ORIGINAL CONTRIBUTION

EXPERIMENTAL ONCOLOGY

https://doi.org/10.15407/exp-oncology.2025.02.197

A. Pavlova [1], O. Mushii [1], V. Bazas [2], I. Karacharova [2], T. Zadvornyi [1], N. Lukianova [1, *]

[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, the NAS of Ukraine, Kyiv, Ukraine.
[2] Kyiv City Clinical Oncology Center, Kyiv, Ukraine

* Correspondence: Email: nataluk10@gmail.com

TUMOR-ASSOCIATED MACROPHAGES: RELATIONSHIP WITH CLINICAL STATUS OF PATIENTS AND MOLECULAR BIOLOGICAL FEATURES OF BREAST CANCER

Background. Infiltration of the tumor microenvironment by macrophages plays a key role in the progression of breast cancer (BC), modulating tumor growth, angiogenesis, immunosuppression, and metastasis. However, the association between macrophage infiltration levels and the clinicopathological characteristics of BC, including the molecular subtype of the neoplasm and receptor status, remains insufficiently studied. **Aim. T**o investigate the relationship between macrophage infiltration in BC tissue and the extent of tumor spread as well as the molecular profile of the neoplasms. **Materials and Methods.** Using immunohistochemistry, the level of infiltration of tumor tissue by $CD68^+$ (total macrophages) and $CD163^+$ (M2 phenotype macrophages) tumor-associated macrophages (TAMs) was assessed in the postoperative samples from 67 patients with BC stage I—II. **Results.** The level of $CD68^+$ macrophage infiltration in BC tissue was associated with the disease stage ($p = 0.004$), tumor size (T category) ($p = 0.026$), and the presence of metastases in regional lymph nodes ($p = 0.047$). The highest number of $CD163^+$ M2-like macrophages was recorded in poorly differentiated BC tissue ($p = 0.024$) and in neoplasms of the basal-like molecular subtype ($p = 0.023$). The lowest numbers of both $CD68^+$ and $CD163^+$ macrophages were detected in HER2/neu-positive tumors ($p = 0.023$). The data indicated that BC tumors classified as T2 and N1—N3 were characterized by an increased content of M1-polarized macrophages, whereas in basal-like BC and poorly differentiated tumors (G3), the M2 macrophage subpopulation predominated. This contributed to the formation of an immunosuppressive tumor phenotype and indicated the potential prognostic significance of macrophage infiltration in malignant breast neoplasms. **Conclusions.** The topology and quantitative characteristics of macrophage infiltration in tumor tissue are closely associated with the extent of BC spread and the molecular profile of the neoplasms. The $CD68^+/CD163^+$ cell ratio may reflect the balance between antitumor and immunosuppressive mechanisms within the microenvironment and be considered a potential prognostic marker.

Keywords: breast cancer, tumor-associated macrophages, disease course.

The beginning of the 21st century marked the final recognition of the necessity for a transition to personalized medicine as a key factor in increasing the effectiveness of cancer treatment [1, 2]. This approach is driven by the desire to move away from the standardized treatment protocols toward indi-

Citation: Pavlova A, Mushii O, Bazas V, Karacharova I, Zadvornyi T, Lukianova N. Tumor-associated macrophages: relationship with clinical status of patients and molecular biological features of breast cancer. *Exp Oncol.* 2025; 47(2): 197-206. https://doi.org/10.15407/exp-oncology.2025.02.197

vidualized therapeutic strategies based on a deep understanding of pathophysiology and carcinogenesis, pharmacogenomics, and individual genetic differences of the patients [3]. Today, cancer is viewed as a complex, heterogeneous disease influenced by a wide range of factors — genetic, molecular, cellular, tissue-specific, population-level, environmental, and socio-economic — that also change over time [4].

While research in the previous decade focused on identifying molecular and genetic diagnostic and prognostic cancer biomarkers, primarily driven by the decoding of the human genome and the availability of relevant technological tools, current attention has significantly shifted toward the tumor microenvironment (TME) [5]. The TME comprises a large variety of cell types (infiltrating immune cells, tumor-associated fibroblasts, endothelial cells, adipocytes, etc.) as well as non-cellular components such as the extracellular matrix (ECM) and various regulatory molecules. The complex network of interactions between tumor cells and TME elements plays a decisive role in tumor growth and progression [6, 7].

It has been established that the most numerous groups of cells in the TME of solid tumors of various histogenesis are tumor-associated macrophages (TAMs) [8]. TAMs are a heterogeneous group in terms of origin and functional activity involved in both innate and adaptive immunity [9, 10]. Based on their morphological, phenotypic, and functional heterogeneity, TAMs are conventionally divided into two distinct subtypes: M1 and M2 macrophages. M1 macrophages play a key role in antitumor immunity and mediate pro-inflammatory processes in the TME, whereas M2 macrophages are characterized by strong pro-tumor properties [11]. It has been shown that M2-type TAMs are involved in tumor growth, metastasis, and the development of resistance to drug therapy [9]. Numerous studies have demonstrated that a high level of TAM infiltration is associated with poor clinical outcomes and low survival rates in patients with tumors of various histogenesis. Moreover, macrophages themselves are considered a promising target for antitumor therapy [12].

Despite the extensive body of research dedicated to the study of TAMs, there is still no definitive answer regarding the role of macrophages in the development and progression of breast cancer (BC), nor the prospects of using them as prognostic or predictive markers. Taking this into account, the aim of the study was to investigate the relationship between macrophage infiltration in BC tissue and both the extent of tumor progression and the molecular profile of the neoplasms.

Materials and Methods

The study was conducted on the postoperative material of 67 patients with BC stage I—II. The patients were treated at the Municipal Non-Profit Enterprise «Kyiv City Oncology Center» during 2019—2022. The Institutional Review Board and Research Ethics Committee of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine approved the work. It was conducted following the Declaration of Helsinki and Good Clinical Practice guidelines. All patients were examined using generally accepted clinical and laboratory methods by the Standards of diagnosis and treatment of oncological patients, approved by the Order of the Ministry of Health of Ukraine No. 396, adopted on 30/06/2015 (registration number GS 2015-396). No patients under study received neoadjuvant treatment. All donors of tumor material provided Informed Consent of Agreement to conduct scientific research. The stage of the tumor process was determined according to the International TNM Classification [13]. Clinical characteristics of the patients are presented in Table 1.

Immunohistochemical study. The samples obtained during surgical treatment were fixed in 10% neutral formalin and embedded in paraffin according to standard histological protocols. Serial 5 μm-thick sections were prepared using a rotary microtome and mounted on adhesive microscope slides.

Immunohistochemical analysis was performed to detect estrogen receptor (ER), progesterone receptor (PR), proliferation marker Ki-67, human epidermal growth factor receptor 2 (HER2/neu), as well as macrophage markers CD68 and CD163. The following monoclonal antibodies were used: ER (clone 1D5, dilution 1:100, Dako Cytomation, Denmark), PR (clone PgR636, dilution 1:100, Dako Cytomation, Denmark), HER2/neu (clone e2-4001, dilution 1:100, Thermo Scientific, USA), Ki-67 (clone MIB-1, dilution 1:100, Dako Cytomation, Denmark), CD68 (clone KP-1, ready to use, Master

Diagnostica, Spain), and CD163 (clone OTI2G12, dilution 1:150, Abcam, USA). The Master Polymer Plus Detection System (Peroxidase) with DAB chromogen (Master Diagnostica, Spain) was used for visualization of the immune reaction. Counterstaining was performed with Mayer's hematoxylin (Thermo Scientific Richard-Allan, USA). Each staining series included appropriate positive and negative controls.

The expression of ER, PR, and Ki-67 was assessed by calculating the percentage of tumor cells with positive nuclear staining in at least five randomly selected high-power fields (×400 magnification). HER2/neu status was evaluated semi-quantitatively based on membrane staining intensity: «+++» – strong complete membrane staining in >10% of cells (positive); «++» – moderate complete staining (equivocal); «+» – weak or incomplete staining (negative); «0» – no staining (negative).

Molecular classification of BC was performed based on the immunohistochemical profile. Luminal A subtype included tumors with positive ER and/or PR expression, negative HER2/neu status, and Ki-67 < 14%. Luminal B subtype was defined by positive ER and/or PR, regardless of HER2/neu status, with Ki-67 > 14%. The HER2-positive subtype was characterized by the absence of ER and PR expression with HER2/neu positivity, irrespective of Ki-67 levels. The basal-like (triple-negative) subtype was defined by the simultaneous negativity for ER, PR, and HER2/neu, with any level of Ki-67 expression [14]. Receptor status and proliferative activity of BC tumors are shown in Table 2.

To assess macrophage infiltration in tumor tissue, $CD68^+$ cells (total macrophages) and $CD163^+$ cells (M2-like macrophages) were counted in five high-power fields with the highest infiltration density in both intratumoral and peritumoral regions. Results were expressed as the number of positive cells per 1 $mm^{2.}$

Statistical analysis. All statistical analyses were performed using GraphPad Prism software, version 10.00 (GraphPad Software Inc., USA). Compari-

Table 1. **Clinical characteristics of BC patients**

Characteristics	Number of patients	
	N	%
Total number of patients	67	100
Average age, years	58.36 ± 1.66	
Age range, years	28—89	
Reproductive status		
Menstrual cycle preserved	21	31.3
Menopause	46	68.7
Clinical stage		
I	22	32.8
II	45	67.2
Tumor size (category T)		
T1	27	40.3
T2	40	59.7
Lymph node involvement (category N)		
N0	44	65.7
N1 -3	23	34.3
Histological type		
Infiltrative ductal adenocarcinoma	49	73.1
Infiltrative lobular adenocarcinoma	18	26.9
Tumor differentiation grade		
G1 (high)	3	4.5
G2 (moderate)	56	83.6
G3 (low)	8	11.9
Molecular subtype		
Luminal A	30	44.8
Luminal B	20	29.9
Triple-negative (basal-like)	8	13.4
HER2/neu-positive	9	11.9

Table 2. **Receptor status and proliferative activity of BC tumors**

Receptor status	Positive (*n*, %)	Negative (*n*, %)
ER	51 (76.1%)	16 (23.9%)
PR	46 (68.7%)	21 (31.3%)
HER2/neu	22 (32.8%)	45 (67.2%)
Ki-67	<14% of positive cells (*n*, %)	>14% of positive cells (*n*, %)
	11 (16.4%)	56 (83.6%)

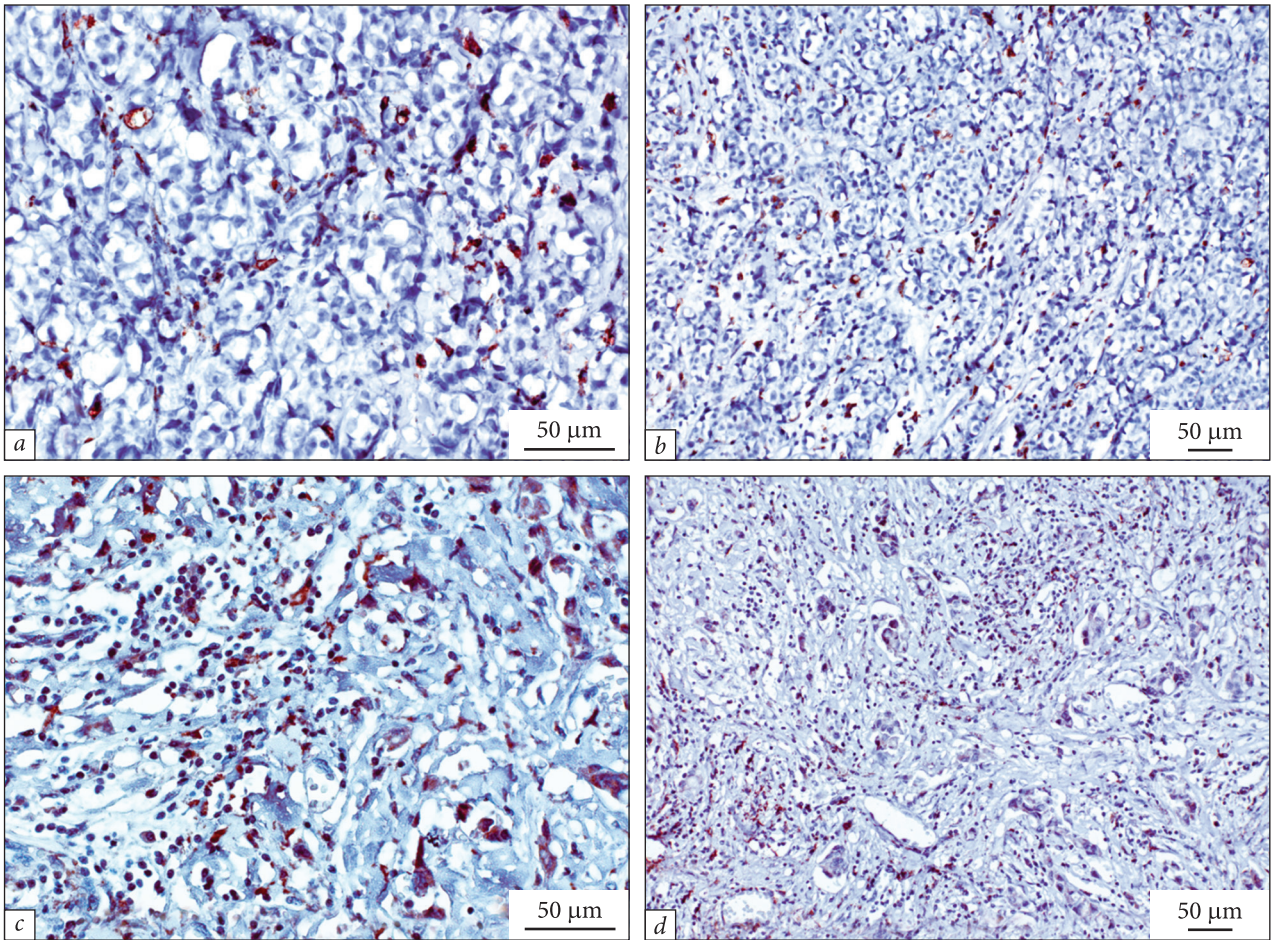

Fig. 1. Representative microphotographs of CD68$^+$ (*a, b)* and CD163$^+$ (*c, d)* cells in BC tissue. Immunohistochemistry with chromogen 3-diaminobenzidine tetrachloride. Counterstaining with Meyer's hematoxylin

sons between two independent groups were conducted using the non-parametric Mann — Whitney U-test. Analysis of variance (ANOVA) was used to compare three or more groups. Quantitative data are presented as mean ± standard error of the mean (M ± m). Box-and-whisker plots were used to visualize the distribution of values across study groups. In these plots, the central line within the box represents the median; the lower and upper edges of the box correspond to the first and third quartiles, respectively. The whiskers extending from the box indicate the minimum and maximum values observed in each group.

Results

In the study of the topology of CD68$^+$ macrophage expression in BC tissue, it was found that these cells predominantly exhibit a round or oval morphology with granular cytoplasmic staining, corresponding to the localization of the lysosomal protein. They are diffusely distributed within the tumor stroma, with moderate to high density in certain areas, particularly near tumor foci and blood vessels.

CD163$^+$ macrophages generally displayed more elongated or amoeboid morphology and were localized in the deeper regions of the tumor stroma or near necrotic areas, as well as in the perivascular and fibrotic zones of BC tissue, which may indicate their immunoregulatory or reparative function. The staining intensity for the CD163 antigen was variable but typically appeared dark brown (Fig. 1).

The quantitative analysis of CD68 expression revealed a significant increase in the number of total macrophages in the tumor tissue of BC patients, depending on the stage of the tumor process (Fig. 2). A 1.9-fold increase in CD68$^+$ macrophage infiltration ($p = 0.004$) was observed in BC tissue samples from patients with stage II compared to stage I.

A direct correlation was established between the level of CD68$^+$ macrophage infiltration and the size of BC tumors: in T2 category tumors, the level of

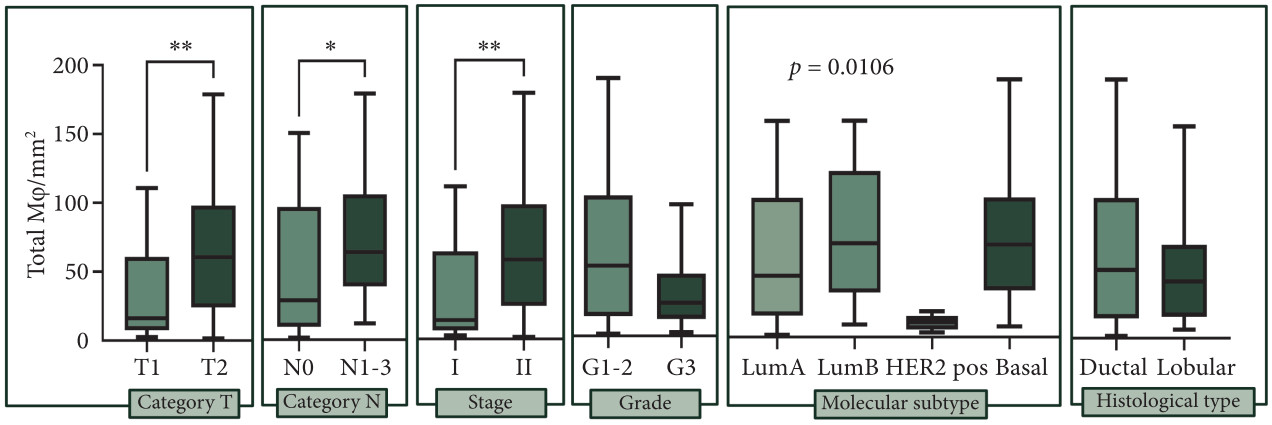

Fig. 2. Dependence of the levels of CD68⁺ macrophage infiltration into BC tissue on the clinical characteristics of the tumor process. * $p < 0.05$, ** $p < 0.005$

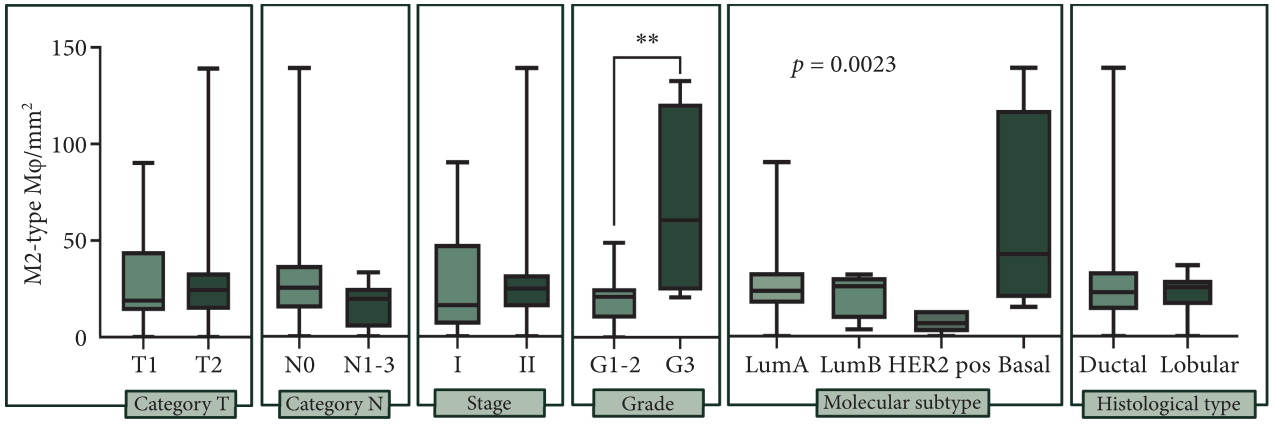

Fig. 3. Dependence of the levels of CD163⁺ macrophage infiltration into BC tissue on the clinical characteristics of the tumor process. ** $p < 0.005$

CD68⁺ cells was 2.0 times higher ($p = 0.026$) compared to T1 category tumors. Additionally, in cases with regional lymph node metastases, the level of CD68⁺ infiltration in BC tissue was 1.4 times higher ($p = 0.047$) than in tumors from patients without metastases. At the same time, no statistically significant differences were observed in the level of CD68⁺ macrophage infiltration in BC tissue across the tumors of different histological types.

The macrophage infiltration intensity also depended on the tumor differentiation grade. Specifically, in samples of poorly differentiated BC, the level of CD163⁺ M2 macrophages was 3.6 times higher ($p = 0.024$) compared to well-differentiated carcinomas (Fig. 3).

In addition, differences in macrophage infiltration levels were found depending on the molecular subtype of BC: the density of CD68⁺ and CD163⁺ cells was significantly higher in tumors of the basal subtype compared to luminal BC and HER2/neu-positive neoplasms.

The lowest number of CD68⁺ macrophages was recorded in the tissue of HER2/neu-positive tumors (10.26 ± 2.309). In the luminal A subtype, the level of total macrophage infiltration was 52.52 ± ± 8.315 cells per mm², which was 5.12 times higher than in HER2/neu-positive BC. The highest numbers of CD68⁺ macrophages were observed in the basal-like and luminal B subtypes — 73.8 ± 18.57 and 71.68 ± 11.28 cells per mm², respectively, amounting to 7.19 and 6.98 times more than in HER2/neu-positive tumors.

The lowest levels of CD163⁺ macrophage infiltration were observed in HER2/neu-positive BC tissue. Meanwhile, in luminal A, luminal B, and basal-like subtypes, the levels of M2 macrophage infiltration were 2.45-, 2.79-, and 5.23-fold higher, respectively, compared to HER2/neu-positive tumors.

No statistically significant differences were found in the CD163⁺ macrophage infiltration levels depending on T or N categories, stage, or histological type of BC.

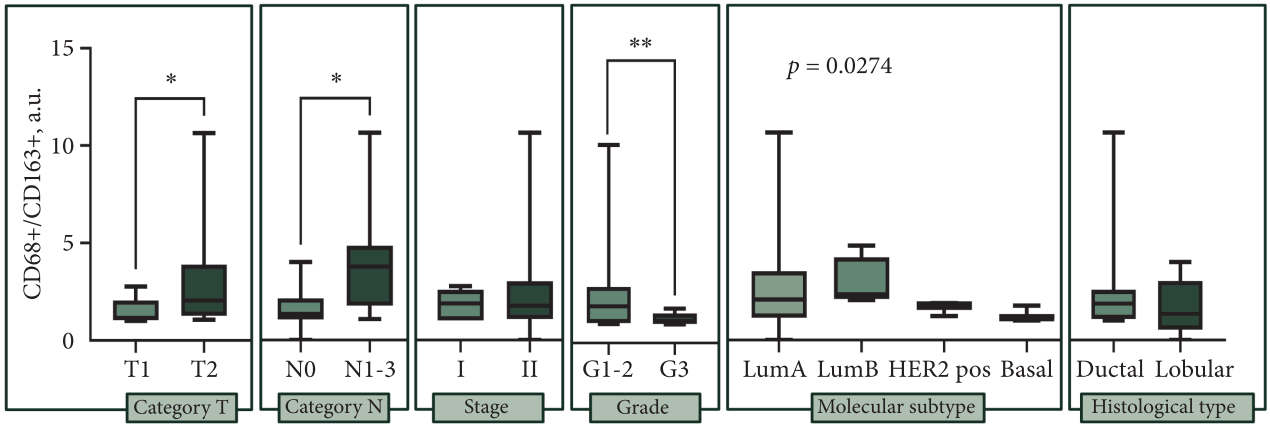

Fig. 4. Dependence of the CD68$^+$ to CD163$^+$ macrophage ratio levels in the BC tissue on the clinical characteristics of the tumor process. * $p < 0.05$, ** $p < 0.005$

Analysis of the CD68$^+$/CD163$^+$ cell ratio in BC tissue revealed its dependence on the disease stage, tumor differentiation grade, and molecular subtype (Fig. 4). Specifically, in the tissue of T2-category tumors, a significant increase in the CD68$^+$/CD163$^+$ ratio was recorded (2.847 ± 0.574; $p = 0.026$) compared to T1-category cases, indicating a predominance of M1-polarized macrophages with potentially antitumor activity. A similar trend was observed in cases with metastatic involvement of regional lymph nodes, where the CD68$^+$/CD163$^+$ ratio reached 4.172 ± 1.214 ($p = 0.019$) compared to the N0 category.

In contrast, in tumors of the basal molecular subtype, the CD68$^+$/CD163$^+$ ratio was significantly lower (1.192 ± 0.117; $p = 0.027$), indicating a predominance of M2-polarized macrophages associated with tumor growth support, angiogenesis, and suppression of antitumor immunity. Similar changes were also observed in poorly differentiated (G3) BC tissue, which likewise showed a reduced CD68$^+$/CD163$^+$ ratio (1.289 ± 0.153; $p = 0.045$).

The CD68$^+$/CD163$^+$ macrophage ratio was the highest in luminal B subtype tissue, reaching 2.34 a.u. In samples of luminal A and HER2/neu-positive BC, this value was lower by 11% and 24%, respectively. A statistically significant reduction in the CD68$^+$/CD163$^+$ macrophage ratio was recorded in tissue of patients with basal-like BC — 2.43 times lower than in luminal B subtype samples.

Analysis of CD163$^+$ macrophage infiltration in tumor tissue demonstrated a direct correlation between the expression of this marker and the receptor status of BC (Fig. 5). In particular, the counts of CD163$^+$ cells in ER-positive and PR-positive BC tissue were 2.9 times ($p = 0.033$) and 2.4 times ($p = 0.012$) higher, respectively, than in ER$^-$ and PR$^-$ tumors. In HER2/neu-negative tumor tissue, the level of CD163$^+$ infiltration was 2.9 times higher ($p = 0.004$) compared to HER2/neu-positive tumors, which may indicate enhanced immunosuppressive activity in the tumor microenvironment.

At the same time, the association between CD163$^+$ macrophage infiltration and tumor proliferative activity, assessed by the Ki-67 index, was insignificant. Similarly, no significant correlation was found between the level of CD68$^+$ macrophage infiltration and the expression of ER, PR, HER2/neu receptors, or the Ki-67 index.

In contrast, samples with a high Ki-67 index (>14%) showed a 1.9-fold increase in the CD68/CD163 ratio compared to tumors with low proliferation levels ($p = 0.004$), which may indicate a predominance of M1-phenotype macrophages in tumors with a more aggressive phenotype. No association was found between the CD68/CD163 ratio and receptor status (ER, PR, HER2/neu) in BC.

Thus, the increased CD68$^+$ macrophage infiltration in BC tissue was associated with higher disease stage and tumor size (T category), as well as the presence of metastases in regional lymph nodes. An elevated level of CD163$^+$ M2-like macrophages was observed in poorly differentiated BC tissue and tumors of the basal molecular subtype. The lowest numbers of both CD68$^+$ and CD163$^+$ macrophages were found in tumors of the luminal and HER2/neu-positive subtypes.

Therefore, a characteristic feature of T2 and N1—3 category BC is an increased presence of M1-polarized macrophages, while in basal-like BC and poorly differentiated (G3) tumors, the dominant subpopula-

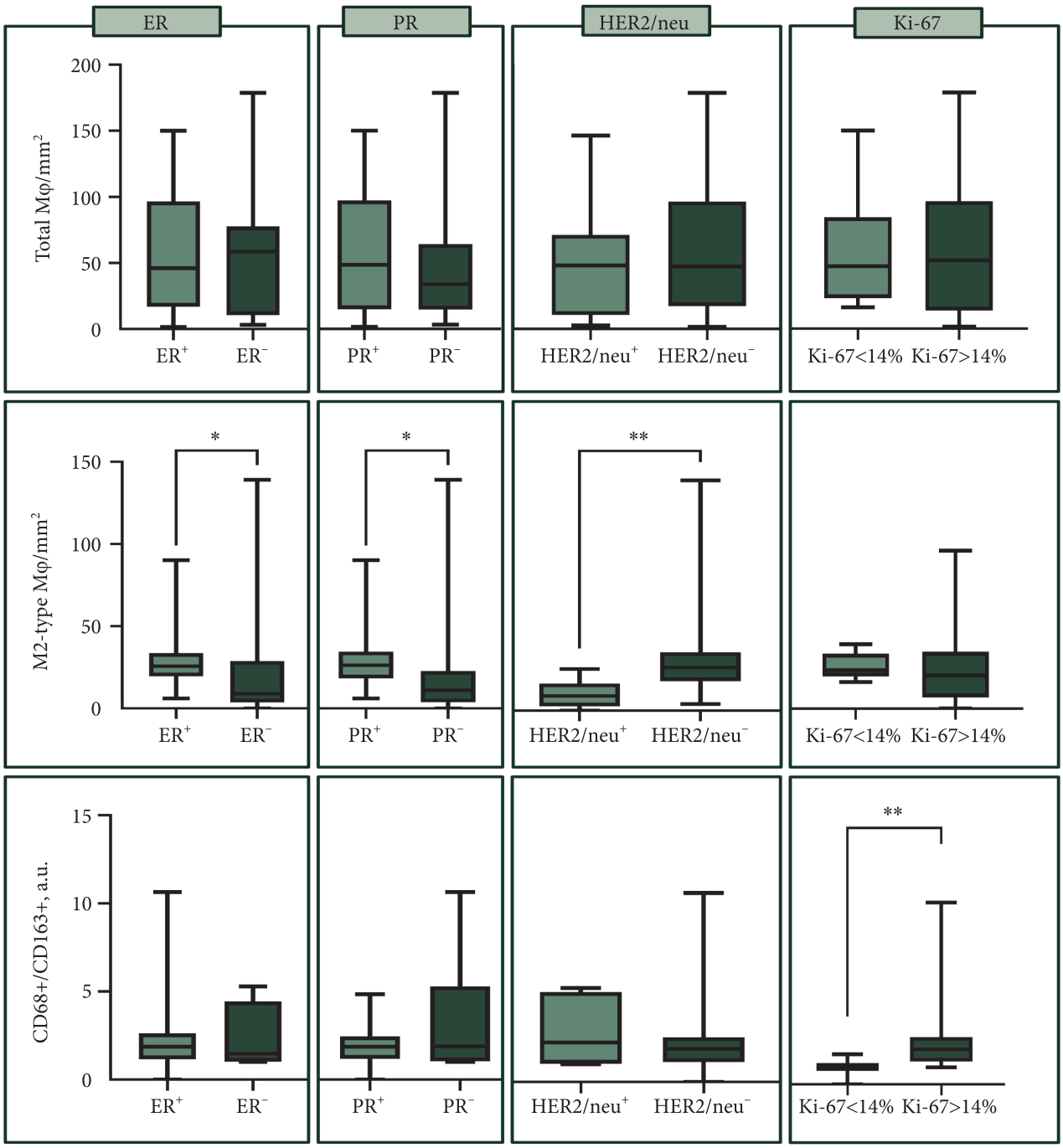

Fig. 5. Association between TAMs infiltration levels and receptor status, as well as Ki-67 expression in BC tissue. * $p < 0.05$, ** $p < 0.005$

tion consists of M2 macrophages. These cells contribute to immunosuppression and tumor progression, indicating the potential role of macrophage infiltration as a prognostic marker of disease aggressiveness.

Discussion

The correlations we identified between $CD68^+$ and $CD163^+$ macrophage infiltration levels and tumor size, regional lymph node involvement, and BC stage are supported by reports from other authors [15, 16]. Specifically, it has been shown that the increased infiltration of $CD68^+$ and $CD47^+$ macrophages in malignant breast tumors is associated with higher proliferative activity, increased migration of tumor-associated lymphocytes into the tumor core, and a greater risk of vascular invasion [17].

Our findings also indicate a direct association between BC proliferation (assessed via Ki-67 index) and the number of both M1- and M2-like macrophages.

However, the inverse correlation we observed between $CD68^+$ and $CD163^+$ macrophage infiltration and ER/PR expression status does not fully align with findings from other researchers [18, 19], reflecting conflicting data in the literature and highlighting the complexity of interactions between immune cells in the TME and the hormonal profile of tumors.

Moreover, Sari et al. [20] reported an increase in M2 macrophages in luminal B subtype tumors, which corresponds with our results and supports the relevance of molecular classification in TAM research in BC.

An interesting approach in TAM studies involves distinguishing between cells infiltrating the tumor core and those in the surrounding stroma. Mwafy et al. [21] demonstrated that the topology of infiltration plays a decisive role in determining the prognostic significance of TAM levels within the tumor mass. Furthermore, Hirschmann et al. [22] showed that infiltration of M2-like macrophages into conditionally normal stromal tissue at the periphery of early-stage BC is, conversely, associated with favorable outcomes and higher patient survival rates.

The tumor stroma is increasingly being viewed not only as a passive substrate for transformed cell growth but also as a key regulator of proliferation, survival, invasion, and therapeutic response. This is supported by our data on the association between spatial organization and histoarchitectural features of the stromal component and the degree of malignancy in both breast [23; 24] and prostate [25] cancers, as well as the infiltration patterns and functional activity of mast cells within the TME [26].

Specifically, mast cells have been shown to modulate the architecture of the collagen framework, influence fiber density and organization, thereby contributing to the formation of a tumor-promoting microenvironment [27]. The stroma not only reflects the state of the tumor process but also creates conditions for tissue remodeling, promotes angiogenesis, and supports the development of an immunosuppressive environment.

Accordingly, our future research will focus on evaluating TAM infiltration levels with consideration of the spatial localization of these cells within both stromal and parenchymal components of the tumor.

In conclusion, our results underscore the need for a comprehensive analysis of both the cellular composition and structural features of the TME, accounting for spatial interactions. Assessing TAMs infiltration concerning their topological distribution between the tumor core and stromal zones in future studies will allow for a deeper understanding of the mechanisms of pathogenesis and tumor progression, considering the molecular and biological characteristics of TAMs.

Funding

This study was supported by the Research Program 'Development and Validation of Complex Treatment Technology for Breast Cancer Patients of Young Age' (No. 0122U201203) financed by the Ministry of Education and Science of Ukraine, and Research Programs 'Stress-induced Tumor Microenvironment Factors as Risk Drivers of Breast Cancer Progression' (No. 0124U000078) funded by the National Academy of Sciences of Ukraine.

REFERENCES

1. Kosorok MR, Laber EB. Precision medicine. *Annu Rev Stat Appl.* 2019;6:263-286. https://doi.org/10.1146/annurev-statistics-030718-105251
2. Garattini L, Curto A, Freemantle N. Personalized medicine and economic evaluation in oncology: all theory and no practice? *Expert Rev Pharmacoecon Outcomes Res.* 2015;15(5):733-738. https://doi.org/10.1586/14737167.2015.1078239
3. Kalia M. Biomarkers for personalized oncology: recent advances and future challenges. *Metabolism.* 2015;64(3 Suppl 1): S16-S21. https://doi.org/10.1016/j.metabol.2014.10.027
4. Su M, Zhang Z, Zhou X, et al. Proteomics, personalized medicine and cancer. *Cancers (Basel).* 2021;13(11):2512. https://doi.org/10.3390/cancers13112512
5. Wang Q, Shao X, Zhang Y. Role of tumor microenvironment in cancer progression and therapeutic strategy. *Cancer Med.* 2023;12:11149–11165. https://doi.org/10.1002/cam4.5698

6. Sabit H, Arneth B, Abdel-Ghany S, et al. Beyond cancer cells: how the tumor microenvironment drives cancer progression. *Cells.* 2024;13(19):1666. https://doi.org/10.3390/cells13191666
7. Brassart-Pasco S, Brézillon S, Brassart B, et al. Tumor microenvironment: extracellular matrix alterations influence tumor progression. *Front Oncol.* 2020;10:397. https://doi.org/10.3389/fonc.2020.00397
8. Munir MT, Kay MK, Kang MH, et al. Tumor-associated macrophages as multifaceted regulators of breast tumor growth. *Int J Mol Sci.* 2021;22(12):6526. https://doi.org/10.3390/ijms22126526
9. Malfitano AM, Pisanti S, Napolitano F, et al. Tumor-associated macrophage status in cancer treatment. *Cancers (Basel).* 2020;12(7):1987. https://doi.org/10.3390/cancers12071987
10. Pan Y, Yu Y, Wang X, et al. Tumor-associated macrophages in tumor immunity. *Front Immunol.* 2020;11:583084. https://doi.org/10.3389/fimmu.2020.583084
11. Kumari N, Choi SH. Tumor-associated macrophages in cancer: recent advancements in cancer nanoimmunotherapies. *J Exp Clin Cancer Res.* 2022;41(1):68. https://doi.org/10.1186/s13046-022-02272-x
12. Fu LQ, Du WL, Cai MH, et al. The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. *Cell Immunol.* 2020;353:104119. https://doi.org/10.1016/j.cellimm.2020.104119
13. Brierley JD, Gospodarowicz MK, Wittekind C, et al. *TNM Classification of Malignant Tumours.* 8th ed. Wiley-Blackwell; 2017. ISBN: 978-1-119-26357-9. 272 p.
14. Britten A, Rossier C, Taright N, et al. Genomic classifications and radiotherapy for breast cancer. *Eur J Pharmacol.* 2013;717(1-3):67-70. https://doi.org/10.1016/j.ejphar.2012.11.069
15. Mwafy SE, El-Guindy DM. Pathologic assessment of tumor-associated macrophages and their histologic localization in invasive breast carcinoma. *J Egypt Natl Canc Inst.* 2020;32(1):6. https://doi.org/10.1186/s43046-020-0018-8
16. Mahmoud SM, Lee AH, Paish EC, et al. Tumour-infiltrating macrophages and clinical outcome in breast cancer. *J Clin Pathol.* 2012;65(2):159-163. https://doi.org/10.1136/jclinpath-2011-200355
17. Chen Y, Klingen TA, Aas H, et al. CD47 and CD68 expression in breast cancer is associated with tumor-infiltrating lymphocytes, blood vessel invasion, detection mode, and prognosis. *J Pathol Clin Res.* 2023;9(3):151-164. https://doi.org/10.1002/cjp2.309
18. Lindsten T, Hedbrant A, Ramberg A, et al. Effect of macrophages on breast cancer cell proliferation, and on expression of hormone receptors, uPAR and HER-2. *Int J Oncol.* 2017;51(1):104-114. https://doi.org/10.3892/ijo.2017.3996
19. Khalili H, Dabiri S, Pourseyedi B, et al. Possible association of CD68-positive macrophages with other prognostic factors (Ki 67, ER, PR, HER2/neu) in primary breast cancer and axillary lymph node metastasis. *J Kerman Univ Med Sci.* 2017;24(6):459-466. ISSN: 1023-9510
20. Sari S, Özdemir Ç, Çilekar M. The relationship of tumour-associated macrophages (CD68, CD163, CD11c) and cancer stem cell (CD44) markers with prognostic parameters in breast carcinomas. *Pol J Pathol.* 2022;73(4):299-309. https://doi.org/10.5114/pjp.2022.125424
21. Mwafy SE, El-Guindy DM. Pathologic assessment of tumor-associated macrophages and their histologic localization in invasive breast carcinoma. *J Egypt Natl Canc Inst.* 2020;32(1):6. https://doi.org/10.1186/s43046-020-0018-8
22. Hirschmann M, Schnellhardt S, Rübner M, et al. Prognostic significance of macrophage phenotypes in peri-tumoral normal tissue of early-stage breast cancer. *Cells.* 2025;14(11):828. https://doi.org/10.3390/cells14110828
23. Lukianova N, Mushii O, Zadvornyi T, et al. Development of an algorithm for biomedical image analysis of the spatial organization of collagen in breast cancer tissue of patients with different clinical status. *FEBS Open Bio.* 2024;14(4):675-686. doi: https://doi.org/10.1002/2211-5463.13773
24. Chekhun V, Mushii O, Zadvornyi T, et al. Features of COL1A1 expression in breast cancer tissue of young patients. *Exp Oncol.* 2023;45(3):351-363. https://doi.org/10.15407/exp-oncology.2023.03.351
25. Zadvornyi T, Lukianova N, Mushii O, et al. Benign and malignant prostate neoplasms show different spatial organization of collagen. *Croat Med J.* 2023;64(6):413-420. https://doi.org/10.3325/cmj.2023.64.413
26. Zadvornyi T, Lukianova N, Borikun T, et al. Mast cells as a tumor microenvironment factor associated with the aggressiveness of prostate cancer. *Neoplasma.* 2022;69(6):1490-1498. https://doi.org/10.4149/neo_2022_221014N1020
27. Mushii O, Pavlova A, Bazas V, et al. Mast cells as a factor in regulation of breast cancer stromal component associated with breast cancer aggressiveness. *Exp Oncol.* 2025;46(4):311-323. https://doi.org/10.15407/exp-oncology.2024.04.311

Submitted: June 9, 2025

А. Павлова [1], О. Мушій [1], В. Базась [2],
І. Карачарова [2], Т. Задворний [1], Н. Лук'янова [1]

[1] Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ, Україна
[2] Київський міський клінічний онкологічний центр, Київ, Україна

ПУХЛИНО-АСОЦІЙОВАНІ МАКРОФАГИ: ЗВ'ЯЗОК ІЗ КЛІНІЧНИМ СТАТУСОМ ПАЦІЄНТІВ ТА МОЛЕКУЛЯРНО-БІОЛОГІЧНИМИ ОСОБЛИВОСТЯМИ РАКУ МОЛОЧНОЇ ЗАЛОЗИ

Стан питання. Інфільтрація пухлинного мікрооточення макрофагами є одним з факторів, що відіграє ключову роль у прогресії раку молочної залози (РМЗ), модулюючи пухлинний ріст, ангіогенез, імуносупресію та метастазування. Однак зв'язок між рівнями макрофагальної інфільтрації та клініко-патологічними характеристиками РМЗ, включаючи молекулярний підтип новоутворень та рецепторний статус, залишається недостатньо вивченим. **Мета.** Дослідити взаємозв'язок між інфільтрацією макрофагами тканини РМЗ та ступенем поширеності пухлинного процесу і молекулярним профілем новоутворень. **Матеріали та методи.** Дослідження проведено на післяопераційному матеріалі 67 хворих на РМЗ I—II стадії. Методом імуногістохімії оцінено рівень інфільтрації пухлинної тканини $CD68^+$ (загальні макрофаги) та $CD163^+$ (макрофаги М2-фенотипу) пухлино-асоційованими макрофагами (ТАМ). **Результати.** Рівень інфільтрації $CD68^+$ макрофагами тканини РМЗ асоціюється із стадією захворювання ($p = 0{,}004$), розмірами новоутворень (категорія Т) ($p = 0{,}026$), а також наявністю метастазів у регіонарні лімфатичні вузли ($p = 0{,}047$). Найбільшу кількість $CD163^+$ М2-подібних макрофагів зафіксовано у тканині низькодиференційованого РМЗ ($p = 0{,}024$) та в новоутвореннях базального молекулярного підтипу ($p = 0{,}023$). Найменшу кількість як $CD68^+$, так і $CD163^+$ макрофагів виявлено в пухлинах HER2/neu-позитивного підтипу ($p = 0{,}023$). Отримані дані свідчать, що характерною ознакою РМЗ категорії Т2 та N1—N3 є підвищення вмісту М1-поляризованих макрофагів, тоді як у тканині базального РМЗ та пухлинах низького ступеня диференціювання (G3) домінує субпопуляція М2-макрофагів, що сприяє формуванню імуносупресивного фенотипу новоутворень та вказує на потенційне прогностичне значення інфільтрації макрофагами злоякісних новоутворень молочної залози. **Висновки.** Топологія та кількісні характеристики макрофагальної інфільтрації пухлинної тканини тісно пов'язані зі ступенем розповсюдженості РМЗ та молекулярним профілем новоутворень. Співвідношення $CD68^+/CD163^+$ клітин може відображати баланс між протипухлинними та імуносупресивними механізмами в мікрооточенні і розглядатися як потенційний прогностичний маркер.

Ключові слова: рак молочної залози, пухлино-асоційовані макрофаги, перебіг захворювання.