	2
Supportive care	Japan	2	0	2	0	0	0	0
	Overseas	6	0	6	0	0	0	0
Miscellaneous	Japan	4	59	0	1	0	3	0
	Overseas	3	41	0	2	1	0	0
Type of application								
NDA	Japan	20	39	3	10	3	3	1
	Overseas	32	36	10	9	9	2	2
EI	Japan	6	19	4	0	1	1	0
	Overseas	8	0	8	0	0	0	0

NDA, new drug application; EI, extension of indications.

patients might experience increased toxicity and show relatively poor survival as compared with younger patients [7, 19–21].

There are some particular barriers in Japan, which differ from those observed overseas. Because of cultural barriers, the implications and necessity of clinical trials are not known to a majority of the Japanese population [22]. Additionally, in Japan, more elderly people live in provinces than in major metropolitan areas [23] and may not have much opportunity to participate in clinical trials as compared with patients living in major metropolitan areas containing most institutions that conduct clinical trials. Elderly patients may not be motivated enough to participate in clinical trials because their medical expenses are quite low owing to the subsidy offered by the national medical insurance system [24]. Additionally, past experiences such as infection caused by the use of HIV- or hepatitis C-contaminated blood products may dissuade elderly patients from enrolling in clinical trials [25, 26]. Furthermore, since the PMDA had not made it mandatory to conduct a phase III trial for NDAs or EIs in oncology until 2006 [27], single-arm phase I/II or II trials with a small sample size were frequently conducted, and researchers might avoid enrolling elderly patients in such trials.

Effective measures against these barriers would include conducting educational exhibits and making announcements via mass media in order to increase the awareness of the importance of clinical trials among the general and elderly population. It is important that 'industrial, government, and academia' emphasize the implications and necessity of clinical trials for the elderly patients.

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disclosure

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Original Article

Disruption of the Blood Brain Barrier by Brain Metastases of Triple-Negative and Basal-Type Breast Cancer But Not *HER2/neu*-Positive Breast Cancer

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BACKGROUND: Generally, the blood-brain barrier (BBB) of brain metastasis was thought to be disrupted. **METHODS:** We retrospectively performed immunohistochemical staining for glucose transporter 1 (GLUT1) and breast cancer resistance protein (BCRP) to evaluate the status of the BBB in resected brain metastases. Associations between expression of GLUT1 and/or BCRP and the immunohistochemical profiles of breast cancers, such as the statuses of hormone receptors, human epidermal growth factor receptor 2 (HER2/*neu*), and a basal-type marker (cytokeratin 5/6, HER1), were also analyzed. **RESULTS:** The study included 29 breast cancer patients with brain metastasis who had undergone brain tumor resections. Among the 29 patients, there was no expression of GLUT1 and BCRP in the intratumor microvessels of 9 (32%) and 11 (38%) patients, respectively. There was no expression of both GLUT1 and BCRP in 8 patients (28%). The expression of GLUT1 was significantly associated with that of BCRP ($P < .001$). A positive correlation was observed between the expression of GLUT1 and/or BCRP and brain metastases of HER2/*neu*-positive breast cancer ($P = .012$), while a negative correlation was observed between the expression of GLUT1 and/or BCRP and brain metastases of triple negative or basal-type breast cancer ($P = .014$ and $P = .003$ for triple negative and basal-type, respectively). **CONCLUSIONS:** Brain metastases of triple negative or basal-type breast cancers may often disrupt the BBB, whereas brain metastases of HER2/*neu*-positive breast cancer tend to preserve the BBB. ***Cancer* 2010;116:302–8.** © *2010 American Cancer Society.*

KEYWORDS: blood brain barrier, brain metastasis, glucose transporter 1, breast cancer resistance protein, and breast cancer.

Brain metastasis is the most common type of malignancy found in the brain and is responsible for a substantial fraction of the total morbidity and mortality of metastatic breast cancer patients. Brain metastasis is generally a late feature in the history of metastatic breast cancer. The incidence of symptomatic brain metastases in metastatic breast cancer ranges from 10% to 16% and has been reported to be even higher in human epidermal growth factor receptor 2 (HER2/*neu*)-positive tumors.[1]

Some recent studies reported that patients with HER2/*neu*-positive metastatic breast cancer had a higher incidence of subsequent brain metastasis than HER2/*neu*-negative patients.[2,3,4] The human epidermal growth factor receptor family is involved in cell proliferation, differentiation, and survival. HER2/*neu* amplification is widely known to indicate an aggressive tumor behavior and a poor clinical outcome in breast cancer patients. HER2/*neu* overexpression occurs in approximately 20% to 30% of breast cancer patients.[5] Trastuzumab, a monoclonal antibody against HER2, has been

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shown to be significantly effective in both adjuvant and metastatic settings.[6,7] One interpretation of these results is that HER2/*neu*-positive breast cancer may have a biological affinity toward the development of brain metastasis. On the other hand, some evidence has indicated that trastuzumab cannot pass through the blood-brain barrier (BBB) and enter the cerebrospinal fluid.[8]

The BBB can create a "sanctuary" for cancer cells, preventing antitumor agents from penetrating in sufficiently high concentrations to have a substantial effect. Current systemic therapy, including hormone therapy, chemotherapy, and molecular-targeted drug therapy, is not effective for the treatment of brain metastasis, and the development of treatment strategies for brain metastasis has become a critical issue. Glucose transporter 1 (GLUT1) is 1 of 14 members of the mammalian facilitative GLUT family of passive carriers that function as an energy-independent system for the transport of glucose down a concentration gradient.[9] GLUT1 is not detectable in a large proportion of cells, including normal tissues and benign lesions, with the exception of erythrocytes, germinal cells of the testis, renal tubules, maternal/fetal barrier in placentas, the perineurium of peripheral nerves, and endothelial cells in BBB vessels.[10,11]

The breast cancer resistance protein (BCRP, also known as ABCG2) was first discovered in a chemotherapy-resistant breast cancer cell line. Extensive research has determined BCRP to be a transporter expressed on the luminal surface of the gastrointestinal tract, bile canniculi, renal tubules, maternal/fetal barrier in placentas, germ cell-blood barrier in testes and ovaries, and endothelial cells in BBB capillaries.[12]

Several immunohistochemical stainings (GLUT1, P-glycoprotein, multidrug resistance protein [MRP] 1 to 6, BCRP) were well-known for evaluation of BBB characteristics.[12] And GLUT1 is selectively localized to the BBB.[13] We, therefore, examined the status of the BBB at the sites of brain metastases in breast cancer patients according to the expression of GLUT1 and/or BCRP and investigated the correlation between the status of the BBB and the immunohistochemical profiles of brain metastases of breast cancer.

MATERIALS AND METHODS

Patients

Twenty-nine patients with brain metastasis were retrospectively identified based on records in the surgical database of the National Cancer Center Hospital, Japan; the patients had undergone surgery between January 1999 and January 2006. All clinical information was collected from the patient charts. The present study was approved by the Institutional Review Board of the National Cancer Center.

Tissue Samples and Microscopic and Immunohistochemical Analyses

Hematoxylin-eosin stained specimens were reviewed by an experienced pathologist (K.T.) and were confirmed to contain an adequate amount of cancer tissue available for use in the present study. All tumor specimens from brain metastasis resections were available for immunohistochemical analysis.

The pathological and immunohistochemical examinations were conducted by an experienced pathologist (K.T.) who was blinded to the clinical statuses of the patients. Formalin-fixed, paraffin-embedded tissue samples were sectioned (4 microns thick) and mounted on charged slides. Immunohistochemical staining for estrogen receptor (ER) (clone 1D5; Dako, Carpinteria, Calif), progesterone receptor (PgR) (clone PgR636; Dako), HER1(EGFR pharmDx kit; Dako), CD34 (1:200, My10; Becton Dickinson, Franklin Lakes, NJ), and BCRP (1:100, clone BXP21, GeneTex SanAntonio, Tex) was performed using the ChemMate ENVISION system (Dako). Immunohistochemical staining for CK5/6 (1:200, clone D5 of 16B4; Dako) and GLUT1 (1:200, polyclonal; Dako) was performed using the streptavidin-biotin method.[14] The HER2 protein status, as assessed using Herceptest (Dako), was scored on a scale of 0 to 3+, according to the Dako scoring system. A HER2/*neu*-positive status was defined as HER2 protein 3+ or HER2 protein 2+ and positive fluorescence using in situ hybridization. Appropriate positive and negative controls were also used for each antibody (for example, red blood cells for GLUT1). ER and PgR staining in the tumor cells were evaluated using the Allred scoring system.[15] HER1 status was evaluated using a sliding scale from 0 to 3+ (0 $\leq$ 1%, 1+ = 1-9%, 2+ = 10-50%, 3+ $\geq$ 50%) to represent the percentage of positive cells among the tumor cells in the brain metastasis; the HER1 status was regarded as positive if 1% or more of the tumor cells were stained. The CK5/6 status was evaluated using a sliding scale from 0 to 3+ (0 $\leq$ 1%, 1+ = 1-9%, 2+ = 10-50%, 3+ $\geq$ 50%) to represent the percentage of positive cells among the tumor cells in the brain metastasis; the CK5/6 status was regarded as positive if 10% or more of the tumor cells were stained.[16]

GLUT1 and BCRP staining were evaluated using a sliding scale from 0 to 3+ (0 ≤ 1%, 1+ = 1-9%, 2+ = 10-50%, 3+ ≥ 50%) to represent the percentage of positive cells among the endothelial cells (indicated by CD34 staining) in the microvessels of the brain metastasis. A disruption in the BBB was defined as the presence of GLUT1- and BCRP-positive cells in less than 10% of the cells in the tumor microvessels, corresponding to a score of 0 or 1+.

Statistical Analysis

Fisher exact test was used to compare the frequency distributions of the ER, PgR, HER1, HER2/*neu*, CK5/6, hormone receptor, triple negative, and basal-type statuses between specimens with no expression of both GLUT1 and BCRP and those with expression of GLUT1 and/or BCRP. The associations were evaluated using the odds ratios (ORs) and 95% confidence intervals (95% CIs). An OR represents odds of the expression of GLUT1 and/or BCRP in patients with positive variable relative to the odds of the expression of GLUT1 and/or BCRP in patients with negative variable. The ORs for ER and HER2/*neu* were calculated after adding 0.5 to each cell of the 2× 2 table because of the presence of zero cell counts.[17]

To further evaluate the stability of the OR for hormone receptor, HER1, HER2/*neu*, triple negative, and basal-type statuses, we calculated their exact ORs using univariate exact conditional logistic regression analyses as sensitivity analyses.

All comparisons were 2-tailed, and $P < .05$ was considered significant. All the analyses were performed using SAS version 9.1.3 for Windows (SAS Institute, Cary, NC).

RESULTS

The present study included 29 patients with brain metastasis, and the median patient age at the time of the diagnosis of brain metastasis was 53 years old (range, 39 to 78 years). The frequency distributions of the characteristics of the brain metastases are shown in Table 1. The median time to brain metastasis from the time of breast cancer diagnosis was 2.9 years (range, 0 to 23.1 years). The median overall survival time was 14.7 months.

In this study, 8 patients had a prior history of receiving chemotherapy containing trastuzumab, 7 of these patients had received trastuzumab-containing chemotherapy in a metastatic setting, and 1 had received trastuzumab-containing neo-adjuvant chemotherapy, 5 patients had a prior history of receiving hormone therapy, 7 patients had a prior history of receiving chemotherapy, 2 patients had a prior history of receiving both hormone therapy and chemotherapy, and 7 patients had received no systemic therapy before the brain tumor resection.

Table 1. Patient Characteristics

	Total
Characteristics of Brain Metastases	
Median size, mm, of brain metastasis (range)	35 (20-60)
No. of brain metastases	
1	24
2	4
3	1
Side (right / left / bilateral)	14 / 14 / 1
Site	
Frontal lobe	1
Parietal lobe	2
Temporal lobe	6
Occipital lobe	3[a]
Cerebellum	16[a]

[a]One patient had brain metastases in both the cerebellum and the occipital lobe.

The frequency distributions of the immunohistochemical profiles and the correlations between the immunohistochemical profiles and the expression of GLUT1 and/or BCRP are shown in Table 2. Among the 29 patients, the brain metastases were immunohistochemically positive for ER, PgR, HER1, HER2, and cytokeratin (CK) 5/6 in 4 (13.8%), 2 (6.9%), 15(51.7%), 11 (37.9%), and 7 (24.1%) patients, respectively. There were 5 patients with hormone receptor-positive breast cancer and 11 patients with HER2/*neu*-positive breast cancer (1 patient was positive for both hormone receptors and HER2/*neu*). Among the 14 patients with both hormone receptor-negative and HER2/*neu*-negative statuses, 10 (34.5%) patients with basal-type (CK5/6 positive and/or HER1 positive) breast cancer were observed.

The intratumor microvessels were immunohistochemically positive for GLUT1 and BCRP in 20 (69.0%, Fig. 1) and 18 (62.1%, Fig. 2) metastases, respectively. The expression of GLUT1 was significantly associated with that of BCRP in the intratumor microvessels (OR, 45.3; 95% CI, 3.3 to 2122.9; Fisher exact test, $P < .001$). Eight patients (27.6%) had brain metastases in which the intratumor microvessels were negative for both GLUT1 and BCRP.

Brain metastases from hormone receptor-positive breast cancer were not correlated with the expression of

Table 2. Correlation Between Immunohistochemical Profiles and the Expression of GLUT1 and BCRP

Variables	Total N=29	No Expression of Both GLUT1 and BCRP	Expression of GLUT1 and/or BCRP	OR (95% CI)	P^a
ER					.552
Negative	25	8	17	1	
Positive	4	0	4	4.371 (0.210, 90.882)	
PgR					.483
Negative	27	7	20	1	
Positive	2	1	1	0.350 (0.004, 31.366)	
HER1					.215
Negative	14	2	12	1	
Positive	15	6	9	0.250 (0.21,1.930)	
HER2/neu					.012
Negative	18	8	10	1	
Positive	11	0	11	18.619 (0.953, 363.768)	
CK5/6					.008
Negative	22	3	19	1	
Positive	7	5	2	0.063 (0.005, 0.673)	
Hormone receptor status					1.000
Negative	24	7	17	1	
ER and/or PgR positive	5	1	4	1.647 (0.127, 92.539)	
Triple negative status[b]					.014
No	15	1	14	1	
Yes	14	7	7	0.071 (0.002, 0.800)	
Basal type[c]					.009
No	19	2	17	1	
Yes	10	6	4	0.078 (0.006, 0.716)	

GLUT1 indicates glucose transporter 1; BCRP, breast cancer resistance protein; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PgR, progesterone receptor; HER1, human epidermal growth factor receptor 1; HER2/neu, human epidermal growth factor receptor 2; CK5/6, cytokeratin 5, 6.
[a]Fisher exact test.
[b]Triple-negative status is defined in the present study as ER-negative, PgR-negative, and HER2/neu-negative.
[c]Basal type is defined in the present study as ER-negative, PgR-negative, and HER2/neu-negative, and CK5/6-positive and/or HER1 positive brain metastasis.

GLUT1 and/or BCRP in the intratumor microvessels (Fisher exact test, $P = 1.000$). However, the other 3 types of brain metastases were significantly correlated with the expression of GLUT1 and/or BCRP in the intratumor microvessels.

A positive correlation was observed between the expression of GLUT1 and/or BCRP and brain metastases of HER2/*neu*-positive breast cancer (OR, 18.619; 95% CI, 0.953 - 363.768; Fisher exact test, $P = .012$). On the other hand, a negative correlation was observed between the expression of GLUT1 and/or BCRP and brain metastases of triple negative breast cancer, including basal-type metastases identified as CK 5/6-positive and/or HER1 positive status (OR, 0.071; 95% CI, 0.002 - 0.800; Fisher exact test, $P = .014$) or brain metastases of basal-type breast cancer (OR, 0.078; 95% CI, 0.006 - 0.716; Fisher exact test, $P = .009$).

The exact ORs for hormone receptor, HER2/*neu*-positive, triple negative, and basal-type statuses are shown in Table 3. The correlations between the expression of GLUT1 and/or BCRP and brain metastases from hormone receptor, HER2/*neu*-positive, triple negative, or basal-type breast cancer that obtained from the univariate exact conditional logistic regression analyses were the same as the above-mentioned results.

DISCUSSION

The present study demonstrated that brain metastases of triple negative breast cancer, especially basal-type breast cancer, were negatively correlated with the expression of GLUT1 and/or BCRP in intratumor microvessels, while brain metastases of HER2/*neu*-positive breast cancer were positively correlated with the expression of GLUT1 and/

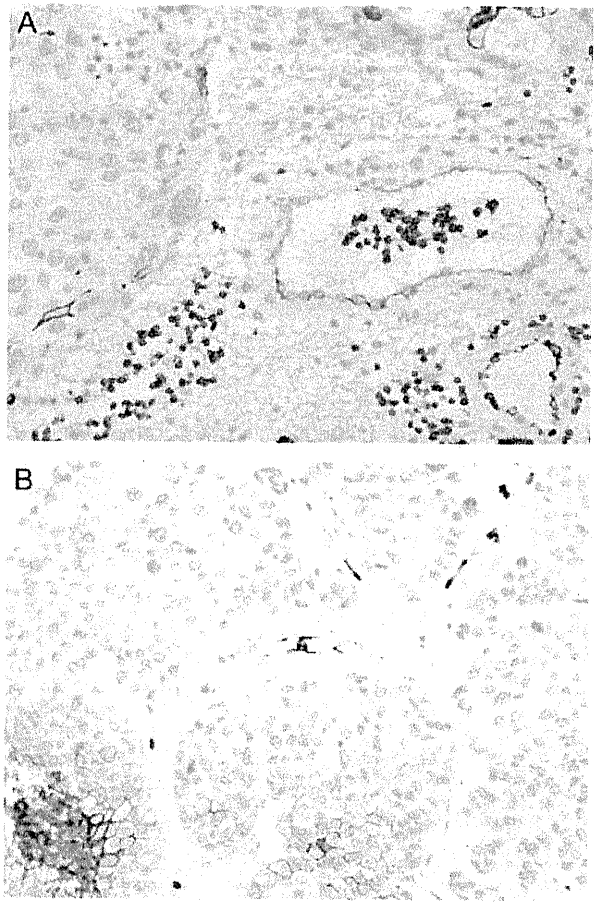

Figure 1. Glucose transporter 1 (GLUT1) immunoreactivity is shown. (A) GLUT1 is strongly expressed (3+) in the endothelial cells of the intratumor microvessels. (B) GLUT1 is weakly expressed (1+) in the intratumor microvessels. GLUT1 was also positive in some tumor cells and red blood cells, which served as a positive control. (×40.)

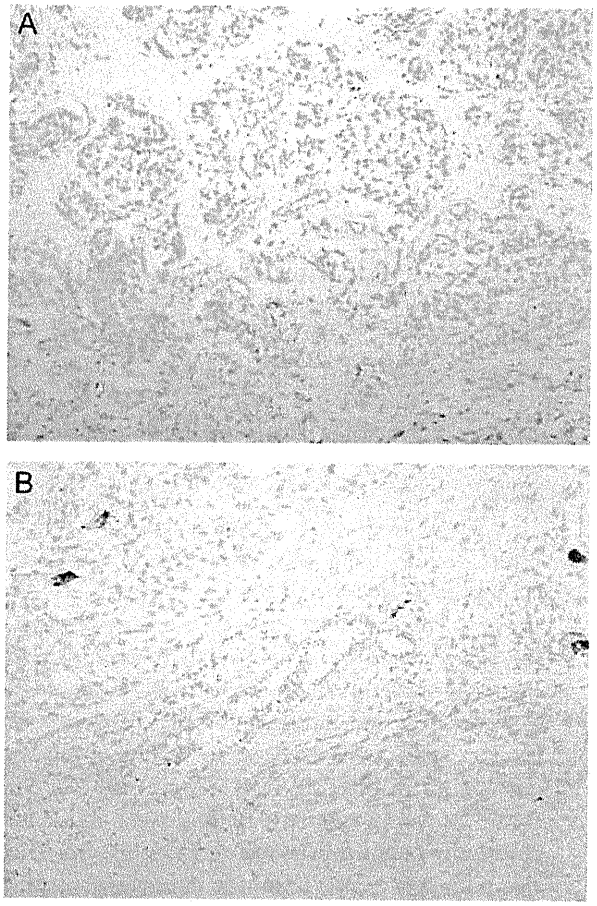

Figure 2. Breast cancer resistance protein (BCRP) immunoreactivity is shown. (A) BCRP is strongly expressed (3+) in the epithelial cells of the intratumor microvessels. (B) Glucose transporter 1 is weakly expressed (1+) in the intratumor microvessels. (×20.)

or BCRP in intratumor microvessels. These results suggest that brain metastases of triple negative breast cancer, but not HER2/*neu*-positive breast cancer, may be associated with a disruption of the BBB based on the observed expression of GLUT1 and/or BCRP. Thus, the present study suggested that the development of brain metastases of HER2/*neu*-positive breast cancer and triple negative breast cancer may differ in terms of the preservation or disruption of the BBB, based on the potential affinity of the cancer cells to brain tissue. Because present study included selected small sample patients who received brain tumor resection, further studies are warranted to address this interpretation in a large cohort of breast cancer patients with brain metastases.

The BBB protects the brain from both endogenous and exogenous toxins. The cellular barriers in the endothelial cells of the central nervous system (CNS) differ from those in the endothelial cells of the periphery, with the cells in the CNS characterized by continuous tight junctions, a lack of fenestrations, and a very low pinocytic activity. Extracellular matrix, pericytes, and astrocyte foot processes further mediate the impermeability of the BBB. Brain capillaries also have a high electrical resistance that increases the impermeability of the BBB to polar and ionic substrates. In addition to these physiologic conditions of the BBB, numerous efflux transporters have been identified in the brain endothelium, including P-glycoprotein, MRP 1 to 6, BCRP, and organic anion and

Table 3. Exact ORs for Hormone Receptor, HER2/neu, Triple-Negative, and Basal-Type Statuses According to Univariate Exact Conditional Logistic Regression Analyses

Variables	Exact OR	Exact 95% CI	P^a
Hormone receptor status	1.621	(0.127, 92.539)	1.000
HER2/neu	10.528	(1.376, + ∞)	.020
Triple negative status[b]	0.079	(0.001, 0.800)	.025
Basal type[c]	0.088	(0.006, 0.716)	.018

OR indicates odds ratio; HER2/neu, human epidermal growth factor receptor 1; CI, confidence interval.
[a]Likelihood ratio test.
[b]Triple negative status is defined in the present study as ER-negative, PgR-negative, and HER2/neu-negative.
[c]Basal type is defined in the present study as ER-negative, PgR-negative, and HER2/neu-negative, and CK5/6-positive and/or HER1-positive brain metastasis.

cation transporters.[12] Thus, the BBB is an efficient barrier against the entry of many drugs, including antitumor agents.

Triple negative breast cancer is a group of primary breast tumors with aggressive clinical behavior that account for 10%-15% of all breast cancers.[18] Most triple negative breast cancer possesses a basal phenotype and shows varying degrees of basal CK and myoepithelial marker expression. Histologically, such cancers are poorly differentiated, and most fall into the basal subgroup of breast cancers, characterized by immunohistochemical staining for markers (ie, CK 5/6, HER1).[19] A previous study, in which primary tumors were immunohistochemically analyzed, reported that patients with ER-negative and PgR-negative tumors either with or without HER2/*neu* over-expression had a high risk of brain metastasis in a case-control study. A multivariate analysis of a database containing 10,782 patients in another study also reported that the independent risk factors for central nervous system metastasis were ER negativity, a young age, and histology of invasive ductal carcinoma.[20] In addition, a previous study revealed that patients with primary tumors that were negative for ER but that expressed basal CK 5/6 and overexpressed HER1 or HER2/*neu* were more likely to develop brain metastasis.[16]

In the present study, 14 patients had brain metastases of triple negative breast cancer, including 10 patients with basal-type (CK5/6-positive and/or HER1-positive). Interestingly, the present study demonstrated a significant association between brain metastasis of triple negative breast cancer, especially basal-type metastases, and disruptions of the BBB. Although preclinical investigations of whether triple negative breast cancer or basal-type cancer cells have a significant affinity to brain tissue remain inconclusive, triple negative breast cancer and basal-type breast cancers may have a different pattern of brain metastasis development and brain tissue affinity compared with HER2/*neu*-positive breast cancer. Patients with triple negative breast cancer are more likely to develop distant metastases earlier than non-triple negative breast cancer patients, develop brain metastases sooner, and have a shorter overall survival.[21] Therefore, the present study suggests that the development of brain metastases of triple negative breast cancer and basal-type breast cancer may be more aggressive with the disruption of the BBB.

Although brain metastasis generally develops during the late phase of breast cancer, HER2/*neu*-positive primary breast cancer has been known to develop brain metastasis as the initial site of recurrence, and it has a shorter brain metastasis-free survival period compared with other types of primary breast cancer.[22] In a preclinical study, HER2/*neu* overexpression increased the metastatic outgrowth of breast cancer cells in the brain, suggesting that HER2/*neu*-positive breast cancer cells have a great affinity to brain tissue.[23] Generally, primary brain tumors, such as high-grade gliomas, infiltrate deeply into the normal brain tissue where the BBB is intact.[24] The present study revealed that the BBB was likely preserved in brain metastases of HER2/*neu*-positive breast cancer; therefore, we speculate that HER2/*neu*-positive breast cancer may develop brain metastases based on its potential affinity for brain tissue, enabling tumor cells to infiltrate the brain tissue across endothelial cells without requiring the disruption of the BBB during an early stage of disease. In an National Surgical Adjuvant Breast and Bowel Project-B31 adjuvant chemotherapy trial for HER2/*neu*-positive patients, no significant difference in CNS metastasis as a first or subsequent event was seen between a trastuzumab group and a control group.[25] Hence, Lin et al concluded that trastuzumab therapy does not increase the risk of CNS relapse; instead, their data suggested that the CNS acts as a

sanctuary site for HER2/*neu*-positive metastases because of the inability of trastuzumab to cross the BBB.[26] The present study may support this interpretation and these results of previous studies.

CONFLICT OF INTEREST DISCLOSURES

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