Clinical verification of the relationship between smoking and the immune microenvironment of breast cancer

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Abstract

Background: The immune tumor microenvironment (iTME) is thought to affect the response to chemotherapy, and tumor-infiltrating lymphocytes (TILs) are often used as an indicator to evaluate the iTME. Smoking is involved in carcinogenesis, the relationship between smoking and the iTME of lung cancer has been reported. We hypothesized that smoking would affect the iTME of breast cancer and aimed to examine this relationship based on the amount of pre-diagnosis smoking and the subsequent effects on treatment response and prognosis.

Methods: This retrospective study evaluated data from 149 patients who underwent preoperative chemotherapy for triple-negative or HER2-enriched breast cancer. TILs were assessed in biopsy specimens at diagnosis. The data of all patients were used to calculate each patient's smoking amount based on pack-years.

Results: Relative to the low smoking group, the high smoking group had a significant greater TILs density (p = 0.043) and a significantly better pathological complete response (pCR) rate (p = 0.042). However, there was no significant difference according to smoking amount in disease-free survival (p = 0.114) or overall survival (p = 0.347).

Conclusions: Smoking may influence the iTME, with an activated iTME being associated with pCR rate. Therefore, controlled activation of the microenvironment in this setting may help improve patients' prognosis.

Keywords: Breast cancer, Smoking, Tumor-infiltrating lymphocytes, Tumor microenvironment, Immune response, Brinkman index

Background

The immune tumor microenvironment (iTME) is thought to affect the response to chemotherapy, and tumor-infiltrating lymphocytes (TILs) are often used as an indicator to evaluate the iTME [1–3]. Many studies have revealed that a high TILs density in breast cancer is associated with good therapeutic effects, such as pathological complete response (pCR), prolonged disease-free survival (DFS), and prolonged overall survival (OS) [4, 5]. It became commonly known that affect TILs density in breast cancer is the cancer subtype, with many reports indicating that a high TILs density is associated with high-risk subtypes, such as triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2-enriched breast cancer (HER2BC) [6, 7]. In recent years, it has also been reported that special genes affect TILs, and it is also important to examine the relationship between genes and the iTME [8, 9].

Smoking is involved in the genesis of many carcinomas, including breast cancer [8], with the carcinogenic substances in tobacco smoke causing chronic inflammatory conditions in the microvessels [10, 11]. Recent studies have also indicated that the iTME is deeply involved in carcinogenesis and that chronic inflammation promotes this process [12, 13]. The relationship between smoking and the iTME of lung cancer has been reported [5, 14], although no reports have examined the relationship indicating that a high TILs density is associated with high-risk subtypes, such as triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2-enriched breast cancer (HER2BC) [6, 7]. In recent years, it has also been reported that special genes affect TILs, and it is also important to examine the relationship between genes and the iTME [8, 9].

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between smoking and the iTME of breast cancer. Therefore, we hypothesized that smoking would affect the iTME of breast cancer and aimed to examine this relationship based on the amount of pre-diagnosis smoking and the subsequent effects on treatment response and prognosis.

Methods

Patient background
This retrospective study evaluated data from 149 patients who underwent preoperative chemotherapy (POC) for resectable TNBC or HER2BC between February 2007 and December 2017 at the Osaka City University Hospital. All patients were questioned regarding their smoking history at the initial visit (cigarettes smoked per day and years of smoking), and the data were used to calculate each patient’s smoking amount based on pack-years (Table 1). The breast cancers were diagnosed pathologically and classified according to subtype based on the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and K-i67. Cases were defined as either HER2BC (ER−, PgR−, and HER2+) or TNBC (ER−, PgR−, and HER2−).

All patients received a standardized outpatient POC regimen that consisted of four courses of FEC100 (5-fluorouracil: 500 mg/m², epirubicin: 100 mg/m², and cyclophosphamide: 500 mg/m²) every 3 weeks, which was followed by 12 courses of weekly paclitaxel (80 mg/m²). The patients with HER2BC also received trastuzumab during the paclitaxel treatment as a weekly dose (2 mg/kg) or tri-weekly dose (6 mg/kg) [15–17]. Staging and therapeutic effect were evaluated using ultrasonography, computed tomography, and bone scintigraphy based on the Response Evaluation Criteria in Solid Tumors [18]. Patients who achieved clinically partial or complete response were categorized as “responders” in the objective response rate (ORR), while patients with clinically stable or progressive disease were defined as “non-responders”. The patients subsequently underwent mastectomy or breast-conserving surgery [19], and the pathological therapeutic effect of the POC was evaluated using the resected specimens. Pathological complete response (pCR) was defined as complete disappearance of the lesion’s invasive components, including the lymph nodes, with or without intraductal components, according to the National Surgical Adjuvant Breast and Bowel Project B-18 protocol [20]. All patients received postoperative radiotherapy delivered to the remnant breast, and the standard postoperative adjuvant therapy was selected based on the cancer subtype. Patients were followed-up after surgery to detect recurrence using physical examinations every 3 months, ultrasonography every 6 months, and computed tomography and bone scintigraphy annually. The DFS interval was calculated from the day of surgery to the first instance of recurrence or death, while OS was calculated from the day of surgery to death.

Histopathological evaluation of TILs density
Specimens that were used to pathologically diagnose breast cancer (obtained via core needle biopsy or vacuum-assisted biopsy) were used to determine the TILs density. In the present study, TILs were defined as lymphocytes infiltrating within the tumor stroma [21]. The TILs density was calculated as the average from five randomly selected fields, and the results were classified as a score of 3 (> 50%), a score of 2 (11–50%), a score of 1 (≤ 10%), or a score of 0 (absent) (Additional file 1: Fig. S1). Based on previous reports [22, 23], we defined a high TILs density as scores of 2–3 (i.e., > 10%) and a low TILs density as scores of 0–1 (≤ 10%).

Statistical analysis

All analyses were performed using JMP software (version 11; SAS Institute, Cary, NC). Differences in the study variables were evaluated using the Chi square test or Fisher’s exact test, as appropriate. The Kaplan–Meier method was used to estimate the DFS and OS outcomes, which were compared using the log-rank test. A Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), and multivariable analysis was performed using a Cox regression model and the backward stepwise selection method. Differences were considered statistically significant at p-values of < 0.05.

Ethics statement

This study was conducted at the Osaka City University Graduate School of Medicine (Osaka, Japan) according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines. The study protocol involved a retrospectively written research, pathological evaluation, and statistical analysis plan [24]. The study complied with the provisions of the Declaration of

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### Table 1 Pack-years of smoking

| Case | Calculation | Value |
|------|-------------|-------|
| 1    | (70 cigarettes/day × 20 cigarettes/pack) × 10 years | 35 pack-years |
| 2    | (35 cigarettes/day × 20 cigarettes/pack) × 20 years | 35 pack-years |
| 3    | (20 cigarettes/day × 20 cigarettes/pack) × 20 years | 20 pack-years |
Helsinki, and all patients provided written informed consent for their treatment and data collection. The study’s retrospective protocol was approved by the ethics committee of Osaka City University (#926).

Results

Clinicopathological features

The clinicopathological features of the 149 women are listed in Table 2. The median age at surgery was 56 years (range 24–75 years old). The median follow-up duration was 1288 days after surgery (range 13–3615 days). The median tumor diameter was 27.6 mm (range 10.2–98.0 mm) and 98 patients (65.8%) were diagnosed with N1–3 lymph node metastasis based on their imaging results. Sixty-two patients had HER2BC (41.6%) and 87 patients had TNBC (58.4%). Ninety-one patients (61.1%) had a high TILs density and 58 patients (38.9%) had a low TILs density at their diagnosis. One hundred and five patients (70.5%) reported never smoking, and 44 patients (29.5%) reported a median smoking amount of 20 pack-years (range 2.5–135 pack-years). Based on the receiver operating characteristic curve analysis, the optimal smoking cut-off value for predicting DFS was defined as 2.5 pack-years, which yielded a distribution of 43 patients (28.9%) in the high smoking group and 106 patients (71.1%) in the low-smoking group (area under the curve: 0.588, sensitivity: 0.325, specificity: 0.846) (Additional file 2: Fig. S2). The ORR was 82.6% and 74 patients (49.7%) achieved a pCR. The therapeutic response was significantly higher among patients with HER2BC than among patients with TNBC (p = 0.023) (Table 3). However, there were no significant differences in the two

Table 2 Clinicopathological features of 149 patients who were treated with preoperative chemotherapy

| Parameters (n = 149) | Number of patients (%) |
|---------------------|------------------------|
| Age (years old)     | 56 (24–75)             |
| Tumour size (mm)    | 27.6 (10.2–98.0)       |
| Skin infiltration   |                        |
| Negative/positive   | 134 (89.9%)/15 (10.1%) |
| Lymph node metastasis | 51 (34.2%)/54 (36.2%)/29 (19.5%)/15 (10.1%) |
| HER2                |                        |
| Negative/positive   | 87 (58.4%)/62 (41.6%)  |
| Ki67                |                        |
| Negative/positive   | 23 (15.4%)/126 (84.6%) |
| ORR                 |                        |
| Non-responders/responders | 11 (7.4%)/138 (92.6%) |
| pCR                 |                        |
| Negative/positive   | 75 (50.3%)/74 (49.7%)  |
| Recurrence          |                        |
| Negative/positive   | 123 (82.6%)/26 (17.4%) |
| TILs                |                        |
| Low/high            | 58 (38.9%)/91 (61.1%)  |
| Smoker              |                        |
| No/yes              | 105 (70.5%)/44 (29.5%) |
| Pack-years of smokers | 20 (2.5–135)        |
| Pack-years          | 106 (71.1%)/43 (28.9%) |

HER human epidermal growth factor receptor, ORR objective response rate, pCR pathological complete response, TILs tumour-infiltrating lymphocytes

Table 3 Comparison of clinicopathological features by subtype

| Parameters | HER2-enriched breast cancer (n = 62) | Triple-negative breast cancer (n = 87) | p value |
|------------|--------------------------------------|---------------------------------------|---------|
| Age (years old) |                                       |                                       |         |
| ≤ 56       | 26 (41.9%)                           | 49 (56.3%)                           | 0.085   |
| > 56       | 36 (58.1%)                           | 38 (43.7%)                           |         |
| Tumour size (mm) |                                     |                                       |         |
| ≤ 27.6     | 30 (48.4%)                           | 45 (51.7%)                           | 0.690   |
| > 27.6     | 32 (51.6%)                           | 42 (48.3%)                           |         |
| Skin infiltration |                                   |                                       |         |
| Negative   | 54 (87.1%)                           | 80 (92.0%)                           | 0.335   |
| Positive   | 8 (12.9%)                            | 7 (8.0%)                             |         |
| Lymph node status |                                  |                                       |         |
| Negative   | 25 (40.3%)                           | 26 (29.9%)                           | 0.188   |
| Positive   | 37 (59.7%)                           | 61 (70.1%)                           |         |
| Ki67       |                                       |                                       |         |
| Negative   | 14 (22.6%)                           | 9 (10.3%)                            | 0.042   |
| Positive   | 48 (77.4%)                           | 78 (89.7%)                           |         |
| ORR        |                                       |                                       |         |
| Non-responders/responders |                  |                                       | 0.023   |
| Negative   | 61 (98.4%)                           | 77 (88.5%)                           |         |
| Positive   | 1 (1.6%)                             | 10 (11.5%)                           |         |
| pCR        |                                       |                                       |         |
| Negative   | 26 (41.9%)                           | 49 (56.3%)                           | 0.085   |
| Positive   | 36 (58.1%)                           | 38 (43.7%)                           |         |
| Recurrence |                                       |                                       |         |
| Negative   | 55 (88.7%)                           | 68 (78.2%)                           | 0.096   |
| Positive   | 7 (11.3%)                            | 19 (21.8%)                           |         |
| TILs       |                                       |                                       |         |
| Low        | 20 (32.3%)                           | 38 (43.7%)                           | 0.206   |
| High       | 42 (67.7%)                           | 49 (56.3%)                           |         |
| Pack-years |                                       |                                       |         |
| Low        | 40 (64.5%)                           | 66 (75.9%)                           | 0.134   |
| High       | 22 (35.5%)                           | 21 (24.1%)                           |         |

HER human epidermal growth factor receptor, ORR objective response rate, pCR pathological complete response, TILs tumour-infiltrating lymphocytes
groups’ pCR rates (p = 0.085), TILs density (p = 0.206), or smoking amount (p = 0.134).

The associations of smoking with clinicopathological features, DFS, and OS
Table 4 shows the results of the associations between smoking and the patients’ clinicopathological features. No significant correlation was found between comparing smokers and never smokers. However, when divided into two groups according to smoking amount, correlation with clinicopathological features was recognized. Relative to the low smoking group, the high smoking group had a significant greater TILs density (p = 0.043) and a significantly better pCR rate (p = 0.042). In the univariate analysis, prolonged DFS was significantly associated with pCR (p < 0.001, HR 0.203, 95% CI 0.068–0.499) and a high TILs density (p = 0.001, HR 0.252, 95% CI 0.107–0.553) (Table 5). In addition, prolonged OS was significantly associated with pCR (p = 0.002, HR 0.183, 95% CI 0.042–0.561) and a high TILs density (p = 0.035, HR 0.357, 95% CI 0.129–0.929) (Table 5). However, there was no significant difference according to smoking amount in DFS (p = 0.114) or OS (p = 0.347) (Fig. 1).

Discussion
Smoking is a risk factor for various carcinomas, including breast cancer [25]. Smoking-related carcinogenesis is linked to various factors, with some of the components in tobacco smoke having estrogenic effects and others having antiestrogenic effects [26, 27]. Moreover, tobacco

Table 4 Difference in clinicopathological features due to pack-years

| Parameters                  | Smoker Yes (n = 44) | Smoker No (n = 105) | p value | Pack-years Smoker High (n = 43) | Smoker Low (n = 106) | p value |
|-----------------------------|---------------------|---------------------|---------|---------------------------------|---------------------|---------|
| Age (years old)             |                     |                     |         |                                 |                     |         |
| ≤ 56                        | 24 (54.5%)          | 51 (48.6%)          | 0.509   | 24 (55.8%)                      | 51 (48.1%)          | 0.398   |
| > 56                        | 20 (45.5%)          | 54 (51.4%)          |         | 19 (44.2%)                      | 55 (51.9%)          |         |
| Tumour size (mm)            |                     |                     |         |                                 |                     |         |
| ≤ 27.6                      | 25 (56.8%)          | 50 (47.6%)          | 0.309   | 25 (58.1%)                      | 50 (47.2%)          | 0.228   |
| > 27.6                      | 19 (43.2%)          | 55 (52.4%)          |         | 18 (41.9%)                      | 56 (52.8%)          |         |
| Skin infiltration           |                     |                     |         |                                 |                     |         |
| Negative                    | 40 (90.9%)          | 94 (89.5%)          | 0.799   | 40 (93.0%)                      | 94 (88.7%)          | 0.428   |
| Positive                    | 4 (9.1%)            | 11 (10.5%)          |         | 3 (7.0%)                        | 12 (11.3%)          |         |
| Lymph node status           |                     |                     |         |                                 |                     |         |
| Negative                    | 18 (40.9%)          | 33 (31.4%)          | 0.269   | 18 (41.9%)                      | 33 (31.1%)          | 0.214   |
| Positive                    | 26 (59.1%)          | 72 (68.6%)          |         | 25 (58.1%)                      | 73 (68.9%)          |         |
| Ki67                        |                     |                     |         |                                 |                     |         |
| Negative                    | 6 (13.6%)           | 17 (16.2%)          | 0.69    | 6 (14.0%)                       | 17 (16.0%)          | 0.752   |
| Positive                    | 38 (86.4%)          | 88 (83.8%)          |         | 37 (86.0%)                      | 89 (84.0%)          |         |
| Intrinsic subtype           |                     |                     |         |                                 |                     |         |
| HER2-enriched               | 22 (50.0%)          | 40 (38.1%)          | 0.181   | 22 (51.2%)                      | 40 (37.7%)          | 0.134   |
| Triple-negative             | 22 (50.0%)          | 65 (61.9%)          |         | 21 (48.8%)                      | 66 (62.3%)          |         |
| ORR                         |                     |                     |         |                                 |                     |         |
| Non-responders              | 3 (6.8%)            | 8 (7.6%)            | 0.866   | 3 (7.0%)                        | 8 (7.5%)            | 0.905   |
| Responders                  | 41 (93.2%)          | 97 (92.4%)          |         | 40 (93.0%)                      | 98 (92.5%)          |         |
| pCR                         |                     |                     |         |                                 |                     |         |
| Negative                    | 17 (38.6%)          | 58 (55.2%)          | 0.065   | 16 (37.2%)                      | 59 (55.7%)          | 0.042   |
| Positive                    | 27 (61.4%)          | 47 (44.8%)          |         | 27 (62.8%)                      | 47 (44.3%)          |         |
| Recurrence                  |                     |                     |         |                                 |                     |         |
| Negative                    | 40 (90.9%)          | 83 (79.0%)          | 0.083   | 39 (90.7%)                      | 84 (79.2%)          | 0.096   |
| Positive                    | 4 (9.1%)            | 22 (21.0%)          |         | 4 (9.3%)                        | 22 (20.8%)          |         |
| TILs                        |                     |                     |         |                                 |                     |         |
| Low                         | 13 (29.6%)          | 45 (42.9%)          | 0.075   | 12 (27.9%)                      | 46 (43.4%)          | 0.043   |
| High                        | 31 (70.5%)          | 60 (57.1%)          |         | 31 (72.1%)                      | 60 (56.6%)          |         |

HER human epidermal growth factor receptor, ORR objective response rate, pCR pathological complete response, TILs tumour-infiltrating lymphocytes
Table 5  Univariate and multivariate analysis with respect to disease-free survival and overall survival

| Parameters                  | Univariate analysis |                  |              |               | Multivariate analysis |              |               |
|-----------------------------|---------------------|------------------|--------------|--------------|----------------------|--------------|--------------|
|                             | Hazard ratio        | 95% CI           | p value      | Hazard ratio  | 95% CI               | p value      |              |
| Disease-free survival       |                     |                  |              |              |                      |              |              |
| Age at operation (year)     |                     |                  |              |              |                      |              |              |
| ≤ 56                        | 0.614               | 0.269–1.335      | 0.220        | 1.556        | 0.455–4.067          | 0.440        |              |
| > 56                        | 1.137               | 0.525–2.503      | 0.744        | 0.394        | 0.180–0.926          | 0.034        | 0.770        | 0.321–1.942 | 0.568        |
| Tumour size (mm)            |                     |                  |              |              |                      |              |              |
| ≤ 27.6                      | 2.440               | 0.933–8.343      | 0.071        | 1.884        | 0.828–4.823          | 0.135        |              |
| > 27.6                      |                      |                  |              |              |                      |              |              |
| Skin infiltration           |                     |                  |              |              |                      |              |              |
| Negative                    | 1.556               | 0.455–4.067      | 0.440        | 0.394        | 0.180–0.926          | 0.034        | 0.770        | 0.321–1.942 | 0.568        |
| Positive                    |                      |                  |              |              |                      |              |              |
| Lymph node status           |                     |                  |              |              |                      |              |              |
| Negative                    | 2.440               | 0.933–8.343      | 0.071        | 2.440        | 0.933–8.343          | 0.071        | 1.677        | 0.617–5.859 | 0.331        |
| Positive                    | 0.394               | 0.180–0.926      | 0.034        | 0.770        | 0.321–1.942          | 0.568        |              |
| HER2-enriched               | 1.884               | 0.828–4.823      | 0.135        |              |                      |              |              |
| Triple-negative             |                      |                  |              |              |                      |              |              |
| ORR                         |                      |                  |              |              |                      |              |              |
| Non-responders              | 0.083               | 0.035–0.210      | < 0.001      | 0.083        | 0.035–0.210          | < 0.001      | 0.154        | 0.059–0.426 | 0.001        |
| Responders                  |                      |                  |              |              |                      |              |              |
| Pathological response       |                     |                  |              |              |                      |              |              |
| Non-pCR                     | 0.203               | 0.068–0.499      | < 0.001      | 0.203        | 0.068–0.499          | < 0.001      | 0.381        | 0.118–1.059 | 0.065        |
| pCR                         |                      |                  |              |              |                      |              |              |
| TILs                         | 0.252               | 0.107–0.553      | 0.001        | 0.252        | 0.107–0.553          | 0.001        | 0.424        | 0.167–1.032 | 0.059        |
| Low                         |                      |                  |              |              |                      |              |              |
| High                        |                      |                  |              |              |                      |              |              |
| Pack-years                  | 0.434               | 0.127–1.134      | 0.092        | 0.434        | 0.127–1.134          | 0.092        | 0.567        | 0.160–1.555 | 0.289        |
| Low                         |                      |                  |              |              |                      |              |              |
| High                        |                      |                  |              |              |                      |              |              |
| Overall survival            |                     |                  |              |              |                      |              |              |
| Age at operation (year)     |                     |                  |              |              |                      |              |              |
| ≤ 56                        | 0.508               | 0.175–1.338      | 0.172        |              |                      |              |              |
| > 56                        | 1.123               | 0.429–2.993      | 0.811        |              |                      |              |              |
| Tumour size (mm)            |                     |                  |              |              |                      |              |              |
| ≤ 27.6                      | 2.778               | 0.781–17.657     | 0.125        |              |                      |              |              |
| > 27.6                      |                      |                  |              |              |                      |              |              |
| Skin infiltration           |                     |                  |              |              |                      |              |              |
| Negative                    | 1.939               | 0.446–5.977      | 0.335        |              |                      |              |              |
| Positive                    |                      |                  |              |              |                      |              |              |
| Lymph node status           |                     |                  |              |              |                      |              |              |
| Negative                    | 1.939               | 0.446–5.977      | 0.335        |              |                      |              |              |
| Positive                    | 1.939               | 0.446–5.977      | 0.335        |              |                      |              |              |
| Ki67                         | 0.638               | 0.234–2.023      | 0.419        |              |                      |              |              |
| Negative                    | (continued)         |                  |              |              |                      |              |              |
| Positive                    | (continued)         |                  |              |              |                      |              |              |
components can be carried through the blood to the mammary gland tissues where they cause DNA damage [10, 11]. Some researchers have indicated that smoking is associated with the development of ER+ breast cancer, while many others have reported that smoking is associated with ER− breast cancer [26–31]. These differences may be related to race [27], which would be consistent with our findings, as all of our patients were Japanese and had ER− cancers. Furthermore, tissue culture and animal experiments have indicated that tobacco smoke components increase proliferative capacity and cause malignant transformation [32–34], which further highlights the relationship between smoking and the development of TNBC or HER2BC.

The present study indicated that the HER2BC and TNBC subtypes were related to smoking and the cancer’s pre-treatment iTME. Interestingly, previous reports have indicated that a high TILs density was significantly associated with prolonged DFS and OS [4, 5], and the present study indicated that TILs density was associated with the pre-diagnosis smoking amount. These results indicate that local microimmune reactions are activated by chronic inflammation in microvessels, which may be related to the release of...
antigens as a result of smoking-related DNA damage. Given that a higher smoking amount was associated with a high TILs density, it is possible that smoking was related to the high pCR rate.

Although no previous studies have evaluated the relationship between smoking and the iTME in breast cancer, that relationship has been studied in lung cancer. For example, in non-small cell lung cancer, smoking was not associated with the expression of CD3, CD4, forkhead box protein 3 (FOXP3), and CD20, although smoking was associated with increased CD8 expression [14, 35]. Furthermore, increased numbers of CD8+ T-cells is associated with a good prognosis among patients with non-small cell lung cancer [14, 36]. Moreover, CD8 is a marker for cytotoxic T-cells, which are associated with an improved prognosis among patients with breast cancer [2, 37]. Although the present study did not directly evaluate the correlation between smoking and DFS or OS, the overall exposure to tobacco smoke is known to be associated with the risks of breast cancer recurrence, breast cancer-related death, and overall mortality [38, 39]. In this context, smoking could activate the iTME and affect the short-term therapeutic effect (i.e., pCR rate), although it might not be associated with the long-term therapeutic effect (i.e., DFS or OS) because it is not correlated with low oxygen levels caused by microangiopathy or deterioration of the iTME.

The present study has several limitations. First, the smoking amount was retrospective determined using self-reported data from at the patient's diagnosis. Second, we did not consider smoking status after diagnosis or second-hand smoke, although passive smoking is an important risk factor for carcinogenesis [25] and lifelong exposure to smoke is more strongly related to the risks of carcinogenesis and recurrence (vs. current smoking status) [38, 39]. It is also reported that special genes, such as MAPKs/TP53, are affecting the iTME [8, 9]. That is, the iTME is also strongly related to genes. Since this result has only been investigated retrospectively, it is necessary to further examine the relationship between smoking and iTME with such as immunohistochemical staining, gene analysis or experiments in vitro. Moreover, it will be important to consider complete smoking-related data to examine the association of smoking with long-term prognosis among patients with breast cancer.

Conclusions
In conclusion, smoking may influence the iTME, with an activated iTME being associated with pCR rate. Therefore, controlled activation of the microenvironment in this setting may help improve patients’ prognosis.

Additional files

Additional file 1: Fig. S1. Histopathological evaluation of tumor-infiltrating lymphocytes (TILs) density. Specimens were obtained to pathologically diagnose breast cancer using core needle biopsy or vacuum-assisted biopsy, and these specimens were evaluated to calculate the TILs density, which was calculated as the average for five randomly selected stromal regions with lymphoplasmacytic infiltration. (A) > 50%, score 3. (B) 11–50%, score 2. (C) < 10%, score 1. (D) Absent, score 0.

Additional file 2: Fig. S2. Receiver operating characteristic curve analysis. The optimal cut-off value for using smoking to predict disease-free survival was identified as 50 pack-years (area under the curve: 0.588, sensitivity = 0.323, specificity = 0.846).

Abbreviations
iTME: immune tumor microenvironment; TILs: tumor-infiltrating lymphocytes; pCR: pathological complete response; DFS: disease-free survival; OS: overall survival; TNBC: triple-negative breast cancer; HER2/BC: human epidermal growth factor receptor 2-enriched breast cancer; POC: preoperative chemotherapy; ER: estrogen receptor; PgR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ORR: objective response rate; pCR: pathological complete response; HR: hazard ratio; CI: confidence interval; REMARK: Reporting Recommendations for Tumor Marker Prognostic Studies; FOXP3: forkhead box protein 3.

Authors’ contributions
All authors were involved in the preparation of this manuscript. KTakada collected the data, and wrote the manuscript. SK and ST summarized the data and revised the manuscript. HF, KH and MO substantial contribution to the study design, performed the operation, and revised the manuscript. HT performed the operation and designed the study. KTakada, SK and ST were involved in the preparation of this manuscript. All authors read and approved the final manuscript.

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

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

Availability of data and materials
The datasets supporting the conclusions of this article is included within the article.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Written informed consent was obtained from all subjects. This research conform to the provisions of the Declaration of Helsinki in 2013. All patients were 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 (926).
Funding
This study was supported in part by Grants-in-Aid for Scientific Research (KAKENHI, Nos. 25461992, 26461957, and 17K10559) from the Ministry of Education, Science, Sports, Culture and Technology of Japan.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 28 September 2018  Accepted: 2 January 2019
Published online: 07 January 2019

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