Differences in tumor-infiltrating lymphocyte density and prognostic factors for breast cancer by patient age

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Abstract

Background: Lymphocytes that surround cancer participate in tumor-related immune responses and are called tumor-infiltrating lymphocytes (TILs). Several recent reports suggest TILs index the tumor microenvironment 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 TIL density than younger BC patients. Here, we retrospectively analyzed the differences in TILs by age and the therapeutic effects of preoperative chemotherapy (POC) in BC patients who were aged either less than 45 years or more than 60 years.

Methods: We retrospectively examined the data of 356 breast cancer patients who underwent POC, including 75 patients aged ≤ 45 years and 116 patients aged > 60 years. Using pre-treatment needle biopsy specimens, TIL density was compared for each age group by Student’s t-test. After analyzing different factors that affect TIL density, prognostic factors were also examined.

Results: Older patients with triple-negative BC had significantly lower TIL density than younger patients, while in human epidermal growth factor receptor 2 (HER2)-enriched BC, TIL density was significantly higher in the younger age group than that in the older age group. In addition, younger patients with HER2-rich breast cancer showed significantly higher complete pathological response rates than older patients with HER2-rich BC. In addition, significant differences in overall survival were observed among these patients with triple-negative BC.

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

Keywords: Breast cancer, Tumor-infiltrating lymphocytes, Tumor-immune microenvironment, Age, Preoperative chemotherapy
complete response (pCR) rate, along with improved disease-free survival (DFS) and overall survival (OS) [5, 6]. Further, the TIL density in breast cancer differs depending on the subtype. For instance, hormone receptor-negative breast cancers (HR-BC), such as triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2-enriched breast cancer (HER2-enriched BC), show high TIL density [7–9]. However, there are fewer reports on factors other than BC subtypes that affect the TIL density.

Currently, the standard treatment is based on the results of various clinical trials. For instance, some clinical trials suggest the prognosis and treatment effect differ depending on the age of the patients [10–12], and several pooled studies have reported differences in the treatment effect due to age [5, 13, 14]. However, until now, only few studies have assessed the relationship between age and TIL density. While increased age may reduce host immunity [15], we can also hypothesize that it affects TIL density. Moreover, clinical trials on the association of TILs and therapeutic effects have not correlated age and TILs [12, 16–19], and most of them have stratified patients into two groups based on TILs or age and performed only t-test analyses to compare the groups.

First, we decided to compare TIL density for each age group, and if the TIL density decreased with age, we hypothesized that omitting the middle-aged group would polarize the younger and the older age groups. We also tested the hypothesis that the therapeutic effect and prognoses of patients may differ with TIL density. Thus, here, we retrospectively analyzed the differences in TIL density by age and analyzed the therapeutic effects in patients with BC ≤ 45 years or > 60 years of age who were treated with preoperative chemotherapy (POC).

### Methods

#### Patient background

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 compared the TIL density in patients aged ≤ 45 years (younger group, \( n = 75 \)) versus those aged > 60 years (older group, \( n = 116 \)). The patients were pathologically diagnosed with BC using 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),

| Parameters | All patients (\( n = 356 \)) (%) | Younger (\( n = 75 \)) (%) | Elderly (\( n = 116 \)) (%) |
|------------|----------------------------------|-----------------------------|-----------------------------|
| Age (years) | 55 (24–78)                       | 41 (24–45)                  | 67 (61–78)                  |
| Tumor size (mm) | 28.7 (9.2–119.8)               | 29.5 (9.2–82.6)             | 27.3 (9.2–89.8)             |
| Skin infiltration |                          |                            |                             |
| Negative/positive | 58 (16.3%)                | 68 (90.7%)/7 (9.3%)        | 90 (77.6%)/26 (22.4%)       |
| Lymph node metastasis |                        |                            |                             |
| 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 |                         |                            |                             |
| Negative/positive | 187 (52.5%)/169 (47.5%)      | 37 (49.3%)/38 (50.7%)      | 67 (57.8%)/49 (42.2%)       |
| Progesterone receptor |                        |                            |                             |
| Negative/positive | 242 (68.0%)/114 (32.0%)     | 42 (56.0%)/33 (44.0%)      | 89 (76.7%)/27 (23.3%)       |
| HER2 |                                |                            |                             |
| Negative/positive | 231 (64.9%)/125 (35.1%)    | 47 (62.7%)/28 (37.3%)      | 69 (59.5%)/47 (40.5%)       |
| Ki67 |                                |                            |                             |
| \( \leq 14 \% \)/ \( > 14 \% \) | 117 (32.9%)/239 (67.1%) | 22 (29.3%)/53 (70.7%) | 40 (34.5%)/76 (65.5%)      |
| Intrinsic subtype |                        |                            |                             |
| 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 |                        |                            |                             |
| Non-responders/responders | 40 (11.2%)/316 (88.8%) | 5 (6.7%)/70 (93.3%) | 17 (14.7%)/99 (85.3%) |
| Pathological response |                        |                            |                             |
| Non-pCR/pCR | 238 (66.9%)/118 (33.1%) | 46 (61.3%)/29 (38.7%) | 78 (67.2%)/38 (32.8%) |
| TILs |                                |                            |                             |
| 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
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 was comprised of four courses of FEC100 (500 mg/m² fluorouracil, 100 mg/m² epirubicin, and 500 mg/m² cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m² 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, patients with clinical partial response (cPR) and complete response (cCR) were defined as “responders” in the objective response rate (ORR), whereas patients with 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 Bowl Project 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 TIL density

TIL 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 classes (3: > 50%; 2: 10–50%; 1: ≤ 10%; or 0: absent) (Supplementary Fig. S1). Further, we defined 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 TIL density was set to 10%.

Statistical analysis

All statistical analyses were performed using the JMP software package (SAS, Tokyo, Japan). The distribution of TIL density by age was evaluated using Student’s t-test. Pearson's chi-square test was used to evaluate the relationship between each categorical variable. 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 statistically significant.

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)                 | ≤ 45                                   | 31 (32.3%)  | 44 (46.3%)  | 0.047   |
|                            | > 60                                   | 65 (67.7%)  | 51 (53.7%)  |         |
| Tumor size (mm)             | ≤ 200                                  | 20 (20.8%)  | 14 (14.7%)  | 0.271   |
|                            | > 200                                  | 76 (79.2%)  | 81 (85.3%)  |         |
| Skin infiltration           | Negative                               | 71 (74.0%)  | 87 (91.6%)  | 0.001   |
|                            | Positive                               | 25 (26.0%)  | 8 (8.4%)    |         |
| Lymph node status           | Negative                               | 33 (34.4%)  | 39 (41.1%)  | 0.341   |
|                            | Positive                               | 63 (65.6%)  | 56 (58.9%)  |         |
| Estrogen receptor           | Negative                               | 37 (38.5%)  | 67 (70.5%)  | < 0.001 |
|                            | Positive                               | 59 (61.5%)  | 28 (29.5%)  |         |
| Progesterone receptor       | Negative                               | 55 (57.3%)  | 76 (80.0%)  | 0.001   |
|                            | Positive                               | 41 (42.7%)  | 19 (20.0%)  |         |
| Hormone receptor            | Negative                               | 35 (36.5%)  | 66 (69.5%)  | < 0.001 |
|                            | Positive                               | 61 (63.5%)  | 29 (30.5%)  |         |
| HER2                       | Negative                               | 69 (71.9%)  | 47 (49.5%)  | 0.002   |
|                            | Positive                               | 27 (28.1%)  | 48 (50.5%)  |         |
| Ki67                       | ≤ 14%                                  | 37 (38.5%)  | 25 (26.3%)  | 0.071   |
|                            | > 14%                                  | 59 (61.5%)  | 70 (73.7%)  |         |
| ORR                        | Non-responders                         | 18 (18.8%)  | 4 (4.2%)    | 0.002   |
|                            | Responders                             | 78 (81.2%)  | 91 (95.8%)  |         |
| Pathological response       | Non-pCR                                | 79 (82.3%)  | 45 (47.4%)  | < 0.001 |
|                            | pCR                                    | 17 (17.7%)  | 50 (52.6%)  |         |

TILs: tumor-infiltrating lymphocytes, HER: human epidermal growth factor receptor, ORR: objective response rate, CR: complete response.
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 on at a median age of 55 years (range, 24–78 years) and the median tumor diameter was 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 TIL density.

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 than 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).

Correlation of TIL density with clinicopathological features and prognosis of patients

First, the 356 patients were divided into high and low TIL density groups, and their correlation with clinicopathological factors was examined (Supplementary Table S1). The following characteristics were significantly different between the low TILs and 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 TIL density group showed significantly better DFS than the low TIL density group in HER2-enriched (P = 0.012) and TNBC (P = 0.002) categories (Supplementary Fig. S2). Therefore, DFS was
| Parameters | All intrinsic subtype (n = 191) | HR+HER2-BC (n = 61) | HR+HER2-BC (n = 27) | HER2BC (n = 48) | TNBC (n = 53) |
|------------|-------------------------------|---------------------|---------------------|----------------|--------------|
|            | 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) | Elderly (n = 30) | p value |
| Tumor size (mm) | | | | | | | | | | | | | | | |
| ≤ 20.0 | 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%) | 8 (26.7%) | 0.225 |
| > 20.0 | 65 (86.7%) | 92 (79.3%) | | 65 (86.7%) | 92 (79.3%) | | 65 (86.7%) | 92 (79.3%) | | 65 (86.7%) | 92 (79.3%) | | 65 (86.7%) | 92 (79.3%) | |
| Skin infiltration | | | | | | | | | | | | | | | |
| Negative | 68 (90.7%) | 90 (77.6%) | 0.020 | 20 (83.3%) | 29 (74.4%) | 0.045 | 14 (87.5%) | 6 (54.5%) | 0.055 | 12 (100.0%) | 29 (80.6%) | 0.098 | 22 (95.7%) | 26 (86.7%) | 0.627 |
| Positive | 7 (9.3%) | 26 (22.4%) | 4 (16.7%) | 10 (56.6%) | 10 (56.6%) | 2 (12.5%) | 5 (45.5%) | 0 (0.0%) | 7 (19.4%) | 1 (4.3%) | 1 (4.3%) | 4 (13.3%) | |
| Lymph node status | | | | | | | | | | | | | | | |
| Negative | 28 (37.3%) | 44 (37.9%) | 0.034 | 8 (33.3%) | 12 (30.8%) | 0.832 | 9 (56.2%) | 2 (18.2%) | 0.048 | 4 (33.3%) | 7 (47.2%) | 0.401 | 7 (30.4%) | 13 (43.3%) | 0.337 |
| Positive | 47 (62.7%) | 72 (62.1%) | 16 (66.7%) | 27 (69.2%) | 16 (66.7%) | 7 (43.8%) | 9 (61.8%) | 8 (66.7%) | 19 (52.8%) | 16 (69.6%) | 17 (56.7%) | |
| Estrogen receptor | | | | | | | | | | | | | | | |
| Negative | 37 (49.3%) | 67 (56.9%) | 0.254 | 2 (83%) | 0 (0.0%) | 0.067 | 0 (0.0%) | 1 (9.1%) | 0.219 | – | – | – | – | – | – |
| Positive | 38 (50.7%) | 49 (43.1%) | 2 (83%) | 39 (100.0%) | 16 (100.0%) | 10 (90.9%) | | – | – | – | – | |
| Progesterone receptor | | | | | | | | | | | | | | | |
| Negative | 42 (56.0%) | 89 (76.7%) | 0.003 | 5 (20.8%) | 16 (41.0%) | 0.099 | 2 (12.5%) | 7 (63.6%) | 0.006 | – | – | – | – | – | – |
| Positive | 33 (44.0%) | 27 (23.3%) | 19 (79.2%) | 23 (59.0%) | 14 (87.5%) | 4 (36.4%) | | – | – | – | – | |
| Hormone receptor- | | | | | | | | | | | | | | | |
| Negative | 35 (46.7%) | 66 (56.9%) | 0.167 | – | – | – | – | – | – | – | – | – | – | – | – |
| Positive | 40 (53.3%) | 50 (43.1%) | – | – | – | – | – | – | – | – | – | – | – | – | – |
| HER2 | | | | | | | | | | | | | | | |
| Negative | 47 (62.7%) | 69 (59.5%) | 0.060 | – | – | – | – | – | – | – | – | – | – | – | – |
| Positive | 28 (37.3%) | 47 (40.5%) | – | – | – | – | – | – | – | – | – | – | – | – | – |
| K67 | | | | | | | | | | | | | | | |
| ≤ 14% | 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%) | 5 (16.7%) | 0.396 |
| > 14% | 53 (70.7%) | 76 (65.5%) | 12 (50.0%) | 18 (46.2%) | 9 (56.2%) | 9 (81.8%) | 11 (91.7%) | 24 (66.7%) | 0.21 | 11 (91.7%) | 25 (83.3%) | 0.21 | |
| ORR | | | | | | | | | | | | | | | |
| Non-responders | 5 (67%) | 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 (28%) | 0.560 | 3 (13%) | 4 (13.3%) | 0.975 |
| 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%) | 0.20 | 11 (91.7%) | 26 (86.7%) | 0.758 |
| Pathological response | | | | | | | | | | | | | | | |
| Non-pCR | 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%) | 17 (56.7%) | 0.758 |
| 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%) | 13 (43.3%) |
Table 3 (continued)

| Parameters | All intrinsic subtype (n = 191) | HR+HER2-BC (n = 61) | HR+HER2+BC (n = 27) | HER2BC (n = 48) | TNBC (n = 53) |
|------------|---------------------------------|---------------------|---------------------|-----------------|---------------|
|            | 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) | Elderly (n = 30) | p value |
| TILs       |                   |                     |         |                |                     |         |             |                     |         |             |                     |         |             |                     |         |
| Low        | 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%)    | 15 (50.0%)         | 0.431   |
| High       | 44 (58.7%)        | 51 (44.0%)          |         | 10 (41.7%)     | 8 (20.5%)          |         | 9 (56.2%)   | 2 (18.2%)         |         | 11 (91.7%)  | 26 (72.2%)          |         | 14 (60.9%)   | 15 (50.0%)         |         |

HER: human epidermal growth factor receptor; ORR: objective response rate; CR: complete response; TILs: tumor-infiltrating lymphocytes
better in the high TIL density group despite no significant difference in HR+ BC ($P = 0.011$). However, the high TIL density group had better OS, although not statistically significant, than the low TIL density group in TNBC category ($P = 0.057$, log-rank), but there was no significant difference between the difference of TIL density (Supplementary Fig. S3). Further, in the univariate analysis for DFS, the high TIL density group was associated with significantly better DFS ($P = 0.010$, HR $= 0.512$) than the low TIL density group (Supplementary Table S2). However, in the multivariate analysis, TIL density was not an significant independent factor for DFS ($P = 0.227$, HR $= 0.699$) and 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 $\leq$ 45 years, 46–60 years, and $\geq$ 61 years, and the distribution of TIL density was analyzed using a $t$-test (Fig. 1). Our analysis did not indicate a significant difference in HR+ BC for any of the age groups. However, for HER2-enriched BC, patients aged $\leq$ 45 years had significantly higher TIL density than patients in the other age groups (vs. 46–60 years: $P = 0.002$, and vs. $\geq$ 61 years: $P = 0.018$). Furthermore, in the TNBC category, the patients aged $\geq$ 61 years had significantly higher TIL density than patients in other age groups (vs. $\leq$ 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 TIL 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 TIL density group were similar to those for all patients: $> 60$ years ($P = 0.047$), skin infiltration ($P < 0.001$, HR $= 0.247$), and pCR ($P < 0.001$, HR $= 0.315$) influenced the DFS. Furthermore, 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 the older patients ($P = 0.009$).

![Fig. 2](image-url) 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.
However, no significant difference was observed in the effect of POC on TNBC.

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 TIL density was observed ($P = 0.346$ and $P = 0.216$, respectively) (Table 5).

**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 show a 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 a 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 was observed 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 the age at onset and pCR rates differ across studies [32–34]. We anticipate that our analysis may have been affected by differences in molecular subtypes.

| **Table 4** | Univariate and multivariate analysis with respect to DFS in younger and elderly patients |
|-------------|---------------------------------------------------------------------------------------------------|
| **Parameters** | **Univariate analysis** | **Multivariate analysis** |
| | **Hazard ratio** | **95% CI** | **p value** | **Hazard ratio** | **95% CI** | **p value** |
| Age at operation (years) | | | | | | |
| ≤ 45 vs > 60 | 0.916 | 0.651–1.300 | 0.619 | | | |
| Tumor size (mm) | | | | | | |
| ≤ 20 vs > 20 | 0.674 | 0.309–1.684 | 0.373 | | | |
| Skin infiltration | | | | | | |
| Negative vs positive | 2.629 | 1.140–5.582 | 0.025 | 2.597 | 1.075–5.385 | 0.035 |
| Lymph node status | | | | | | |
| Negative vs positive | 4.935 | 1.756–20.600 | 0.001 | 3.981 | 1.385–16.828 | 0.008 |
| Estrogen receptor | | | | | | |
| Negative vs positive | 0.738 | 0.358–1.469 | 0.390 | | | |
| Progesterone receptor | | | | | | |
| Negative vs positive | 0.733 | 0.322–1.524 | 0.418 | | | |
| Hormone receptor | | | | | | |
| Negative vs positive | 0.675 | 0.327–1.344 | 0.265 | | | |
| HER2 | | | | | | |
| Negative vs positive | 0.237 | 0.070–0.602 | 0.001 | 0.479 | 0.130–1.423 | 0.193 |
| Intrinsic subtype | | | | | | |
| Not TNBC vs TNBC | 2.710 | 1.356–5.392 | 0.005 | 2.418 | 1.080–5.456 | 0.032 |
| Ki67 | | | | | | |
| ≤ 14% vs > 14% | 2.339 | 1.066–5.872 | 0.033 | 2.489 | 1.089–6.417 | 0.030 |
| Objective response rate | | | | | | |
| Non-responders vs responders | 0.309 | 0.145–0.734 | 0.010 | 0.381 | 0.159–0.984 | 0.047 |
| Pathological response | | | | | | |
| Non-pCR vs pCR | 0.195 | 0.058–0.499 | < 0.001 | 0.238 | 0.065–0.685 | 0.006 |
| TILs | | | | | | |
| 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
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 rates 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 $\geq$ 65 years and those aged 40–50 or 51–65 years, but no significant difference between patients aged $\geq$ 65 years and those aged < 40 years. Furthermore, studies reporting a higher pCR rate in younger patients did not observe a significant difference in prognosis between 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 the advent of newer biological treatments, the number of clinical trials claiming prognosis to differ with age has decreased.

Here, when we studied TILs at all ages, we observed a 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 TIL 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 the 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 were not well-defined and that the clinicopathological factors other than TIL density differed with age. In addition, genetic predisposition, medications such as steroids, and lifestyle may also affect the immune microenvironment, but these factors could not be examined because this was a retrospective study. Furthermore, in this study, TILs were collectively examined, but they have various subclasses. As a typical example, CD8-positive cytotoxic

![Fig. 3](image.png)

**Fig. 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.
T cells are reported to have a better prognosis as they are highly expressed [46–48], on the other hand, regulatory T cells, which were famous for being positive for FOXP3, were reported to be involved in poor prognosis [46]. PD-1 / PD-L1, which is also a target molecule in clinical treatment, might also affect TILs and prognosis [47, 49]. In addition, a study has reported that the host's immune environment itself affects the pCR of preoperative chemotherapy [50]. In the future, it will be necessary to study immunohistochemical staining in our research as well. However, it was important to know the difference depending on the age in the evaluation of TILs by hematoxylin and eosin staining. And our study is considered to be the key study to show the reason why the therapeutic effect by age was different. The change with age in TME 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 [51–53]. 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 TIL 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; CI: Confidence intervals; DFS: Disease-free survival; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; HR: Hormone receptor; ORR: Objective response rate; OS: Overall survival; pCR: Pathological complete response; PgR:

| Parameters | Univariate analysis | Multivariate analysis |
|------------|---------------------|-----------------------|
|            | Hazard ratio 95% CI | p value | Hazard ratio 95% CI | p value |
| Age at operation (years) | | | | |
| ≤ 45 vs > 60 | 0.813 0.524–1.255 | 0.346 | | |
| Tumor size (mm) | | | | |
| ≤ 20 vs > 20 | 1.188 0.402–5.074 | 0.778 | | |
| Skin infiltration | | | | |
| Negative vs positive | 5.034 1.940–12.433 | 0.002 | 6.899 2.467–18.908 | < 0.001 |
| Lymph node status | | | | |
| Negative vs positive | 4.239 1.227–26.631 | 0.019 | 2.999 0.815–19.389 | 0.106 |
| Estrogen receptor | | | | |
| Negative vs positive | 0.474 0.169–1.167 | 0.107 | | |
| Progesterone receptor | | | | |
| Negative vs positive | 0.475 0.137–1.285 | 0.151 | | |
| Hormone receptor | | | | |
| Negative vs positive | 0.441 0.157–1.085 | 0.076 | | |
| HER2 | | | | |
| Negative vs positive | 0.283 0.066–0.844 | 0.021 | 0.721 0.149–2.809 | 0.645 |
| Intrinsic subtype | | | | |
| Not TNBC vs TNBC | 3.966 1.640–10.130 | 0.002 | 3.703 1.323–11.575 | 0.012 |
| Ki67 | | | | |
| ≤ 14% vs >14% | 2.730 1.004–9.518 | 0.049 | 2.271 0.768–8.314 | 0.144 |
| Objective response rate | | | | |
| Non-responders vs responders | 0.244 0.097–0.692 | 0.010 | 0.259 0.090–0.797 | 0.020 |
| Pathological response | | | | |
| Non-pCR vs pCR | 0.241 0.056–0.718 | 0.009 | 0.384 0.082–1.332 | 0.137 |
| TILs | | | | |
| 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.
Progestosterone receptor; POC: Pre-operative chemotherapy; TN: Triple-negative; TILs: Tumor-infiltrating lymphocytes; TIME: Tumor microenvironment; US: Ultrasound; VAB: Vacuum-assisted biopsy.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12957-022-02513-5.

Additional file 1: Supplementary Figure S1. Histopathological analysis of TIL density. The TIL density was calculated as average of infiltrating lymphocytes in the tumor stroma from five random fields, and graded as: a) 3 (>50%), b) 2 (10–50%), c) 1 (10%), and d) 0 (absent).

Additional file 2: Supplementary Figure S2. Comparison of disease-free survival (DFS) between high and low TIL density 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.

Additional file 3: Supplementary Figure S3. Comparison of overall survival (OS) between high and low TIL density 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.

Additional file 4: Supplementary Table S1. Difference in clinicopathological features due to TILs in all patients.

Additional file 5: Supplementary Table S2. Univariate and multivariate analysis with respect to DFS in all patients.

Additional file 6: Supplementary Table S3. Univariate and multivariate analysis with respect to OS in all patients.

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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, 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. TM helped with study data collection and manuscript preparation. MS, HT, KH, and MO conceived the study and drafted the manuscript. SK and MO conceived the study and participated in its design and manuscript editing. All authors have read and approved the final manuscript.

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

Declarations
Ethics approval and consent to participate
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.

Competing interests
The authors declare that they have no competing interests.

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References
1. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71. https://doi.org/10.1093/annonc/mdu450.
2. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol. 2011;8(3):151–60. https://doi.org/10.1038/reclonc.2010.225.
3. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune context in human tumors: impact on clinical outcome. Nat Rev Cancer. 2012;12(1):29–30. https://doi.org/10.1038/nrc3245.
4. Couzin-Frankel J. Breakthrough of the year 2013. Cancer Immunother Sci. 2013;3(26(165)):1432–3. https://doi.org/10.1111/sce.12665.1432.
5. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014;32(27):2599–606. https://doi.org/10.1002/jco.2013.35.0491.
6. Denkert C, von Minckwitz G, Brasc JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33(9):983–91. https://doi.org/10.1200/JCO.2014.58.1967.
7. Ohtani H, Mori-Shiraishi K, Nakajima M, Ueki H. Defining lymphocyte-predominant breast cancer by the proportion of lymphocyte-rich stroma and its significance in routine histopathological diagnosis. Pathol Int. 2015;65(12):644–51. https://doi.org/10.1111/pin.12355.
8. Stanton SE, Adams S, D’Ulivieri CL. Variation in the incidence of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review. JAMA Oncol. 2016;2(10):1354–60. https://doi.org/10.1001/jamaoncol.2016.1061.
9. Asano Y, Kashiwagi S, Goto W, Takada K, Takahashi K, Hatanou T, et al. Prediction of treatment response to neoadjuvant chemotherapy in breast cancer by subtype using tumor-infiltrating lymphocytes. Anticancer Res. 2018;38(4):2311–21. https://doi.org/10.21873/anticancer.12476.
10. Hruber J, von Minckwitz G, Denkert C, Tisch H, Weiss E, Zahn DM, et al. Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study. Breast Cancer Res Treat. 2010;124(1):133–40. https://doi.org/10.1007/s10549-010-1103-9.
11. Loibl S, Untch M, Burchardt N, Hüber J, Sinn BV, Blommer JU, et al. A randomised phase II study investigating durvalumab in addition to an anthracycline-taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. Ann Oncol. 2019;30(8):1279–88. https://doi.org/10.1093/annonc/mdz158.
12. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50. https://doi.org/10.1093/annonc/mdu112.
13. von Waldenfels G, Loibl S, Furlanetto J, MachHeidt A, Lederer B, Denkert C, et al. Outcome after neoadjuvant chemotherapy in elderly breast cancer patients - a pooled analysis of individual patient data from eight prospectively randomized controlled trials. Oncotarget. 2018;9(20):15168–79. https://doi.org/10.18632/oncotarget.24586.
14. Loibl S, Jackisch C, Lederer B, Untch M, Paepke S, Kummer S, et al. Outcome after neoadjuvant chemotherapy in young breast cancer patients: a pooled analysis of individual patient data from eight prospectively randomized controlled trials. Breast Cancer Res Treat. 2015;152(2):377–87. https://doi.org/10.1007/s10549-015-3479-z.

15. Yaqoob P. Ageing alters the impact of nutrition on immune function. Proc Nutr Soc. 2017;76(3):347–51. https://doi.org/10.1017/S0032660317000670.

16. Salgado R, Denkert C, Campbell C, Savas P, Nuciforo P, Aura C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with neoadjuvant lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. JAMA Oncol. 2015;1(14):448–54. https://doi.org/10.1001/jamaoncol.2015.0830.

17. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eeuw F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31(7):886–97. https://doi.org/10.1001/jco.2011.41.0902.

18. Luem SJ, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, et al. Tumor-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol. 2017;18(1):51–61. https://doi.org/10.1016/S1470-2045(16)30313-9.

19. Perez EA, Ballman KV, Tenner KS, Thompson EA, Badve SS, Bailey H, et al. Very young women (<35 years) with operable breast cancer: features of one. 2009;4(11):e7695. https://doi.org/10.1371/journal.pone.0007695.

20. Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst. 2005;97(3):188–94. https://doi.org/10.1093/jnci/dji021.

21. Mieog S, van der Hage JA, van der Velde CJ. Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev. 2007;2:CD005002. https://doi.org/10.1002/14651858.CD005002.pub2.

22. Kawajiri H, Takashima T, Onoda N, Kashiwagi S, Noda S, Ishikawa T, et al. Efficacy and feasibility of neoadjuvant chemotherapy with FEC 100 followed by weekly paclitaxel for operable breast cancer. Oncol Lett. 2012;4(4):612–6. https://doi.org/10.3892/ol.2012.801.

23. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47. https://doi.org/10.1016/j.ejca.2008.10.026.

24. Kashiwagi S, Onoda N, Asano Y, Kurata K, Morisaki T, Noda S, et al. Partial mastectomy using manual blunt dissection (MBD) in early breast cancer. BMC Surg. 2015;15:117. https://doi.org/10.1186/s12893-015-0102-5.

25. Wolmark N, Wang J, Mamounas E, Vujanic G, Bailey H, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst Monogr. 2001;30:96–102. https://doi.org/10.1093/oxfordjournals.jncimonographs.a03469.

26. Kashiwagi S, Asano Y, Goto W, Takada K, Takahashi K, Noda S, et al. Use of tumor-infiltrating lymphocytes (TILs) to predict the treatment response to endocrine chemotherapy in breast cancer. PLoS One. 2017;12(2):e0170634. https://doi.org/10.1371/journal.pone.0170634.

27. Asano Y, Kashiwagi S, Goto W, Takada K, Hatano T, et al. Prediction of survival after neoadjuvant chemotherapy for breast cancer by evaluation of tumor-infiltrating lymphocytes and residual cancer burden. BMC Cancer. 2017;17(1):888. https://doi.org/10.1186/s12885-017-3927-8.

28. Gennari R, Cunilongo G, Rotmensz N, Robertson C, Colleoni M, Zurrida S, et al. Breast carcinoma in elderly women: features of disease presentation, choice of local and systemic treatments compared with younger, postmenopausal patients. Cancer. 2004;101(6):1302–10. https://doi.org/10.1002/cncr.20353.

29. Wildiers H, Kunkler I, Biganzoli L, Fracheboud J, Viale G, Bernard-Marty C, et al. Management of breast cancer in elderly individuals: recommendations of the International Society of Geriatric Oncology. Lancet Oncol. 2015;16(12):1041–53. https://doi.org/10.1016/S1470-2045(15)30378-9.

30. Crivellari D, Aapro M, Leonard R, von Minckwitz G, Brain E, Goldhirsch A, et al. Breast cancer in the elderly. J Clin Oncol. 2007;25(14):1882–90. https://doi.org/10.1200/JCO.2006.10.2079.
cancer patients with residual tumor burden after neoadjuvant chemotherapy. World J Surg Oncol. 2021;19(1):264. https://doi.org/10.1186/s12957-021-02361-9.

50. Lingfeng T, Xiujie S, Gang T. Exploring the influencing factors of the pathologic complete response in estrogen receptor-positive, HER2-negative breast cancer after neoadjuvant chemotherapy: a retrospective study. World J Surg Oncol. 2022;20(1):27. https://doi.org/10.1186/s12957-022-02492-7.

51. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540–50. https://doi.org/10.1016/S0140-6736(15)01281-7.

52. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373(2):123–35. https://doi.org/10.1056/NEJMoa1504627.

53. Nishijima TF, Muss HB, Shachar SS, Moschos SJ. Comparison of efficacy of immune checkpoint inhibitors (ICIs) between younger and older patients: a systematic review and meta-analysis. Cancer Treat Rev. 2016;45:30–7. https://doi.org/10.1016/j.ctrv.2016.02.006.

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