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ORIGINAL PAPER

EXPRESSION OF CD8, CD68 AND HER2 MARKERS IN BREAST CARCINOMA IN DIABETES PATIENTS

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Abstract

Introduction. Type 2 diabetes mellitus (T2DM) is recognized as a risk factor for breast cancer, potentially influencing tumour aggressiveness and immune response through the tumour microenvironment (TME).
The objective of the study was to evaluate the expression of immune markers such as CD8, CD68 and HER2/neu receptor in the TME of invasive breast carcinoma in diabetes compared to non-diabetes patients.
Material and methods. The study included invasive breast carcinoma patients with T2DM (n=29) and without T2DM (n=43), from the Oncological Institute of Republic of Moldova, between 2018–2020. Immunohistochemical (IHC) analysis evaluated the expression of CD8, CD68, HER2/neu markers. Statistical analysis aimed the mean, median and associations with clinicopathological features.
Results. In patients with T2DM, the TME showed a significantly lower density of intratumoural CD8+ T lymphocytes (mean 15.2 vs 17.5 in non-diabetics, p=0.0005) and a significantly higher infiltration of intratumoural CD68+ macrophages (mean 10.61 vs 9.5 in non-diabetics, p=0.02). Although overall HER2 expression was comparable between the groups,

Résumé

Expression des marqueurs CD8, CD68 et HER2 dans le carcinome du sein chez diabétiques

Introduction. Le diabète de type 2 (DT2) est reconnu comme un facteur de risque de cancer du sein, influençant potentiellement l'agressivité tumorale et la réponse immunitaire via le microenvironnement tumoral (MET).
L'objectif de l'étude a été d'évaluer l'expression de marqueurs immunitaires tels que CD8, CD68 et le récepteur HER2/neu dans le MET du carcinome mammaire invasif chez les patientes diabétiques par rapport aux patientes non diabétiques.
Materiel et méthodes. L'étude a inclus des patientes atteintes d'un carcinome mammaire invasif, diabétiques (n = 29) et non diabétiques (n = 43), dans l'Institut d'Oncologie de la République de Moldova, pendant 2018-2020. L'analyse immunohistochimique (IHC) a évalué l'expression des marqueurs CD8, CD68 et HER2/neu. L'analyse statistique a porté sur la moyenne, la médiane et les associations avec les caractéristiques clinicopathologiques.

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correlation analysis revealed significant, diabetes-specific associations. In the T2DM cohort, intratumoural CD8+ cells positively correlated with perineural invasion (r=0.42, p=0.01), and intratumoural CD68+ macrophages strongly correlated with nodal extension (pN) (r=0.42, p=0.01).

Conclusions. T2DM induces significant alterations in immune cell populations and molecular interactions within the breast carcinoma TME. These diabetes-specific changes suggest distinct tumour biology, emphasizing the necessity for personalized therapeutic strategies in diabetes patients with breast cancer.

Keywords: HER2/neu, tumour microenvironment, type 2 diabetes mellitus, breast carcinoma.

List of abbreviations:

AGE = advanced glycation end-products
CD = cluster of differentiation
CD8it = intratumoural CD8+ T lymphocytes
CD8pt = peritumoural CD8+ T lymphocytes
CD68it = intratumoural CD68+ macrophages
CD68pt = peritumoural CD68+ macrophages
DAB = 3,3′-diaminobenzidine
HE = hematoxylin-eosin
HER2/neu = human epidermal growth factor receptor 2
IHC = immunohistochemistry
IGF-1 = insulin-like growth factor 1
M1 = classically activated macrophage phenotype
M2 = alternatively activated macrophage phenotype
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells = natural Killer cells
NST = no special type (ductal carcinoma)
pM = distant metastases status (TNM classification)
pN = regional lymph node status (TNM classification)
pT = primary tumour size/extent (TNM classification)
PnI = perineural invasion
RAGE – receptor for advanced glycation end-products
SD = standard deviation
STAT3 = signal transducer and activator of transcription 3
T2DM = type 2 diabetes mellitus
TAMs = tumour-associated macrophages
TCR = T cell receptor
TME = tumour microenvironment
WHO = World Health Organization

Résultats. Chez les patients atteints de DT2, le TME a montré une densité significativement plus faible de lymphocytes T CD8+ intratumoraux (moyenne de 15,2 contre 17,5 chez les non-diabétiques, p = 0,0005) et une infiltration significativement plus élevée de macrophages CD68+ intratumoraux (moyenne de 10,61 contre 9,5 chez les non-diabétiques, p = 0,02). Bien que l'expression globale de HER2 ait été comparable entre les groupes, l'analyse de corrélation a révélé des associations significatives spécifiques au diabète. Dans la cohorte DT2, les cellules CD8+ intratumorales étaient positivement corrélées à l'invasion périneurale (r = 0,42, p = 0,01), et les macrophages CD68+ intratumoraux étaient fortement corrélés à l'extension ganglionnaire (pN) (r = 0,42, p = 0,01).

Conclusions. Le DT2 induit des altérations significatives des populations de cellules immunitaires et des interactions moléculaires au sein du TME du carcinome du sein. Ces modifications spécifiques au diabète suggèrent une biologie tumorale distincte, soulignant la nécessité de stratégies thérapeutiques personnalisées chez les patientes diabétiques atteintes d'un cancer du sein.

Mots-clés: HER2/neu, microenvironnement tumoral, diabète de type 2, carcinome du sein

Introduction

Breast cancer is a major global public health concern, representing the most common malignancy in women and one of the leading causes of cancer-related mortality. Its incidence continues to rise worldwide, with multifactorial and interrelated risk factors, including genetic predisposition, hormonal influences, lifestyle behaviors, and comorbidities. According to GLOBOCAN 2018 and 2022 estimates, breast cancer accounts for more than 2.3 million new cases and nearly 685,000 deaths annually, underscoring not only its medical relevance but also its substantial socioeconomic impact on healthcare systems and societies worldwide[1–3]. Among various risk factors, type 2 diabetes mellitus (T2DM) has been recognized as an independent risk factor for the development of breast cancer, also being associated with a poorer prognosis and increased tumour aggressiveness[4]. Diabetic women face a 20–27% higher risk of developing breast cancer, and this comorbidity is linked to higher recurrence and mortality rates[5].

The tumour microenvironment (TME) plays a crucial role in the initiation, progression, and response to therapy of breast cancer[5]. It is a complex ecosystem composed of cancer cells, stromal cells (including fibroblasts, endothelial cells), a variety of immune cells, and the extracellular matrix[6]. Dynamic interactions between these components modulate tumour growth, angiogenesis, invasion, and metastasis. In the context of T2DM, systemic metabolic dysregulations, such as chronic hyperglycemia, hyperinsulinemia, insulin resistance, and low-grade chronic inflammation, can profoundly remodel the TME, influencing both cancer cells and immune system components. Molecular mechanisms include the activation of insulin/IGF-1 signaling pathways, which promote cell proliferation and angiogenesis[5], and the creation of a pro-tumourigenic microenvironment through the activation of pro-survival pathways like NF-κB and STAT3. Oxidative stress induced by hyperglycemia contributes to DNA damage and genomic instability[5], and the advanced glycation end-products (AGE) – receptor for AGE (RAGE) axis is also implicated in activating pro-inflammatory and pro-oncogenic pathways[4,5]. There is growing evidence that metabolic alterations related to T2DM may facilitate tumour initiation and fuel evolution toward more aggressive phenotypes by sustaining chronic inflammation and immune dysregulation[5,7,8].

CD8+ cytotoxic T lymphocytes are key players in antitumour immunity, capable of recognizing and destroying cancer cells, and their density in the TME is often correlated with a favorable prognosis[9]. Tumour-associated macrophages (TAMs), frequently identified by the CD68+ marker, can exhibit plasticity from an antitumour M1 role to a pro-tumour M2 role, with high M2 density associated with tumour progression[10]. The human epidermal growth factor receptor 2 (HER2/neu) is overexpressed in 15-20% of breast cancers, associated with an aggressive phenotype. Other immune cells like CD4+ T helper cells, CD3+ total T cells, CD45+ total leukocytes, and CD56+ natural killer (NK) cells also modulate the TME[11]. Recent studies highlight the importance of analyzing a broad panel of immune and stromal markers, as TME complexity cannot be understood by investigating single markers in isolation.

Evidence suggests T2DM can alter CD8+ T lymphocyte function [12], promote a pro-tumour phenotype of macrophages, and influence oncogenic signaling pathways. However, the specific interactions among the CD8+ T cell immune infiltrate, CD68+ macrophages, HER2/neu expression, and an extended panel of other immune and angiogenic markers, in breast carcinoma patients with T2DM compared to non-diabetics, are still insufficiently elucidated[8,12]. Most available studies have focused on single cell types or limited endpoints, leaving a gap in understanding the comprehensive TME remodeling in diabetic versus non-diabetic breast cancer patients[13,14].

The objective of the study was to investigate and compare the expression patterns of immune markers (CD8, CD68), and HER2/neu, within the TME of invasive breast carcinoma in patients with T2DM versus non-diabetic patients. The research hypothesis was that T2DM alters the density, distribution and interrelationships of these markers, contributing to distinct tumor biology.

Material and methods

Study design and patient selection. This descriptive and retrospective study included specimens collected from patients diagnosed with invasive breast carcinoma of ductal no special (NST) type, who underwent surgical treatment at the Oncological Institute of the Republic of Moldova between 2018-2020. The patients did not undergo preoperative chemo- or radiotherapy. Two study groups were formed: group I included 43 patients with breast carcinoma without T2DM; group II included 29 patients with breast carcinoma and T2DM. The serum glycemia was evaluated in all patients pre-operatively, fasting, in venous blood, by the colorimetric method (Selectra Pro XL, NL). The control group consisted of 30 normal breast tissue samples, collected from women aged 50-86 years (who died accidentally, with material collected within the first 24 hours post-mortem

Table 1. Summary of antibodies and IHC protocols used

Marker	*Clone/Code/ Source/ Dilution/ Incubation time*	*Antigen retrieval solution*	*Detection system/ Dilution / Incubation time*
HER2/neu	Polyclonal rabbit/ A0485/ Dako/ 1:600/ 20 min	Dako EnVision™ FLEX Target Retrieval Solution, Low pH (Link), K800521-2, (1:50, 20 min)	Dako Real™ EnVision™ Detection Kit (K5007)/ 1:1000/ 30 min
CD8	C8/144B/GA62362-2/ Dako/ RTU/ 20 min	Dako Target Retrieval Solution, High pH (Link), K800421-2, (1:50, 20 min)	Dako EnVision FLEX (DM842)/ HRP/ RTU/ 20 min
CD68	KP1/M081401-2/ Dako/ RTU/ 20 min		Dako EnVision FLEX+, Mouse, High pH (K8002)/ 1:100/ 30 min

Note: Immunohistochemistry was performed using HER2/neu (polyclonal rabbit, Dako A0485, 1:600, 20 min, low pH retrieval), CD8 (clone C8/144B, Dako GA62362-2, RTU, 20 min, high pH retrieval), and CD68 (clone KP1, Dako M0814, RTU, 20 min, high pH retrieval). Detection was carried out with Dako EnVision™ FLEX or FLEX+ HRP systems, with incubation times ranging from 20–30 minutes. CD – cluster of differentiation; HER2/neu – human epidermal growth factor receptor 2; HRP – horseradish peroxidase; IHC – immunohistochemistry; min – minutes; pH – potential of hydrogen; RTU – ready to use; Dako EnVision™ FLEX – detection system based on a dextran polymer conjugated with horseradish peroxidase.

from the Center of Forensic Medicine of the Republic of Moldova). Relevant clinicopathological data (patients' age, histological type, grade of differentiation, tumour size (pT), regional lymph node status (pN), presence of distant metastases (pM) and perineural invasion (PnI) were collected from clinical observation charts and histopathological reports.

Informed consent and ethical approval. The study was conducted in accordance with the principles of the Declaration of Helsinki. The approval for the research was obtained from the Research Ethics Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova (no. 7/70 of 12.11.2021). Written informed consent was obtained from all participants prior to enrollment, and the confidentiality of personal data was strictly maintained throughout the study.

Tissue sample processing and histopathological analysis. Specimens were fixed in 10% buffered formalin solution (pH 7.2-7.4) for 6-36 hours. Subsequently, selected tissue fragments were dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin (BioWax Plus 56/58, Biognost, HR), according to standard laboratory protocols. Paraffin blocks were sectioned at a thickness of 3-5 µm using a rotary microtome (Accu-Cut® SRM™ 200 Rotary Microtome, Sakura, JP). For initial histopathological diagnosis and evaluation of morphological characteristics, sections were stained with hematoxylin-eosin (HE). The histological type of breast carcinoma was determined according to the World Health Organization (WHO) classification ed. 5-th. Tumour differentiation grade was assessed using the Nottingham grading system (Elston-Ellis), which considers tubule formation, nuclear pleomorphism, and mitotic activity.

Immunohistochemical analysis. Paraffin sections were immunostained for HER2/neu, CD8 and CD68 (Dako Autostainer Link 48). After PT Link deparaffinization, rehydration and heat-induced epitope retrieval (Table 1), mono/polyclonal antibodies (Dako/Agilent) were applied. Dako detection systems with DAB were used. Nuclei were counterstained with Mayer–Lille hematoxylin. Positive and negative controls accompanied each run.

Evaluation of immunostaining and quantification of markers. Two experienced pathologists independently performed the immunohistochemical evaluation using light microscopes (Olympus BX53, equipped with a digital camera Olympus SC180, integrated into the OLYMPUS cellSens dimension analysis system, version 4.2, JP). Image evaluation and quantitative analysis were performed using the dedicated software with the Count & Measure Full module. The expression of CD8 and CD68 was quantified by counting positive cells in at least 10 microscopic fields at 400x magnification, within the tumor and in the adjacent stroma. The results were reported as the average number of positive cells. HER2/neu expression was scored based on the updated College of American Pathologists/ American Society of Clinical Oncology guidelines, ranging from 0 to 3+. A score of 3+ was considered HER2-positive, while scores of 0, 1+, and 2+ were considered HER2-negative. Hybridization *in situ* techniques were not used.

Statistical analysis. A MS Access database was created (MS Access 2016) and data were analyzed (SPSS Statistics 23.0). Continuous variables were reported as mean±SD, median and the range. Group differences were determined using Welch's t or Mann–Whitney U test, depending on data type. Associations were explored with Pearson (r) or Spearman coefficients (rs), interpreted as negligible

Table 2. Expression of CD8, CD68 and HER2 in case of breast NST tumors

Marker		Breast cancer	Breast cancer with T2DM	p-value (differences between groups)
		X±SD; M	X±SD; M	
CD8	it	17.5±13.4; 14	15.2±11.3; 12	0.0005
	pt	29.4±15.2; 28	28.0±18.3; 25	0.73
CD68	it	9.5±7.5; 6	10.61±9.85; 9	0.02
	pt	12.4±12.2; 11	16.3±16.1; 13	0.27
HER2+	-	18.6%	16.7%	>0.05

Note: Values are presented as mean±standard deviation (X±SD) and median (M). Comparisons between groups were performed using p-values to indicate statistical significance.CD – cluster of differentiation; HER2/neu – human epidermal growth factor receptor 2; it – intratumoral; NST – no special type; p – probability value; pt – peritumoral; T2DM – type 2 diabetes mellitus.

(<0.12), low (0.12–0.24), moderate (0.24–0.41) or strong (>0.41). Significance was set at p≤0.05.

Results

Expression of CD8, CD68 and HER2 in control breast tissue. CD8+ T lymphocytes demonstrated similar mean densities intraepithelially (10.8±6.7, median=11.5, range 2-18) and periductally (10.2±5.6, median=8.5, range 4-17), without significant regional differences (p=0.25). CD68+ cells exhibited higher densities in the periductal stroma (29.5±10.3, median=29, range 16-41) compared to intraepithelial compartments (11.8±8.1, median=12.5, range 0-24, p=0.03). HER2 expression was not detected in control breast tissue, consistent with expected findings for normal, non-pathological breast samples.

Patient cohort characteristics and tumour morphology. The study cohort consisted of 43 patients with invasive NST breast carcinoma without T2DM (mean age 63.7±6.9 years) and 29 patients with similar tumour and T2DM (mean age 64.5±7.9 years). Comparative morphological analysis of tumours revealed certain trends. Although the difference was not statistically significant (p=0.089), the mean tumour size was apparently bigger in patients with T2DM (2.9±2.2 cm) compared to those without T2DM (2.1±0.8 cm). Patients with T2DM showed a numerically higher, but statistically non-significant (p=0.35), frequency of advanced tumours. In the non-diabetic group, the tumour distribution was: pT1 in 23 cases (53.5%), pT2 in 18 cases (41.9%), and pT3 in 2 cases (4.7%). In contrast, the diabetic group presented with: pT1 in 13 cases (44.8%), pT2 in 12 cases (41.4%), pT3 in 3 cases (10.3%), and pT4 in one case (3.4%). Regarding nodal metastases (pN), although most cases in both groups were pN0 or pN1, a higher incidence was observed in diabetic group (7 cases, 24.1%) compared to the non-diabetic group (6 cases, 14.3%) (p=0.16). Reduced mitotic activity (Mt 1) was more frequent (p=0.02) in patients with T2DM (58.6%) than in those without T2DM (48.8%), while moderate mitotic activity (Mt 2) was predominant in patients without T2DM (39.5% *vs* 24%, p=0.001). Increased mitotic activity (Mt 3) was slightly higher in patients with T2DM (17.2% *vs* 11.6%, p=0.05). G2 tumours predominated in both groups, accounting for 22 cases (52.4%) in the non-diabetic group and 19 cases (65.5%) in the T2DM group, but G3 tumours were more frequent (p=0.01) in patients without T2DM (38.1%) compared to those with T2DM (27.6%). The correlation between tumour grade and mitotic activity was strong in both groups but more pronounced in patients with T2DM (r=0.69, p=0.00002 *vs* r=0.54, p=0.0002 in non-T2DM).

Immunohistochemical expression of markers and comparison between cohorts. The immunohistochemical analysis revealed notable distinctions in the TME of compared groups. The density of intratumoural CD8+ T lymphocytes of diabetic cohort was found to be significantly lower compared to the non-diabetic group (15.2±11.3, M=12, range 0-40 vs 17.5±13.4, M=14, range 0-57, p = 0.0005), suggesting a compromised cytotoxic immune response in this kind of tumours. In contrast, the infiltration of CD68+ macrophages within the tumour mass and in the surrounding peritumoural tissue was higher among patients with T2DM. Specifically, intratumoural CD68+ cells showed a modest, but statistically significant increase in the diabetic cohort (10.6±9.85, M=9, range 0-27 *vs* 9.5±7.5, M=6, range 1-27, p = 0.02), while peritumoural CD68+ macrophages also tended to be more abundant in the diabetic group (16.3±16.1, M=13, range 0-36) compared to the non-diabetic group (12.4±12.2, M=11, range 0-34), though this difference did not reach statistical significance (p>0.05) (Fig. 1).

Regarding HER2 expression, no substantial difference was observed between the two groups, with the percentage of tumours exhibiting strong (3+) HER2

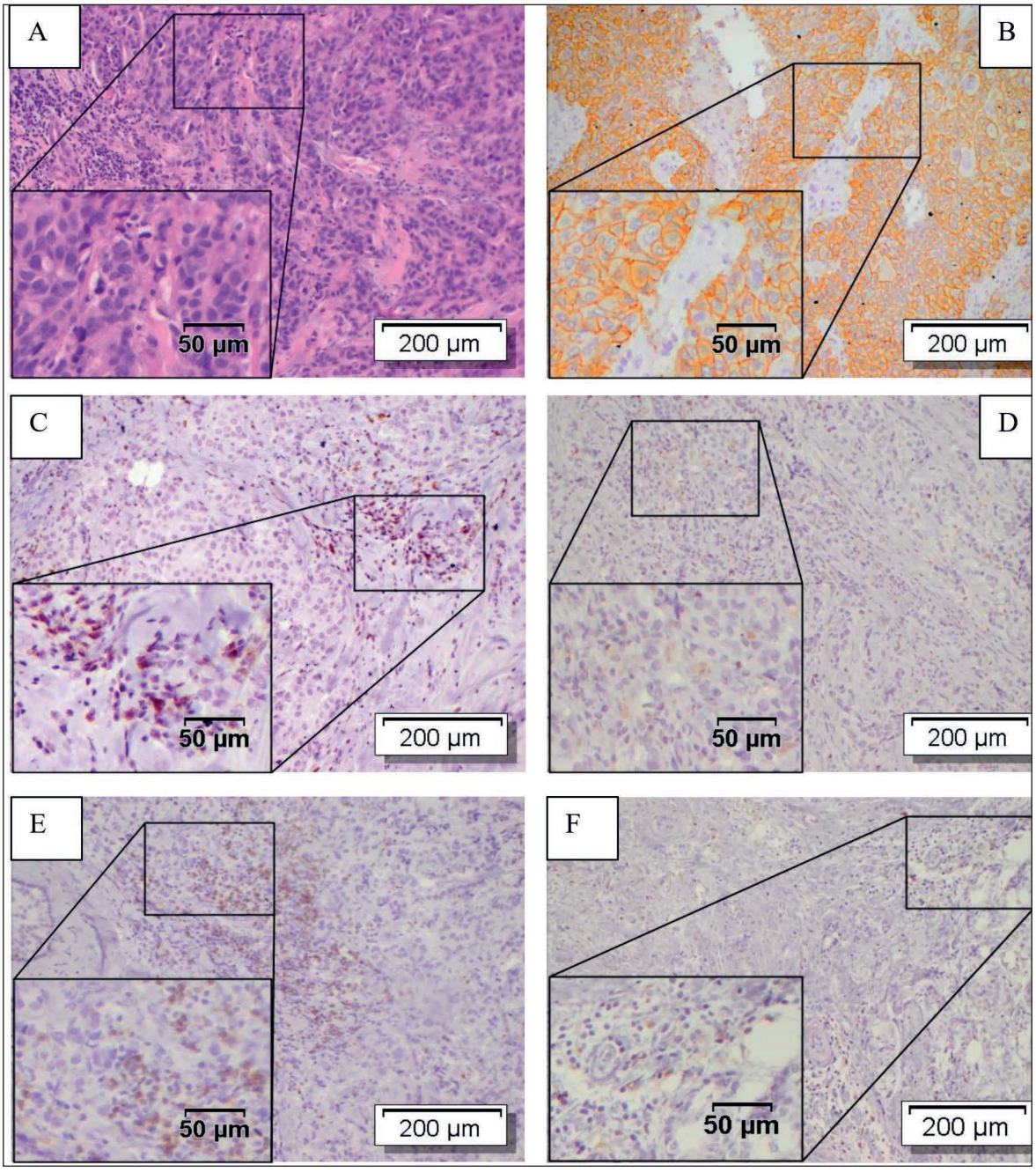


Figure 1. Representative micrographs of tumour tissue stained with HE (A) and IHC: B – HER2/neu expression in breast cancer with T2DM; C – CD8+ cells in breast cancer with T2DM; D – CD68+ cells in breast carcinoma. E– CD8+ cells in breast cancer. F – CD68+ cells in breast carcinoma with T2DM; Mayer's haematoxylin counterstained.

positivity being statistically similar (18.6% in non-diabetic patient's *vs* 16.7% in those with T2DM, p=0.51).

Taken together, these findings indicate that breast carcinomas arising in the context of T2DM are characterized by a relative reduction in cytotoxic T cell infiltration and a concurrent increase in TAMs, whereas HER2 status remains largely unaffected by diabetic status. This altered immune landscape may have important implications for tumour progression and the response to therapy in this patient population.

Correlations with clinicopathological characteristics and markers correlations. T2DM-induced alterations in the TME become more pronounced when examining the correlations between immune markers, clinicopathological parameters and inter-marker relationships (Table 3). CD8+ intratumoural T lymphocytes (CD8it) displayed notable shifts in their associations. In the non-diabetic cohort, CD8it correlated significantly with blood glucose levels ($r = 0.37$, $p = 0.02$), a relationship which was not

Table 3. Statistical associations (r) between CD8, CD68, HER2/neu and clinicopathological characteristics of breast tumors.

	Sugar level	Patient's age	PnI	pT	pN	Mitotic activity	Grade	CD8it	CD8pt	CD68it	CD68pt	HER2/ neu
Sugar level	–	0.37* –0.15	–0.15 0.42*	0.30* 0.23*	–0.08 0.23*	0.06 –0.29	–0.10 –0.05	0.37* -0.15	0.30* –0.08	0.06 –0.10	–0.05 0.08	0.20* –0.08
Patient's age	0.37* –0.15	–	–0.06 0.42*	0.19* 0.23*	–0.10 0.23*	0.02 –0.29	0.19* –0.05	–0.06 0.19*	–0.10 0.26*	0.19 0.40*	0.02 0.14*	–0.15* 0.04
PnI	–0.15 0.42*	–0.06 0.42*	–	0.01 0.23*	–0.04 0.23*	0.02 –0.29	0.03 –0.05	0.01 0.42*	–0.04 0.20*	0.02 0.22*	0.03 0.02	–0.17 –0.13
pT	0.30* 0.23*	0.19* 0.23*	0.01 0.23*	–	–0.23 0.25*	0.02 –0.28	0.51* –0.22	–0.23 0.23*	0.02 0.25*	0.51* 0.09	0.33* –0.03	0.06 0.03
pN	–0.08 0.23*	–0.10 0.23*	–0.04 0.23*	–0.23 0.25*	–	–0.06 –0.28	0.05 –0.22	–0.06 0.23*	0.05 0.27*	–0.06 0.42*	–0.24 0.17	–0.04 –0.11
Mitotic activity	0.06 –0.29	0.02 –0.29	0.02 –0.29	0.02 –0.28	–0.06 –0.2	–	0.20* –0.22	0.20* –0.29*	–0.28 –0.28	0.33* –0.22	0.44* 0.09	0.21* 0.23*
Grade	–0.10 –0.05	0.19* –0.05	0.03 –0.05	0.51* –0.22	0.05 –0.2	0.20* –0.22	–	0.12 –0.05	–0.04 –0.23	–0.05 –0.22	0.13 –0.12	0.15* 0.06
CD8it	0.37* –0.15*	–0.06 0.19*	0.01 0.42*	–0.23 0.23*	–0.06 0.23*	0.20* –0.29*	0.12 –0.05	–	0.68* 0.37*	–0.28 0.45*	–0.21 –0.09	0.17* –0.22
CD8pt	0.30* –0.08	–0.10 0.26*	–0.04 0.20*	0.02 0.25*	0.05 0.27*	–0.28 –0.28	–0.04 –0.23	0.68* 0.37*	–	0.02 0.37*	0.09 0.31*	0.33* 0.15*
CD68it	0.06 –0.10	–0.19 0.40*	0.02 0.22*	0.51* 0.09	–0.06 0.42*	0.33* –0.22	–0.05 –0.22	–0.28 0.45*	0.02 0.37*	–	0.40* 0.38*	0.12 –0.15
CD68pt	–0.05 0.08	0.02 0.14*	0.03 0.02	0.33* –0.03	–0.24 0.17	0.44* 0.09	0.13 –0.12	–0.21 –0.09	0.09 0.31*	0.40* 0.38*	–	0.34* 0.29*
HER2/ neu	0.20* –0.08	–0.15* 0.04	–0.17 –0.13	0.06 0.03	–0.04 –0.11	0.21* 0.23*	0.15* 0.06	0.17* –0.22	0.33* 0.15*	0.12 –0.15	0.34* 0.29*	–

Note: Correlation coefficients (r) between immune markers (CD8, CD68, HER2/neu) and clinicopathological parameters in breast carcinoma (alone (first row) and associated with T2DM (second row)). Significant correlations are marked with an asterisk (*). CD8it – intratumoral CD8+ T lymphocytes, CD8pt – peritumoral CD8+ T lymphocytes, CD68it – intratumoral CD68+ macrophages, CD68pt – peritumoral CD68+ macrophages, HER2/neu – human epidermal growth factor receptor 2, pT – primary tumor size, pN – regional lymph node status, PnI – perineural invasion, r – Pearson or Spearman correlation coefficient, * – statistically significant (p≤0.05).

proved in patients with T2DM (r = −0.15, p = 0.22). Conversely, in the T2DM group, a significant positive correlation emerged between CD8it and perineural invasion (PnI) (r = 0.42, p = 0.01), a pattern absent in non-diabetics (r = 0.01, p = 0.47).

For CD68it, correlations with nodal extension (pN) and patient age became prominent only in the diabetic cohort (pN: r = 0.42, p = 0.01; age: r = 0.40, p = 0.02), while remaining non-significant in non-diabetics (pN: r = –0.06, p = 0.38; age: r = 0.19, p = 0.16). Interestingly, the correlation of CD68it with mitotic activity was significant only in the non-diabetic cohort (r = 0.33, p = 0.04), in comparison to T2DM group (r = −0.22, p = 0.13). Similarly, in the non-diabetic cohort, peritumoural macrophages (CD68pt) correlated positively with mitotic activity (r = 0.44, p = 0.01), suggesting a pro-tumoural role. In contrast, in patients with T2DM, these significant associations disappeared; the correlation with mitotic activity weakened and became non-significant (r = 0.09, p = 0.33).

These findings, summarized in Table 3, indicate that T2DM not only influences the quantity and spatial distribution of immune cells but also reshapes their functional and molecular interactions with tumour characteristics.

Discussion

This study investigated the complex interactions between CD8+ T lymphocyte populations, CD68+ macrophages and HER2/neu expression in the TME of breast carcinoma in patients with and without T2DM. The findings underscore that T2DM is not merely comorbidity but an active factor that significantly modulates the tumour's immune and molecular landscape, with potential prognostic and therapeutic implications.

Impact of T2DM on CD8+ T-lymphocytes infiltrate. It is well known that diabetic microenvironment is characterized by chronic low-grade inflammation, hyperglycemia and altered cytokine profiles, all of

which can negatively impact T-cell homeostasis and function[15,16]. Recent research indicates that hyperglycemia and insulin resistance, hallmarks of T2DM, may impair T-cell receptor signaling and reduce T-cell migration into tumour tissues[17]. Our study supports these conclusions, by presenting a lower content of CD8+ cells in the tumours associated with T2DM.

Additionally, a study by Tobe et al.[18] demonstrated that in diabetic breast cancer patients, immune infiltrates are less prognostically informative than in non-diabetics, likely due to altered immune cell functionality. Moreover, in patients with T2DM, our study revealed a different pattern of correlations for intratumoural CD8+ T lymphocytes (CD8it) compared to the non-T2DM cohort. In the T2DM group, while CD8it did not show significant correlations with most clinicopathological parameters, it notably demonstrated a significant positive association with perineural invasion (PnI) ($r = 0.42$, $p = 0.01$). This contrasts with the non-T2DM group, where the only significant correlation of CD8it was with glucose levels ($r = 0.37$, $p = 0.02$). These findings suggest that T2DM may alter the clinicopathological relevance of CD8+ T cell infiltration in breast cancer, shifting its association from metabolic factors to features of tumour invasion (Table 3).

Role of CD68+ macrophages in the T2DM context. The study revealed an infiltration of CD68+ macrophages into the TME in T2DM patients. Moreover, the intratumural content of CD68it positively correlated with nodal extension (pN) ($r = 0.42$, $p = 0.01$) and patient's age ($r = 0.40$, $p = 0.02$) in the diabetic group only. These data suggest a pro-tumoural role for TAMs in the diabetic context. T2DM, by inducing a chronic pro-inflammatory environment and metabolic alterations, can favor TAM polarization towards an M2-like phenotype, which supports tumour growth, angiogenesis, invasion, and immunosuppression[19,20]. The strong correlation found between CD8it and CD68it ($r = 0.45$ in T2DM) is interesting, while it may indicate co-recruitment, it could also reflect complex interactions where macrophages modulate CD8+ T lymphocyte function, possibly in an immunosuppressive manner. Recent evidence[21] further supports these observations, showing that the infiltration of CD68+ macrophages is increased both in peritumoural and intratumoural sites in breast cancers associated with T2DM, compared to non-diabetic cases, results similar to our data.

Previous studies have shown that the abundance of TAMs is associated with poor prognosis and increased metastatic burden in breast cancer, especially in the presence of metabolic syndrome or T2DM[19,22]. Thus, our results further strengthen the hypothesis that T2DM modifies the tumour immune microenvironment, fostering conditions favorable for tumour progression and immune escape via macrophage-mediated mechanisms.

Trialogical interaction (CD8, CD68, HER2) and clinical implications. Our data suggest a complex trialogical interaction between CD8+, CD68+ and HER2/neu in breast carcinoma, which seems to be altered by the diabetic status. The previous clinical and experimental data show that CD68+ TAMs (TAMs) accumulate preferentially in HER2-positive tumours and correlate with nodal spread, underscoring a bidirectional HER2–macrophage axis that can be amplified by the diabetic status[23,24]. Our findings partially confirmed this, validating a significant correlation between intratumoural CD68+ macrophages and nodal spread in patients with T2DM, but not a significant association with HER2 status. The metabolic context of T2DM appears to create a distinct tumour microclimate, characterized by a possibly inefficient adaptive immune response (CD8+), an increased presence of macrophages with a potential pro-tumour role (CD68+), and a particular interaction with HER2-positive tumours, which might be more proliferative[5].

Study limitations. Limitations of this study include its retrospective design and the relatively small size of the T2DM patient cohort, which may influence the statistical power of some correlations. Also, detailed characterization of macrophage phenotypes (M1 *vs* M2) or the functional status (e.g., exhaustion markers) of CD8+ T lymphocytes was not performed. These aspects represent important directions for future research.

Future research directions. Prospective studies with a larger number of patients and more in-depth molecular analyses are needed to validate these findings and elucidate the precise molecular mechanisms. Close collaboration between oncologists and endocrinologists is essential for the optimal management of patients with breast carcinoma and T2DM. Strategies aimed at modulating the immune response and optimal metabolic control could be particularly relevant.

Conclusions

The study provides additional evidence of the substantial impact of T2DM on the tumour microenvironment in breast carcinoma. T2DM was associated with a reduced intratumoural density of $CD8^+$ T lymphocytes, suggesting a potential impairment of adaptive immune responses. Furthermore, distinct correlations were observed between T-cell

subtypes and clinicopathological parameters such as patient age, Nottingham score, and HER2 status in the context of T2DM, indicating a complex reorganization of adaptive immunity. T2DM also influenced the infiltration pattern of $CD68^+$ macrophages, with increased macrophage density being correlated with nodal extension, thereby pointing to a potential pro-tumourigenic role. Collectively, these findings highlight that interactions among immune cell populations and molecular markers are distinct in the setting of T2DM, reflecting a multifaceted reconfiguration of cellular and molecular networks within the TME under diabetic conditions.

Author contributions

D.B. conceived the study, contributed to its design, performed the processing of specimens and tissue culture methods, conducted the immunohistochemical and statistical analyses, and drafted the initial version of the manuscript. V.F. and L.S. were involved in the conception and design of the study, provided supervision of the research process, and contributed to the critical revision of the manuscript. E.C., V.D., and E.F. participated in the study design, supervised the molecular immunology components, and contributed to the critical revision of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
2. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 2019;157:107843.
3. Ferlay J, Ervik M, Lam F, et al. Cancer Today. Lyon: International Agency for Research on Cancer; 2022. Available from: https://gco.iarc.who.int/today/
4. Karimi MA, Nasiri M, Emami H, Shahidi M. The impact of sodium-glucose cotransporter-2 inhibitors on breast cancer and cancer-related mortality: a systematic review and meta-analysis of randomized controlled trials. *BMC Cancer*. 2025;25:991.
5. Smith J, Doe A. The interplay between breast cancer and diabetes mellitus: shared mechanisms, risk factors, and integrated management strategies. *Aroc J*. 2025. Available from: https://arocjournal.com/journal/the-interplay-between-breast-cancer-and-diabetes-mellitus-shared-mechanisms-risk-factors-and-integrated-management-strategies/
6. Gamde MS, Ogenyi OD. Angiogenesis in breast cancer: a review. *Asian Pac J Cancer Biol*. 2024;9(2):97-103.
7. Ribatti D, editor. *Angiogenesis in Cancers*. Basel: MDPI; 2023. doi:10.3390/books978-3-0365-6917-8
8. Qiu J, Zheng Q, Meng X. Hyperglycemia and chemoresistance in breast cancer: from cellular mechanisms to treatment response. *Front Oncol*. 2021;11: [Article ID].
9. DeCensi A, Puntoni M, Goodwin P, et al. Differential effects of metformin on breast cancer proliferation according to markers of insulin resistance and tumor subtype in a randomized presurgical trial. *Breast Cancer Res Treat*. 2014;148(1):81-90.
10. Stan MC, Mirestean C, Badulescu F. Metabolic syndrome and breast cancer development: a durable friendship. *Med Discoveries*. 2023. Available from: https://www.meddiscoveries.org/articles/1065.html
11. Xie H, Xi X, Lei T, Liu H, Xia Z. CD8+ T cell exhaustion in the tumor microenvironment of breast cancer. *Front Immunol*. 2024;15:1507283.
12. Zhao Z, Alcaraz A, Cordon-Cardo C, et al. Association of genetic susceptibility variants for type 2 diabetes with breast cancer risk in women of European ancestry. *Cancer Causes Control*. 2016;27(5):679-93.
13. Li F, Wang S, Zhang H, et al. Unraveling the breast cancer tumor microenvironment: crucial factors influencing natural killer cell function and therapeutic strategies. *Int J Biol Sci*. 2025;21(7):2606-20.
14. Gross A, Blot W, Visvanathan K. Type II diabetes and subtype-specific breast cancer risk in medically underserved black and white women. *Cancer Res*. 2019;79(13 Suppl):P1-10-02.
15. Hotamisligil GS. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. 2017;17(2):117-30.
16. Wensveen FM, Valentić S, Šestan M, Turk Wensveen T, Polić B. The 'Big Bang' in obese fat: events initiating obesity-induced adipose tissue inflammation. *Eur J Immunol*. 2015;45(9):2446-56.
17. Brown KK. The immunopathogenesis of fibrosis in systemic sclerosis. *Clin Exp Immunol*. 2019;197(2):146-60.
18. Tobe A, Horimoto Y, Kobayashi K, Kamisada N, Hirano M. Impact of diabetes on patient outcomes in breast cancer patients. *Breast Care (Basel)*. 2022;17(5):480-5.
19. Obeid E, Nanda R, Fu YX, Olopade OI. The role of TAMs in breast cancer progression: a review. *Int J Oncol*. 2013;43(1):5-12.
20. Chen Y, Song Y, Du W, Gong L, Chang H, Zou Z. TAMs: an accomplice in solid tumor progression. *J Biomed Sci*. 2019;26(1):78.
21. Foca E, Brinza D, Portnoi E, et al. The influence of diabetes mellitus on blood vessels amount in case of breast cancer. *Mold J Health Sci*. 2023;10(1):3-8.

22. Yung RL, Ligibel JA. Obesity and breast cancer: risk, outcomes, and future considerations. *Clin Adv Hematol Oncol*. 2016;14(12):790-7.
23. Jääskeläinen MM, Rilla K, Salo S, et al. High numbers of CD163+ TAMs predict poor prognosis in HER2+ breast cancer. *Cancers (Basel)*. 2024;16(3):634.
24. Foca E, Brinza D, Portnoi E, et al. Role of CD68+ cells in the progression of breast cancer associated with diabetes mellitus. *Arch Balk Med Union*. 2024. Available from: https://umbalk.org/role-of-cd68-cells-in-the-progression-of-breast-cancer-associated-with-diabetes-mellitus/