eISSN 2255-0569

ORIGINAL

Tumor infiltrating lymphocytes density as the chemoresistance and relapse factor for advanced breast cancer patients

Densidad de linfocitos infiltrantes tumorales como factor de quimiorresistencia y recaída en pacientes con cáncer de mama avanzado

Nataliia Babchenko[1], Oleksii Volodimirovich Movchan[2], Ivan Ivanovich Smolanka[2], Andriy Oleksandrovich Lyashenko[2], Anton Dmitrovich Loboda[2], Dosenko Irina Viktorivna[2], Ivankova Oksana Mykolaivna[2]

1. Otorhinolaryngology department, Bogomolets National Medical University, Kyiv,
2. State Non-Commercial Enterprise "National Cancer Institute of Ukraine" of Ministry of Health of Ukraine, Kyiv, Department of breast cancer and reconstructive surgery, Ukraine

Corresponding author
Nataliia Babchenko
E-mail: nazonex27@gmail.com

Received: 25 - X - 2023
Accepted: 27 - XI - 2023

doi: 10.3306/AJHS.2024.39.02.123

Abstract

Introduction: Recent studies on more reliable predictors, such as TIL density and spatial localization, are associated with clinical and pathological features.
Aim: - to improve comprehensive treatment of advanced breast cancer patients by focusing on tumor infiltrating lymphocytes density.
Methods: We examined TILs in 150 advanced breast cancer (ABC) and non-ABC patients, focusing on their spatial distribution in the tumor.
Results: TIL decline following neo-adjuvant chemotherapy (NC) is associated with the ABC (OR: 0.25, 95% CI: 0.013-0.56, p = 0.018). The difference in peripheral immunological markers between ABC and non-ABC was not statistically significant. 75 individuals had remaining status after NC. In this group, having a low number of TIL before NC (HR: 0.23, 95% CI: 0.05-1.02, p = 0.05) was associated with a longer OS, whereas having a high number of TIL after NC (HR: 0.29, 95% CI: 0.10-0.97, p = 0.047) and a low answer of cancer cell to therapy (HR: 0.20, 95% CI: 0.11-0.98, p = 0.044) (RFS), that leaded to chemoresistance and relapses.
Conclusions: ABC patients with a higher number of TIL following NC associated with a poor outcome. The quantity of TIL was considerably decreased following NC in both groups.

Key words: Breast cancer, tumor-infiltrating lymphocytes, chemoresistance, recurrence.

Resumen

Introducción: Estudios recientes sobre predictores más fiables, como la densidad de TIL y la localización espacial, se asocian con características clínicas y patológicas.
Objetivo: mejorar el tratamiento integral de las pacientes con cáncer de mama avanzado centrándose en la densidad de linfocitos infiltrantes tumorales.
Metodología: Examinamos los TIL en 150 pacientes con cáncer de mama avanzado (ABC) y no ABC, centrándonos en su distribución espacial en el tumor.
Resultados: La disminución de TIL tras la quimioterapia neoadyuvante (NC) está asociada al ABC (OR: 0,25; IC 95%: 0,013-0,56; p = 0,018). La diferencia en los marcadores inmunológicos periféricos entre ABC y no ABC no fue estadísticamente significativa. 75 individuos tenían estado remanente después de NC. En este grupo, tener un número bajo de TIL antes de la NC (HR: 0,23; IC 95%: 0,05-1,02; p = 0,05) se asoció con una SG más larga, mientras que tener un número alto de TIL después de la NC (HR: 0,29; IC 95%: 0,10-0,97; p = 0,047) y una baja respuesta de las células cancerosas a la terapia (HR: 0,20; IC 95%: 0,11-0,98; p = 0,044) (RFS), que condujo a quimiorresistencia y recaídas.
Conclusiones: Los pacientes con ABC con un mayor número de TIL tras la NC se asociaron a un mal pronóstico. La cantidad de TIL disminuyó considerablemente tras la NC en ambos grupos.

Palabras clave: Cáncer de mama, linfocitos infiltrantes de tumores, quimiorresistencia, recurrencia.

Cite as: Babchenko N, Movchan OV, Smolanska II, Lyashenko AO, Loboda AD, Viktorivna DI, Mykolaivna IO. Tumor infiltrating lymphocytes density as the chemoresistance and relapse factor for advanced breast cancer patients. *Academic Journal of Health Sciences 2024*; 39 (2):123-129 doi: 10.3306/AJHS.2024.39.03.123

Introduction

Although the clinical outcomes of most breast cancer subtype have improved, the prognosis for advanced breast cancer (ABC) remains poor, with a 5-year overall survival rate of 39%[1] and recurrence-free survival 21% and chemoresistance rate about 34 %[2].

Current treatment guidelines for stage III ABC recommend preoperative chemotherapy followed by surgical resection and adjuvant radiation therapy[3]. Currently, the National Cancer Network (NCCN) guidelines recommend that patients who respond appropriately to neo-adjuvant chemotherapy (NC) undergo total mastectomy with level I/II axillary lymph node dissection[4]. ABC patients have an approximate 43% increased risk of death compared to those with non-ABC[5]. There is increasing evidence that the tumor microenvironment (TME) is of paramount importance in ABC pathological biology. However, despite this well-documented association, the composition of ABC TME is very poorly described, especially when compared to other types of breast cancer. Histologically, ABC tumors show extensive involvement of lymph and blood vessels, cancer-related fibroblasts and their associated extracellular matrix, and invasive immune cells[6].

This suggests that ABC clinical and research studies need to consider TIL in addition to stage. This results in better results for patients with high TIL, than for patients with low TIL. Is an immune checkpoint molecule that is more abundant in both ABC tumor cells and TIL than non-ABC and is a potential predictive biomarker for immunotherapy[7].

Previous studies have shown that high numbers of tumor-infiltrating lymphocytes (TILs) do not consistently guarantee good results in all ABC patients. In Luminal HER2-negative patients, high TIL numbers are considered a prognostic factor that is detrimental to survival. TIL ought to be considered from an unused viewpoint in arrange to comprehensively get it the tumor microenvironment[8]. Recent studies on more reliable predictors, such as TIL density and spatial localization, are associated with clinical and pathological features. However, the association between clinical pathological features and para-neoplastic infiltrating lymphocytes (PIL) in the lobular region of para-neoplasm remains unclear[9].

Like other cancers, the immune infiltrate of the ABC TME appears to be heterogeneous. However, immune-related gene expression in ABC appears to be distinct from non-ABC tumors, independent of molecular subtype. To date, most studies have focused on the resident lymphocytes of ABC tumors. For instance, it has been reported that up to 41% of ABC tumors harbor dense aggregates of CD8+ cytotoxic T-lymphocytes. This was paralleled by a recent, larger study of 150 treatment naïve ABC patients, which reported that, on average, tumor infiltrating lymphocytes (TILs) comprise roughly 18% of the tumor stroma. Tumors with stroma composed of more than 10% leukocytes had a significant life-prolonging effect. This study also looked at molecular subtypes and investigated the status of hormone receptors and HER2. There was no significant difference in TIL infiltration between ABC and non-ABC tumors in the luminal and triple negative subgroup, but HER2-positive ABC tumors had TIL compared to HER2-positive non-ABC tumors[10].

Here we summarize the clinical evidence that explains the potential role of TIL in the pathological biology of ABC and past and present attempts to improve the treatment of ABC. Discuss the current status of ABC TME, where increased presence of TIL is associated with better response to chemotherapy. This decisively suggests TIL density as an important prognostic factor.

Materials and methods

The SNE "National Cancer Institute of Ukraine's" institutional Ethics Committee authorized this study (Minutes No. 221 of December 23, 2020).

We presented findings from a study of ABC patients who were diagnosed and treated at the SNE "National Cancer Institute of Ukraine" between December 31, 2020, and December 31, 2022. In this study, we looked at all stage patients (n = 150) - the first group (75 patients) had ABC; the second group (75 patients) with non-ABC. Examine the progression of TIL between ABC and non-ABC. Anthracycline/taxane-based neo-adjuvant chemotherapy (NC) followed by a lumpectomy or mastectomy was also required for participation. Trastuzumab was given to HER2 positive individuals (n = 26 non-ABC/31 ABC).

Results based on clinical data, biomarker data, and surgical and resected tissue discoveries.

The estimated 2-year overall survival (OS) and recurrence-free survival (RFS) values are reported, with statistical confidence intervals (CI). TILs were evaluated for prognostic significance in patients with ABC who were treated with chemotherapy in the neo-adjuvant context using the technique for reading TILs (as per International Immune-Oncology Biomarker Working Group criteria)[11].

Three distinct researchers scored the TIL following NC on hematoxylin and eosin stained 5-mm slices of formalin-fixed paraffin-embedded tumor tissue. The International TILs Working Group's precise guidelines for rating TIL in remaining tumor tissue following neo-adjuvant treatment were followed[12]. With hematoxylyn and eosin slides containing the most invasive tumors surviving, a semi-quantitative evaluation of the fraction of stromal compartments impacted by TIL in all locations harboring invasive tumor cells was done. TIL was also assessed in the tumor bed for all ABC patients with pCR.

TIL had an interclass correlation coefficient (ICC) of 0.827 (95% CI: 0.776-0.882, p < 0.001), indicating high agreement. The mean was computed and utilized as a continuous and categorical variable: 10% (category 1), 10%-40% (category 2), and 40% (category 3). After speaking with additional pathologists, a consensus score was produced if the results differed. Cell counts were reported: the proportion of remaining tumor beds occupied by invasive cancer cells following microscopic examination of slides containing the greatest number of remaining tumors and also analyzed by TIL. Estrogen (ER) and progesterone receptor (PR) expression were measured using approved immune-histo-chemical techniques and were considered positive if the Alfred score was 3/8 or higher. When a fluorescence in situ hybridization (FISH) test revealed amplification, tumor samples were designated HER2-positive.

Statistical Analysis

A Mann-Whitney U test for continuous parameters was used to compare the two ABC and non-ABC groups. A Paired Wilcoxon signed-rank test was used to compare the evolution of the parameters before and after neo-adjuvant chemotherapy. A multivariate logistic regression model was built using significant parameters from univariate analysis. The median value was utilized for distribution, and two survival endpoints were measured: The time between pathological diagnosis and cancer recurrence is defined as recurrence-free survival (RFS), while the interval between pathological diagnosis and death is defined as overall survival (OS). Patients who had not relapsed or died at the time of analysis were censored at the date of their final follow-up visit, with the survival data last updated on December 31st 2022.

The log-rank test was used to compare survival curves computed by Kaplan-Meier. A multivariate analysis proportional hazard model was used to assess the impact of all important clinical and pathological variables on survival. When p-values were less than 0.05, they were considered statistically significant.

Overall survival curves of advanced breast cancer patients treated with neo-adjuvant chemotherapy, according on pathological prognostic stage and TILs.

Results

Tumor samples were considered HER2-positive (+) when a fluorescence in situ hybridization (FISH) test documented amplification. Systemic therapy altered over the research period, however the majority of HER2+ patients (n = 26 non-ABC/31 ABC) received target therapy. The absence of carcinoma in the resected breast material and all collected regional lymph nodes following completion of neo-adjuvant chemotherapy was classified as partial or complete response (PCR).

Table I describes the tumor features. The majority of the ABC patients had a hormone receptor (HR) positive malignancy (n = 39, 52.3%), and 50 exhibited PCR following neo-adjuvant treatment (75.0%). Aside from having more poorly differentiated tumors (p = 0.001) and a higher stage (p < 0.001), which are inherent to the classification of ABC, no significant clinical and pathological changes were detected between the ABC and non-ABC cohorts.

Evolution of TIL after neo-adjuvant chemotherapy

Following neo-adjuvant chemotherapy, the amount of TIL was reduced in both the ABC (median TIL: 11%, p = 0.002) and non-ABC (median TIL: 12.5%, p = 0.007) groups, however the decline was considerably greater in the ABC cohort (p = 0.039). **Figure 1**.

Table I: Clinical and Pathological indicators non-ABC and ABC patients.

Indicators	non-ABC	ABC	p
Hormonal receptor status			**0.48**
Negative	34	31	
Positive	41	46	
HER2 status			0.89
Negative	49	44	
Positive	26	31	
Neo-adjuvant chemotherapy response			0.78
RS	26	25	
PCR	49	50	
Before neo-adjuvant chemotherapy TIL			0.24
<12.5%	32	27	
≥12.5%	43	48	
After neo-adjuvant chemotherapy TIL in RS (%)			0.52
<5%	42	54	
≥5%	33	21	
Difference in TIL before and after neo-adjuvant chemotherapy			0.022
Increase (>5%)	6	7	
No change	60	50	
Decrease (<5%)	9	18	

non-ABC-non-advanced breast cancer, ABC - advanced breast cancer, RS – remaining status, pCR – complete (partial) pathological response. Bold values denote statistical significance at the p < 0.05 level.

ABC patients showed a larger decline in TILs following NC (OR: 0.24, 95% CI: 0.21-0.66, p = 0.016) in a multivariate model containing all patients. A higher decline in TIL was related with a high number of TIL before to NC (OR: 0.029, 95% CI: 0.0018-0.175, p = 0.002) and a low number of TIL after NC (OR: 23.12, 95% CI: 3.57-489.76, p = 0.008) in a model including just ABC patients – **Table II**.

Figure 1: Evolution of TIL after neo-adjuvant chemotherapy.

Figure 1 (A): Boxplot graph of the evolution of TIL after neo-adjuvant chemotherapy: In both ABC (median TIL: 11%, p < 0.001) and non-ABC (median TIL: 12,5%, p = 0.06) the number of TIL are lower after neo-adjuvant chemotherapy.

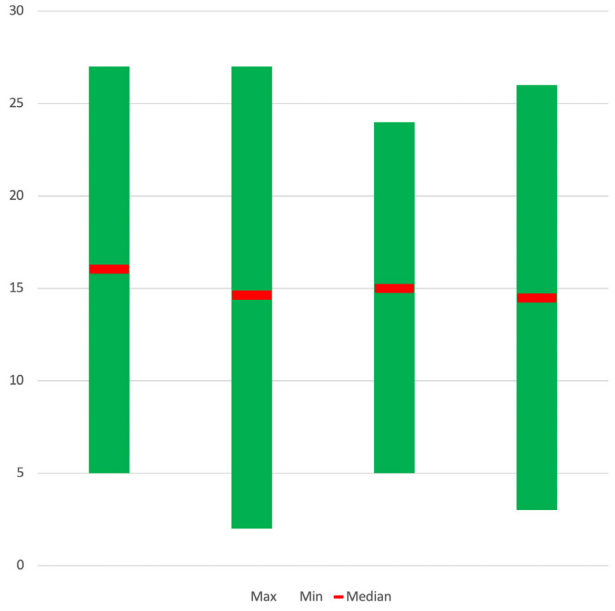
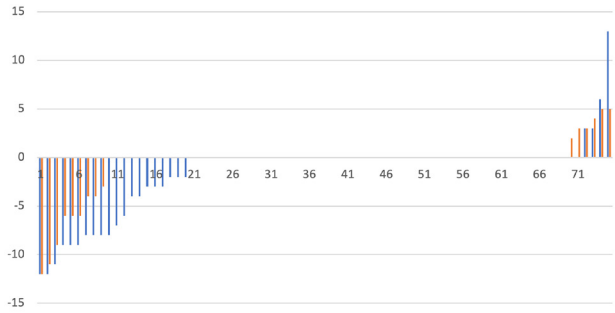


Figure 1 (B): ABC Non-ABC



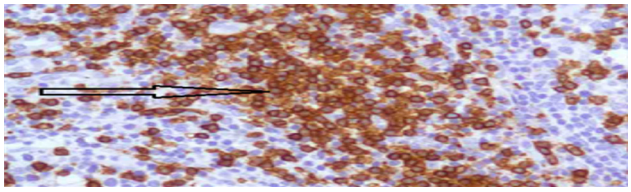
In the overall cohort (including both ABC and non-ABC patients) the number of TIL after NC seemed to largely depend on the number of TIL (OR: 2.023, 95% CI: 1.18–3.125, p = 0.04) and the remaining cancer cell count (OR: 2.05, 95% CI: 1.60-6.98, p = 0.004) -**Table II**.

In the ABC cohort, the number of TIL after neo-adjuvant chemotherapy was only significantly associated with higher remaining cancer cell count (OR: 12.64, 95% CI: 4.88-15.65, p < 0.001) - **Picture 1a** and **1b** and worse clinical outcome (RECIST 1.1 – stabilization (without regression) after NC – **Picture 2**.

TIL decline following NC is associated with the ABC (OR: 0.25, 95% CI: 0.013-0.56, p = 0.018).

The difference in peripheral immunological markers between ABC and non-ABC was not statistically significant. 75 individuals had remaining status after NC. In this group, having a low number of TIL before NC (HR: 0.23, 95% CI: 0.05-1.02, p = 0.05) was associated with a longer OS, whereas having a high number of TIL after NC (HR: 0.29, 95% CI: 0.10-0.97, p = 0.047) and a low answer of cancer cell to therapy (HR: 0.20, 95% CI: 0.11-0.98, p = 0.044) (RFS) - **Figure 2a**; **b**.

Picture 1a: Cell counts were reported: the proportion of remaining tumor beds occupied by invasive cancer cells following microscopic examination of slides containing the greatest number of remaining tumors and also analyzed by TILs before neo-adjuvant chemotherapy.



Picture 1b: Cell counts were reported: the proportion of remaining tumor beds occupied by invasive cancer cells following microscopic examination of slides containing the greatest number of remaining tumors and also analyzed by TILs after neo-adjuvant treatment and a low answer of cancer cell to therapy..

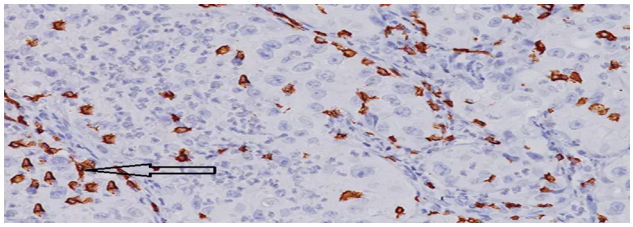


Table II: Uni- and multivariate analysis for decrease of TIL after neo-adjuvant chemotherapy HR - hormone receptor status, Bold values denote statistical significance at the p < 0.05 level.

Indicators	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Non-ABC opposite ABC	0.22 (0.162–0.583)	**<0.001**	0.235 (0.0217–0.759)	**0.02**
TIL before neo-adjuvant chemotherapy: <12.5% opposite ≥12.5%	0.079 (0.047–0.232)	**<0.003**	0.028 (0.014–0.087)	**<0.001**
TIL after neo-adjuvant chemotherapy: <5% opposite ≥5%	2.023 (1.18–3.125)	**0.04**	12.64 (4.88–15.65)	**<0.001**
Remaining cancer cell count: <20% opposite >20%	2.021 (1.60–4.31)	0.065		

Boxplot depicting the evolution of median TIL after neo-adjuvant chemotherapy in individual ABC patients and nom-ABC patients: Out of 75 patients, 7 had an increase, 50 had no change and 18 patients had a decrease; non-ABC, out of 75, 6 had an increase, 60 had no change and 9 patients had a decrease.

Picture 2: Spiral computer scan left breast gland: a – skin edema and umbilication, tumor 3 cm, before neo-adjuvant chemotherapy; b – remaining skin edema and umbilication, tumor 2 cm, after neo-adjuvant chemotherapy.

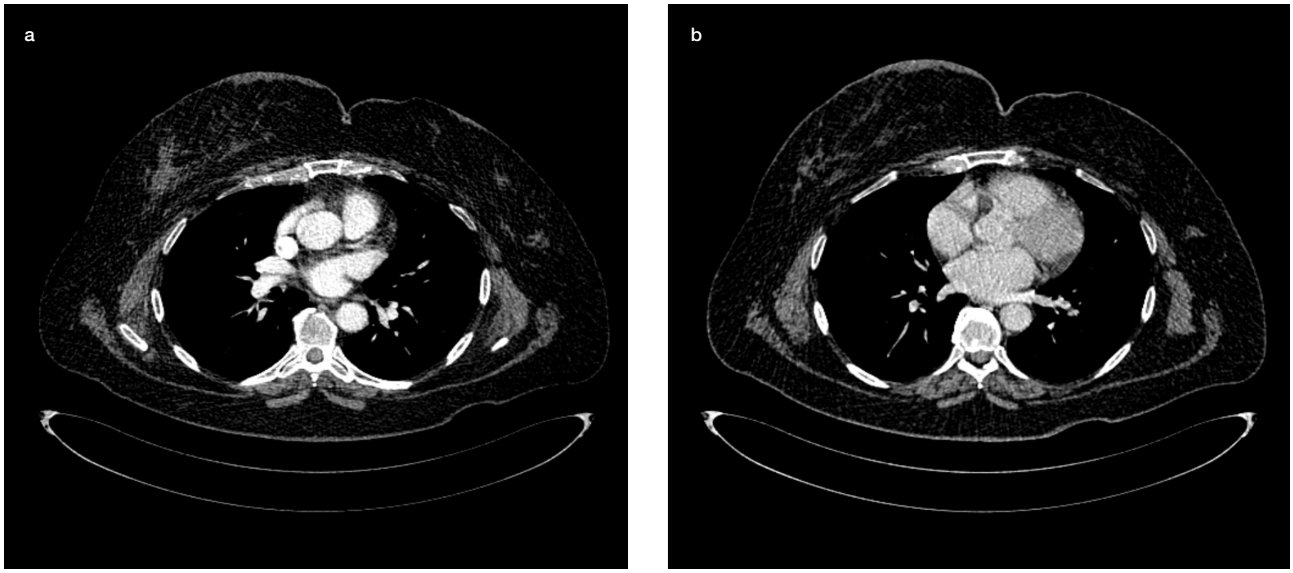
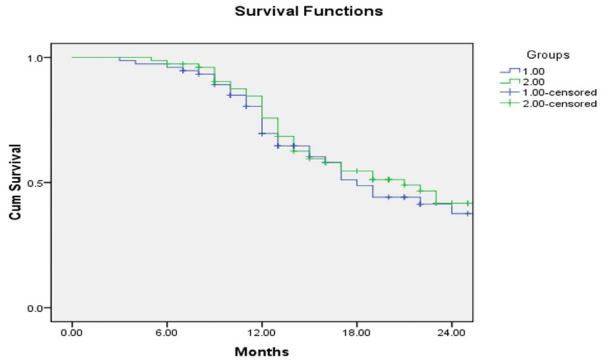
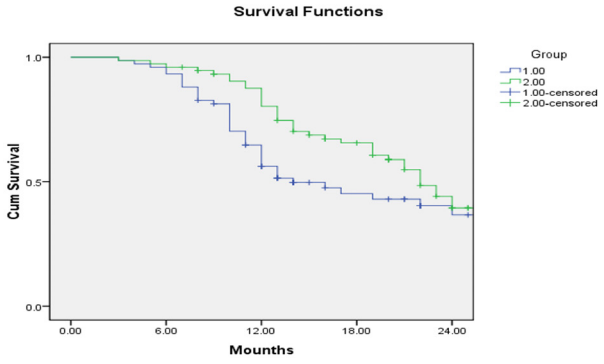


Figure 2: Kaplan–Meier curves for RFS.



A. Patients with ≥4% sTIL after neo-adjuvant chemotherapy have a significant shorter RFS: Median survival of 18.0 months (95% CI: 15.1–20.9) opposite 20.9 months (95% CI: 15.7–26.3), p = 0.002.



B.A higher remaining cell count (≥17.5%) in the tumor bed is associated with a shorter RFS: Median survival of 14,3 months (95% CI: 7.7–20.3) opposite21.8 months (95% CI: 19.4–24.6), p < 0.001.

In the ABC population, HR and HER2 status had no effect on OS or RFS.

A low TIL score before NC (p = 0.028) remained a significant predictor of longer OS in the HR+ group, while a larger remaining cancer cell count (p = 0.018) and a rise in TIL after NC (p = 0.033) were associated with shorter RFS. In the HER-positive group, an increase in TIL after NC was related with a lower RFS (p = 0.048), but only a lower pre-neo-adjuvant chemotherapy TIL was associated with a longer OS (p = 0.028) in the TN patients - **Table III**.

Table III: Uni- and multivariate analysis for RFS in the group of ABC patients without pCR after neo-adjuvant chemotherapy. pCR – complete (partial) pathological response.

Indicators	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p	HR (95% CI)	p
HR negative opposite HR positive	0.35 (0.24–1.14)	0.06		
HER2 negative opposite HER2positive	1.15 (0.53–1.85)	0.07		
TIL before neo-adjuvant chemotherapy: <10% opposite ≥10%	1.32 (0.74–1.91)	0.09		
TIL after neo-adjuvant chemotherapy: <4% opposite ≥4%	0.33 (0.15–0.98)	**0.003**	0.33 (0.15–0.98)	**0.003**
Remaining cell count: <17.5% opposite ≥17.5%	0.12 (0.05–0.29)	**<0.002**	0.24 (0.12–0.56)	**<0.002**
Change: increase opposite decrease	2.11 (0.93–3.23)	0.03	2.15 (0.85–4.23)	0.11

Discussion

Similarly, to the Ochi T study[13], we discovered that following neo-adjuvant treatment, TIL decreased in more patients. This was true in both the ABC (median TIL: 11%, p< 0.001) and non-ABC groups, although the median decline in the ABC cohort was larger (OR: 0.24, 95% CI: 0.21-0.66, p = 0.016). Following NC, a high number of TIL was associated with a high number of residual cancer cells in our ABC cohort, indicating that more surviving tumor cells may attract more infiltrating immune cells.

According to Vagia E[14], a larger number of TIL following neo-adjuvant treatment correlates with decreased tumor burden as measured by tumor size and nodal status. This might explain why, in several studies, a larger number of TIL was linked to a better prognosis, like Van Berckelaer C[15], notwithstanding our findings. Indeed, a greater number of TIL following NC was associated with a shorter OS (HR: 0.24, 95% CI: 0.04-1.12, p < 0.05) and RFS (HR: 0.33, 95% CI: 0.11-0.98, p = 0.046) in our ABC population.

While we found a link between cancer cell count and TIL count following neo-adjuvant treatment, both were independent prognostic indicators in the multivariate model. As a result, it appears that the predictive effect of TIL in our ABC cohort is more than just a reflection of tumor burden. The number of remaining tumor cells is not the only indicator of remaining tumor burden or response to neo-adjuvant chemotherapy, but chemoresistance and following relapses. According to Murthy R et al., a combination of RCB (residual cancer burden) and TIL is a more sensitive predictor of RFS than TIL alone[16].

TIL following NC had no effect on RFS in HER2+ BC, but had a borderline significant effect in TNBC, according to other researchers, so the HR state has an influence on the number of TIL - Zhang H[17], however in univariate analysis, we only found a relationship between HR status and a more TIL. Even though the patient numbers for the different genetic subtypes were limited in our study, an increase in TIL was related with a shorter RFS in both the HR+ and HER2+ ABC cohorts, but not in the TN ABC cohort. The immune response to the tumor is influenced by the composition of the immune infiltrate as well as the functional state of immune cells. According to Loi S et al [8] a large number of CD8+ cells is advantageous in terms of both chemotherapeutic response and survival.

Rufell et al.[18] demonstrated, for example, an increase in CD8+ cells and a decrease in CD20+ lymphocytes after chemotherapy. In the study of Gracia-Martinez et al.[19], patients with high TIL after neo-adjuvant chemotherapy had a worse RFS, which was partially explained by the presence of many CD68+ macrophages that have been associated with tumor progression[20]. The unfavorable predictive effect of TIL following neo-adjuvant chemotherapy, as well as the greater decline in TIL in ABC, might thus be explained by a distinct immune infiltrate composition in ABC compared to non-ABC illness. It is critical to do more study to investigate the makeup and role of the various immune cells in ABC[21].

The timing of surgery and the time since the previous chemotherapy session may alter the amount and composition of immune infiltrates, and hence the prognostic effect. In this study, we managed to explore the evolution of TIL ABC and compare this with a molecular subtype-matched cohort of non-ABC patients.

We showed that a low number of TIL after neo-adjuvant chemotherapy was associated with a longer RFS and that TIL tended to decrease in ABC compared to non-ABC.

There was neither significant difference in TIL score between ABC and non-ABC, nor the quantity of TIL was considerably decreased following NC in both groups.

Despite recent breakthroughs in other types of breast cancer, ABC continues to pose a considerable clinical challenge and is frequently resistant to standard therapy. Though immunotherapy has transformed the treatment paradigm for a variety of malignancies, such methods have yet to demonstrate significant therapeutic benefit in ABC. As previously stated, the reasons for immunotherapy's greater failure in ABC are unknown. Many of the same hurdles to treatment success that have hampered immune therapy in other breast tumors exist in ABC; nevertheless, there have been relatively few investigations investigating ABC's intrinsic resistance to such techniques. Furthermore, the immunological milieu of the ABC TME is still being explored, with several results that are very context-specific and even inconsistent.

This is an important and relatively unexplored area of breast cancer research that deserves more investigation, especially given that ABC has a far poorer prognosis than stage-matched non-ABC breast cancer[22].

Conclusions

ABC is linked with a considerably lower TIL following neo-adjuvant chemotherapy. In ABC patients with a higher number of TIL following neo-adjuvant treatment associated with a poor outcome.

There was no significant difference in TIL score between ABC and non-ABC, and the quantity of TIL was considerably decreased following neo-adjuvant chemotherapy in both groups.

Acknowledgments
This work is dedicated to our friend prof. Smolanka I.I., who is courageously fighting advanced breast cancer.

Conflict of Interest
None

References

1. Yang F, He Q, Dai X, Zhang X, Song D. The potential role of nanomedicine in the treatment of breast cancer to overcome the obstacles of current therapies. Front Pharmacol. 2023;14:1143102. doi:10.3389/fphar.2023.1143102. https://pubmed.ncbi.nlm.nih.gov/36909177/

2. Meegdes M, Geurts S, Erdkamp F, Dercksen M, Vriens B, Aaldering K et al. Real-world time trends in overall survival, treatments and patient characteristics in HR+/HER2- metastatic breast cancer: an observational study of the SONABRE Registry. Lancet Reg Health Eur. 2023;26:100573. doi:10.1016/j.lanepe.2022.100573. https://pubmed.ncbi.nlm.nih.gov/28870934/

3. Flood E, Krasnow A, Orbegoso C, Karantzoulis S, Bailey J, Bayet S, et al. Using qualitative interviews to identify patient-reported clinical trial endpoints and analyses that are the most meaningful to patients with advanced breast cancer. PLoS One. 2023;18(1):e0280259. doi: 10.1371/journal.pone.0280259 https://pubmed.ncbi.nlm.nih.gov/36649275/

4. Hyder T, Bhattacharya S, Gade K, Nasrazadani A, Brufsky A. Approaching Neoadjuvant Therapy in the Management of Early-Stage Breast Cancer. Breast Cancer (Dove Med Press). 2021; 13:199-211. https://doi.org/10.2147/BCTT.S273058

5. Cecil K, Huppert L, Mukhtar R, Dibble EH, O'Brien SR, Ulaner GA, et al. Metabolic Positron Emission Tomography in Breast Cancer. PET Clin. 2023 Jun 25:S1556-8598(23)00036-6. doi: 10.1016/j.cpet.2023.04.004. https://pubmed.ncbi.nlm.nih.gov/37369614/

6. Raman D, Cimpean AM, De Miglio MR. Editorial: Drug resistance in breast cancer-mechanisms and approaches to overcome chemoresistance. Front Oncol. 2023;12:1080684.doi:10.3389. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9847496/

7. Luen S, Salgado R, Dieci M, Vingiani A, Curigliano G, Gould RE, et al. Prognostic implications of remaining disease tumor-infiltrating lymphocytes and remaining cancer burden in triple-negative breast cancer patients after neo-adjuvant chemotherapy. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol./ESMO. 2019;30:236–24 https://pubmed.ncbi.nlm.nih.gov/30590484/

8. Loi S, Salgado R, Adams S, Pruneri G, Francis PA, Lacroix-Triki M,. et al. Tumor infiltrating lymphocyte stratification of prognostic staging of early-stage triple negative breast cancer. NPJ Breast Cancer.2022; 8:3-14. https://doi.org/10.1038/s41523-021-00362-1

9. Du X, Zhou Z, Shao Y, Qian K, Wu Y, Zhang J, et al. Immunoarchitectural patterns as potential prognostic factors for invasive ductal breast cancer. NPJ Breast Cancer. 2022;8:26-34. https://doi.org/10.1038/s41523-022-00389-y

10. Bagmut I, Movchan O, Sheremet M, Smolanka I, Lyashenko A, Dosenko I, et al. Characteristics of certain genetical and biological properties of carcinogenesis in the development of breast cancer with type 2 diabetes mellitus and tumor relapse. Rom J Diabetes Nutr Metab Dis 2022;29 (2): 245-252. https://doi.org/10.46389/rjd-2022-1082

11. Song X, Ma J, Zhang H, Zhang Q. Prognostic significance of the primary tumor site and immune indexes in patients with estrogen receptor-positive, human epidermal growth factor receptor-2-negative breast cancer. Gland Surg. 2020 Oct;9(5):1450-1468. doi: 10.21037/gs-20-622. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7667077/

12. Locy H, Verhulst S, Cools W, Waelput W, Brock S, Cras L, et al. Assessing Tumor-Infiltrating Lymphocytes in Breast Cancer: A Proposal for Combining Immunohistochemistry and Gene Expression Analysis to Refine Scoring. Front.Immunol. 2022;13:794175.doi:10.3389/fimmu.2022.794175. https://www.frontiersin.org/article/10.3389/fimmu.2022.794175

13. Ochi T, Bianchini G, Ando M, Nozaki F, Kobayashi D, Criscitiello C, et al. Predictive and prognostic value of stromal tumor-infiltrating lymphocytes before and after neoadjuvant therapy in triple negative and HER2-positive breast cancer. Eur. J.Cancer.2019;118:41-8. https://www.sciencedirect.com/science/article/abs/pii/S095980491930317X

14. Vagia E, Cristofanilli M. New Treatment Strategies for the Inflammatory Breast Cancer. Curr Treat Options Oncol. 2021; 22(6):50-61. https:// doi: 10.1007/s11864-021-00843-2. PMID: 33893888

15. Van Berckelaer C, Vermeiren I, Vercauteren L, Rypens C, Oner G, Trinh XB, et al. The Evolution and Prognostic Role of Tumour-Infiltrating Lymphocytes and Peripheral Blood-Based Biomarkers in Inflammatory Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. Cancers (Basel). 2021;13(18):4656-4566. doi: 10.3390/cancers13184656. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8471511/

16. Murthy RK, Song J, Raghavendra AS, Li Y, Hsu L, Hess KR, et al. Incorporation of clinical and biological factors improves prognostication and reflects contemporary clinical practice. NPJ Breast Cancer .2020;6:1–9. https://www.researchgate.net/publication/340142835_Incorporation_of_clinical_and_biological_factors_improves_prognostication_and_reflects_contemporary_clinical_practice

17. Zhang H, Ma G, Du S, Sun J, Zhang Q. Nomogram for predicting cancer specific survival in inflammatory breast carcinoma: a SEER population-based study. Peer J. 2019;7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6752187/

18. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. Proc. Natl. Acad. Sci. USA. 2012;109:2796–2801. doi: 10.1073/pnas.1104303108. https://pubmed.ncbi.nlm.nih.gov/21825174/

19. Garcia-Martinez E, Gil G, Benito A, Gonzalez-Billalabeitia E, Conesa M, Garcia Garcia T, et al. Tumor-infiltrating immune cell profiles and their change after neoadjuvant chemotherapy predict response and prognosis of breast cancer. Breast Cancer Res. 2014;16:488. doi: 10.1186/s13058-014-0488-5. https://pubmed.ncbi.nlm.nih.gov/25432519/

20. Eiró N, Pidal I, Fernandez-Garcia B, Junquera S, Lamelas ML, del Casar JM, et al. Impact of CD68/(CD3+CD20) ratio at the invasive front of primary tumors on distant metastasis development in breast cancer. PLoS ONE. 2012;7:e52796.doi:10.1371/journal.pone.0052796. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0052796

21. Poddar A, Rao S, Prithviraj P, Kannourakis G, Jayachandran A. Crosstalk between Immune Checkpoint Modulators, Metabolic Reprogramming and Cellular Plasticity in Triple-Negative Breast Cancer. Current Oncology. 2022; 29(10):6847-6863. https://doi.org/10.3390/curroncol29100540

22. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4)†. Ann Oncol. 2018 Aug 1;29(8):1634-1657. doi: 10.1093/annonc/mdy192. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7360146/