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***Observational Study***

**Clinico-pathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy**

Galvez M *et al.* Biomarkers in BC with neoadjuvance

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**Abstract**

***AIM***

To investigate the survival impact of clinical-pathological factors including pathological complete response (pCR) and tumor-infiltrating lymphocytes (sTIL) levels according to subtypes in breast cancer (BC) patients who received neo-adjuvant chemotherapy (NAC).

***METHODS***

We evaluated 435 BC patients who came and received NAC at the Instituto Nacional de Enfermedades Neoplasicas from 2003 to 2014. sTIL was analyzed as the proportion of tumor stroma occupied by lymphocytes, and was prospectively evaluated on hematoxylin and eosin-stained sections of the pre-NAC core biopsy. pCR was considered in the absence of infiltrating cancer cells in primary tumor and axillary lymph nodes. Analysis of statistical association between clinical pathological features, sTIL, pCR and survival were carried out using SPSSvs19.

***RESULTS***

Median age was 49 years (range 24-84) and the most frequent clinical stage was IIIB (58.3%). Luminal A, Luminal B, HER2-enriched and (triple-negative) TN phenotype was found in 24.6%, 37.9%, 17.7% and 19.8%, respectively. pCR was observed in 11% and median percentage of sTIL was 40% (2%-95%) in the whole population. pCR was associated to Ct1-2 (*P* = 0.045)and to high sTIL (*P* = 0.029) in the whole population. There was a slightly trend to be significant for sTIL (*P* = 0.054) in Luminal A. sTIL was associated to grade III (*P* < 0.001), no-Luminal A subtype (*P* < 0.001), RE-negative (*P* < 0.001), PgR-negative (*P* < 0.001), HER2-positive (*P* = 0.002) and pCR (*P* = 0.029) in the whole population. Longer DFS was associated to grade I-II (*P* = 0.006), cN0 (*P* < 0.001), clinical stage II (*P* = 0.004), ER-positive (*P* < 0.001), PgR-positive (*P* < 0.001), luminal A (*P* < 0.001) and pCR (*P* = 0.002). Longer DFS was associated to grade I-II in Luminal A (*P* < 0.001), N0-1 in Luminal-A (*P* = 0.045) and TNBC (*P* = 0.01), clinical stage II in Luminal-A (*P* = 0.003) and TNBC (*P* = 0.038), pCR in TNBC (*P* < 0.001). Longer overall survival was associated to grade I-II (*P* < 0.001), ER-positive (*P* < 0.001), PgR-positive (*P* < 0.001), Luminal A (*P* < 0.001), cN0 (*P* = 0.002) and pCR (*P* = 0.002) in whole population. OS was associated to clinical stage II (*P* = 0.017) in Luminal-A, older age (*P* = 0.042) in Luminal B and pCR in TNBC (*P* = 0.005).

***CONCLUSION***

Predictive and prognostic value of clinic-pathological features like pCR and sTIL differ depending on the evaluated molecular subtype.

**Key words:** Breast cancer; Subtype; Tumor-infiltrating lymphocytes; Neoadjuvant therapy; Pathological complete response; Survival

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**Core tip:** The authors evaluated a series of 435 breast cancer (BC) cases who received neoadjuvant chemotherapy. They evaluated the association between stromal tumor-infiltrating lymphocytes levels and pathological complete response (pCR) in pre-neoadjuvant chemotherapy samples according to molecular subtypes. The results confirm differences in the predictive and prognostic role of stromal TILs and pCR depending on the tumor subtype. Additionally, the authors evaluate value of traditional prognostic features in every BC subset. Our results increase the understanding of biomarkers in the heterogeneous scenario of BC.

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**INTRODUCTION**

Breast cancer (BC) is the second most common cancer in the world and the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers); and is the fifth cause of death from cancer overall (522000 deaths)[1]. Neoadjuvant chemotherapy (NAC) is the standard therapy for locally advanced BC and could improve both surgical options and long-term outcome[2]. Response to NAC is considered an in vivo test of tumor sensitivity to NAC and the achievement of a complete pathological response (pCR) is associated with longer disease free survival and greater overall survival [3-7]. Tumor-infiltrating lymphocytes (TILs) evaluate host immune system against tumor and also constitutes a valuable predictive biomarker of NAC response and survival[8-11].

Breast cancer is a heterogeneous disease, and intrinsically different subtypes of breast cancer have been identified in the past years based on gene expression profiles and on combination of immunohistochemical (IHC) status of hormone and HER2 receptors. Responsiveness to preoperative therapies and the outcome after surgery can be predicted by breast cancer subtypes[12-14].

In this study, we investigated the survival impact of different clinico-pathological factors including pCR and TIL levels according to the subtypes in breast cancer patients who received NAC. Predictive role of different clinicopathological features for having high density TIL and obtaining pCR according to subtypes was also performed.

**MATERIALS AND METHODS**

We found 435 patients diagnosed with BC at clinical stage IIB to IIIC at medical department of the Instituto Nacional de Enfermedades Neoplasicas from 2003 to 2014. Eligibility criteria for this retrospective study were to have a histological diagnosis based in a core needle biopsy, to have received NAC regimen and to have undergone surgery after NAC. Patient characteristics such as age, clinical stage, histological subtype and grade, presence of estrogen receptors (RE), progesterone (PgR) receptors and HER-2 and molecular subtype was obtained from pathology report of pre-NAC core biopsy. Complete pathological response was defined as absence of invasive cancer in the breast and axillary nodes, irrespective of carcinoma in situ (ypT0/is ypN0), as previously described[4,15]. Phenotype classification was prospectively concluded through the evaluation of RE, PgR, HER2 and ki67 as well as histological grade (in cases without ki67 information): Luminal A (ER ≥ 10%, PgR ≥ 20%, HER2-negative and ki67 < 15% or HG-I-II), Luminal B (ER ≥ 10% and any PgR < 20%, HER2-positive, ki67 < 15% or HG-III), HER2-enriched (ER < 10%, PgR < 10% and HER2-positive) and TN (ER < 10%, PgR < 10% and HER2-negative). Stromal lymphocytic infiltration (TIL) was prospectively evaluated in pre-NAC core biopsy and was defined as percentage of stromal area covered by lymphocytes[16].

Follow-up and recurrence information (date and location) was obtained from patient files. Time-From-Last-Chemotherapy-To-Surgery (TLCS) was considered the amount of months from the date of the last NAC administration to surgery of the primary tumor. OS is calculated from surgery date of primary breast tumor to death or last follow-up date, and disease free survival (DFS) is calculated from surgery date of primary breast tumor to recurrence or last follow-up date.

***Statistical analysis***

Categorical comparisons and association analysis between clinical pathological features and pCR were carried out using the chi-square statistic or Fisher exact test.

Survival analysis, regarding OS and DFS, was calculated using the Kaplan-Meier method, and differences between curves were estimated by log-rank test. In all cases, the level of alpha was set at 0.05 a priori. Statistical analysis was performed using SPSSvs19.

**RESULTS**

***Clinicopathological description***

There were 435 patients included for this study, median age at diagnosis was 49 years (range 24-84), median tumor size was 6.5 cm (range 1.0-24.0), T3 was found in 27.8% and T4 was found in 63.9%. Inflammatory disease was found in 29.2%. Most frequent clinical stage were IIIB (60.5%) and IIIA (18.6%). Ductal histology was found in 93.3%, high grade in 52.2%, ER+ status in 62.8%, PgR+ status in 51% and HER2+++ in 32.4%. Luminal A, Luminal B, HER2-enriched and TN phenotype was found in 24.6%, 37.9%, 17.7% and 19.8%, respectively. Most frequent neoadjuvant chemotherapy were doxorubicin- cyclophosphamide by 4 cycles followed by 12 weekly paclitaxel (67.18%), doxorubicin-cyclophosphamide by 4 cycles followed by every 3 wk paclitaxel by 4 cycles (18.85%) and doxorubicin-cyclophosphamide by 4 cycles alone (7.32%). The median time from the last chemotherapy to surgery (TLCS) was 63 days (max 982 d). Complete pathological response (pCR) was observed in 48 (11%) patients. Median percentage of sTIL was 40% (2%-95%) in the whole population and was 70% (60%-95%) in patients with pCR. Recurrence was found in 35.7%, median DFS was 7.54 and median OS was 5.16 (95%CI: 4.16- 6.15) years (Table 1).

***Clinicopathological factors associated to pCR according to*** BC ***subtypes***

Association analysis found that pCR was associated to T1-2 (*P* = 0.045) and to high sTIL (*P* = 0.029) in the whole population (Table 1). Higher sTIL had a slightly trend to be associated to pCR (*P* = 0.054) in Luminal A, and smaller tumor size had a trend to be associated to pCR (*P* = 0.098) in Luminal A. Clinical involvement of axillary lymph nodes was not associated to variation of pCR (Table 2). An additional analysis by level of axillary involvement found that N2-3 had lower rates of pCR than N0-1 only in TNBC (*P* = 0.018).

***Clinicopathological factors associated to sTIL according to BC subtypes***

Association analysis found that sTIL was associated to grade III (*P* < 0.001), no- luminal A subtype (*P* < 0.001), ER-negative (*P* < 0.001), PgR-negative (*P* < 0.001), HER2-positive (*P* = 0.002) and pCR (*P* = 0.029) in the whole population (Table 1). Within each BC subtype, sTIL remained associated to grade III in Luminal B (*P* = 0.011) and TN (*P* = 0.006) subtypes, as well as cN+ in Luminal B (*P* = 0.02) (Table 3).

***Prognostic clinicopathological factors according to BC subtypes***

Survival analysis found longer DFS was associated to grade I-II (*P* = 0.006), cN0 (*P* < 0.001), clinical stage II (*P* = 0.004), ER-positive (*P* < 0.001), PgR-positive (*P* < 0.001), luminal A (*P* < 0.001) and pCR (*P* = 0.002). Longer DFS was associated to grade I-II in Luminal A (*P* = 0.033), N0-1 in Luminal-A (*P* = 0.045) and TNBC (*P* = 0.01), clinical stage II in Luminal-A (*P* = 0.003) and TNBC (*P* = 0.038), and pCR in TNBC (*P* = 0.001) (Table 1).

Longer OS was associated to grade I-II (*P* < 0.001), ER-positive (*P* < 0.001), PgR-positive (*P* < 0.001), Luminal A (*P* < 0.001), cN0 (*P* = 0.007) and pCR (*P* = 0.002) in whole population. It was also associated to older age in luminal B (*P* = 0.042), to clinical stage II in Luminal-A (*P* = 0.017), to cN0 (*P* = 0.045) and pCR in TNBC (*P* = 0.005) (Figure 1). Differences in TILs did not affect survival in the whole nor molecular subtype population (Table 1 and Figure 2).

**DISCUSSION**

The biological heterogeneity of BC has been extensively described, and differences between intrinsic subtypes have been confirmed in the recent decade. We explored differences in the survival impact of tumor features including pCR, TIL levels in every of the four molecular subtypes. Rates of pCR is lower in Luminal- (9.2%), HER2-enriched (13%) and TNBC (15.3%) subtype. pCR is also associated to longer survival in the whole population as well as in TNBC (pCR = 92.3% *vs* not pCR = 26.5% 5 year OS, *P* = 0.005) (and trend in Luminal-A, Luminal-B and HER2-enriched) phenotypic subsets of our series. It is widely assumed that patients who achieve pCR have significantly better DFS and OS rates in all molecular subtypes[12-14,17-19]. von Minckwitz *et al*[6] found pCR was not associated to prognosis only in Luminal-A tumors ina series of 6377 patients with Anthracycline-Taxane-based NAC from 7 randomized trials and some authors claim it is related to the observed continuous tumor shrinkage occurred in their ER-positive tumor group during extended NAC different than early and short-period tumor shrinkage observed in the ER-negative group[6,18-24].

pCR was more frequent in small tumor in both the whole population and in Luminal-A subtype in our series. It is concordant with the previously mentioned idea that the effect of chemotherapy in Luminal A is slower than in other subtypes. By other side, Baron *et al*[18] found a similar lower rate of pCR in tumor size larger than 5 cm (*P* = 0.022) in the whole series (*n* = 608), but not association in the Luminal setting (*P* = 0.411). Higher grade of axillary involvement (cN2-3) was associated to lower rates of pCR only in the TNBC subset of our series. This lower response in bulky metastases could explain the previously described TNBC paradox phenomena of higher pCR rates but also higher distant relapse[21].

pCR was associated to higher percentage of sTIL in the whole population and also within the HER2-enriched subtype (*P* = 0.02). A trend to the association was found in Luminal A, Luminal B and TNBC. Different studies find that high TIL levels in pre-NAC samples are associated to higher pCR rates in the whole BC population[26-28]. Wang *et al*[28] performed a meta-analysis with 23 studies including 13100 BC patients and similarly found that high TIL levels was associated with improved pCR rate in the whole population, and in HER2 and TNBC. A high TIL level significantly predicted longer OS in the whole population (*P* < 0.001) and in patients with HER2-positive (*P* = 0.005) BC and in TNBC patients (*P* < 0.001)[29].

TIL showed association with grade III tumors in whole population and in Luminal B and TNBC subsets in our series. Similarly, Pruneri *et al*[30] describes that higher TIL levels have a trend to be associated with HG3 (*P* = 0.052) and was associated to ki67 ≥ 50% (*P* < 0.0001) in a series of 897 TNBC cases and could be reflect the appearance of larger amount of neoantigens that elicit an immunomediated response. Involvement of axillary lymph nodes was associated to higher TIL levels only in Luminal B subset. High density of TIL have previously been described as associated to absence of lymph node involvement in whole population of BC, and our results indicate that this association could differ by some subtypes[31]. Higher levels of sTIL was not associated to longer survival in the whole population nor in any subtype in our series. The reason for this finding could be explained by the small size of our series and because the highest impact of TILs is over pCR instead of survival.

Our study has some limitations. First, because of the retrospective design of the study, different chemotherapy schemas were used depending on the oncologist decision and surgical election depending on surgeon. Second, the sample sizes of each BC subgroup are rather small, so the prognostic impact of every clinicopathological feature in each BC subtype should be investigated in a larger population in subsequent studies. Despite these limitations, this is the first comprehensive report of the NAC effect over breast molecular subtype in Latin-American population.

**ARTICLE HIGHLIGHTS**

***Research background***

Breast cancer can be classified into Luminal A, Luminal B, HER2-enriched and Triple-Negative. Clinicopathological features can identify breast cancer prognosis and include pathological complete response (tumor sensibility to chemotherapy) and Tumor Infiltrating Lymphocytes (host activity against the tumor).

***Research motivation***

Discussion and new information about molecular breast cancer subtypes have been included in the most relevant cancer related meeting and more than 30000 articles have been published in the last 2 years. Two biomarkers: Pathological complete response (pCR) and tumor-infiltrating lymphocytes (TIL) have been re-defined and gained pathologist acceptance in the last 3 years.

***Research objectives***

The main objective is to evaluate the survival impact of different clinico-pathological factors including pCR and TIL levels according to the subtypes in breast cancer patients who received neo-adjuvant chemotherapy.

***Research methods***

Evaluation of TIL levels was prospectively performed following international guidelines. Breast cancer cases were classified according to 2017 St Gallen Breast Cancer Meeting guidelines.

***Research results***

pCR was associated to cT1-2 (*P* = 0.045) and to high sTIL (*P* = 0.029) in the whole population. However, this relationship was not found inside every molecular subtype probable because small sample size. pCR was associated to longer DFS in whole population (*P* = 0.002) and in TNBC (*P* < 0.001); as well as to longer OS in whole population (*P* = 0.002) and in TNBC (*P* = 0.005).

***Research conclusions***

Predictive and prognostic value of clinic-pathological features like pCR and sTIL differ depending on the evaluated molecular subtype. Identification of pCR and TIL roles in every molecular subtype will allow to identify those who need more intense chemotherapy and those who will benefit from an immune modulator treatment.

***Research perspectives***

No information about relevance of pCR and TILs have been published in South-American breast cancer women. An increase of the knowledge about prognosis impact of pCR and TIL in every molecular breast cancer subtype will allow to obtain more effective personalized therapies. Furthermore, similar analysis need to be done with more precise methods to evaluate response to chemotherapy and host immune activity like tumor residual burden and CD3/CD8 ratio, respectively.

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**Figure 1 Overall survival regarding tumor-infiltrating lymphocytes (cut-off: 50%) for Luminal A (A), Luminal B (B), HER2-enriched (C) and Triple Negative group (D).**



**Figure 2 Overall survival regarding pathological complete response for Luminal A (A), Luminal B (B), HER2-enriched (C) and triple negative group (D).**

**Table 1 Clinical-pathological features**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Cases *n* = 435 (%)** | **sTIL ≥ 50%*****n* = 181 (%)** | ***P*** | **pCR*****n* = 48 (%)** | ***P*** | **Overall Survival at 5 yr (OS = 50.1%)** | ***P*** | **Progression free survival at 5 yr****(DFS = 57.8%)** | ***P*** |
| Age (yr), median (range) | 49 (24-84) | 49 (24-84) | 0.923 | 47 (28-80) | 0.472 |  | 0.512 |  | 0.833 |
| < 50 | 231 (53.1) | 96 (35.2) |  | 28 (12.1) |  | 48.8% |  | 59.7% |  |
| ≥ 50 | 204 (46.9) | 85 (36.7) |  | 20 (9.8) |  | 51.7% |  | 55.9% |  |
| Histological subtypes |  |  | 0.928 |  | 0.234 |  | 0.512 |  | 0.497 |
| Ductal | 406 (93.3) | 169 (43.6) |  | 43 (10.6) |  | 49.0% |  | 57.5% |  |
| Lobular | 21 (4.8) | 7 (36.8) |  | 2 (9.5) |  | 61.0% |  | 55.2% |  |
| Others | 8 (1.8) | 5 (62.5) |  | 3 (37.5) |  | - |  | - |  |
| Histological grade |  |  | < 0.001 |  | 0.170 |  | 0.001 |  | 0.006 |
| G1-G2 | 200 (46) | 59 (32.6) |  | 17 (8.5) |  | 57.1% |  | 64.6% |  |
| G3 | 227 (52.2) | 119 (65.7) |  | 29 (12.8) |  | 42.8% |  | 52.2% |  |
| NR | 8 (1.8) | 3 (1.7) |  | 2 (25) |  | 83.3% |  | 45.7% |  |
| ER |  |  | < 0.001 |  | 0.098 |  | < 0.001 |  | 0.000 |
| No | 162 (37.2) | 89 (57.8) |  | 23 (14.2) |  | 36.1% |  | 47.1% |  |
| Yes | 273 (62.8) | 92 (35.2) |  | 25 (9.2) |  | 58.2% |  | 64.3% |  |
| PgR |  |  | 0.003 |  | 0.246 |  | < 0.001 |  | 0.000 |
| No | 213 (49) | 104 (51) |  | 27 (12.7) |  | 41.0% |  | 50.0% |  |
| Yes | 222 (51) | 77 (36.5) |  | 21 (9.5) |  | 58.4% |  | 64.8% |  |
| HER2 |  |  | 0.002 |  | 0.135 |  | 0.334 |  | 0.135 |
| No | 294 (67.6) | 106 (38.3) |  | 28 (9.5) |  | 53.7% |  | 60.4% |  |
| Yes | 141 (32.4) | 75 (54.3) |  | 20 (14.2) |  | 40.8% |  | 52.3% |  |
| Molecular subtypes |  |  | < 0.001 |  | 0.233 |  | < 0.001 |  | < 0.001 |
| Luminal A | 107 (24.6) | 30 (29.7) |  | 13 (12) |  | 72% |  | 76.1% |  |
| Luminal B | 165 (37.9) | 61 (38.4) |  | 12 (7) |  | 50.6% |  | 57.7% |  |
| HER2-enriched | 77 (17.7) | 50 (66.7) |  | 10 (13) |  | 41.5% |  | 54.9% |  |
| Triple-Negative | 86 (19.8) | 40 (50) |  | 13 (15) |  | 32.5% |  | 40.3% |  |
| Tumor size (cm) |  |  | 0.183 |  | 0.019 |  | 0.490 |  | 0.250 |
| Median (range) | 6.5 (1-24) | 6.5 (1-16) |  | 6.0 (2-15) |  |  |  |  |  |
| cT |  |  |  |  |  |  |  |  |  |
| cT1-cT2 | 36 (8.3) | 19 (54.3) |  | 8 (22.2) |  | 55.0% |  | 69.2% |  |
| cT3-cT4 | 399 (91.7) | 162 (42.6) |  | 40 (10) |  | 49.6% |  | 56.8% |  |
| cN |  |  | 0.084 |  | 0.743 |  | 0.007 |  | 0.001 |
| cN0 | 83 (19.1) | 28 (35) |  | 10 (12) |  | 65.8% |  | 77.0% |  |
| cN1-cN2-cN3 | 352 (80.9) | 153 (45.7) |  | 38 (10.8) |  | 47.2% |  | 54.2% |  |
| Clinical stage |  |  | 0.192 |  | 0.088 |  | 0.155 |  | 0.004 |
| II | 72 (16.6) | 26 (36.6) |  | 12 (16.7) |  | 62.1% |  | 74.3% |  |
| III | 363 (83.4) | 155 (45.1) |  | 36 (9.9) |  | 48.1% |  | 55.4% |  |
| sTIL% |  |  |  |  | 0.002 |  | 0.598 |  | 0.747 |
| Median (range) | 40 (2-95) | 70 (60-95) |  | 65 (5-95) |  |  |  |  |  |
| <50% | 266 (61.1) | 0 (0) |  | 20 (7.5) |  | 49.6% |  | 55.7% |  |
| >=50% | 149 (34.3) | 181 (100) |  | 26 (17.4) |  | 53.9% |  | 63.1% |  |
| Missing data | 20 (4.6) | 20 (0) |  | 2 (10) |  | - |  | - |  |
| TLCS (days) |  |  | 0.411 |  | 0.633 |  | 0.317 |  | 0.156 |
| Median (range) | 63 (5-982) | 58 (8-982) |  | 65 (8-281) |  |  |  |  |  |
| Shorter than median | 207 (47.6) | 91 (45.5) |  | 22 (10.6) |  | 48.5% |  | 55.0% |  |
| Longer than median | 211 (48.5) | 82 (41.4) |  | 26 (12.3) |  | 56.7% |  | 61.2% |  |
| Missing data | 17 (3.9) | 8 (47.1) |  | 0 (0) |  | 17.6% |  | 46.3% |  |
| pCR |  |  | 0.029 |  |  |  | 0.002 |  | 0.002 |
| No | 387 (89) | 154 (41.7) |  | 0 (0) |  | 47.4% |  | 55.1% |  |
| Yes | 48 (11) | 27 (58.7) |  | 48 (100) |  | 85.1% |  | 84.9% |  |
| Relapse |  |  | 0.895 |  | <0.001 |  | <0.001 |  |  |
| No | 284 (65.3) | 118 (43.4) |  | 42 (14.8) |  | 81.6% |  | - |  |
| Yes | 151 (34.7) | 63 (44.1) |  | 6 (4) |  | 8.58% |  | - |  |

TIL: Tumor-infiltrating lymphocytes; pCR: Pathological complete response; OS: Overall survival; DFS: Disease free survival; PgR: Progesterone; TLCS: Time-From-Last-Chemotherapy-To-Surgery.

**Table 2 Association between response and Clinical-pathological features regarding molecular subtype**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Lum A** | **Lum B** | **HER2** | **TN** |
|  | **Total** | **pCR** |  | **Total** | **pCR** |  | **Total** | **pCR** |  | **Total** | **pCR** |  |
|  | ***n* = 107 (%)** | ***n* = 13 (%)** | ***P*** | ***n* = 165 (%)** | ***n* = 12 (%)** | ***P*** | ***n* = 77 (%)** | ***n* = 10 (%)** | ***P*** | ***n* = 86 (%)** | ***n* = 13 (%)** | ***P*** |
| Age (yr) |  |  | 1.000 |  |  | 0.315 |  |  | 0.507 |  |  | 0.157 |
| median (range) | 47 (28-75) | 46 (28-62) |  | 51 (25-84) | 52 (39-69) |  | 51 (28-80) | 46 (29-80) |  | 49 (26-73) | 45 (28-68) |  |
| < 50 | 72 (67) | 9 (13) |  | 78 (48) | 4 (5) |  | 37 (48) | 6 (16.2) |  | 44 (48) | 9 (20) |  |
| ≥ 50 | 35 (33) | 4 (11) |  | 87 (52) | 8 (9) |  | 40 (52) | 4 (10) |  | 42 (52) | 4 (10) |  |
| Histological subtypes |  |  | 0.349 |  |  | 1.000 |  |  | 0.434 |  |  | 0.392 |
| Ductal | 97 (91) | 11 (11) |  | 153 (93) | 11 (7) |  | 73 (95) | 9 (12.3) |  | 83 (97) | 12 (14) |  |
| Lobular and others | 10 (9) | 2 (20) |  | 12 (7) | 1 (8) |  | 4 (5) | 1 (25) |  | 3 (3) | 1 (33) |  |
| Histological grade |  |  | - |  |  | 0.213 |  |  | 0.266 |  |  | 1.000 |
| G1-G2 | 103 (97) | 12 (12) |  | 61 (39) | 2 (3) |  | 23 (30) | 1 (4.3) |  | 13 (15) | 2 (15) |  |
| G3 | - | - |  | 102 (61) | 10 (10) |  | 53 (69) | 9 (17) |  | 72 (85) | 10 (14) |  |
| NR | 4 (3) | 1 (25) |  | 2 (1) | 0 (0) |  | 1 (1) | 0 (0) |  | 1 (0) | 1 (100) |  |
| Tumor size (cm) |  |  | 0.102 |  |  | 0.213 |  |  | 0.511 |  |  | 0.620 |
| Median (range) | 6 (2-15) | 5 (2-9) |  | 7 (2-20) | 6 (2-12) |  | 7 (2.5-14) | 6 (4-12) |  | 7 (1-24) | 8 (3-15) |  |
| cT1-cT2 | 10 (7) | 3 (30) |  | 12 (7) | 2 (17) |  | 5 (6) | 1 (20) |  | 9 (10) | 2 (22) |  |
| cT3-cT4 | 97 (93) | 10 (10) |  | 153 (93) | 10 (7) |  | 72 (94) | 9 (12.5) |  | 77 (90) | 11 (14) |  |
| cN |  |  | 0.306 |  |  | 0.222 |  |  | 0.270 |  |  | 0.0213 |
| cN0 | 27 (23) | 5 (19) |  | 28 (18) | 0 (0) |  | 53 (69) | 5 (9.4) |  | 14 (14) | 4 (29) |  |
| cN1-cN2–cN3 | 80 (77) | 8 (10) |  | 137 (82) | 12 (9) |  | 24 (31) | 5 (20.8) |  | 72 (86) | 9 (13) |  |
| Clinical stage |  |  | 0.471 |  |  | 0.652 |  |  | 1.000 |  |  | 0.122 |
| EC II | 23 (20) | 4 (17) |  | 21 (12) | 2 (10) |  | 11 (14) | 1 (9.1) |  | 17 (16) | 5 (29) |  |
| EC III | 84 (80) | 9 (11) |  | 144 (88) | 10 (7) |  | 66 (86) | 9 (13.6) |  | 69 (84) | 8 (12) |  |
| sTIL% |  |  | 0.054 |  |  | 0.750 |  |  | 0.150 |  |  | 1.000 |
| Median (range) | 30 (2-90) | 50 (10-90) |  | 40 (5-90) | 30 (8-90) |  | 60 (5-95) | 80 (30-95) |  | 45 (2-90) | 50 (5-80) |  |
| <50 | 71 (69) | 6 (8) |  | 98 (60) | 6 (6) |  | 25 (32) | 1 (4) |  | 40 (47) | 6 (15) |  |
| >=50 | 30 (24) | 7 (23) |  | 61 (37) | 5 (8) |  | 50 (66) | 9 (18) |  | 40 (47) | 6 (15) |  |
| Missing data | 6 (6) | 0 (0) |  | 6 (3) | 1 (17) |  | 2 (3) | 0 (0) |  | 6 (7) | 1 (17) |  |
| TLCS (d) |  |  | 0.233 |  |  | 0.238 |  |  | 0.744 |  |  | 0.500 |
| Median (range) | 67 (14-458) | 80 (16-281) |  | 61 (5-412) | 54 (8-140) |  | 60 (11-240) | 66 (37-106) |  | 64 (8-982) | 66 (14-122) |  |
| Shorter than median | 49 (48) | 4 (8) |  | 77 (45) | 8 (10) |  | 41 (53) | 5 (12.2) |  | 40 (48) | 5 (13) |  |
| Longer than median | 57 (51) | 9 (16) |  | 76 (47) | 4 (5) |  | 33 (43) | 5 (15.2) |  | 45 (51) | 8 (18) |  |
| Missing data | 1 (1) | 0 (0) |  | 12 (8) | 0 (0) |  | 3 (4) | 0 (0) |  | 1 (1) | 0 (0) |  |
| Relapse |  |  | 0.121 |  |  | 0.753 |  |  | 0.300 |  |  | <0.001 |
| No | 87 (79) | 13 (15) |  | 109 (65) | 9 (8) |  | 46 (60) | 8 (17.4) |  | 42 (41) | 12 (29) |  |
| Yes | 20 (21) | 0 (0) |  | 56 (35) | 3 (5) |  | 31 (40) | 2 (6.5) |  | 44 (59) | 1 (2) |  |

TIL: Tumor-infiltrating lymphocytes; TLCS: Time-From-Last-Chemotherapy-To-Surgery.

**Table 3 Association between percentage of tumor-infiltrating lymphocytes and clinical-pathological features regarding molecular subtype**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Lum A** | **Lum B** | **HER2** | **TN** |
|  | **< 50%** | ≥ **50%** | ***P*** | **< 50%** | ≥ **50%** | ***P*** | **< 50%** | ≥ **50%** | ***P*** | **< 50%** | ≥ **50%** | ***P*** |
|  | ***n* = 71 (%)** | ***n* = 30 (%)** | ***n* = 98 (%)** | ***n* = 61 (%)** | ***n* = 25 (%)** | ***n* = 50 (%)** | ***n* = 40 (%)** | ***n* = 40 (%)** |
| Age (yr) |  |  | 0.181 |  |  | 0.783 |  |  | 0.624 |  |  | 0.074 |
| Median (range) | 47 (28-75) | 47 (36-74) |  | 52 (28-73) | 50 (25-84) |  | 52 (28-66) | 49 (29-80) |  | 51 (26-73) | 45 (27-73) |  |
| < 50 | 50 (70) | 17 (57) |  | 46 (47) | 30 (49) |  | 11 (44) | 25 (50) |  | 16 (40) | 24 (60) |  |
| ≥ 50 | 21 (30) | 13 (43) |  | 52 (53) | 31 (51) |  | 14 (56) | 25 (50) |  | 24 (60) | 16 (40) |  |
| Histological subtypes |  |  | 0.445 |  |  | 1.000 |  |  | 0.597 |  |  | 1.000 |
| Ductal | 66 (93) | 26 (87) |  | 91 (93) | 57 (93) |  | 23 (92) | 48 (96) |  | 39 (98) | 38 (95) |  |
| Lobular and others | 5 (7) | 4 (13) |  | 7 (7) | 4 (7) |  | 2 (8) | 2 (4) |  | 1 (3) | 2 (5) |  |
| Histological grade |  |  | - |  |  | 0.011 |  |  | 0.514 |  |  | 0.006 |
| G1-G2 | 69 (97) | 28 (93) |  | 43 (44) | 15 (25) |  | 9 (36) | 14 (28) |  | 11 (28) | 2 (5) |  |
| G3 | 0 (0) | 0 (0) |  | 53 (54) | 46 (75) |  | 16 (64) | 35 (71) |  | 29 (73) | 38 (95) |  |
| NR | 2 (3) | 2 (7) |  | 2 (2) | 0 (0) |  | 0 (0) | 1 (2) |  | 0 (0) | 0 (0) |  |
| Tumor size (cm) |  |  |  |  |  |  |  |  |  |  |  |  |
| Median (range) | 6 (3-13) | 6 (2-15) |  | 6 (3-20) | 7 (2-15) |  | 7 (3-14) | 7 (3-14) |  | 7 (4-24) | 7 (1-16) |  |
| cT |  |  | 1.000 |  |  | 0.538 |  |  | 0.659 |  |  | 0.263 |
| cT1-cT2 | 7 (10) | 3 (10) |  | 6 (6) | 6 (10) |  | 1 (4) | 4 (8) |  | 2 (5) | 6 (15) |  |
| cT3-cT4 | 64 (90) | 27 (90) |  | 92 (94) | 55 (90) |  | 24 (96) | 46 (92) |  | 38 (95) | 34 (85) |  |
| cN |  |  | 0.890 |  |  | 0.02 |  |  | 0.631 |  |  | 0.762 |
| cN0 | 18 (25) | 8 (27) |  | 22 (22) | 5 (8) |  | 6 (24) | 8 (16) |  | 6 (15) | 7 (18) |  |
| cN1-cN2-cN3 | 53 (75) | 22 (73) |  | 76 (78) | 56 (92) |  | 11 (44) | 27 (54) |  | 34 (85) | 33 (83) |  |
| Clinical Stage |  |  | 0.666 |  |  | 0.141 |  |  | 0.742 |  |  | 0.576 |
| EC II | 17 (24) | 6 (20) |  | 16 (16) | 5 (8) |  | 3 (12) | 8 (16) |  | 9 (23) | 7 (18) |  |
| EC III | 54 (76) | 24 (80) |  | 82 (84) | 56 (92) |  | 22 (88) | 42 (84) |  | 31 (78) | 33 (83) |  |
| TLCS (d) |  |  | 0.631 |  |  | 0.882 |  |  | 0.502 |  |  | 0.141 |
| Median (range) | 64 (14-449) | 70 (19-458) |  | 61 (5-412) | 58 (8-285) |  | 68 (16-234) | 56 (11-240) |  | 74 (24-230) | 51 (14-982) |  |
| Shorter than median | 34 (48) | 13 (43) |  | 48 (49) | 28 (46) |  | 12 (48) | 28 (56) |  | 15 (38) | 22 (55) |  |
| Longer than median | 36 (51) | 17 (57) |  | 44 (45) | 27 (44) |  | 12 (48) | 20 (40) |  | 24 (60) | 18 (45) |  |
| Missing data | 1 (1) | 0 (0) |  | 6 (6) | 6 (10) |  | 1 (4) | 2 (4) |  | 1 (3) | 0 (0) |  |
| pCR |  |  | 0.054 |  |  | 0.750 |  |  | 0.150 |  |  | 1.000 |
| No | 65 (92) | 23 (77) |  | 92 (94) | 56 (92) |  | 24 (96) | 41 (82) |  | 34 (85) | 34 (85) |  |
| Yes | 6 (8) | 7 (23) |  | 6 (6) | 5 (8) |  | 1 (4) | 9 (18) |  | 6 (15) | 6 (15) |  |
| Relapse |  |  | 0.450 |  |  | 0.201 |  |  | 0.737 |  |  | 0.502 |
| No | 59 (83) | 23 (77) |  | 61 (62) | 44 (72) |  | 16 (64) | 30 (60) |  | 18 (45) | 21 (53) |  |
| Yes | 12 (17) | 7 (23) |  | 37 (38) | 17 (28) |  | 9 (36) | 20 (40) |  | 22 (55) | 19 (48) |  |
| %sTIL was performed over 415 cases. There 20 missed values. TIL: Tumor-infiltrating lymphocytes; TLCS: Time-From-Last-Chemotherapy-To-Surgery.  |