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HER2/NEU AND CD3 EXPRESSION IN BREAST CANCER ASSOCIATED WITH DIABETES

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Rezumat. HER2/neu și expresia cd3 în cancerul de sân asociat cu diabetul.

Cancerul de sân reprezintă una dintre cele mai frecvente neoplazii la nivel global, fiind responsabil pentru o mortalitate ridicată, mai ales în prezența comorbidităților precum diabetul zaharat de tip 2. În acest context, markerii moleculari precum CD3 și HER2/neu joacă un rol esențial. CD3 este o proteină crucială pentru funcționarea și activarea celulelor T, având un rol central în imunitatea antitumorală. În paralel, receptorul HER2/neu, un membru al familiei receptorilor factorului de creștere epidermică (EGFR), este implicat direct în procesele de proliferare celulară, angiogeneză și agresivitate tumorală în cancerul de sân. Scopul acestui studiu a fost evaluarea expresiei markerilor CD3 și HER2/neu în carcinoamele mamare NST (non-special type) la pacientele cu și fără DZ de tip 2. Analiza urmărește corelarea nivelului de expresie al acestor markeri cu prezența diabetului zaharat și cu caracteristicile clinico-patologice ale tumorilor.

Cuvinte-cheie: Cancer de sân, diabet zaharat, CD3, HER2/neu, carcinoame NST.

Abstract.

Breast cancer is one of the most common neoplasms globally, being responsible for a high mortality rate, especially in the presence of comorbidities such as diabetes mellitus (DM). In this context, molecular markers such as CD3 and HER2/neu play an essential role. CD3 is a crucial protein for the function and activation of T cells, playing a central role in antitumor immunity. Similarly, the HER2/neu receptor, a member of the epidermal growth factor receptor (EGFR) family, is directly involved in processes of cellular proliferation, angiogenesis and tumor aggressiveness in breast cancer. The aim of this study was to evaluate the expression of CD3 and HER2/neu markers in NST (non-special type) breast carcinomas in patients with and without type 2 DM. The analysis focuses on correlating the expression levels of these markers with the presence of diabetes mellitus and the clinicopathological characteristics of the tumors.

Keywords: Breast cancer, diabetes mellitus, CD3, HER2/neu, NST carcinomas.

Резюме. Экспрессия HER2/NEU и CD3 при раке молочной железы, ассоциированном с диабетом.

Рак молочной железы является одним из самых распространенных новообразований в мире, отвечающих за высокий уровень смертности, особенно при наличии сопутствующих заболеваний, таких как сахарный диабет. В этом контексте молекулярные маркеры, такие как CD3 и HER2/neu, играют ключевую роль. CD3 рецептор является жизненно важным для функции Т-клеток, исполняя важную роль в противоопухолевом иммунитете. Рецептор HER2/neu, являющийся членом семейства рецепторов эпидермального фактора роста (EGFR), напрямую участвует в процессах клеточной пролиферации, ангиогенеза и опухолевой агрессивности при раке молочной железы. Целью данного исследования было оценить экспрессию маркеров CD3 и HER2/neu в карциномах NST (неспецифического типа) молочной железы у пациенток с сахарным диабетом 2 типа и без него. Анализ направлен на корреляцию уровня экспрессии этих маркеров с наличием сахарного диабета и клинико-патологическими характеристиками опухолей.

Ключевые слова: Рак молочной железы, сахарный диабет, CD3, HER2/neu, карциномы NST.

Introduction.

Cancer represents a complex disease that affects individuals regardless of age, ethnicity, or geographic location. A notable example is breast cancer, which originates in the glandular tissue of the breast. This pathology is characterized by distinct features at the microscopic, clinical, and imaging levels, as well as in its therapeutic management. According to data published by GLOBOCAN 2020, breast cancer constitutes approximately 10% of all cancer cases worldwide, including in the Republic of Moldova [1], [2].

On the other hand, diabetes mellitus is a chronic metabolic disorder with a complex and multifactorial etiology, stemming from genetic or acquired dysfunctions related to insulin secretion and/or peripheral tissue resistance to its action. This condition causes significant disturbances in carbohydrate, lipid, protein, and ionic metabolism and is often accompanied by immune dysfunctions. The chronic complications of diabetes are primarily the result of microangiopathy, associated with sustained and elevated levels of hyperglycemia. The impact of diabetes is substantial, affecting approximately 9% of the population both globally and nationally [2], [3], [4].

CD3 is an essential protein in the functioning of T cells, playing a central role in their activation. [5], [6]. Structurally, CD3 is a transmembrane protein located on the surface of T cells, where it forms a complex with the T-cell receptor (TCR). Its structure comprises four distinct membrane protein isoforms: CD3-delta (δ), CD3-epsilon (ε), CD3-gamma (γ), and CD3-zeta (ζ). These subunits are organized into three sets of dimers: CD3-epsilon-delta, CD3-epsilon-gamma, and CD3-zeta-zeta. Understanding this structure is critical due to its impact on T cell functionality, as structural deficiencies, particularly in the delta and epsilon subunits, can severely impair T lymphocyte differentiation. According to Fischer et al. (2005), such structural anomalies compromise cellular immunity and are associated with numerous clinical consequences, underscoring the pivotal role of CD3 in immune homeostasis. [7].

The HER2/neu receptor, also known as ErbB2, is a transmembrane receptor with a molecular weight of approximately 185 kDa and is part of the epidermal growth factor receptor (EGFR) family. Its function involves activating intracellular signaling pathways that regulate cellular growth and survival processes. [8].

Current evidence indicates that HER2/neu contributes to mammary tissue development and the maintenance of its homeostasis. However, in approximately 20% of breast carcinoma cases, HER2/neu is amplified, leading to a significant increase in tumor aggressiveness [9], [10].

HER2/neu overexpression is associated with accelerated cellular proliferation, increased tumor invasiveness, and a poorer prognosis [11], [12]. Current evidence demonstrates that tumor cells with high levels of HER2/neu expression become more susceptible to immune system attacks through T cell activation, highlighting the importance of assessing this marker's expression in immune therapeutic strategies [12].

Recent studies in the field have demonstrated the efficacy of bispecific antibodies targeting both HER2/neu and CD3, paving the way for new therapeutic strategies in the treatment of HER2-positive breast cancer. These bispecific antibodies function by actively recruiting cytotoxic T cells into the tumor microenvironment, thereby stimulating a specific immune response against the carcinoma. A notable example is the bispecific anti-HER2/anti-CD3 antibody, known as BiHC, which has shown remarkable cytotoxic activity. BiHC facilitates the formation of an immune synapse between T cells and HER2-positive tumor cells, leading to their lysis through cell-mediated mechanisms. Preclinical data have reported a significant reduction in tumor mass in experimental models, underscoring the clinical potential of these approaches. [13], [14], [15], [16].

The interactions between HER2/neu and CD3 in the tumor microenvironment play a critical role in regulating the immune response and can directly influence tumor progression. For instance, T cell activation via CD3 can stimulate the secretion of pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), which, in turn, can modulate HER2/neu expression on tumor cells (Li et al., 2018; Zhou et al., 2015). This alteration in HER2/neu expression establishes an immunological feedback loop that simultaneously impacts tumor cell behavior and promotes further recruitment of T cells to the tumor microenvironment. [16], [17].

The presence of CD3+ T cells within the tumor is well-documented and is considered a positive prognostic factor in HER2-positive breast cancer. Studies by Okabe et al. (2017) and Hou et al. (2018) have shown that a higher density of T cells, particularly CD8+ T cells, is associated with improved survival, suggesting that their activation via CD3 plays a significant role in controlling tumor progression. This correlation highlights the importance of simultaneously assessing HER2/neu and CD3 expression to stratify risks, identify patients eligible for immunotherapy, and personalize treatment strategies [18], [19].

To date, there is limited evidence regarding how associated diabetes mellitus may influence the

presence of CD3+ T cells and HER2/neu receptor expression in the tumor microenvironment and peritumoral area of breast cancer. The aim of this study was to elucidate how diabetes mellitus, a condition that affects multiple tissues, can alter the immune and molecular landscape of breast tumors. Thus, we investigated the distribution of CD3+ T cells and the level of HER2/neu expression in both unaffected mammary parenchyma and breast carcinomas obtained from patients with and without type 2 diabetes mellitus. Our analysis revealed that diabetic status does not significantly impact the density of CD3+ T lymphocytes in intra- and peritumoral regions or the level of HER2/neu expression by malignant cells. However, the interaction between CD3-mediated immune activation and HER2/neu signaling remains a critical aspect in understanding tumor progression and optimizing therapeutic strategies for breast cancer, regardless of the presence of diabetes mellitus. Further studies are needed to explore potential subtle differences and their implications for targeted therapies and immunotherapy.

Materials and methods.

This descriptive, retrospective study was conducted on a sample of 58 cases of invasive ductal breast carcinomas of NST (non-special type) registered between 2021 and 2022 at the Oncology Institute of the Republic of Moldova. In 29 of these cases, the tumor was associated with type 2 diabetes mellitus (T2DM). The mean age of patients in the T2DM group was 63.2±6.5 years, while the mean age in the non-diabetic group was 64.5±7.9 years. Preoperative blood glucose levels were determined using the colorimetric method with the Selectra Pro XL biochemical analyzer (Netherlands).

The control group consisted of 30 mammary tissue samples obtained from women who had died accidentally, with no history of oncological diseases. The mean age in the control group was 64.2±6.2 years.

Immunohistochemical procedures were performed to evaluate the expression of CD3 and HER2/neu. The analysis of CD3+ lymphocytic infiltration was conducted following the methodology proposed by Salgado et al. (2015), which specifies the use of appropriate magnifications (20x–40x) for a detailed assessment of lymphocytic infiltration [20].

HER2/neu assessment was performed according to the guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).

The histopathological evaluation was performed by two experienced pathologists, who carefully selected the cases included in the immunohistochemical analysis to ensure the quality and relevance of the data.

Quantification and statistical analysis. Quantitative assessments were performed in ten microscopic fields (intra-/peritumoral or periductal/periacinar in the control group, x200), selecting areas with the highest density of immunohistochemical expression.

The cases were divided into two groups (tumors associated with diabetes and tumors without diabetes) based on clinical and morphological data, using MS Access 2007. Descriptive statistical analysis was conducted using WINSTAT 2012.1 software (R. Fitch Software, Bad Krozingen, Germany), with X±SD values and medians calculated. Associations between variables were determined using Spearman's correlation coefficient (r_s).

CD3 and HER2/neu values in the two groups were compared using the t-Student test for paired and unpaired samples. A significance threshold of $p \leq 0.05$ was considered statistically significant for all tests.

The study was approved by the Ethics Committee of the "Nicolae Testemițanu" State University of Medicine and Pharmacy (no. 7, 12.11.2021), which respects the ethical standards for clinical research and patient data protection.

Results.

In the unaffected mammary gland parenchyma, CD3+ T cells were identified as infiltrates localized in the intralobular, interlobular, and intraepithelial stroma, with a noticeable concentration around glandular ducts and acini. In the extralobular stroma, the presence of CD3+ T cells was less prominent, being observed mainly around the ducts. Within the mammary stroma, CD3 marker reactivity was mild, indicating a predominantly periductal and periacinar distribution around glandular structures.

The average number of intraepithelial CD3+ T cells was 22.2±18.4 (ranging from 6 to 55, with a median of 17.5). CD3 expression in the ductal and periacinar areas averaged 22.2±15.7 (ranging from 14 to 54, with a median value of 16.5). To assess the relationship between CD3 expression in intraepithelial ductal and periductal areas, the correlation coefficient was calculated. Statistical analysis revealed that this association was not statistically significant ($r_s = -0.35$, $p = 0.25$).

In cases of invasive ductal carcinoma without associated diabetes, CD3+ T cells were unevenly distributed. The peritumoral areas showed a higher density of CD3+ cells, particularly around medium-sized blood vessels. At the interface between the tumor and cancer-free areas, a sharp decrease in CD3+ lymphocytic infiltration was observed. The content of CD3+ T cells within the tumor was evaluated at 16.6±14.5 (ranging from 0 to 57, with a median of

15). In peritumoral areas, the number of CD3+ T cells was 33.6±16.3 (ranging from 0 to 62, with a median of 20).

Comparing intra- and peritumoral areas, statistically significant differences were identified using both the independent t-test (t = -4.19, p = 0.0001) and the paired t-test (t = -4.19, p = 0.00025). Statistically significant differences were also observed in cases associated with diabetes mellitus between the density of CD3+ T lymphocytes in intra- and peritumoral areas. These differences were confirmed using both the independent t-test (t = -3.87, p = 0.00028) and the paired t-test (t = -3.53, p = 0.00145) Figure 1.

Examining the relationship between the absence of diabetes, HER2/neu marker expression, and intra- and peritumoral CD3 levels, the following results were obtained: for HER2/neu and intratumoral CD3, the correlation did not reach statistical significance (r_s = 0.01, p = 0.48). Similarly, for HER2/neu and peritumoral CD3, the correlation was negative and low but also not statistically significant (r_s = −0.21, p = 0.14).

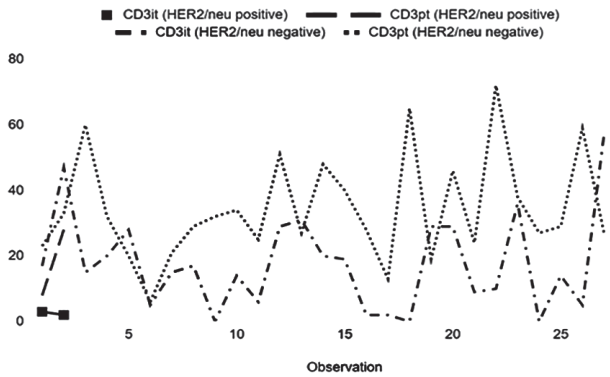

Figure 1. Trends in CD3it (intratumoral site) and CD3pt (peritumoral) expression based on HER2/neu status in non-diabetic CGM. This chart highlights CD3 values according to HER2/neu status (positive or negative) for non-diabetic patients. Clear differences between CD3it and CD3pt values can be observed, with more pronounced variations in patients with HER2/neu-negative status.

In the analysis of statistical associations in cases with associated diabetes, it was found that the correlation between HER2/neu and intratumoral CD3 was negative but not statistically significant (r_s = −0.27, p = 0.07). However, for HER2/neu and peritumoral CD3, the correlation was positive and statistically significant (r_s = 0.33, p = 0.04).

These results indicate an inverse statistical relationship between the presence of diabetes and intratumoral CD3 infiltration, suggesting a potential reduction in the density of CD3+ T lymphocytes within the tumor in the presence of metabolic dysregulation. Conversely, the moderate and positive correlation observed for peritumoral CD3 suggests that diabetes may promote the recruitment of CD3+ T lymphocytes to the tumor margins. The statistical significance achieved for peritumoral CD3 in diabetes highlights the importance of further studies to elucidate the mechanisms underlying these differences Figure 2.

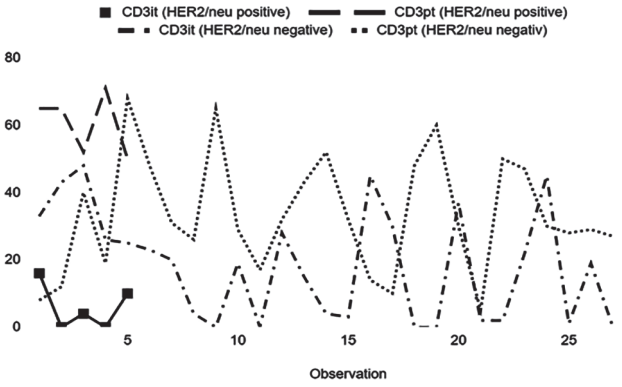

Figure 2. Trends in CD3it (intratumoral) and CD3pt (peritumoral) expression based on HER2/neu status in diabetic CGM. This chart provides a visual representation of the impact of HER2/neu expression on CD3 values among diabetic patients. The differences between CD3it and CD3pt highlight the role of HER2/neu expression in shaping the immune response, an aspect that warrants further investigation.

Discussions.

The immune system plays a crucial role in monitoring and maintaining tissue homeostasis, including in the mammary parenchyma. CD3+ T cells predominantly accumulate in the intralobular stroma and around glandular structures, indicating active presence near ducts and acini. However, in the extralobular stroma, the density of these cells is visibly lower, which may reflect differing defense needs in this region.

This seemingly harmonious distribution is disrupted in the presence of invasive ductal carcinoma. The current study highlighted that in cases without associated diabetes, CD3+ T lymphocytes predominantly cluster in the peritumoral areas, particularly around medium-caliber blood vessels. This morphological pattern suggests the presence of a "protective barrier" at the tumor margin, which, for various reasons, fails to penetrate the tumor interior. Furthermore, the sharp reduction in CD3+ infiltration density at the interface between the tumor and cancer-free tissue clearly underscores this phenomenon. The tumor microenvironment, known for its sophisticated mechanisms of immune evasion, appears to create an invisible yet powerful barrier that prevents T cell infiltration into the carcinoma.

Additionally, the analysis of CD3+ density between intra- and peritumoral areas confirmed a

statistically significant difference. This pattern is observed in both cases without diabetes and those associated with diabetes mellitus, leading to an important question: what is the role of diabetes in this immunological dynamic?

Our study highlights that diabetes influences the density of peritumoral CD3+ T cells, with these cells being more numerous in the presence of diabetes. Interestingly, however, diabetes does not significantly increase CD3+ density within the tumor itself. It is possible that the systemic inflammation associated with diabetes amplifies immunological activity at the tumor periphery but fails to overcome the internal immunosuppressive barriers created by the tumor. In this study, we also analyzed the relationship between HER2/neu markers and the density of CD3+ lymphocytes. Surprisingly, we did not identify a significant correlation between HER2/neu and CD3 density in either intra- or peritumoral regions. These results suggest that HER2/neu status does not directly influence the local immune response, although previous studies have suggested that HER2 overexpression can modulate the tumor microenvironment [21]. However, when we analyzed the impact of diabetes on the relationship between HER2/neu and CD3 density, we observed a significant positive correlation between diabetes and peritumoral CD3 density. This finding raises important questions about how metabolic status influences local immunological activity.

The results of this study contribute to the existing data on the complexity of interactions between the immune response, metabolic status, and tumor biology. While the increased peritumoral lymphocytic infiltration may indicate an active immune response [22], it is evident that immune escape mechanisms succeed in limiting this response, particularly within the carcinoma itself [23], [24]. In addition, diabetes, known for its systemic pro-inflammatory effects, appears to play a paradoxical role: on one hand, it amplifies lymphocyte density at the tumor periphery, while on the other, it contributes to maintaining a low immune infiltration within the tumor itself.

In conclusion, this study adds new pieces to a complex puzzle, highlighting the importance of the local immune response in breast cancer and how it is influenced by factors such as diabetes mellitus. The findings suggest that the tumor microenvironment can block the local immune response, while the metabolic alterations induced by diabetes may influence the immune response around the tumor. The intra- and peritumoral content of CD3+ lymphocytes differs depending on the presence or absence of metabolic dysregulation caused by diabetes. Moreover, the increased expression of HER2/neu in neoplasms associated with diabetes enhances the infiltration of CD3+ T lymphocytes into the peritumoral stroma. These observations open new fields for research, focusing on the mechanisms that regulate intra- and peritumoral immune responses, as well as the development of novel therapies targeting these complex interactions, taking into account the systemic dysregulation induced by diabetes mellitus.

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