Breast Cancer (2019) 26:386–396
https://doi.org/10.1007/s12282-018-00937-0

ORIGINAL ARTICLE

Invasive breast cancers in adolescent and young adult women show more aggressive immunohistochemical and clinical features than those in women aged 40–44 years

Ai Hironaka-Mitsuhashi[1,2,3] · Hitoshi Tsuda[4] · Masayuki Yoshida[1] · Chikako Shimizu[5,6] · Sota Asaga[7,8] · Takashi Hojo[7,9] · Kenji Tamura[5] · Takayuki Kinoshita[7] · Toshikazu Ushijima[3,10] · Nobuyoshi Hiraoka[1] · Yasuhiro Fujiwara[5]

Received: 24 July 2018 / Accepted: 30 November 2018 / Published online: 11 December 2018

Abstract

Background Limited knowledge exists concerning the clinicopathological features of breast cancers (BCs) occurring in adolescent and young adult (AYA) women. We evaluated tumor characteristics in AYA women in comparison with those in middle-aged premenopausal women.

Methods From consecutive AYA patients (< 35-year-old) with invasive BC in a single institute, 82 patients first treated with surgery were examined. As the control group, 82 tumors from middle-aged premenopausal patients (40–44 years) were selected by matching pathological T and N factors. We compared habitual factors, immunohistochemical parameters, and patient outcome between the two groups.

Results Most of the study population (148 of 164, 90.2%) were in the early clinical stages (stage I or II). In the AYA group, the number of childbirths was smaller ($p < 0.0001$), while the volume of alcohol consumption was larger ($p < 0.0001$), and palpable primary tumors were more frequent ($p < 0.01$) than in the control group. The positivities of estrogen receptor, progesterone receptor, and androgen receptor were lower ($p < 0.001$, $p = 0.03$, and $p < 0.001$, respectively), and the triple-negative (TN) BCs rates were higher ($p < 0.01$) in the AYA group. Distant recurrence-free survival (DRFS) curves were different in the whole population ($p = 0.02$) and in hormone receptor-positive cases ($p = 0.01$).

Conclusions We confirmed that BCs occurring in AYA women had more aggressive features than those of the older premenopausal women in terms of a high proportion of TN subtypes and a lower DRFS.

Keywords Adolescent and young adult women · Breast cancer · Pathological features

✉ Hitoshi Tsuda
htsuda@ndmc.ac.jp

[1] Department of Pathology, National Cancer Center Hospital, Tokyo 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

[2] Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

[3] Course of Advanced Clinical Research of Cancer, Juntendo Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

[4] Department of Basic Pathology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan

[5] Department of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

[6] National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

[7] Department of Breast Surgery, National Cancer Center Hospital, Tokyo 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

[8] Department of Breast Surgery, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan

[9] Department of Breast Surgery, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

[10] Division of Epigenomics, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Introduction

The peak incidence of breast cancer (BC) in Japan occurs in women in their 40s and 50s, and BCs occurring in young adult women < 35 years of age account for only 2.7% of all BCs [1]. Nonetheless, BC is the second most frequent epithelial malignancy, and is one of the leading causes of death in this age group [2, 3], that is, the adolescent and young adult (AYA) age group. The management of cancers in the AYA generation is socially recognized as an important issue in terms of a high frequency of familial cancer, fertility conservation during and after treatment, and patients' long-term survivorship [3].

In BC patients, being younger than 35 years of age was shown to be an independent predictor of higher rates of local recurrence and cancer-related death in comparison with older age groups [3–6]. BCs occurring in young women tend to be diagnosed at advanced stages and/or present themselves as having unfavorable biological characteristics, including high histological grades (HGs), high proliferation rates, high-level lymphovascular invasion, and hormone receptor (HR) negativity [1, 3–9]. Consequently, physicians tend to offer young BC patients intensive treatment which can be their further burden.

Clinically, the evaluation of both histological parameters and immunohistochemical parameters, including HR, human epidermal growth factor receptor 2 (HER2), Ki-67, and basal-like markers, form the baseline for treatment decision-making. For example, the surrogate intrinsic subtype is defined as a combination of the status of HR, HER2, and a cell proliferation marker, e.g., Ki-67 labeling index (Ki-67 LI) evaluated using immunohistochemistry (IHC). This immunohistochemical subtyping enables an approximate determination of tumor characteristics [4, 7–11]. The triple-negative breast cancer (TNBC), defined as a lack of both HR and HER2 status, usually shows a worse prognosis than other BC subtypes. More specifically, TNBC with basal-like features has distinct characteristics that correlate with a poor prognosis. Several studies have shown a higher occurrence of TNBC and basal-like types in the younger population than in the older population [3, 4, 7, 9, 10], which also explains the worse prognosis in AYA BC patients [9, 10].

However, there is limited information regarding the clinicopathological features of BC arising in AYA women compared with BC arising in older patients within Japanese premenopausal women. In this study, which mainly comprised early-stage BCs, we compared habitual factors, immunopathological parameters including surrogate intrinsic subtypes, and patient outcomes between BCs occurring in women aged < 35 years and those occurring in women aged from 40 to 44 years through matching pathological T and N factors.

Patients and methods

Patients

This study was approved by the internal review board of the National Cancer Center, Tokyo, Japan (No. 2011-015 and 2014-386). Patient cohorts in the present study are presented in Fig. 1. Briefly, clinical data were obtained for 193 women aged < 35 years, who had been diagnosed with BC at the National Cancer Center Hospital, Tokyo, Japan, between September 1997 and April 2011. Five patients declined to provide a tissue sample for use in the research. From the patients who received informed consent, we excluded 20 BCs, including 10 cases at advanced stages, six bilateral BCs and four BCs with concurrent cancer in another organ to be treated. In total, 168 women who had been diagnosed with primary operable BC at clinical stages I to III were identified from the institutional pathology database. Next, we excluded 70 patients who had received neoadjuvant systemic therapies, and 12 patients whose BC comprised multiple lesions. We also excluded one T0N1 patient, one T4N2 patient, and two T3N0 patients, as no counterparts in the control group were identified within the same period. A total of 82 patients comprised the AYA patient group. As the control group consisted of BCs that matched pathological T and N factors, 82 older premenopausal patients (40–44 years old) who were first treated with surgery in the same period were identified. Tumor-node-metastasis status was re-staged according to the 7th edition of the American Joint Committee on Cancer staging manual [12]. In total, 164 cases were entered for analyses.

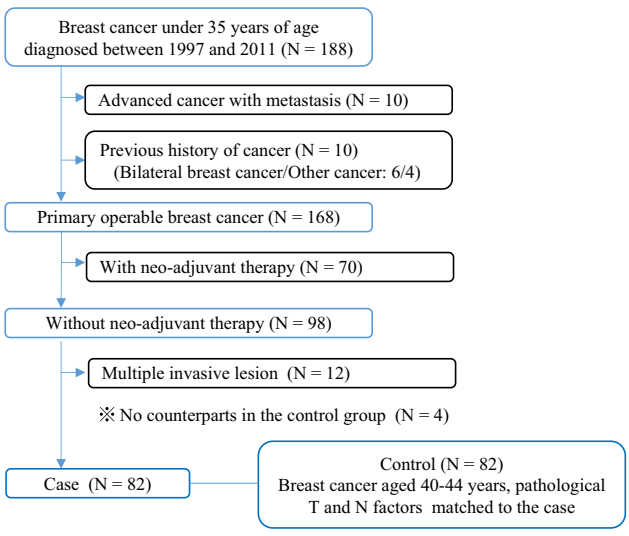

Fig. 1 Patient cohort selection in this study. Flow diagram shows derivation of the analytic cohort of patients enrolled in this study

The clinical information consisted of the chief complaint, family history, smoking history, alcohol consumption, body mass index (BMI), and menstrual and reproductive factors, and the contents of adjuvant therapies were obtained from medical records. Alcohol consumption over the previous year was evaluated through self-assessment. The questionnaire included the average consumption of beer, wine, and spirits, and the frequency of drinking responses were either "never," "hardly ever to three times or less a week," and "four times or more a week." Those who consume alcohol four or more times a week tend to drink more than those who consume alcohol three times or less; therefore, we considered this classification correlated with the volume of alcohol consumption.

Immunohistochemical analysis

A histopathological review was performed for routinely processed hematoxylin and eosin (HE)-stained pathology slides of the main tumor. Parameters assessed were the invasive size of the primary tumor, histological type, HG of both invasive and intraductal components, tumor infiltrating lymphocytes (TILs), lymphovascular invasion, necrosis of tumor, and comedo necrosis in the intraductal component. Histological type was determined based on the World Health Organization classification, 4th Edition [13]. HG was evaluated according to Elston and Ellis [14]. TILs were graded as follows: 0 if mononuclear cells including lymphocytes and plasma cells were absent inside the tumor; 1+ if mononuclear cells were present in ≦10% of tumor cells; and 2+ if mononuclear cells were present in > 10%. Tumors that scored 0 and 1+ were considered low TILs, and tumors that scored 2+ were considered high TILs. Lymphovascular invasion and necrosis were classified as negative or positive.

Formalin-fixed paraffin-embedded tumor blocks from each patient were used for the construction of tissue microarrays (TMAs). Two cores 2 mm in diameter were enucleated from a representative tumor tissue block and transferred to recipient paraffin blocks with an arrayer (Azumaya, Tokyo, Japan). These TMA blocks were cut into 3-μm-thick sections. We confirmed the presence of tumor tissues on HE-stained sections. If both cores from a tumor were not informative, due to loss or no inclusion of tumor tissue, the whole section from the representative block was also assessed.

We examined estrogen receptor (ER), progesterone receptor (PgR), androgen receptor (AR), HER2, Ki-67, p53, epidermal growth factor receptor (EGFR), and cytokeratin 5/6 (CK5/6) expression status using IHC. Antibodies used, and details of assay conditions are presented in Table 1. The BC tissues that had been previously confirmed as positive for these eight molecules were used as positive controls. These tissues, without loading the primary antibody, were used as negative controls.

The expression status of ER, PgR, AR, and p53 was recorded using the Allred scoring system [15]. Tumors that scored three or more were judged to be positive for ER, PgR, and AR, and those that scored four or more were judged to be positive for p53. HER2 status was classified following criteria recommended by the American Society of Clinical Oncology/College of American Pathologists in 2013 [16]. HER2 was judged to be positive if membranous staining was 3+ or 2+ with HER2 gene amplification through dual color in situ hybridization using a Ventana Inform Dual ISH HER2 kit (Roche Diagnostics. Tokyo, Japan). Ki-67 LI was calculated by dividing the number of Ki-67 positive tumor cells by the number of all tumor cells calculated, usually 1,000 in the invasive component. We regarded Ki-67 LI < 14% as low, and Ki-67 LI ≧ 14% as high, in accordance with Cheang et al. [17]. EGFR expression was also scored according to the criteria developed for HER2. Scores of 1+, 2+ and 3+ were regarded as EGFR-positive. A strong cytoplasmic stain was considered as positive for CK5/6 regardless of the proportion. All slides were evaluated independently by two investigators (A.H. and H.T. or M.Y.). Discrepancies between observers were resolved through reviewing the slide together and discussing it until consensus was reached.

Table 1 Antibodies used for immunohistochemistry

Antibody	Clone	Dilution	Source	Detection	Pretreatment
ER	Rabbit mono (SP1)	Ready to use	Roche Diagnostics	ultraView	None
PgR	Rabbit mono (1E2)	Ready to use	Roche Diagnostics	ultraView	None
HER2	Rabbit poly	Not applicable	Dako	HercepTest™	None
Ki-67	Mouse mono (MIB-1)	1:100	Dako	EnVision™ FLEX	Citric acid
AR	Mouse mono (AR441)	1:50	Dako	EnVision™ FLEX	TRS9 (Dako)
p53	Mouse mono (DO-7)	1:100	Dako	EnVision™ FLEX	Citric acid
EGFR	Mouse mono (2-18C9)	Not applicable	Dako	EGFR pharmDx	Proteinase K
CK5/6	Mouse mono (D5/16 B4)	1:400	Dako	EnVision™ FLEX	TRS9 (Dako)

AR androgen receptor, *CK5/6* cytokeratin 5/6, *EGFR* epidermal growth factor receptor, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *PgR* progesterone receptor

The definition for each molecular subtype was based on the 2013 modified St. Gallen International Expert Consensus recommendations [18]. These definitions included luminal A-like: ER and/or PgR positive, HER2 negative, and Ki-67 low; luminal B-like (HER2-negative): ER and/or PgR positive, HER2 negative, and Ki-67 high; luminal B-like (HER2-positive): ER and/or PgR positive, HER2 overexpressed or amplified, and any Ki-67 LI; HER2-enriched: HER2 overexpressed or amplified, and ER/PgR negative; TNBC: ER, PgR, and HER2 negative. The expression of either EGFR or CK5/6 was regarded as a basal-like feature of TNBC [19].

Statistical analysis

The statistical calculations were performed using JMP®9.02 software (SAS 2009). Clinicopathological parameters were compared using the Chi-square test. Distant recurrence-free survival (DRFS) and overall survival curves were drawn using the Kaplan–Meier method, and compared using a log-rank test. DRFS was defined as the time from surgery to the event of distant relapse. We did not consider local recurrence and metachronous contralateral BC as an event. Overall survival was defined as the time from surgery until the date of death from any cause, or the date of the last follow-up. All statistical tests were two-sided and the significance level was defined as $p < 0.05$.

Results

Habitual and clinical characteristics

The median ages were 32.0 years (range 22–34) and 42.0 years (range 40–44) in the AYA patient and control groups, respectively. Comparisons of habitual and clinical characteristics between these two groups are shown in Table 2.

Compared with the control group, the AYA group had a significantly fewer number of childbirths ($p < 0.0001$). Additionally, 20% of the young women answered "four or more times a week" to the question regarding the frequency of alcohol consumption, and this percentage was higher than that of the older women who provided the same answer (9%). Therefore, the volume of alcohol consumption was considered to be higher in the AYA patient group ($p < 0.0001$).

On the other hand, there was no significant difference between the two groups with respect to BMI, the age of menarche, smoking patterns, and family history of BCs. The rate of women with a family history of other cancers was higher in the AYA patient group (66%) than in the control group (52%), although the difference was not significant ($p = 0.08$).

Clinical stages of the disease were Ia, IIa, IIb, IIIa, and IIIc in 41, 23, 10, six, and two patients in the AYA patient group and 44, 20, 10, six and two patients in the control group, respectively. Therefore, the majority of all the patients (148 of 164, 90%) were at either stage I or II.

The rate of cancer detection by mass, pain, or nipple discharge was higher in the AYA patients (91%) than in the older patients (74%) ($p < 0.01$). In the AYA patient group, 93% of women received adjuvant therapies, as follows: 26% received hormone therapy only, 27% received chemotherapy only, and 40% received both hormone therapy and chemotherapy. In the control group, these rates were 87%, 38%, 9%, and 40%, respectively. There was no disproportion in these rates between the two groups ($p = 0.20$).

Immunohistopathological characteristics

The histopathological features of the two groups are presented in Table 3. There was no significant difference in pT, pN, and histological type between the two groups. The mean invasive tumor sizes were 1.99 cm in the AYA patient group and 2.02 cm in the control group. The most frequent type was invasive carcinoma of no special type in both groups (72% versus 73%). There was no statistical difference in HG of both the invasive and noninvasive components between these two groups. The HG of the invasive carcinoma component was not assessed in four patients due to only a small number of invasive cells present, and the HG of the intraductal carcinoma component was not evaluated in 18 patients where no intraductal component was observed. There were also no significant differences between the two groups for TILs, lymphovascular invasion, necrosis, or the comedo necrosis in the intraductal component.

The results of positivity of biomarkers are presented in Table 4. In comparison with tumors in the control group, tumors in the AYA patient group were more frequently ER negative (28% versus 6%, $p < 0.001$), PgR negative (22% versus 10%, $p = 0.03$), AR negative (23% versus 5%, $p < 0.001$), and EGFR positive (28% versus 15%, $p = 0.04$). The percentages of HR-negative tumors were also significantly higher in the AYA patient group (21%) than in the control group (5%, $p < 0.01$).

HER2, Ki-67 LI, p53, and CK5/6 were not significantly different between the two groups. The HER2 status was evaluated in the invasive carcinoma component. We excluded four patients in the AYA patient group because the invasive component was lost in the slides. Ki-67 was mainly examined in the invasive component. We excluded 12 patients where the number of tumor cells was under 1000 cells (8 patients in the AYA patient group, 4 patients in the control group). The mean Ki-67 LI was 29.2% in the AYA patient group, and 28.2% in the control group.

Table 2 Comparison of clinical characteristics between the AYA patient group and the control group

Parameter	Number of patients (%)		*p*-value
	AYA group (N = 82)	Control group (N = 82)	
BMI (kg/m^2) (mean ± SD)	20.9 ± 3.5	21.2 ± 3.3	0.53
Menarche (years) [mean (range, median)]	12.3 (10-16, 12)	12.4 (10-15, 12)	0.59
Number of children			
0	55 (67)	27 (33)	<0.0001
1	19 (23)	11 (13)	
2	5 (6)	33 (40)	
3	3 (4)	10 (12)	
4	0 (0)	1 (1)	
Smoking habit			
Never	60 (73)	60 (73)	0.46
Previous	10 (12)	6 (7)	
Current	12 (15)	16 (19)	
Alcohol consumption			
Never	35 (43)	65 (79)	<0.0001
Hardly ever to <4 times a week	31 (38)	10 (12)	
Four or more times a week	16 (20)	7 (9)	
Family history of breast cancer			
Yes	20 (24)	16 (20)	0.45
No	62 (76)	66 (80)	
Family history of other cancer			
Yes	54 (66)	43 (52)	0.08
No	28 (34)	39 (48)	
Initial detection of cancer			
Palpable mass	72 (88)	58 (71)	<0.01
Nipple discharge	3 (4)	2 (2)	
Breast pain	0 (0)	1 (1)	
Detection by screening	7 (9)	21 (26)	
Adjuvant therapy			
Not received	6 (7)	11 (13)	0.20
Hormonal therapy	21 (26)	31 (38)	
Chemotherapy	22 (27)	7 (9)	
Hormone + chemotherapy	33 (40)	33 (40)	

SD standard deviation

On evaluation of the surrogate intrinsic subtype, four patients without HER2 data were excluded. All of the TNBCs showed basal-like features. Therefore, TNBCs and basal-like subtypes were more frequent in the AYA patient group than in the control group (13 versus two patients) ($p < 0.01$).

Survival

Mean follow-up periods were 8.5 years (range 0.9–16.4 years) for the AYA patient group and 8.7 years (range 0.2–16.5 years) for the control group. During these periods, 18 patients showed distant recurrences, and 10

Table 3 Comparison of histopathological features between the AYA patient group and the control group

Parameter	Number of patients (%)		*p*-value
	AYA group (N = 82)	Control group (N = 82)	
pT factor			
pT1	57 (70)	58 (71)	0.98
pT2	22 (27)	21 (26)	
pT3	3 (4)	3 (4)	
pN factor			
pN0	51 (62)	51 (62)	0.99
pN1	23 (28)	24 (29)	
pN2	6 (7)	5 (6)	
pN3	2 (2)	2 (2)	
Histological type			
Microinvasion	1 (1)	1 (1)	0.55
Invasive, no special type	59 (72)	60 (73)	
Invasive lobular	2 (2)	6 (7)	
Mucinous	2 (2)	2 (2)	
With medullary features	7 (9)	3 (4)	
With apocrine differentiation	0 (0)	1 (1)	
Invasive micropapillary	4 (5)	3 (4)	
Invasive papillary	2 (2)	0 (0)	
Cribriform	3 (4)	6 (7)	
Metaplastic	1 (1)	0 (0)	
Secretory	1 (1)	0 (0)	
Histological grade of invasive component			
1	8 (10)	9 (11)	0.44
2	27 (34)	34 (43)	
3	45 (56)	37 (46)	
Not Assessed*	2	2	
Histological grade of intraductal component			
1	17 (24)	22 (29)	0.67
2	32 (45)	34 (45)	
3	22 (31)	19 (25)	
No component*	11	7	
Tumor infiltrating lymphocytes			
Low	24 (29)	18 (22)	0.28
High	58 (71)	64 (78)	
Lymphovascular invasion			
Negative	42 (51)	42 (51)	1.00
Positive	40 (49)	40 (49)	
Necrosis			
Negative	49 (60)	52 (63)	0.63
Positive	33 (40)	30 (37)	
Comedo necrosis			
Negative	31 (44)	43 (57)	0.10
Positive	40 (56)	32 (43)	
Not Assessed**	11	7	

Table 3 (continued)

[a]Histological grade was not assessed in four cases because of small amount of tumor volume

[b]These 18 cases did not have intraductal component

Table 4 Comparison of biomarker status between the AYA patient group and the control group

Parameter	Number of patients (%)		*p*-value
	AYA group (N = 82)	Control group (N = 82)	
Estrogen receptor			
Negative	23 (28)	5 (6)	< 0.001
Positive	59 (72)	77 (94)	
Progesterone receptor			
Negative	18 (22)	8 (10)	0.03
Positive	64 (78)	74 (90)	
Androgen receptor			
Negative	19 (23)	4 (5)	< 0.001
Positive	63 (77)	78 (95)	
HER2			
Negative	67 (86)	74 (90)	0.40
Positive	11 (14)	8 (10)	
No data*	4	2	
Ki-67			
High	61 (82)	60 (77)	0.40
Low	13 (18)	18 (23)	
Not included**	8	4	
p53			
Negative	42 (51)	39 (48)	0.64
Positive	40 (49)	43 (52)	
Epidermal growth factor receptor			
Negative	59 (72)	70 (85)	0.04
Positive	23 (28)	12 (15)	
Cytokeratin 5/6			
Negative	64 (78)	70 (85)	0.23
Positive	18 (22)	12 (15)	
Surrogate subtype			
Luminal A-like	14 (17)	18 (22)	< 0.01
Luminal B-like (HER2-negative)	40 (49)	54 (66)	
Luminal B-like (HER2-positive)	8 (10)	6 (7)	
HER2-enriched	3 (4)	2 (2)	
Triple-negative	13 (16)	2 (2)	
Not Assessed*	4 (5)	0	

[a]HER2 was not assessed in four cases because invasive component was lost in the slide of immunohistochemistry

[b]Ki-67 data were not analyzed in 12 cases because the number of invasive carcinoma cells was < 1000

patients died in the AYA patient group. In contrast, distant recurrences in six patients and deaths in six patients were observed in the control group. All deaths in both groups were due to BC.

The DRFS curves showed a significant difference between the two groups ($p=0.02$) (Fig. 2a). Stratified by HR status, the difference in the DRFS curves was larger in the HR-positive tumors ($p=0.01$), while there was no significant difference in HR-negative tumors ($p=0.97$). (Fig. 3a, c).

However, overall survival curves did not show a significant difference between the two groups in the whole population ($p=0.20$) (Fig. 2b). Even when stratified by HR status, overall survival did not show significant differences between the two groups (Fig. 3b, d).

Discussion

In this study, we compared habitual factors, background factors, and clinicopathological and immunohistochemical features between the BCs that occurred in the AYA generation and those that occurred in women between 40 and 44 years of age as controls. Compared with older patients, the AYA group was characterized with a smaller number of childbirths, a larger volume of alcohol consumption, and symptomatic lesions. In the present study, in patients primarily at clinical stages I or II, we could show that BCs occurring in the AYA generation women had more aggressive immunohistochemical features and biological properties than the older women in terms of a higher proportion of TNBC and a lower DRFS.

Habitual and background features shown to be characteristic in the present study support the results of previous studies [1, 20, 21]. A smaller number of childbirths in the AYA group than in the older group might reflect the differences among the generation. For example, the younger generation should have less children than older generation even in women without BC because an increasing number of women give births after the age of 35 years in Japan. The likelihood of alcohol consumption and the volume of alcohol consumption were considered to be higher in the AYA patient group in our study. From National Health and Nutrition Survey of e-stat, the official statistics of Japan (http://www.e-stat.go.jp/), habitual drinking is more frequent in the middle-aged women than in the AYA women in general population. Therefore, the present result may be of significance. Alcohol consumption has been linked to BC risk in multiple studies [20, 21]. Chen et al. reported that alcohol consumption in early adult life was related to BC risk [21], but it is still unknown if the volume and duration of alcohol consumption has an influence on tumorigenicity in the breast. Further detailed epidemiological cohort studies are necessary to clarify the relationship between patient characteristics and breast carcinogenesis.

Using the BC registration data of the Japanese Breast Cancer Society, Kataoka et al. reported that young patients (< 35 years) had a more frequent family history of BCs and lower BMIs than the older (35 years or older) patients [1]. The absence of a BC family history relationship to BCs in our study might be due to the exclusion of patients with bilateral BCs and having excluded patients with concurrent cancers in other organs from the analyses. The rate of women who had a family history of other cancers was higher in the AYA patient group than in the older patient group (66% vs 52%, respectively). On the other hand, the different significance in BMI found in Kataoka's study

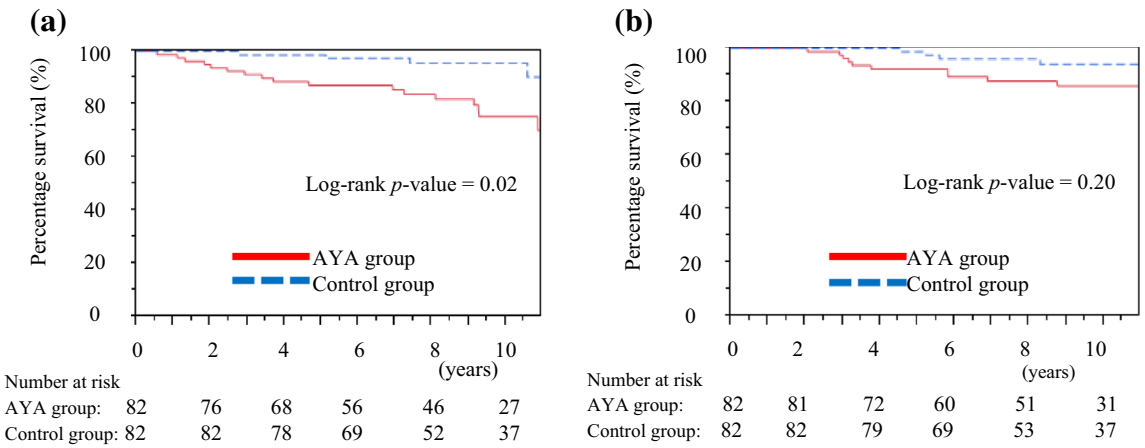

Fig. 2 Distant recurrence-free survival (DRFS) curves and overall survival curves for the adolescent and young adult (AYA) patient group and the control group. **a** DRFS curves. Two curves show significant difference ($p=0.02$). **b** Overall survival curves. Two curves do not show a significant difference, but then clinical outcome of the AYA group (red) tends to be worse than that of the control group (blue) ($p=0.13$)

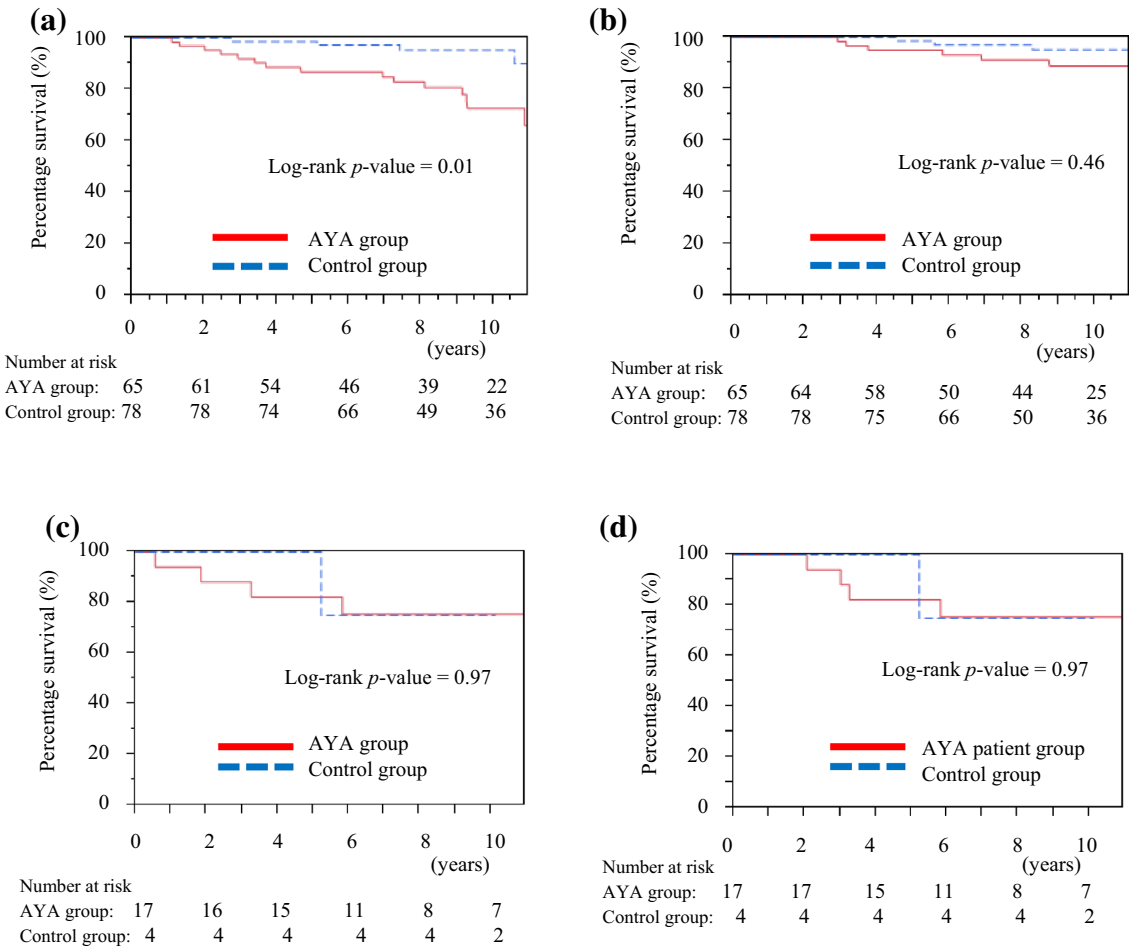

Fig. 3 Distant recurrence-free survival (DRFS) curves and overall survival curves for the young patient group and the control group stratified by hormone receptor (HR) status. **a** DRFS curves for the patients with HR-positive tumors. **b** Overall survival curves for the patients with HR-positive tumors. DRFS curves in **a** show a significant difference ($p=0.01$), but overall survival curves in **b** do not show a significant difference ($p=0.29$). **c** DRFS curves for the patients with HR-negative tumors. **d** Overall survival curves for the patients with HR-negative tumors. In **c** and **d**, DRFS and overall survival curves do not show significant differences ($p=0.49$, and 0.97)

may have been due to the difference in the age distribution of the control group, which was 35 years old or older and included postmenopausal women in Kataoka's study [1] and was 40–44 years old in the present study.

With regard to clinical findings, the rate of cancer detection by mass, pain or nipple discharge was higher in the AYA patients than in the older patients, despite these two groups having had almost the same pT factor. The same findings have been previously reported by Kataoka et al. [1], and as one reason of these results, the lower screening receiving rate of the young generation was considerable in Japan. On the other hand, there was no statistical difference in the HG of both the invasive and noninvasive components between these two groups, which was contrary to most previous studies [3, 6, 8]. There were no distinct histopathological characteristics in AYA BC patients.

In our results, TNBCs and basal-like subtypes were more frequent in the AYA patient group than in the control group. These results are compatible with the results of many other studies [3, 4, 7, 9, 10]. At the mRNA level, Anders et al. [6] showed that the mean expression level of EGFR was higher in the patient group aged < 45 years than in those aged 65 years or older. Contrary to these results, Lin et al. reported a higher prevalence of HR-positive tumors and a lower prevalence of basal-like subtypes in young patients than in older patients in a Taiwanese cohort by IHC [11].

We demonstrated that both ER-negative and PgR-negative tumors and AR-negative tumors were more frequent in the AYA patient group than in the control group. These results are compatible with previous studies [4, 7–9]. Niemeier et al. reported that AR was positive in 95% and 34% of ER-positive and ER-negative cases, respectively, and that AR and ER statuses were strongly associated with each other

[22]. Recently, AR has drawn interest as the target of therapy in TNBCs [23]. In this cohort, all TNBC cases had basal-like features, and one of these TNBCs was also AR positive.

Although most previous studies have demonstrated that the rate of HER2 overexpressing BCs was higher in the younger patients than in the control patient groups [1, 4, 6, 7], we could not identify such a positive relationship between HER2 overexpression and the AYA patient group in this study. However, some previous studies have been in line with our results [7, 11]. HER2 overexpression rates differ according to tumor progression. The rate increases in high-grade ductal carcinoma in situ (DCIS) and high-grade invasive carcinoma with extensive intraductal spread [24]. In the present study, no DCIS cases were included, and the number of advanced stage cases was very small. It is necessary to consider that these characteristics of the study population may have influenced the results.

In our study, almost the same clinical stages between the AYA and the control groups were taken into consideration, the AYA patient group showed a higher rate of distant metastases than the control group. Our findings are in line with those of previous studies [3, 5–7]. We showed that there were significant differences in the DRFS curves. Such differences were observed not only in the whole cases but also in the HR-positive cases. Different significance in DRFS curves according to HR status is in line with the recent studies that reported different effects of subtypes on biological propertied of breast cancer among age groups [7, 25]. The difference in DRFS rate between the AYA patient group and the control group appear have derived not only from higher incidences of HR-negative cases and TNBCs, but also from more aggressive nature of HR-positive cases in the former because of unknown reasons. As one possible reason for high incidence of HR-negative cases, TNBCs, or basal-like subtypes, we may be able to speculate distribution of patients with germline mutation *BRCA1* or other genes in the AYA patient group. However, we could not find a difference in overall survival between the two groups in the whole population even when we stratified the cases by HR status. Taking into consideration the relatively long follow-up periods in this study, the lack of differences in overall survival curves between these groups or between these subgroups might be attributed to recent advances of effective drugs for young BC patients. Therefore, further studies are warranted for treatment strategy concerning young BC patients.

This study has some limitations. First, we conducted this study in a single institute as a retrospective study. Generally, young BC patients under 35 years of age are rare. As such, our research cohort was not large. Second, we excluded patients who had received neoadjuvant therapies. However, these exclusions enabled us to avoid the therapeutic effects over primary cancers on the evaluation of immunohistochemical features [26]. Third, there were limitations in our study design. We evaluated the parameters as a case–control study and used TMAs for biomarker assessment. Although the assessment using TMAs might raise issues regarding intratumor heterogeneity [27], we had to select our patients accordingly, given the high costs of immunochemical analysis. However, taking into consideration the need for data without missing values, we succeeded in obtaining information on the clinicopathological and immunohistochemical features in almost all patients, which allowed this study to elucidate tumor characteristics more effectively.

In summary, we confirmed that invasive BCs occurring in patients aged < 35 years old showed more aggressive biological properties than those that occurred in older premenopausal patients in terms of immunohistochemical subtype and DRFS. Our results may add significant information that will contribute towards understanding the characteristics of BCs in the AYA generation.

Acknowledgements We thank to Sachiko Miura, M.T., Chizu Kina, M.T., Toshiko Sakaguchi, M. T., Yasuo Shibuki, M. T., and Ms. Chinami Onuma for technical assistance.

Compliance with ethical standards

Conflict of interest Y. F. reports grants and other from Japan Agency for Medical Research and Development, grants and other from the Ministry of Health Labor and Welfare, Japan, during the conduct of the study; others from Astra Zeneca KK, other from Eisai Co., Ltd, other from Daiichi Sankyo Co., Ltd, other from Taiho Pharmaceutical Co., Ltd, grants from Takeda Pharmaceutical Co., Ltd, grants and other from Chugai Pharmaceutical Co., Ltd, other from Eli Lilly Japan KK, other from Novartis Pharma KK, outside the submitted work. Other authors have stated that they have no conflicts of interest. All authors have approved the final article.

References

1. Kataoka A, Tokunaga E, Masuda N, Shien T, Kawabata K, Miyashita M. Clinicopathological features of young patients (< 35 years of age) with breast cancer in a Japanese Breast Cancer Society supported study. Breast Cancer. 2014;21(6):643–50. https://doi.org/10.1007/s12282-013-0466-2.
2. The editorial board of the cancer statistics in Japan. Cancer statistics in Japan 2017. Tokyo: Foundation for promotion of cancer research; 2017.
3. Ribnikar D, Ribeiro JM, Pinto D, Sousa B, Pinto AC, Gomes E, et al. Breast cancer under age 40: a different approach. Curr Treat Options Oncol. 2015;16(4):16. https://doi.org/10.1007/s11864-015-0334-8.
4. Cancello G, Maisonneuve P, Rotmensz N, Viale G, Mastropasqua MG, Pruneri G, et al. Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (< 35 years) with operable breast cancer. Ann Oncol. 2010;21(10):1974–81. https://doi.org/10.1093/annonc/mdq072.
5. Purushotham A, Shamil E, Cariati M, Agbaje O, Muhidin A, Gillett C, et al. Age at diagnosis and distant metastasis in breast cancer—a surprising inverse relationship. Eur J Cancer. 2014;50(10):1697–705. https://doi.org/10.1016/j.ejca.2014.04.002.

6. Anders CK, Hsu DS, Broadwater G, Acharya CR, Foekens JA, Zhang Y, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol. 2008;26(20):3324–30. https://doi.org/10.1200/JCO.2007.14.2471.
7. Kim EK, Noh WC, Han W, Noh DY. Prognostic significance of young age (< 35 years) by subtype based on ER, PR, and HER2 status in breast cancer: a nationwide registry-based study. World J Surg. 2011;35(6):1244–53. https://doi.org/10.1007/s00268-011-1071-1.
8. Colleoni M, Rotmensz N, Robertson C, Orlando L, Viale G, Renne G, et al. Very young women (< 35 years) with operable breast cancer: features of disease at presentation. Ann Oncol. 2002;13(2):273–9.
9. Keegan TH, DeRouen MC, Press DJ, Kurian AW, Clarke CA. Occurrence of breast cancer subtypes in adolescent and young adult women. Breast Cancer Res. 2012;14(2):R55.
10. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295(21):2492–502.
11. Lin CH, Liau JY, Lu YS, Huang CS, Lee WC, Kuo KT, et al. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. Cancer Epidemiol Biomark Prev. 2009;18(6):1807–14. https://doi.org/10.1158/1055-9965.EPI-09-0096.
12. Edge S, Byrd DR, Compton CC, Fritz AG, Fritz AG, Greene FL, et al. AJCC cancer staging handbook from the AJCC cancer staging manual. New York: Springer; 2010.
13. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. WHO classification of tumors of the breast. 4th ed. Lyon: IARC; 2012.
14. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19(5):403–10.
15. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998;11(2):155–68.
16. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31(31):3997–4013. https://doi.org/10.1200/JCO.2013.50.9984.
17. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101(10):736–50. https://doi.org/10.1093/jnci/djp082.
18. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ, et al. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22(8):1736–47. https://doi.org/10.1093/annonc/mdr304.
19. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10(16):5367–74.
20. Park SY, Kolonel LN, Lim U, White KK, Henderson BE, Wilkens LR. Alcohol consumption and breast cancer risk among women from five ethnic groups with light to moderate intakes: the Multiethnic Cohort Study. Int J Cancer. 2014;134(6):1504–10. https://doi.org/10.1002/ijc.28476.
21. Chen WY, Rosner B, Hankinson SE, Colditz GA, Willett WC. Moderate alcohol consumption during adult life, drinking patterns, and breast cancer risk. JAMA. 2011;306(17):1884–90. https://doi.org/10.1001/jama.2011.1590.
22. Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. Mod Pathol. 2010;23(2):205–12. https://doi.org/10.1038/modpathol.2009.159.
23. Fioretti FM, Sita-Lumsden A, Bevan CL, Brooke GN. Revising the role of the androgen receptor in breast cancer. J Endocrinol. 2014;52(3):R257-65. https://doi.org/10.1530/JME-14-0030.
24. Tsuda H, Hirohashi S. Multiple developmental pathways of highly aggressive breast cancers disclosed by comparison of histological grades and c-erbB-2 expression patterns in both the non-invasive and invasive portions. Pathol Int. 1998;48(7):518–25.
25. Sheridan W, Scott T, Caroline S, Yvonne Z, Vanessa B, David V, et al. Breast cancer in young women: have the prognostic implications of breast cancer subtypes changed over time? Breast Cancer Res Treat. 2014;147(3):617–29. https://doi.org/10.1007/s10549-014-3125-1.
26. Pinder SE, Rakha EA, Purdie CA, Bartlett JM, Francis A, Stein RC, et al. Macroscopic handling and reporting of breast cancer specimens pre- and post-neoadjuvant chemotherapy treatment: review of pathological issues and suggested approaches. Histopathology. 2015;67(3):279–93. doi. https://doi.org/10.1111/his.12649.
27. Ruiz C, Seibt S, Al Kuraya K, Siraj AK, Mirlacher M, Schraml P, et al. Tissue microarrays for comparing molecular features with proliferation activity in breast cancer. Int J Mol Cancer. 2006;118(9):2190–4.