# Artificial intelligence (AI) based image analysis of PD-L1, TIL, immune signature for prediction of long term outcomes of patients who received neoadjuvant chemotherapy in breast cancer

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## Introduction

Tumor-infiltrating lymphocytes (TILs) and tumor-stroma ratio (TSR) have been recognized as prognostic and predictive biomarkers in breast cancer. However, challenges such as interobserver variability and lack of standardized cut-off values have limited their clinical implementation. Recent advancements in artificial intelligence (AI)-based pathology have shown potential to enhance reproducibility and objectivity in TIL quantification. This study aims to: (1) evaluate the predictive value of AI-quantified stromal TILs from H&E-stained slides; and (2) validate the clinical utility of TIL biomarkers long term prognosis in breast cancer patients after NAC.

## Methods and Materials

TILs were assessed from pre-treatment tumor H&E slides using a validated AI-powered WSI analysis tool. Immune phenotypes were classified as inflamed, immune-excluded, or desert. TIL cut-off was set at 30%. Formalin-fixed paraffin-embedded samples (FFPEs) were obtained from biopsy tissue, and plasma samples were collected at baseline, pre-surgery (after NAC). Forty-seven breast cancer-related genes were analyzed using next-generation sequencing. The diagnostic performance of TIL and TSR was evaluated, and logistic regression analyses were conducted to assess the impact of clinical and molecular factors on TIL levels.

**Table 1.** Baseline patient characteristics

| Patients' characteristics | Total patients (N=101) | |
|---|---|---|
| | Patients with genetic work-up for FFPE and baseline ctDNA (N=99) | Patients with genetic work-up for baseline and post-NAC ctDNA (N=45) |
| Demographics | | |
| Female (%) | 99 (100%) | 45 (100%) |
| Age, years (median [range]) | 51 (32–73) | 51 (32–73) |
| Histologic findings | | |
| Tumor size, mm (median [IQR]) | 40.0 (27.0–60.0) | 40.0 (28.0–60.0) |
| Immunohistochemical subtypes (%) | | |
| HR+/HER2- | 77 (24.6%) | 22 (26.7%) |
| HR+/HER2+ | 64 (20.1%) | 29 (34.1%) |
| HR-/HER2+ | 15 (14.8%) | 11 (13.3%) |
| HR-/HER2- | 24 (34.8%) | 30 (36.1%) |
| unknown | 1 (1.4%) | -- |
| Ki-67 index, % (median [IQR]) | 35.5 (17.9–44.1) | 30.2 (18.5–46.3) |
| Lymph node metastasis (%) | 69 (71.8%) | 60 (72.3%) |
| Pathological complete remission (%) | 29 (40.3%)* | 31 (37.3%) |
| Samples with mutated gene | | |
| PIK3R1 | 65 (64.2%) | -- |
| Baseline ctDNA | 43 (42.2%) | 48 (57.8%) |
| Post-NAC ctDNA | -- | 22 (26.5%) |

**Figure 1. TSR and TIL by recurrence status**

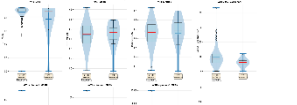
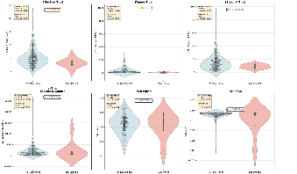


**Figure 2. TIL analysis by recurrence vs non-recurrence**



## Conclusions

Reduced lymphocytic infiltration, especially tumor-specific eTILs, is associated with reoccrence and may reflect impaired anti-tumor immune response. Although none of the markers reached statistical significance due to small smaple size, the consistent directional trends across all TIL markers-but not TSR- suggest that immune microenvironment characteristics may be more informative than stromal proportion alone in predicting recurrence risk

## Results and Discussion

A total of 101 patients with EBC were enrolled. All patients were female, with a median age of 51 years (range, 32–73). Median tumor size was 40.0 mm (interquartile range [IQR], 27.0–60.0 mm). IHC subtype results categorized 17 patients (24.6%) as HR +/ HER2 -, 14 patients (20.3%) as HR +/HER2 +, and 24 patients (34.8%) as HR-/HER2- (triple-negative breast cancer, TNBC). Median Ki-67 index was 31.5% (IQR, 17.9%–44.1%). Also, 83 patients underwent genetic work-up for baseline and post-NAC ctDNA, and 31 (37.3%) showed pCR after NAC.

TSR values were high in both groups (>0.60) and did not significantly differ between recurrence and non-recurrence cohorts. eTIL and sTIL markers showed lower medians in recurrence, indicating a potential association between diminished lymphocyte infiltration and poorer outcomes. eaTILs (cell density) differences were more pronounced, with recurrence cases showing substantially lower immune-cell density (Figure 1).

Across, all TIL-related features (eTILS, eTILs, esTILs) recurrence cases consistently demonstrated lower immune infiltration, with eaTILs showing the strongest rend toward statistically significance (p= 0.05. In contrast, TSR did not differ between groups and showed no statistical difference (all P >0.45) (Figure 2).

Aggressive tumor phenotypes (ER-, PR-, higher stage) tend to have higher TSR values, reflecting greater stromal enrichment. TIL markers (eTILs, esTILs, eaTILs) are consistently elevated in ER- and PR- subtype, supporting their known immunogenicity. Absolute TIL density (eaTILs) diminishes with advancing T or N stage, even when percentage-based TIL measure remain elevated, suggesting structural or stromal constraints in later-stage disease (Figure 3).

2.1 Association with Distant Metastasis

eTILs and esTILs showed the strongest associations, with –log10(p-value = 1.25–1.30), corresponding to p ≈ 0.045–0.055, approaching or meeting the p < 0.05 threshold. These results suggest that higher TIL density is significantly associated with distant metastasis, with eTILs showing the most pronounced signal among TIL variables.

2.2 Association with Recurrence

eTILs also demonstrated near-significant or significant association with recurrence (–log10(p-value > 1.2; p ≈ 0.05). esTILs and eaTILs showed weaker but still notable effects. These findings support the role of TIL burden as a potential predictor of recurrence risk.

2.3 Association with T and N Stage

For T and N stage, TIL variables generally fell in the –log10(p-value 0.4–0.8) range, not reaching the p < 0.1 threshold. Although nonsignificant, these comparisons consistently showed greater variability and stronger trends than TSR. This pattern suggests that TIL levels may be more responsive to advanced clinical events (recurrence, metastasis) than to anatomical staging.(Figure 4).

**Figure 3. Comprehensive comparison of TSR and TIL across Prognostic variables**

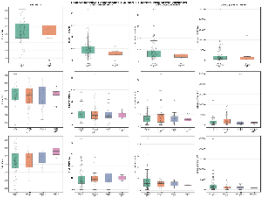
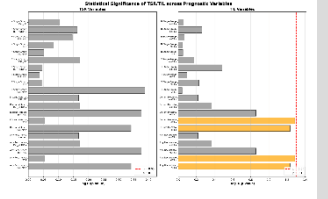


**Figure 4. TSR and TIL score across prognostic variables**



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