# Patient Age Associates With Tumor-Infiltrating Lymphocytes Density In Breast Cancer

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**Research Article**

**Keywords:** breast cancer, tumor-infiltrating lymphocytes, tumor immune-microenvironment, Age, preoperative chemotherapy

**Posted Date:** May 13th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-486109/v1

## Abstract

***Purpose***: Lymphocytes surrounding the cancer participate in tumor-related immune responses, and are called tumor-infiltrating lymphocytes (TILs). Several recent reports suggest TILs to index the tumor-microenvironment (TME), and predict the therapeutic effect of chemotherapy. However, only few studies have studied the relationship between age and TILs. Aging reduces host immunity, and we predict that it may also affect TILs. Thus, we hypothesized that older breast cancer (BC) patients may have low TILs density than younger BC patients. Here, we retrospectively analyzed the differences in TILs by age and the therapeutic effects of pre-operative chemotherapy (POC) in BC patients aged less than 45 years and more than 60 years.

***Methods***: POC was administered to 356 patients. We confirmed and compared TILs density and therapeutic effects in patients aged < 45 years (n=75) and those aged >61 years (n=116). TIL density was evaluated using pre-treatment needle biopsy specimens. Definition and evaluation of TILs was based on the International TILs Working Group 2014.

***Results***: Based on subtype, younger patients showed significantly higher pathological complete response rates than older patients with hormone receptor (HR)+ human epidermal growth factor receptor 2 (HER2)+ and HER2-enriched BC. In HR+HER2+BC, the objective response rate was significantly high in younger than in older patients. Further, a significant difference was observed for overall survival between these patients with triple-negative BC.

***Conclusions***: Our study suggests that younger BC patients possess significantly high TILs density than older patients. These differences may influence the therapeutic efficacy in highly immunogenic subtypes.


## Introduction

The tumor-infiltrating lymphocytes (TILs) surround the cancer tissue and are involved in tumor-related immune responses [1]. Moreover, the TILs, as component of the tumor-microenvironment (TME), allow prediction of the therapeutic efficacy of chemotherapy [2–4]. In patients with breast cancer (BC), an increase in TILs density correlated with increase in the rate to pathologic complete response (pCR), along with extension of the disease-free survival (DFS) and OS [5, 6]. Further, the TILs density in breast cancer differs depending on the subtype. For instance, the HR- BC, such as the TNBC and HER2-enriched BC, show high TILs density [7–9]. However, there are fewer reports on factors other than BC subtypes that affect the TILs density.

Current standard treatment is based on the results of various clinical trials. For instance, some clinical trials suggest the prognosis and treatment effect to differ depending on the age of the patients [10–12], and several pooled studies have reported differences in the treatment effect due to age [13, 14, 5]. However, till now, only few studies have mentioned the relationship between age and TILs. While increased age may reduce host immunity [15], we can anticipate it to also affect TILs. Moreover, the clinical trials studying association of TILs and therapeutic effects have not correlated age and TILs [12, 16–19], and most of them have divided patients into two groups based on TILs or age, with only *t*-test analyses of each group.

Therefore, we set to test the hypothesis that TILs density decreases with age of BC patients, we compared the TILs density in younger and the older BC patients after omitting the patients in the middle age group. We also tested the hypothesis that the therapeutic effect and prognoses of patients may differ with TILs density. Thus, here, we retrospectively analyzed the differences in TILs density by age and the therapeutic effects in BC patients aged < 45 years and > 61 years receiving pre-operative chemotherapy (POC).

## Materials And Methods

### Patients

A total of 356 patients with BC received POC between February 2007 and March 2018 at the Osaka City University Hospital, Japan, and were retrospectively recruited in the study. Further, we confirmed the TILs density and therapeutic effects, based on which we compared the TILs in patients with age < 45 years (younger, n=75) versus those with age > 61 years (older, n=116). The patients were pathologically diagnosed with BC by core needle biopsy (CNB) or vacuum-assisted biopsy (VAB), and by immunohistochemical staining of the specimen to evaluate the expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki67. Based on the results, the subtypes were classified as follows: HER2-enriched BC (ER-, PgR-, and HER2+); TNBC (ER-, PgR-, and HER2-); HR+HER2+BC (ER+ and/or PgR+, and HER2+); and HR+HER2-BC (ER+ and/or PgR+, and HER2-). Before chemotherapy, the staging of BC was evaluated using ultrasonography (US), computed tomography (CT), and bone scintigraphy. POC was administered in BC patients diagnosed with stage IIA (T1, N1, M0 or T2, N0, M0), IIB (T2, N1, M0 or T3, N0, M0), IIIA (T1-2, N2, M0 or T3, N1-2, M0), IIIB (T4, N0-2, M0), or IIIC (T1-4, N3, M0). The POC regimen comprised of four courses of FEC100 (500 mg/m$^2$ fluorouracil, 100 mg/m$^2$ epirubicin, and 500 mg/m$^2$ cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m$^2$ paclitaxel administered weekly. For HER2+ BC patients, an additional weekly (2 mg/kg) or tri-weekly (6 mg/kg) dosage of trastuzumab was administered during paclitaxel treatment [20-22]. The anti-tumor effects of POC were evaluated according to the Response Evaluation Criteria in Solid Tumors [23]. Further, the clinical partial response (cPR) and complete response (cCR) were defined as “Responders” in the objective response rate (ORR). Whereas, clinical stable disease (cSD) and clinical progressive disease (cPD) were defined as “Non-responders”. After POC, all the patients underwent mastectomy or breast-conserving surgery [24]. A pathologic complete response (pCR) was defined as the complete disappearance of the invasive components of the lesion with or without intraductal components, including that in the lymph nodes according to the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 protocol [25].

Post-surgery, standard adjuvant therapy was administered according to each subtype and surgical procedure. During adjuvant therapy, all the patients were evaluated for tumor recurrence by physical examination, US, and CT and bone scintigraphy every 3, 6, and 12 months, respectively. The median follow-up time was 1281 days (range, 13-3675 days) after surgery.

### Histopathological evaluation of TILs density

TILs density was evaluated using pretreatment specimens obtained by CNB or VAB. The TILs were defined and evaluated based on the International TILs Working Group 2014 [1] as the average of the infiltrating lymphocytes within the tumor stroma at five randomly selected fields. Next, the results were classified into four class (3: >50%; 2: >10–50%; 1: ≤10%; or 0: absent) (Supplementary Fig. S1). Further, we defined the scores 2 and 3 as “High”, and scores 1 and 0 as “Low” according to previous reports [26,27]. Thus, in brief, the cut-off value of TILs density was set to 10%.

### Statistical analysis

All statistical analyses were performed using the JMP software package (SAS, Tokyo, Japan). The distribution of TILs density by age was evaluated using Student's *t*-test. The Pearson’s chi-square test was used to evaluate the relationship between each factor. Prognostic analyses, such as DFS or OS, were examined using the Kaplan–Meier method and log-rank test. The hazard ratio (HR) and 95% confidence interval (CIs) were calculated using the Cox proportional hazards model. Multivariable analysis was performed using the Cox regression model. A *P*-value < 0.05 was considered to be statistically significant.

## Results

# Clinicopathological features of BC patients

The clinicopathological features of patients (n = 356) treated with POC have been summarized in Table 1. The patients were operated at median age of 55 years (range, 24–78 years) and the median tumor diameter 28.7 mm (range, 9.2–119.8 mm). Skin infiltration was observed in 58 patients (16.3%). Further, imaging methods of diagnosis did not indicate lymph node metastasis in 121 patients (34.0 %). The number of ER-negative, PgR-negative, and HER2-positive patients was 187 (52.5 %), 242 (68.0 %), and 125 (35.1 %), respectively. Moreover, Ki67-high (above 14%) was observed in 239 patients (67.1 %). Based on these results, the BC subtypes were classified as follows– HR + HER2-: 126 patients (35.4 %), HR + HER2+: 47 patients (13.2 %), HER2-enriched: 78 patients (21.9 %), and TNBC: 105 patients (29.5 %). Furthermore, the Responders for ORR reached 88.8%, the rate of pCR post-operative pathology was 33.1%, and 161 patients (45.2%) showed high TILs density.

Table 1
Clinicopathological features of 356 patients who were treated with preoperative chemotherapy

| Parameters | All patients ($n$ = 356) (%) | Younger ($n$ = 75) (%) | Elderly ($n$ = 116) (%) |
|---|---|---|---|
| Age (years old) | 55 (24–78) | 41 (24–45) | 67 (61–78) |
| Tumor size (mm) | 28.7 (9.2–119.8) | 29.5 (9.9–82.6) | 27.3 (9.2–89.8) |
| Skin infiltration<br>Negative / Positive | 298 (83.7%) / 58 (16.3%) | 68 (90.7%) / 7 (9.3%) | 90 (77.6%) / 26 (22.4%) |
| Lymph node metastasis<br>N0 / N1 / N2 / N3 | 121 (33.9%) / 133 (37.4%) / 68 (19.1%) / 34(9.6%) | 28 (37.3%) / 28 (37.3%) / 14 (18.7%) / 5 (6.7%) | 44 (37.9%) / 36 (31.0%) / 22 (19.0%) / 14(12.1%) |
| Estrogen receptor<br>Negative / Positive | 187 (52.5%) / 169 (47.5%) | 37 (49.3%) / 38 (50.7%) | 67 (57.8%) / 49 (42.2%) |
| Progesterone receptor<br>Negative / Positive | 242 (68.0%) / 114 (32.0%) | 42 (56.0%) / 33 (44.0%) | 89 (76.7%) / 27 (23.3%) |
| HER2<br>Negative / Positive | 231 (64.9%) / 125 (35.1%) | 47 (62.7%) / 28 (37.3%) | 69 (59.5%) / 47 (40.5%) |
| Ki67<br>≤ 14 % / >14 % | 117 (32.9%) / 239 (67.1%) | 22 (29.3%) / 53 (70.7%) | 40 (34.5%) / 76 (65.5%) |
| Intrinsic subtype<br>HR + HER2-BC / HR + HER2 + BC / HER2BC / TNBC | 126 (35.4%) / 47 (13.2%) / 78 (21.9%) / 105 (29.5%) | 24 (32.0%) / 16 (21.3%) / 12 (16.0%) / 23 (30.7%) | 39 (33.6%) / 11 (9.5%) / 36 (31.0%) / 30 (25.9%) |
| Objective response rate<br>Non-Responders / Responders | 40 (11.2%) / 316 (88.8%) | 5 (6.7%) / 70 (93.3%) | 17 (14.7%) / 99 (85.3%) |
| Pathological response<br>Non-pCR / pCR | 238 (66.9%) / 118 (33.1%) | 46 (61.3%) / 29 (38.7%) | 78 (67.2%) / 38 (32.8%) |
| TILs<br>Low / High | 195 (54.5%) / 161 (45.2%) | 31 (41.3%) / 44 (58.7%) | 65 (56.0%) / 51 (44.0%) |

HER: human epidermal growth factor receptor. CR: complete response. TILs: tumor- infiltrating lymphocytes.

Further, while most of the clinicopathological factors were not significantly different, the rate of skin infiltration and PgR-negative status were significantly higher in the older that in the younger patients ($P$ = 0.002 and $P$ = 0.003, respectively) (Table 2). Moreover, the ORR, although statistically insignificant, was found to be higher in the younger than in the older patients ($P$ = 0.091).

Table 2
Difference in clinicopathological features due to TILs in younger and elderly patients

| Parameters | tumor- infiltrating lymphocytes (*n* = 191) | | |
|---|---|---|---|
| | Low (*n* = 96) | High (*n* = 95) | *p* value |
| Age (years old) | 31 (32.3%) | 44 (46.3%) | 0.047 |
| ≤ 45 | 65 (67.7%) | 51 (53.7%) | |
| > 60 | | | |
| Tumor size (mm) | 20 (20.8%) | 14 (14.7%) | 0.271 |
| ≤ 20.0 | 76 (79.2%) | 81 (85.3%) | |
| > 20.0 | | | |
| Skin infiltration | 71 (74.0%) | 87 (91.6%) | 0.001 |
| Negative | 25 (26.0%) | 8 (8.4%) | |
| Positive | | | |
| Lymph node status | 33 (34.4%) | 39 (41.1%) | 0.341 |
| Negative | 63 (65.6%) | 56 (58.9%) | |
| Positive | | | |
| Estrogen receptor | 37 (38.5%) | 67 (70.5%) | < 0.001 |
| Negative | 59 (61.5%) | 28 (29.5%) | |
| Positive | | | |
| Progesterone receptor | 55 (57.3%) | 76 (80.0%) | 0.001 |
| Negative | 41 (42.7%) | 19 (20.0%) | |
| Positive | | | |
| Hormone receptor | 35 (36.5%) | 66 (69.5%) | < 0.001 |
| Negative | 61 (63.5%) | 29 (30.5%) | |
| Positive | | | |
| HER2 | 69 (71.9%) | 47 (49.5%) | 0.002 |
| Negative | 27 (28.1%) | 48 (50.5%) | |
| Positive | | | |
| Ki67 | 37 (38.5%) | 25 (26.3%) | 0.071 |
| ≤14 % | 59 (61.5%) | 70 (73.7%) | |
| >14 % | | | |
| ORR | 18 (18.8%) | 4 (4.2%) | 0.002 |
| Non-Responders | 78 (81.2%) | 91 (95.8%) | |
| Responders | | | |
| Pathological response | 79 (82.3%) | 45 (47.4%) | < 0.001 |
| Non-pCR | 17 (17.7%) | 50 (52.6%) | |
| pCR | | | |

TILs: tumor- infiltrating lymphocytes. HER: human epidermal growth factor receptor. ORR: objective response rate. CR: complete response.

## Correlation of TILs density with clinicopathological features and prognosis of patients

First, the 356 patients were divided into high and low TILs density groups, and their correlation with clinicopathological factors was examined (Supplementary Table S1). Following characteristics were observed in the low TILs than the high TILs group: ≥ 45 years ($P = 0.008$), skin invasion ($P = 0.001$), ER-positive ($P < 0.001$), PgR-positive ($P < 0.001$), HER2-negative ($P = 0.011$), Ki67-high ($P < 0.001$), low ORR ($P = 0.001$), and low pCR rate ($P < 0.001$).

Further, the high TILs density group showed significantly better DFS than the low TILs density group in HER2-enriched ($P = 0.012$, log-rank) and TNBC ($P = 0.002$, log-rank) categories (Supplementary Fig. S2). Therefore, DFS was better in the high TILs density group despite no significant difference in HR + BC ($P = 0.011$, log-rank). However, the high TILs density group had better OS, although not statistically significant, than the low TILs density group in TNBC category ($P = 0.057$, log-rank), but there was no significant difference between the difference of TILs density (Supplementary Fig. S3). Further, in the univariate analysis

for DFS, high TILs density group associated with significantly better DFS ($P = 0.010$, HR = 0.512) (Supplementary Table S2). However, in the multivariate analysis for DFS, TILs density was not an independent factor ($P = 0.227$, HR = 0.699), since skin invasion ($P = 0.012$, HR = 2.180), lymph node metastasis ($P = 0.001$, HR = 2.918), HER2-positive ($P = 0.020$, HR = 0.498), Responders in ORR ($P < 0.001$, HR = 0.247), and pCR ($P < 0.001$, HR = 0.315) influenced the DFS. Additionally, difference in OS due to TILs was insignificant even in the univariate analysis ($P = 0.214$, HR = 0.660) (Supplementary Table S3).

Further, the patients were classified based on age as < 45 years, 46–60 years, and ≥ 61 years, and the distribution of TILs density was analyzed using *t*-test (Fig. 1). Our analysis did not indicate significant difference in HR + BC for any of the age groups. However, in HER2-enriched BC, the patients aged < 45 years had significantly higher TILs density than patients in other age groups (vs. 46–60 years: $P = 0.002$, and vs. ≥ 61 years: $P = 0.018$). Furthermore, in the TNBC category, the patients aged ≥ 61 years had significantly higher TILs density than patients in other age groups (vs. ≤ 40 years: $P = 0.035$, and vs. 46–60 years: $P = 0.047$).

**Examination of clinicopathological factors and prognosis in the younger and older BC patients**

First, we studied the correlation between TILs density and clinicopathological factors in the younger and older patients (Table 2). Although patients aged 46–60 years were excluded from the analysis, the characteristics of the high TILs density group were similar to those for all patients: > 60 years ($P = 0.047$), skin infiltration ($P = 0.001$), ER-positive ($P < 0.001$), PgR-positive ($P = 0.001$), HER2-negative ($P = 0.002$), lower ORR ($P = 0.002$), and lower pCR rate ($P < 0.001$).

Further, younger patients showed significantly higher pCR rates than older patients in the HR + HER2- and HER2-enriched BC category ($P = 0.021$ and $P = 0.048$, respectively) (Table 3). Moreover, in HR + HER2 + BC, the responder rate for ORR was significantly higher in the younger patients than in older patients ($P = 0.009$). However, no significant difference was observed in the effect of POC on TNBC.

Table 3
Difference in clinicopathological features due to age

| Parameters | All intrinsic subtype (*n* = 191) | | | HR + HER2-BC (*n* = 61) | | | HR + HER2 + BC (*n* = 27) | | | HER2BC (*n* = 48) | | | TNBC (*n* = |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Young (*n* = 75) | Elderly (*n* = 116) | *p* value | Young (*n* = 24) | Elderly (*n* = 39) | *p* value | Young (*n* = 16) | Elderly (*n* = 11) | *p* value | Young (*n* = 12) | Elderly (*n* = 36) | *p* value | Young (*n* = 23) |
| Tumor size (mm) | 10 (13.3%) | 24 (20.7%) | 0.194 | 2 (8.3%) | 7 (17.9%) | 0.290 | 3 (18.8%) | 2 (18.2%) | 0.970 | 2 (16.7%) | 7 (19.4%) | 0.831 | 3 (13.0%) |
| ≤ 20.0 | 65 (86.7%) | 92 (79.3%) | | 22 (91.7%) | 32 (82.1%) | | 13 (81.2%) | 9 (81.8%) | | 10 (83.3%) | 29 (80.6%) | | 20 (87.0%) |
| > 20.0 | | | | | | | | | | | | | |
| Skin infiltration | 68 (90.7%) | 90 (77.6%) | 0.020 | 20 (83.3%) | 29 (74.4%) | 0.405 | 14 (87.5%) | 6 (54.5%) | 0.055 | 12 (100.0%) | 29 (80.6%) | 0.098 | 22 (95.7%) |
| Negative | 7 (9.3%) | 26 (22.4%) | | 4 (16.7%) | 10 (25.6%) | | 2 (12.5%) | 5 (45.5%) | | 0 (0.0%) | 7 (19.4%) | | 1 (4.3%) |
| Positive | | | | | | | | | | | | | |
| Lymph node status | 28 (37.3%) | 44 (37.9%) | 0.934 | 8 (33.3%) | 12 (30.8%) | 0.832 | 9 (56.2%) | 2 (18.2%) | 0.048 | 4 (33.3%) | 17 (47.2%) | 0.401 | 7 (30.4%) |
| Negative | 47 (62.7%) | 72 (62.1%) | | 16 (66.7%) | 27 (69.2%) | | 7 (43.8%) | 9 (81.8%) | | 8 (66.7%) | 19 (52.8/%) | | 16 (69.6%) |
| Positive | | | | | | | | | | | | | |
| Estrogen receptor | 37 (49.3%) | 67 (57.8%) | 0.254 | 2 (8.3%) | 0 (0.0%) | 0.067 | 0 (0.0%) | 1 (9.1%) | 0.219 | - | - | | - |
| Negative | 38 (50.7%) | 49 (42.2%) | | 22 (91.7%) | 39 (100.0%) | | 16 (100.0%) | 10 (90.9%) | | - | - | | - |
| Positive | | | | | | | | | | | | | |
| Progesterone receptor | 42 (56.0%) | 89 (76.7%) | 0.003 | 5 (20.8%) | 16 (41.0%) | 0.099 | 2 (12.5%) | 7 (63.6%) | 0.006 | - | - | | - |
| Negative | 33 (44.0%) | 27 (23.3%) | | 19 (79.2%) | 23 (59.0%) | | 14 (87.5%) | 4 (36.4%) | | - | - | | - |
| Positive | | | | | | | | | | | | | |
| Hormone receptor | 35 (46.7%) | 66 (56.9%) | 0.167 | - | - | | - | - | | - | - | | - |
| Negative | 40 (53.3%) | 50 (43.1%) | | - | - | | - | - | | - | - | | - |
| Positive | | | | | | | | | | | | | |
| HER2 | 47 (62.7%) | 69 (59.5%) | 0.660 | - | - | | - | - | | - | - | | - |
| Negative | | | | - | - | | - | - | | - | - | | - |
| Positive | 28 (37.3%) | 47 (40.5%) | | | | | | | | | | | |
| Ki67 | 22 (29.3%) | 40 (34.5%) | 0.458 | 12 (50.0%) | 21 (53.8%) | 0.767 | 7 (43.8%) | 2 (18.2%) | 0.166 | 1 (8.3%) | 12 (33.3%) | 0.091 | 2 (8.7%) |
| ≤14 % | | | | | | | | | | 11 (91.7%) | | | |
| >14 % | 53 (70.7%) | 76 (65.5%) | | 12 (50.0%) | 18 (46.2%) | | 9 (56.2%) | 9 (81.8%) | | | 24 (66.7%) | | 21 (91.3%) |
| ORR | 5 (6.7%) | 17 (14.8%) | 0.091 | 2 (8.3%) | 8 (20.5%) | 0.199 | 0 (0.0%) | 4 (36.4%) | 0.009 | 0 (0.0%) | 1 (2.8%) | 0.560 | 3 (13.0%) |
| Non-Responders | 70 (93.3%) | 99 (85.2%) | | 22 (91.7%) | 31 (79.5%) | | 16 (100.0%) | 7 (63.6%) | | 12 (100.0%) | 35 (97.2%) | | 20 (87.0%) |
| Responders | | | | | | | | | | | | | |
| Pathological response | 46 (61.3%) | 78 (67.2%) | 0.403 | 18 (75.0%) | 37 (94.9%) | 0.021 | 13 (81.2%) | 10 (90.9%) | 0.488 | 1 (8.3%) | 14 (38.9%) | 0.048 | 14 (60.9%) |
| Non-pCR | 29 (38.7%) | 38 (32.8%) | | 6 (25.0%) | 2 (5.1%) | | 3 (18.8%) | 1 (9.1%) | | 11 (91.7%) | 22 (61.1%) | | 9 (39.1%) |
| pCR | | | | | | | | | | | | | |
| TILs | 31 (41.3%) | 65 (56.0%) | 0.047 | 14 (58.3%) | 31 (79.5%) | 0.071 | 7 (43.8%) | 9 (81.8%) | 0.048 | 1 (8.3%) | 10 (27.8%) | 0.165 | 9 (39.1%) |
| Low | | | | | | | | | | 11 (91.7%) | | | |
| High | 44 (58.7%) | 51 (44.0%) | | 10 (41.7%) | 8 (20.5%) | | 9 (56.2%) | 2 (18.2%) | | | 26 (72.2%) | | 14 (60.9%) |

HER: human epidermal growth factor receptor. ORR: objective response rate. CR: complete response. TILs: tumor- infiltrating lymphocytes.

Next, when DFS was compared between the younger and older patients, no significant difference was found overall or in any subtype (Fig. 2). Moreover, our analysis indicated that age or TILs was not a predictor of DFS in the univariate analysis ($P = 0.619$ and $P = 0.066$, respectively) (Table 4). Although upon

comparison of OS, a significant difference was observed between younger and older patients with TNBC ($P = 0.039$, log-rank) (Fig. 3), the results were contrasting and suggested better OS in older patients than in younger patients. Additionally, in univariate analysis with OS, no significant difference in age and TILs density was observed ($P = 0.346$ and $P = 0.216$, respectively) (Table 5).

Table 4
Univariate and multivariate analysis with respect to DFS in younger and elderly patients

| | Univarite analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| Parameters | Hazard ratio | 95% CI | *p* value | Hazard ratio | 95% CI | *p* value |
| Age at opetation (yr)<br>≤ 45 vs > 60 | 0.916 | 0.651-1.300 | 0.619 | | | |
| Tumor size (mm)<br>≤ 20 vs > 20 | 0.674 | 0.309–1.684 | 0.373 | | | |
| Skin infiltration<br>Negative vs Positive | 2.629 | 1.140–5.582 | 0.025 | 2.597 | 1.075–5.858 | 0.035 |
| Lymph node status<br>Negative vs Positive | 4.935 | 1.756–20.600 | 0.001 | 3.981 | 1.385–16.828 | 0.008 |
| Estrogen receptor<br>Negative vs Positive | 0.738 | 0.358–1.469 | 0.390 | | | |
| Progesterone receptor<br>Negative vs Positive | 0.733 | 0.322–1.524 | 0.418 | | | |
| Hormone receptor<br>Negative vs Positive | 0.675 | 0.327–1.344 | 0.265 | | | |
| HER2<br>Negative vs Positive | 0.237 | 0.070–0.602 | 0.001 | 0.479 | 0.130–1.423 | 0.193 |
| Intrinsic subtype<br>Not TNBC vs TNBC | 2.710 | 1.356–5.392 | 0.005 | 2.418 | 1.080–5.456 | 0.032 |
| Ki67<br>≤14 % vs > 14 % | 2.339 | 1.066–5.872 | 0.033 | 2.489 | 1.089–6.417 | 0.030 |
| Objective response rate<br>Non-Responders vs Responders | 0.309 | 0.145–0.734 | 0.010 | 0.381 | 0.159–0.984 | 0.047 |
| Pathological response<br>Non-pCR vs pCR | 0.195 | 0.058–0.499 | < 0.001 | 0.238 | 0.065–0.685 | 0.006 |
| TILs<br>Low vs High | 0.523 | 0.253–1.045 | 0.066 | 0.991 | 0.431–2.231 | 0.982 |

DFS: Disease-free survival. CI: confidence intervals. HER: human epidermal growth factor receptor. pCR: pathological complete response. TILs: tumor-infiltrating lymphocytes.

Table 5
Univariate and multivariate analysis with respect to OS in younger and elderly patients

| | Univarite analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| Parameters | Hazard ratio | 95% CI | *p* value | Hazard ratio | 95% CI | *p* value |
| Age at opetation (yr)<br>≤ 45 vs > 60 | 0.813 | 0.524–1.255 | 0.346 | | | |
| Tumor size (mm)<br>≤ 20 vs > 20 | 1.188 | 0.402–5.074 | 0.778 | | | |
| Skin infiltration<br>Negative vs Positive | 5.034 | 1.940-12.433 | 0.002 | 6.899 | 2.467–18.908 | < 0.001 |
| Lymph node status<br>Negative vs Positive | 4.239 | 1.227–26.631 | 0.019 | 2.999 | 0.815–19.389 | 0.106 |
| Estrogen receptor<br>Negative vs Positive | 0.474 | 0.169–1.167 | 0.107 | | | |
| Progesterone receptor<br>Negative vs Positive | 0.475 | 0.137–1.285 | 0.151 | | | |
| Hormone receptor<br>Negative vs Positive | 0.441 | 0.157–1.085 | 0.076 | | | |
| HER2<br>Negative vs Positive | 0.283 | 0.066–0.844 | 0.021 | 0.721 | 0.149–2.809 | 0.645 |
| Intrinsic subtype<br>Not TNBC vs TNBC | 3.966 | 1.640–10.130 | 0.002 | 3.703 | 1.323–11.575 | 0.012 |
| Ki67<br>≤14 % vs > 14 % | 2.730 | 1.004–9.518 | 0.049 | 2.271 | 0.768–8.314 | 0.144 |
| Objective response rate<br>Non-Responders vs Responders | 0.244 | 0.097–0.692 | 0.010 | 0.259 | 0.090–0.797 | 0.020 |
| Pathological response<br>Non-pCR vs pCR | 0.241 | 0.056–0.718 | 0.009 | 0.384 | 0.082–1.332 | 0.137 |
| TILs<br>Low vs High | 0.578 | 0.232–1.380 | 0.216 | | | |

OS: Overall survival. CI: confidence intervals. HER: human epidermal growth factor receptor. pCR: pathological complete response. TILs: tumor- infiltrating lymphocytes.

## Discussion

The characteristics of BC in the older patients have been often reported. For example, large tumor size [13, 28–30], frequent skin infiltration [29, 31], infrequent lymph node metastasis [28, 30], high rate of HR positivity [13, 28], and fewer HER2-positive tumors [28–30] have been reported in older patients. The clinicopathological characteristics of older BC patients in our study shows strong correlation to the decision of administering POC or not, though some features similar to those reported by others were identified.

While age-related differences in pCR rates have not been reported in several clinical trials, a pooled analysis observed high pCR rate in younger BC patients [14]. Moreover, reports suggest that the pCR rate decreased with age [10, 13]. Analysis of BC based on subtype in these studies suggested a strong correlation between HR + HER2- and TNBC, whereas no significant difference with age in HER2-positive BC, which differed in our study, and the exact reason remains to be identified. Further, there are various molecular subtypes of TNBC, and their age at onset and pCR rates have been observed to differ across studies [32–34]. We anticipate that our analysis may have been affected by differences in molecular subtypes of TNBC, or due to differences in the chemotherapy regimen. Furthermore, reports suggest that the expression of androgen receptor (AR) increases with age in BC patients [35–37], and that the AR-positive cases show low pCR rate than the AR-negative cases [38]. Additionally, newer biomarkers may also affect these outcomes.

Moreover, von Waldenfels et al. have reported that prognosis worsens with age in BC patients [13]. However, their study observed significant differences in prognoses between patients aged ≥ 65 years and those aged 40–50 or 51–65 years, but no significant difference between patients aged ≥ 65 years and those aged < 40 years. Furthermore, studies reporting higher pCR rate in younger patients did not observe a significant difference in prognosis in TNBC [14]. In

contrast, studies reported more than 10 years back suggest poor prognosis [39–41], and aggressive cellular properties in the younger BC patients [39, 42–44]. AR expression also affects prognosis and may contribute [38]. Additionally, with advent of newer biological treatments, the number of clinical trials claiming prognosis to differ with age have decreased.

Here, when we studied TILs at all ages, we observed correlation between TILs and clinicopathological factors, treatment effects, and prognosis similar to those reported previously. Moreover, our analysis suggests that younger BC patients had significantly higher TILs density than older BC patients. Additionally, age-related ORR and pCR rates differed in HER2-positive BC. Moreover, a pooled analysis for TNBC alone reported that the older patients had significantly lower TILs than in younger patients [45]. This result can be attributed to the decrease in host immunity due to aging, and to the inherent cellular characteristics of BC that vary with age.

However, this study has a limitation that the criteria for dividing patients into younger and older patients was not well-defined, and that the clinicopathological factors, other than TILs density, differed with age. However, the change in TME with age suggests that it may have influenced the therapeutic effect due to the characteristics of the host's immune system, and the differences in cancer itself depending on the age. Additionally, in lung cancer, it has been reported that the therapeutic effect of the immune checkpoint inhibitors (ICIs) decreases in the older patients [46–48]. Therefore, age may also serve as an important clinical factor in deciding the course of treatment of BC patients with ICIs.

## Conclusions

The analysis presented in this study suggests that younger BC patients show significantly higher TILs density than older patients, along with differences in prognoses between the groups. Moreover, these differences may allow selection of better treatment modalities for the highly immunogenic subtypes of BC.

## Abbreviations

AR: androgen receptor, BC: breast cancer, CIs: confidence intervals, cCR: clinical complete response, CNB: core needle biopsy, CT: computed tomography, cPD: clinical progressive disease, cPR: clinical partial response, cSD: clinical stable disease, DFS: disease-free survival, ER: estrogen receptor, HER2: human epidermal growth factor receptor 2, HR: hormone receptor, NSABP: National Surgical Adjuvant Breast and Bowel Project, ORR: objective response rate, OS: overall survival, pCR: pathological complete response, PgR: progesterone receptor, POC: pre-operative chemotherapy, TN: triple-negative, TILs: tumor-infiltrating lymphocytes, TIME: tumor-microenvironment, US: ultrasonography, VAB: vacuum-assisted biopsy.

## Declarations

### Ethics approval and consent to participate

A written informed consent to participate in the study was obtained from each subject in accordance with the declaration of Helsinki principles. Each patient or the patient’s family was fully informed of the investigational nature of this study and provided their written, informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (approve number #926).

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This study was supported in part by Grants-in-Aid for Scientific Research (KAKENHI**,** Nos. 17K10559, 19K18067 and 20K08938) from the Ministry of Education, Science, Sports, Culture and Technology of Japan.

### Authors’ contributions

KT participated in the design of the study and drafted the manuscript. SK participated in the design of the study and manuscript editing. YA, WG, SI, and TM helped with study data collection and manuscript preparation. MS, HT, KH and MO conceived the study, and participated in its design and coordination and helped to draft the manuscript. All authors have read and approved the final manuscript.

### Acknowledgements

We thank Yayoi Matsukiyo and Tomomi Okawa (Department of Breast and Endocrine Surgery, Osaka City University Graduate School of Medicine) for helpful advice regarding data management.

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## Figures

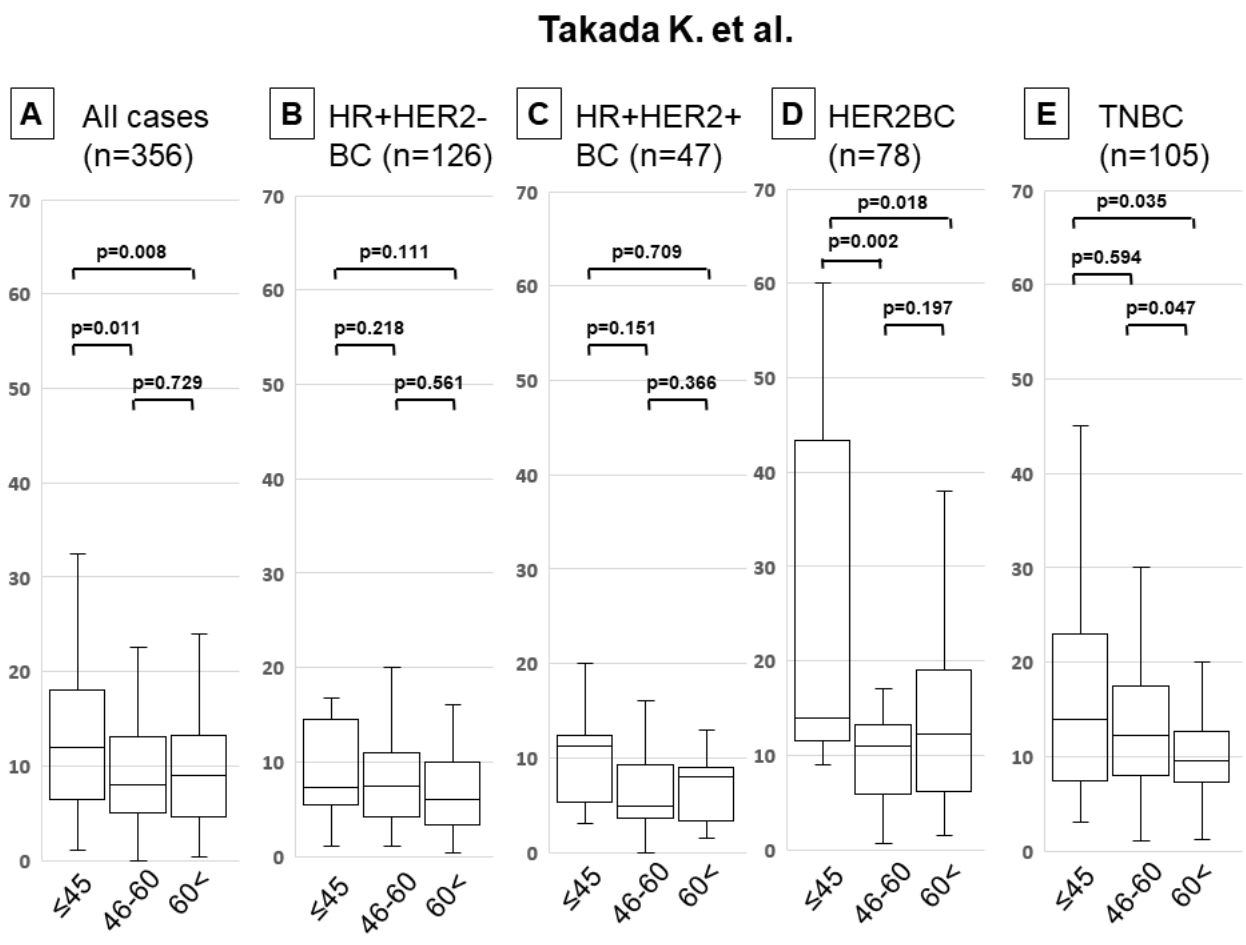


**Figure 1**

Correlation of TILs density with age of BC patients. Patients were grouped based on their BC subtype as: a) all cases, b) HR+HER2-, c) HR+HER2+, d) HER2-enriched, and e) TNBC. The TILs density in each age-group in each subtype has been indicated using box-plot distribution analysis. P-values in the figure indicate statistical significance for each comparison obtained using t-test

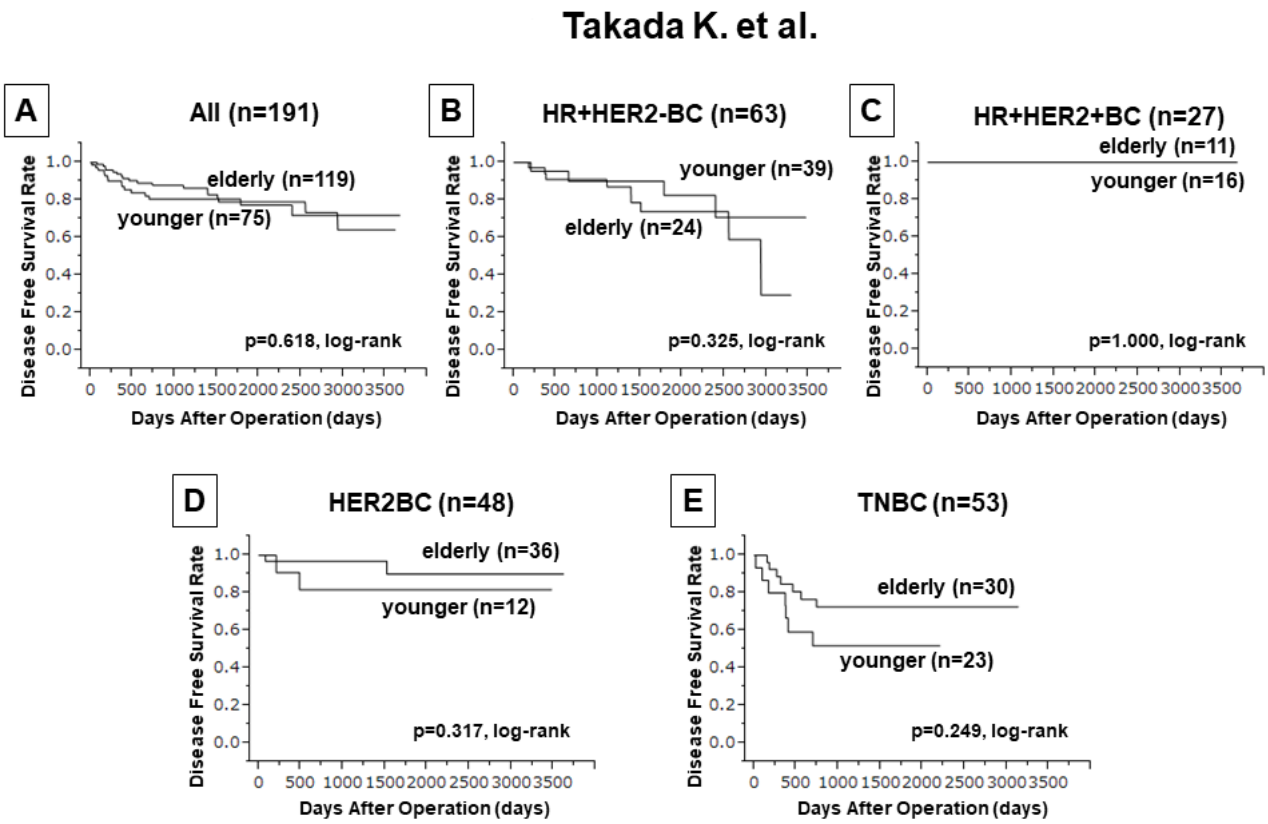


Figure 2

Comparison of disease-free survival (DFS) between younger and older patients with varied BC subtypes. Kaplan-Meier DFS analysis has been indicated for patients grouped based on their BC subtype as: a) all cases, b) HR+HER2-, c) HR+HER2+, d) HER2-enriched, and e) TNBC. P-values in the figure indicate statistical significance for each comparison obtained using log-rank test

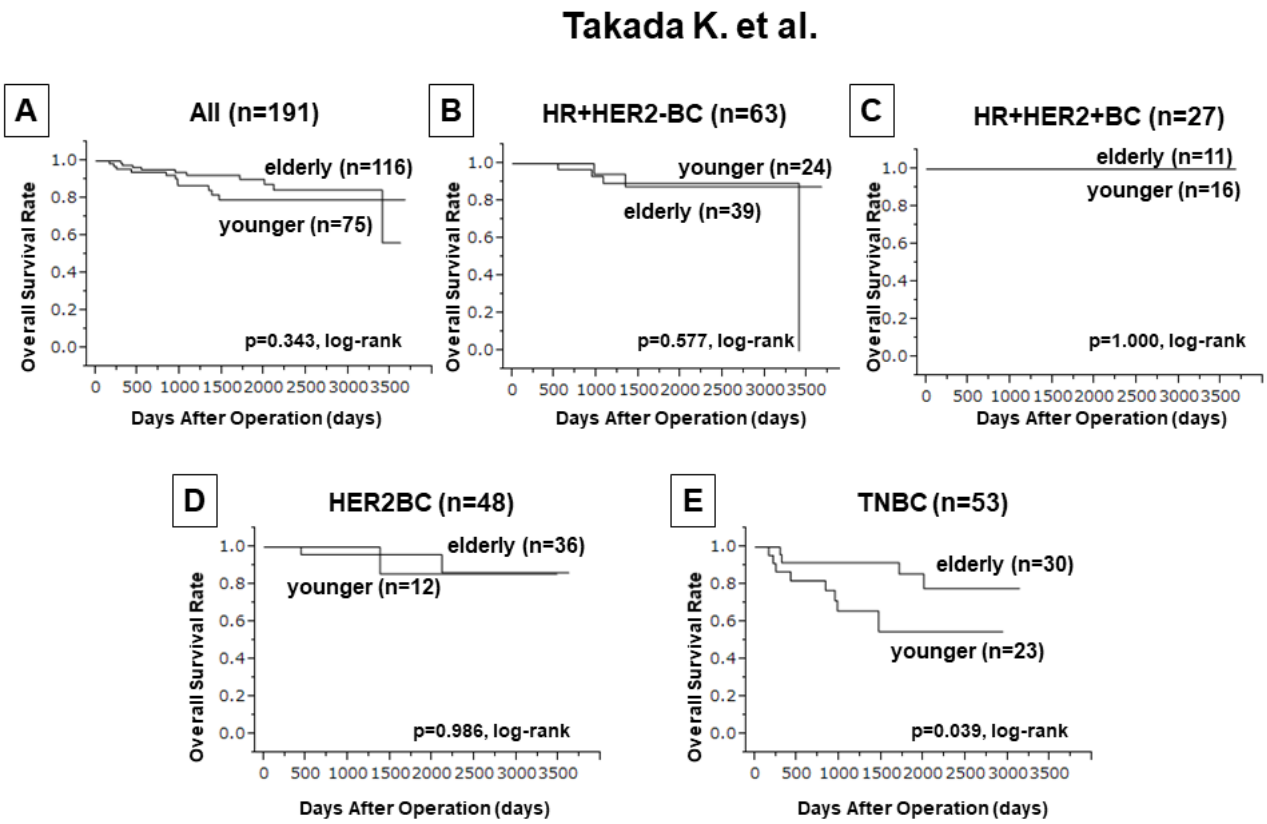


Figure 3

Comparison of overall survival (OS) between younger and older patients with varied BC subtypes. Kaplan-Meier OS analysis has been indicated for patients grouped based on their BC subtype as: a) all cases, b) HR+HER2-, c) HR+HER2+, d) HER2-enriched, and e) TNBC. P-values in the figure indicate statistical significance for each comparison obtained using log-rank test

## Supplementary Files

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