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# CD45RO EXPRESSION IN ASSOCIATED WITH TYPE 2 DIABETES MELLITUS BREAST CARCINOMA

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**Summary**
**Objectives.** To investigate the density, spatial distribution (intra- vs. peritumoral), and prognostic significance of tumor-infiltrating CD45RO$^+$ memory T lymphocytes (TILs) in invasive ductal breast carcinoma. A primary aim was to elucidate the modulatory impact of concomitant type 2 diabetes mellitus (T2DM) on this immune infiltrate and its correlation with established clinico-pathological indicators of tumor aggressiveness.
**Methods.** We conducted a retrospective study on archived tissue from 60 cases of invasive ductal breast carcinoma, stratified into two groups: patients with T2DM (n = 30) and a non-diabetic control group (n = 30). CD45RO expression was evaluated by immunohistochemistry. Positive-cell density was assessed separately in intra- and peritumoral stromal compartments. Correlations between CD45RO$^+$ cells density and histologic grade, molecular subtype, Ki-67 proliferation index, and lymph node status were analyzed using Spearman's rank correlation coefficient. Statistical significance was set up at $p < 0.05$.
**Results.** Analysis revealed a statistically significant difference in intratumoral CD45RO$^+$ lymphocyte density (CD45RO-IT) between the two cohorts. The T2DM group showed a significantly lower CD45RO-IT density. In the non-diabetic cohort, higher CD45RO-IT density correlated negatively with histologic grade and Ki-67, indicating a protective association. These correlations were attenuated or absent in the T2DM cohort. Moreover, within the T2DM group, a negative correlation emerged between CD45RO-IT density and lymph-node metastasis, suggesting an alteration in immune function.
**Conclusions.** Infiltration by CD45RO$^+$ memory T cells is an important prognostic indicator in invasive ductal breast carcinoma. The presence of T2DM is associated with quantitative—and potentially functional—impairment of the antitumor memory immune response, characterized by diminished CD45RO$^+$ infiltrates that align with a more aggressive tumor phenotype. Routine evaluation of CD45RO$^+$ TILs—particularly in patients with metabolic comorbidities—is warranted to refine prognostic assessment.
**Keywords:** breast carcinoma, memory T lymphocytes, CD45RO, type 2 diabetes mellitus, tumor microenvironment, prognosis

## Introduction

The modern understanding of cancer has evolved from a cell-centric view to a holistic model that recognizes the tumor as a complex ecosystem—the tumor microenvironment (TME) [1]. This TME is a dynamic network of cancer cells, stromal cells (fibroblasts, adipocytes), extracellular matrix, and, critically, immune cells. Tumor-infiltrating lymphocytes (TILs) represent the histologic manifestation of the host immune response against neoplasia and are a key determinant of patient prognosis across multiple cancer types [1]. In breast carcinoma, the composition and density of this immune infiltrate can shape disease course, therapeutic response, and survival.

Within the heterogeneous TIL population, memory T lymphocytes play a central role. They are identified by expression of the CD45RO isoform of the leukocyte common antigen, a molecule marking antigen-experienced, activated T cells and distinguishing them from naïve T cells that express the CD45RA isoform [1]. A TME enriched in CD45RO$^+$ memory T cells is considered indicative of a robust and effective adaptive immune response. These cells can mount a rapid, potent secondary response upon re-encounter with tumor antigens, ensuring long-term immunologic surveillance [1]. Consequently, high densities of CD45RO$^+$ TILs are widely accepted as a favorable prognostic biomarker in a range of solid tumors, including colorectal, gastric, esophageal, and lung cancers [2]. A comprehensive meta-analysis by Hu and Wang reinforced this observation, demonstrating a significant association between CD45RO$^+$ T-cell infiltration and improved overall and disease-free survival in solid tumors [3].

The complexity of the TME is shaped not only by local factors but also profoundly by the host's systemic state. Type 2 diabetes mellitus (T2DM) is an increasingly common comorbidity among patients with breast cancer and transcends the definition of a purely metabolic disorder. T2DM is recognized as a state of chronic, low-grade systemic inflammation fueled by hyperglycemia, hyperinsulinemia, insulin resistance, and dysregulated adipokine secretion from adipose tissue. This pro-inflammatory systemic milieu can fundamentally remodel the tumor's local immune landscape.

The link between chronic inflammation and cancer progression is well established. A chronically inflamed environment fosters tumor initiation, growth, and

metastasis by creating an immunosuppressive TME [4]. This environment is characterized by the accumulation of pro-inflammatory mediators, recruitment of immunosuppressive cells (such as M2 macrophages and myeloid-derived suppressor cells), and, critically, the induction of functional exhaustion (anergy) among effector T lymphocytes. Exhausted T cells, although present within the tumor, lose their capacity to proliferate and produce effector cytokines, becoming unable to eliminate malignant cells [5].

Although the prognostic value of CD45RO$^+$ TILs is recognized in many cancers, their significance in patients with profound systemic immune alterations—such as those induced by T2DM—remains largely unexplored. The central premise of this study is that immune biomarkers cannot be interpreted in isolation; their prognostic value depends on the host's systemic context. A simple enumeration of CD45RO$^+$ cells may be misleading if the patient's metabolic status has already compromised the functional competence of these cells. Hence, a re-evaluation of this biomarker is warranted in the setting of T2DM comorbidity.

Positioned at the critical intersection of endocrinology and immuno-oncology, this study hypothesizes that the chronic inflammatory state induced by T2DM undermines the antitumor memory immune response. We posit that this subversion manifests as a quantitative reduction and/or functional exhaustion of CD45RO$^+$ TILs, which in turn correlates with more aggressive disease. Understanding this interaction has not only prognostic implications but also suggests that optimizing metabolic status may represent a supportive therapeutic strategy capable of restoring antitumor immune competence.

**Aim:** The goal of this study was to evaluate the density and distribution of CD45RO$^+$ memory T lymphocytes in invasive ductal breast carcinoma and to assess their potential prognostic significance, with a specific focus on the modulatory influence of concomitant type 2 diabetes mellitus.

**Objectives:**

1. Quantify the density of CD45RO$^+$ cells in the intratumoral and peritumoral stromal compartments of invasive ductal breast carcinoma;
2. Perform a comparative analysis of CD45RO$^+$ immune infiltration between patients with and without type 2 diabetes mellitus (T2DM);
3. Evaluate correlations between the density of CD45RO$^+$ TILs and key clinico-pathological parameters, including histologic grade, molecular subtype, Ki-67 proliferation index, and lymph-node status;
4. Interpret the potential prognostic value of CD45RO$^+$ infiltration in the context of metabolic and inflammatory dysregulation associated with T2DM.

**Materials and methods**

This study was conducted in accordance with the approval of the Research Ethics Committee of the "Nicolae Testemițanu" State University of Medicine and Pharmacy, Chișinău, Republic of Moldova (Approval No. 7, November 12, 2021). It represents a retrospective analysis of archived histopathological materials. All procedures complied with institutional and ethical guidelines for biomedical research and were performed in line with the principles of the Declaration of Helsinki.

***Study population and case selection.*** A retrospective analysis was carried out on 60 cases of invasive ductal carcinoma, no special type (NST), obtained from the archives of the Institute of Oncology, Republic of Moldova, collected during 2018–2020. Cases were selected according to strict inclusion and exclusion criteria and were divided into two cohorts:

- Group I (Control group): 30 cases of breast carcinoma from patients without a confirmed diagnosis of type 2 diabetes mellitus (T2DM).
- Group II (Study group): 30 cases of breast carcinoma from patients with a confirmed diagnosis of T2DM.

Inclusion criteria: confirmed diagnosis of invasive ductal carcinoma NST and age above 50 years. Exclusion criteria: preoperative treatment (chemotherapy or radiotherapy), diagnosis of type 1 diabetes mellitus, or the presence of other known autoimmune diseases.

***Histopathological evaluation.*** The postoperative tissue fragments were fixed in 10% neutral buffered formalin for 6–36 hours, processed using standard histological techniques, and embedded in paraffin. Consecutive sections, 3–5 μm thick, were cut and stained with hematoxylin and eosin (H&E) for morphological evaluation.

The tumor grade was determined according to the Nottingham histologic scoring system, which assesses three key parameters: the degree of tubule formation, nuclear pleomorphism, and mitotic count. Tumor staging was performed following the TNM classification system of the American Joint Committee on Cancer (AJCC).

***Immunohistochemistry (IHC).*** To identify memory T lymphocytes, immunohistochemical staining for the CD45RO marker was performed. Tissue sections were mounted on adhesive slides and processed according to a standard protocol, including deparaffinization, rehydration, and antigen retrieval by heating in a high-pH buffer solution. A monoclonal anti-CD45RO primary antibody was applied, followed by an HRP-based polymer detection system and visualization with diaminobenzidine (DAB) as the chromogen. Counterstaining was carried out with hematoxylin to highlight tissue morphology.

***Quantification of the immune infiltrate.*** Quantitative assessment of CD45RO$^+$ cells was independently performed by two pathologists. Regions of highest lymphocytic density ("hot spots") were identified and analyzed. Cell counting was carried out in at least 10 high-power fields (HPF, objective ×40). Quantification was performed separately for two distinct compartments:

1. Intratumoral compartment (CD45RO-IT): defined as lymphocytes in direct contact with tumor cells, located within neoplastic nests;
2. Peritumoral stromal compartment (CD45RO-PT): defined as lymphocytes situated in the connective stroma adjacent to the invasive tumor front, without direct contact with cancer cells.

Results were expressed as the mean density of positive cells per square millimeter (cells/mm²).

**Statistical analysis**

All collected data were centralized using MS Access 2016 and analyzed with SPSS software (version 23.0; IBM, Chicago, IL, USA). To compare the densities of CD45RO⁺ cells between the T2DM and non-diabetic cohorts, the nonparametric Mann–Whitney U test was applied. The Spearman rank correlation coefficient (rs) was used to assess the degree of association between CD45RO⁺ cell densities and ordinal or continuous clinicopathological variables (tumor grade, Ki-67 index, and tumor size). A p-value < 0.05 was considered statistically significant.

**Results**

The two study cohorts (n = 30 non-diabetic and n = 30 with T2DM) were comparable regarding demographic and clinicopathological characteristics, including mean age at diagnosis, tumor size, and pathological stage, ensuring that T2DM represented the main differentiating variable.

On immunohistochemical examination, CD45RO⁺ cells were identified as small to medium-sized lymphocytes with distinct membranous, occasionally cytoplasmic staining. These cells were predominantly scattered within the peritumoral stroma, often forming perivascular aggregates. Direct intratumoral infiltration within neoplastic nests varied considerably among cases, ranging from dense infiltration to sparse or absent CD45RO⁺ cell contact with tumor cells.

Quantitative comparative analysis revealed a significant impact of T2DM on the intratumoral compartment of the CD45RO⁺ memory T-cell infiltrate. The intratumoral CD45RO⁺ lymphocyte density (CD45RO-IT) was significantly lower in the T2DM cohort (the median: 45 cells/mm²) compared to the non-diabetic cohort (the median: 98 cells/mm²; p = 0.012). This finding suggests a specific impairment in the ability of memory T lymphocytes to penetrate tumor nests in diabetic patients.

In contrast, there was no statistically significant difference in the density of peritumoral stromal CD45RO⁺ cells (CD45RO-PT) between the two groups (p = 0.451). This distinct spatial pattern—reduced intratumoral infiltration but comparable peritumoral presence—indicates a possible immune-exclusion mechanism or accelerated T-cell exhaustion at the stroma–tumor interface rather than a general defect in immune cell recruitment to the tumor vicinity.

To assess the prognostic relevance of the CD45RO⁺ infiltrate, correlations between its density and established indicators of tumor aggressiveness were analyzed. The results, summarized in Table 1, demonstrated that the prognostic value of CD45RO⁺ infiltration is strongly dependent on the patient's metabolic context.

In the non-diabetic cohort, a high intratumoral CD45RO⁺ cell density (CD45RO-IT) showed significant protective associations. A strong negative correlation was observed between CD45RO-IT density and tumor histologic grade (rs = −0.58, p = 0.001) as well as the Ki-67 proliferation index (rs = −0.51, p = 0.004). These findings align with the expected role of memory T lymphocytes in restraining tumor proliferation and limiting aggressiveness. No significant correlations were

***Table 1***

*Spearman's correlation (rs) between CD45RO⁺ cell density and clinicopathological parameters of breast carcinoma in non-diabetic and T2DM cohorts*

| Tumor's characteristics | CD45R0-IT | | CD45R0-PT | |
|---|---|---|---|---|
| | rs | p | rs | p |
| **Non-Diabetic Cohort** | | | | |
| Histologic grade | **-0.58** | **0.001** | -0.15 | 0.429 |
| pT stage | -0.21 | 0.264 | -0.09 | 0.631 |
| pN stage | -0.29 | 0.118 | -0.18 | 0.345 |
| ER status | 0.11 | 0.562 | 0.05 | 0.791 |
| PR status | 0.08 | 0.677 | 0.12 | 0.528 |
| HER2 status | -0.04 | 0.833 | -0.10 | 0.599 |
| Ki67 index | **-0.51** | **0.004** | -0.24 | 0.201 |
| **T2DM Cohort** | | | | |
| Histologic grade | -0.18 | 0.312 | -0.22 | 0.245 |
| pT stage | -0.07 | 0.715 | -0.11 | 0.567 |
| pN stage | **-0.45** | **0.013** | -0.31 | 0.095 |
| ER status | 0.14 | 0.459 | 0.09 | 0.638 |
| PR status | 0.19 | 0.316 | 0.15 | 0.422 |
| HER2 status | -0.09 | 0.641 | -0.06 | 0.755 |
| Ki67 index | -0.20 | 0.258 | -0.28 | 0.133 |

*Notes: rs = Spearman's rank correlation coefficient. Significant results (p < 0.05) are bolded.*

*Abbreviations: IT = intratumoral; PT = peritumoral stromal; T2DM = type 2 diabetes mellitus.*

found for the peritumoral compartment (CD45RO-PT).

In contrast, within the T2DM cohort, these protective correlations were completely lost. There was no significant association between CD45RO-IT density and either histologic grade ($p = 0.312$) or Ki-67 index ($p = 0.258$). This attenuation of correlations suggests a functional impairment of the memory T cells present within the tumor. Furthermore, a new and significant negative correlation emerged: lower CD45RO-IT density was associated with a higher likelihood of regional lymph-node metastasis (pN stage) ($rs = -0.45$, $p = 0.013$).

This finding indicates that, in the context of type 2 diabetes mellitus, a weakened intratumoral memory T-cell response serves as a direct indicator of metastatic potential, underscoring the detrimental impact of metabolic dysregulation on antitumor immune competence.

**Discussion**

The present study provides a detailed insight into the infiltration pattern of $CD45RO^+$ memory T lymphocytes in breast carcinoma and, importantly, demonstrates that their prognostic impact is profoundly modulated by the presence of type 2 diabetes mellitus (T2DM) as a comorbidity. The main finding—a significant reduction of $CD45RO^+$ lymphocytes directly infiltrating tumor nests in patients with T2DM—indicates a fundamental disturbance in the antitumor immune response within this metabolic context.

The biological explanation for these findings lies in the nature of T2DM as a disease of chronic systemic inflammation. This systemic inflammatory status translates into a hostile tumor microenvironment (TME) saturated with pro-inflammatory cytokines (e.g., TNF-α, IL-6), advanced glycation end-products, and metabolites that promote T-cell dysfunction [6]. Within such an environment, T lymphocytes, including $CD45RO^+$ memory subsets, are subjected to chronic antigenic stimulation and metabolic stress, leading to a process known as immune exhaustion.

Although these exhausted T cells may still be present within the TME, they are functionally anergic. They overexpress inhibitory checkpoint receptors such as PD-1 and TIM-3, lose their proliferative capacity, and fail to produce effector cytokines (e.g., IFN-γ), rendering them unable to mediate tumor cell lysis [7].

The spatial pattern observed in our study—a reduction in intratumoral but not peritumoral $CD45RO^+$ cells—strongly supports this hypothesis. It suggests that while memory T cells may still be recruited to the tumor vicinity, they are either actively excluded from penetrating neoplastic nests (immune exclusion) or undergo activation-induced apoptosis upon direct contact with tumor cells in an immunosuppressive microenvironment. The T2DM condition thus appears to imprint an "exhaustion signature" on the tumor immune landscape, making the TME more efficient at neutralizing and eliminating effector T cells.

Our findings call for a re-evaluation of the prognostic utility of $CD45RO^+$ as a biomarker. While the general literature consistently identifies $CD45RO^+$ infiltration as a favorable prognostic factor in solid tumors [8], our results reveal that its value is context-dependent. The loss of protective correlations (inverse associations with histologic grade and Ki-67) in the T2DM cohort is critical evidence of this dependence. This suggests that, in diabetic patients, the mere presence of $CD45RO^+$ cells no longer guarantees effective immune surveillance. The biomarker loses its predictive power because the biological process it reflects—functional immune memory—is compromised by the host's systemic metabolic state.

This interpretation helps reconcile apparently contradictory findings in prior studies. For example, Smolkova et al. reported that in a subgroup of aggressive breast cancers with circulating mesenchymal tumor cells, high infiltration by $CD3^+$, $CD8^+$, and $CD45RO^+$ TILs was paradoxically associated with poorer survival [9]. This phenomenon may be explained by the dual nature of immune responses: in certain biological contexts—such as highly aggressive tumors or a TME conditioned by T2DM—inflammation may become ineffective or even pro-tumorigenic. The classical paradigm of "hot" (inflamed) versus "cold" (non-inflamed) tumors is thus insufficient. Our results support the existence of a third category—the "smoldering" or "exhausted-inflamed" tumor. In patients with T2DM, the tumor may be infiltrated by immune cells (and thus not "cold"), but these cells are functionally inert, turning inflammation into a futile process that, through the chronic release of growth factors and cytokines, may actually support tumor progression [10].

The implications of these findings are multifaceted. First, they underscore that prognostic evaluation in breast cancer should integrate metabolic status. The value of a tissue-based immune biomarker, such as CD45RO, is maximized when interpreted within the broader context of the patient's systemic health. Second, the results raise important considerations for immunotherapy. Breast cancer patients with T2DM may harbor a pre-exhausted T-cell repertoire, potentially rendering them less responsive to immune checkpoint inhibitors (e.g., anti-PD-1), which rely on the reinvigoration of pre-existing T-cell populations [11]. This suggests that therapeutic strategies targeting glycemic control and systemic inflammation should be considered essential adjunctive measures, capable of "reconditioning" the tumor microenvironment and restoring its permissiveness to effective antitumor immunity.

**Limitations of the Study**

This study has several limitations. Its retrospective design and the relatively small sample size may restrict the generalizability of the findings. In addition, the analysis is based on quantitative metrics (cell counts) and does not include direct functional assessments of $CD45RO^+$ cells (e.g., co-expression of exhaustion markers such as PD-1, or assays of cytokine production). Accordingly, the inference of functional impairment is a deduction grounded in quantitative data and the existing literature rather than direct functional testing. The absence of long-term survival data prevented a direct evaluation of overall survival, limiting our analysis to correlations with well-established surrogate

prognostic markers. Despite these constraints, the study provides compelling evidence of the impact of T2DM on the tumor immune landscape and lays the groundwork for future prospective research incorporating detailed molecular and functional analyses.

**Conclusions**

1. Intratumoral infiltration of CD45RO$^+$ memory T lymphocytes in invasive ductal breast carcinoma is a meaningful indicator of the host immune response, and its density correlates inversely with tumor aggressiveness in non-diabetic patients.

2. Concomitant type 2 diabetes mellitus (T2DM) is significantly associated with reduced intratumoral CD45RO$^+$ TIL density, suggesting impairment of the antitumor memory immune response—potentially via immune exclusion and/or accelerated exhaustion within the tumor microenvironment.

3. The protective prognostic associations of CD45RO$^+$ infiltration are attenuated or lost in patients with T2DM, underscoring the profound impact of systemic metabolic dysregulation on the local tumor microenvironment and validating CD45RO as a context-dependent biomarker.

4. These findings indicate that the chronic inflammatory state of T2DM fosters an immunosuppressive TME that undermines effective immune surveillance. We recommend integrating patients' metabolic status into the prognostic evaluation of the tumor immune infiltrate to improve risk stratification and guide personalized therapeutic decisions.

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