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Research Article

Ratio of T-cell Subsets Predicts Prognosis in Triple-Negative Breast Cancer Patients

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Abstract

Background: Triple-negative breast cancer (TNBC) is an aggressive form of cancer characterized by the absence of estrogen, progesterone, and HER2 receptors. Current therapies are ineffective for long-term control and have side effects. Recent research highlights the role of tumor-infiltrating lymphocytes (TILs), specifically various T-cell subsets, in influencing TNBC prognosis. Understanding the interactions between CD4+, CD8+, Treg, TH1, and TH2 cells within the tumor microenvironment could pave the way for personalized immunotherapies, improving the survival and quality of life of TNBC patients.

Methods: The dataset consisted of 204 chemotherapy-treated patients with TNBC from the METABRIC and TCGA datasets. T-cell subset ratios were calculated on the basis of gene expression data. Statistical tests, including logistic regression, Kaplan-Meier survival analysis, and principal component analysis, were conducted to assess the relationship between T-cell ratios and patient prognosis. Statistical validation was performed via confusion matrices, odds ratios, and the Mann-Whitney U test to confirm the model's performance in predicting patient outcomes.

Results: Initial analyses revealed differences in T-cell subset ratios between living and deceased patients with TNBC. Logistic regression confirmed that higher ratios of CD4/CD8, CD4/Treg, CD8/Treg, and CD4/TH1 cells were linked to a better prognosis, whereas higher ratios of TH1/TH2, TH2/Treg, and CD8/TH1 cells were associated with worse outcomes. Statistical validation revealed that higher CD8+ T-cell ratios and certain T-cell subset ratios such as CD4+/CD8+, and CD8+/TH1 ratios, were correlated with improved overall survival. These findings highlight the potential of T-cell subset ratios as prognostic biomarkers in TNBC.

Discussion: These findings support prior research linking immune system composition with TNBC prognosis. The heterogeneity of triple-negative breast cancer and the dynamic nature of the tumor microenvironment suggest that longitudinal studies with larger, more diverse cohorts are needed to refine these findings.

Conclusion: The model offers a promising approach to personalize treatment plans and optimize resource allocation for high-risk patients. Personalized treatment plans can result in higher survival rates by tailoring treatments to individual patient characteristics. Additionally, optimal resource allocation can lower costs and increase access to care for more patients.

Keywords: *Triple-negative Breast Cancer; T-cells; CD4+ T cells; Tumor-Invading Lymphocytes; Principal component analysis*

Introduction

Triple-negative breast cancer (TNBC) is an especially aggressive form of breast cancer characterized by the lack of expression of three key receptors: estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2). This absence of receptors makes TNBC unresponsive to certain targeted therapies that are effective for other types of breast cancer [1]. As a result, TNBC patients have a poorer prognosis and higher rates of recurrence and mortality than other breast cancer subtype patients do. The 5-year survival rate for all TNBC stages combined was approximately 77%, whereas it was approximately 91% for all stages combined for the breast cancer patients in the Surveillance, Epidemiology, and End Results (SEER) database. [2]. Not only this but, the recurrence rate of TNBC in a five-year post-treatment follow-up period has been found to be around 52% [3, 4]

Currently, the standard treatment options for TNBC primarily involve cytotoxic chemotherapy, surgery, and sometimes radiation therapy. While these treatments can effectively shrink the initial tumor, they are often associated with severe side effects and may not be successful in preventing long-term recurrence [5]. Additionally, the lack of targeted therapies limits options for patients whose cancer progresses or becomes resistant to chemotherapy.

This highlights the urgent need for new and effective treatment strategies for TNBC. Recent research has focused on unraveling the complex interplay between tumor-infiltrating lymphocytes (TILs) and the prognosis of TNBC [6]. TILs are a type of immune cell that infiltrates and attacks tumor cells, and their presence and activity have been linked to a favorable prognosis in various cancers, including TNBC [7].

Researchers have observed how the interaction between a pair or an individual TIL influences the prognosis of patients with TNBC [8, 9]. Using multiple statistical tests, the goal of this research was to determine correlations between T-cell subsets, namely CD4+ T cells, CD8+ T-cells, Regulatory T-cells (Tregs), T-helper 1 cells (TH1), and T-helper 2 cells (TH2) and the prognosis of TNBC patients [10, 11, 33, 34].

Many recent studies have focused on either one or two of these TILs, but it is crucial to gain a more holistic understanding of the immune system by understanding how the interaction between all these immune cells either amplifies or mitigates the immune response.

T-cells play many individual roles. For example, Th1 cells activate macrophages and cell-mediated immunity against pathogens. TH2 T cells stimulate immune cells to attack pathogens and thus activate the B-cell arm of the immune response. Tregs are a regulatory class of T cells that prevent a runaway immune response, which itself can lead to severe adverse consequences such as a runaway autoimmune response. Finally, CD8+ T cells directly interact with the cell and release cytotoxins which eventually cause the infected cells to die [10,11]. What has not been fully explored is how the roles change due to the interaction between T cells when a patient has TNBC. By investigating the interplay between various T-cell subsets, including CD4+, CD8+, Tregs, TH1, and TH2 cells, a more comprehensive understanding of the immune microenvironment in TNBC can be achieved. This may lead to immunotherapies that utilize the power of the immune system to fight TNBC more effectively.

In conclusion, this research underscores the critical role of TILs in influencing the prognosis of TNBC patients. Delving deep into these complex interactions holds immense potential for the development of personalized immunotherapies. Such therapies could utilize the power of a patient's immune system to effectively target and eliminate TNBC cells, potentially leading to improved long-term survival and a reduced treatment burden for patients with this aggressive form of breast cancer. Further research with larger patient cohorts and functional assays is warranted to validate these findings and translate them into clinically effective immunotherapeutic strategies for TNBC.

Methods

Patient Cohort Data from METABRIC and TCGA

Patients with triple-negative breast cancer were selected from the METABRIC [12] and TCGA [13] datasets. These two datasets are ideal for this project as they have been analyzed and discussed in the literature, which makes it easier to compare the results obtained. The datasets are also continuously updated which provides the largest public deidentified dataset possible for TNBC. The inclusion criteria covered the patients who had Triple-Negative breast cancer, underwent chemotherapy, and were between 20 and 40 years old. The dataset collected the read count values for a patient.

The data was retrieved from a biopsy and analyzed the survival rates of the patients after a 10-year period. The refined dataset included 204 patients. 68 patients died after 10 years, and 136 patients were still living. The acquired datasets were manually examined and cleaned for any inconsistencies or missing values. Finally, the datasets were merged into one via the Pandas library functions [14]. The read count values were scaled via a logarithmic scaling method [15].

Visualization of dataset Using Seaborn Library

The ratios of various T-cell subsets were calculated within the merged dataset. To analyze the expression of these T cells, the read count values of specific genes were calculated. For CD4+ T cells, the expression of the CD4 gene was used. For CD8+ T cells, CD8A gene expression was measured. For TH1 T cells TBX21 gene expression was measured. For TH2 T cells, GATA3 gene expression was measured [16]. For Treg T cells, the expression of FOXP3 was measured. The Seaborne library was used to perform hierarchical clustering and visualize the data via cluster maps [17]. Hierarchical clustering allows for the identification of groups of samples with similar expression patterns.

Table 1. List of the specific T cell subtypes and corresponding prototypical genes.

T-cell subset	Gene
CD4+	*CD4*
CD8+	*CD8A*
TH1	*TBX21*
TH2	*GATA3*
Treg	*FOXP3*

Statistical Tests

Logistic regression analysis was subsequently conducted using the calculated T-cell subset ratios (TH1/TH2, CD4+/CD8+, CD4+/Treg, CD8+/Treg, CD4/TH1, CD4/TH2, CD8/TH1, TH1/Treg, and TH2/Treg) as independent variables with patient prognosis as the dependent variable. Kaplan-Meier analysis was used to measure the overall survival probability [18]. Correlational analysis was carried out between favorable T-cell ratios and the survival period of the patients who eventually died. The datasets were separated into data from TCBA and data from METABRIC and graphs were generated using R programming [19]. The cell population percentages were analyzed via PCA in R version 4.2.1. Statistical analysis was also conducted where the T-cell percentages were compared to the patient sample groups using Kruskal-Wallis tests and the correlation between T-cell percentages was compared using Spearman Rank correlation [20, 21]. In this case, the Kruskal-Wallis test is used to compare the distribution of T-cell subset percentages between different patient groups. The Spearman Rank Correlation is used to assess the correlation between different T-cell subset ratios and identify potential associations. P values less than 0.05 were deemed to be significant and multiple testing was conducted using the method of Benjamini and Hochberg. The test is used to control the false discovery rate when conducting multiple hypothesis tests. The Benjamini-Hochberg method adjusts the p-value threshold to account for multiple comparisons [22].

Statistical Validation

A confusion matrix was generated to evaluate the performance of the logistic regression model in predicting patient outcomes [23]. After this step, odds ratios were determined. To statistically validate the model, the results were confirmed using the Mann-Whitney U test [24].

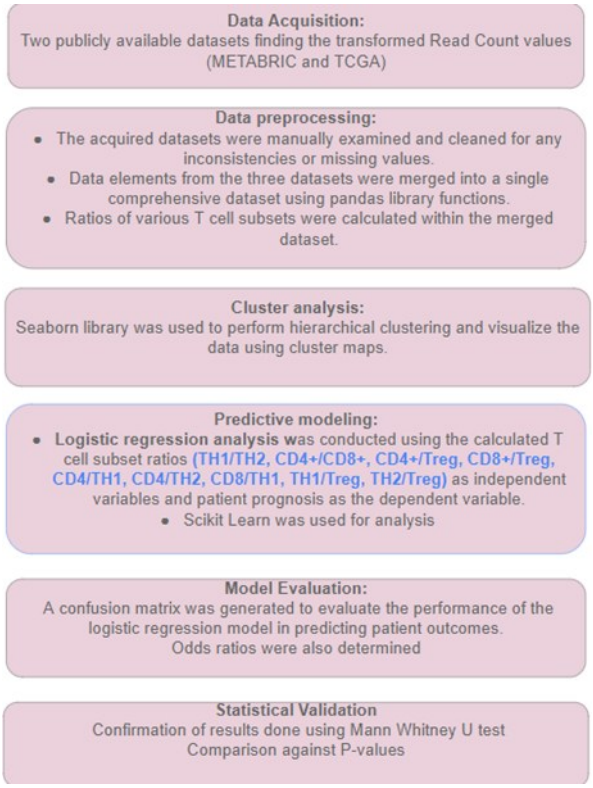

Figure 1. *Diagram of workflow describing the steps taken to analyze the T cell subset ratios.*

Results

Expression Profile Value Differences Between Different T-cell Subtype Ratios

The cluster map revealed differences in the ratios of CD8+/TH1, CD8+/Treg, TH2/Treg, CD4+/TH1, CD4+/Treg, TH1/Treg, CD8+/TH2, CD4+/CD8+, and CD4/TH2 in cells between living patients and deceased patients (Figure 2). A higher ratio of CD8+ to TH1 cells, TH2 to Treg cells, CD4+ to TH1 cells, CD4+ to Treg cells, and CD4+ to CD8+ cells is associated with living patients. A lower ratio of CD8+ to Treg cells, TH1 to TH2 cells, TH1 to Treg cells, CD8+ to TH2 cells, and CD4+ to TH2 cells is associated with living patients.

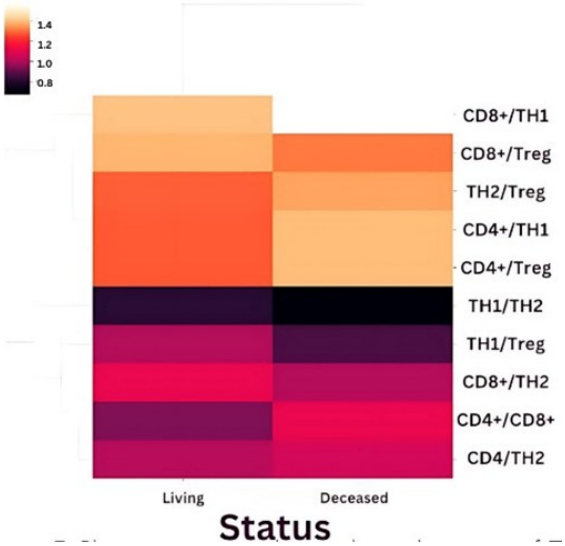

Figure 2. *Hierarchical clustering exhibiting the effect of T-cell ratios in living vs deceased patients.*

An additional statistical test, principal component analysis (PCA), was performed [25]. The PCA plots show that the living individuals are quite spread out whereas the deceased individuals form a compact cluster (Figure 3). This is especially evident in PC1 where CD4+ and TH2+ are contributing to the spread. This finding is reinforced by the results of the Kruskal-Wallis test, which revealed that the numbers of CD4+, TH2, and Treg cells were significantly different between the sample groups. CD4+, TH2, and Treg-related measures are largely independent of each other on the basis of the Spearman rank correlation results showing that each of these cell populations is independent of their association with the sample group.

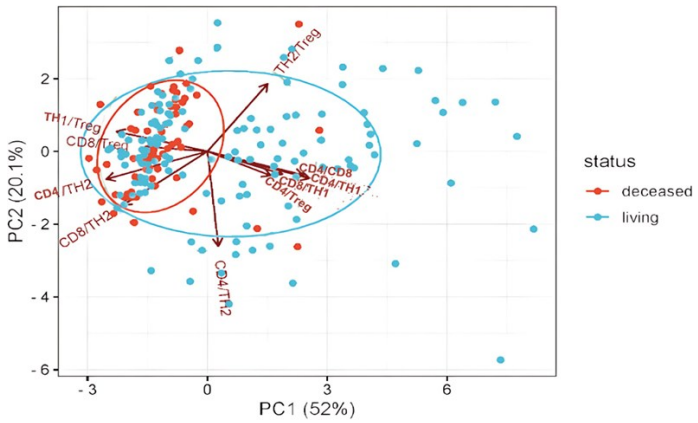

Figure 3. *PCA analysis of T-cell ratio subsets and their significance in prognosis.*

P-values Below 0.01 for Differences in T-cell Subset Ratios Using the Mann-Whitney U Test.

The confusion matrix indicated that the logistic regression algorithm was able to successfully find a grouping between the ratio of the T-cell subsets and status, indicating whether the person was deceased or living. The confusion matrix revealed only one false negative and two false positives. A higher ratio of CD4/CD8, CD4/Treg, CD8/Treg, and CD4/TH1 cells indicates a better prognosis. A higher ratio of TH1/TH2, TH2/Treg, and CD8/TH1 cells indicates a worse prognosis according to the odds ratio. Given the nonnormality of the read count data, a nonparametric test was necessary. A Mann-Whitney U test was conducted to determine if there was a significant difference in the read count ratios between the living and deceased patient groups (Figure 4).

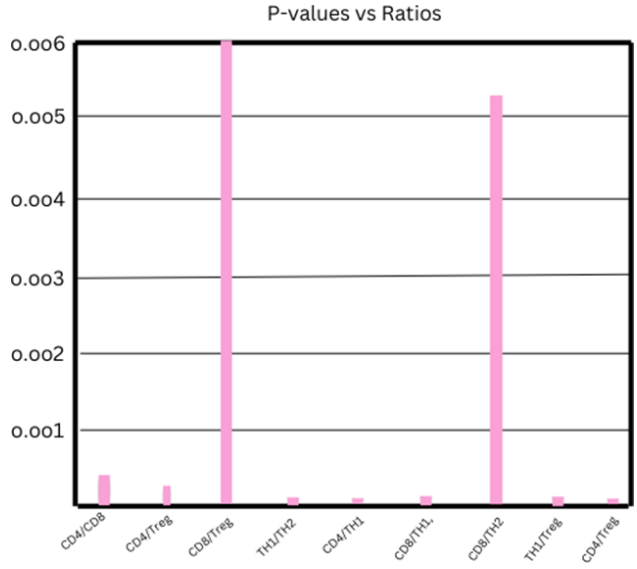

Figure 4. *Graphical representation of P-values of the ratios of the T-cell subsets between living patients and deceased patients.*

The P-values of these ratios were less than 0.01 which indicates statistical significance. The values are shown above (Figure 4).

For all of these ratios, there was a significant difference between the values of these ratios in living patients vs deceased patients which reinforced the logistic regression model. Finally, a Kaplan-Meier analysis was performed for each set. The findings revealed that CD8+ T-cells are significantly correlated with better survival. CD4+ T-cells, TH1, and TH2 mRNA levels were not significantly correlated with better survival. In terms of the ratio analysis, a high ratio of CD4+ to CD8+ T cells was strongly correlated with overall survival (OS). A lower ratio of TH2 cells to Tregs is better correlated with OS. A low ratio of CD8+ T cells to TH1 cells was strongly correlated with OS. A low ratio of CD8+ T-cells to TH2 cells was strongly correlated with OS. A low ratio of CD8+ to TH1 was strongly correlated with OS. A low ratio of CD8+ T-cells to TH1 was strongly correlated with OS. A lower ratio of CD4+ T-cells to Tregs was better correlated with OS. A greater number of CD4+ to CD8+ T-cells indicates a better prognosis.

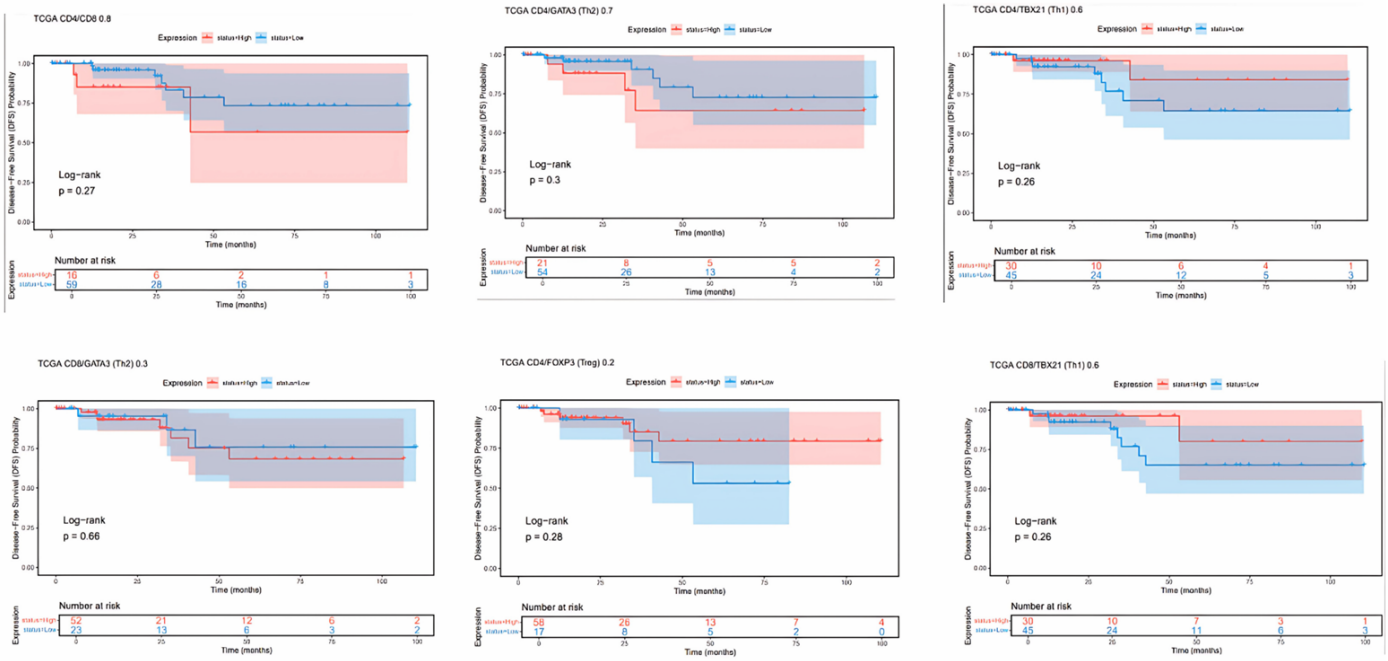

Figure 5. *Graphical representation of the disease-free survival probability in CD4+/CD8+, CD4+/TH2, CD4+/TH1, CD4+/Treg, CD8+/TH1, CD8+/TH2, and CD8+/Treg T-cells*

Discussion

Our study advances the understanding of TNBC by developing a logistic regression model that leverages T-cell subset ratios to predict patient outcomes more accurately. By examining a broader spectrum of T-cell interactions, including CD4+, CD8+, Tregs, TH1, and TH2 cells, this model provides a comprehensive view of the immune landscape, which could lead to more personalized treatment approaches.

Compared to previous models, which often focused solely on CD4+ and CD8+ T-cell interactions, our approach examines multiple T-cell subset ratios [8]. This method enhances the ability to identify patients at higher risk of poor outcomes, allowing for tailored treatment strategies. Our findings align with existing research showing that a higher ratio of cytotoxic T-cells to Tregs is linked to better prognoses, while a high CD4+/Treg ratio suggests poorer outcomes due to the pro-inflammatory role of CD4+ cells potentially facilitating tumor growth [24, 5].

The ratio of immune cells plays a critical role in determining the prognosis of breast cancer patients. Specifically, CD4 T-cells enhance the antitumor response of CD8 T-cells, while Tregs contribute to tumor progression by suppressing immune responses or promoting protumor inflammation [27]. These findings emphasize the prognostic importance of immune cell ratios in guiding therapy decisions, particularly for metastatic TNBC (mTNBC), where the CD8/Treg ratio can be a decisive factor. Interestingly, PD-L1 expression does not significantly affect 1-year OS, highlighting the dominant role of immune cell ratios over PD-L1 in influencing patient outcomes [27].

Th1 and Th2 cells, two subsets of CD4+ T-helper cells, are pivotal in modulating the tumor microenvironment. Th1 cells, characterized by their production of cytokines such as IFN-γ and IL-2, drive cell-mediated immunity by activating cytotoxic CD8+ T-cells and macrophages. This pro-inflammatory response is central to antitumor immunity, with Th1 dominance generally associated with improved tumor control [28]. Conversely, Th2 cells, which produce cytokines such as IL-4, IL-10, and IL-13, mediate humoral immunity but also contribute to an immunosuppressive tumor microenvironment. Th2-dominated responses suppress Th1 activity, recruit Tregs, and support M2 macrophage polarization, which collectively promote tumor progression [28].

In the tumor microenvironment, the Th1/Th2 balance is a critical determinant of cancer outcomes. A Th1-dominated response is linked to enhanced antitumor immunity, while a Th2-dominated environment correlates with immune suppression and metastasis [27]. For instance, in hepatocellular carcinoma, a higher Treg/Th2 ratio promotes metastasis, highlighting the importance of these immune subsets in shaping disease progression. The Th1/Th2 ratio is a prognostic factor for breast cancer and was statistically significant in LumA and Basal-like breast cancer survival analysis. Downregulation of immune-related gene sets and pathways affects the balance of Th1/Th2 towards Th2 polarization and leads to poor outcomes [29].

Tumor-infiltrating Tregs (regulated by factors such as CTLA-4, FOXP3, and PD-L1) further contribute to immune suppression, and immune checkpoint inhibitors (ICPI) have been employed to disrupt these mechanisms. ICPI not only potentiates effector T-cell responses but also reduces Treg-mediated suppression [26]. For example, PD-L1 enhances FOXP3 expression, augmenting Treg suppressive functions, while its blockade prevents naive Th cells from converting into Tregs, thereby favoring a Th1-dominant response [27].

The interplay between CD4+ subsets, including Th1, Th2, Th17, and other helper T cells, shapes the immune landscape of tumors. Identification of Th17 cells, which rely on STAT3 and RORγt for differentiation, has further advanced our understanding of CD4+ T-cell contributions to cancer [29]. Similarly, enhancing antigen uptake by tumor-infiltrating antigen-presenting cells (APCs) could amplify CD4-mediated tumor rejection. These interactions underscore the complexity of tumor immunity, where immune cell ratios and their functional states dictate therapeutic outcomes [30]. By leveraging the unique capacities of Th1 and CD8 cells to orchestrate antitumor responses, alongside strategies to counteract Th2 and Treg influences, immunotherapy continues to evolve as a cornerstone of cancer treatment. Understanding and modulating these immune dynamics will pave the way for more effective, personalized cancer therapies [31].

Limitations

Despite its promise, the study's limitations include a small, homogeneous sample consisting mostly of Caucasian patients, which restricts the generalizability of the findings. TNBC's heterogeneity and the dynamic nature of tumor microenvironments further complicate the ability to predict patient outcomes accurately. Future research should focus on larger, more diverse cohorts and standardizing methodologies for measuring T-cell gene expression to validate and extend these findings.

The potential for T-cell subset ratios as prognostic biomarkers offers a promising avenue for developing personalized immunotherapies, ultimately aiming to improve long-term survival and reduce the treatment burden for patients with this aggressive cancer type. Further studies are warranted to confirm these results and translate them into clinically effective strategies across diverse populations and cancer subtypes.

Conclusion

This study revealed a significant correlation between specific T-cell subset ratios and patient prognosis in TNBC patients. By analyzing the interplay of CD4+, CD8+, Treg, TH1, and TH2 cells, a logistic regression model was developed that was capable of predicting patient outcomes on the basis of their T-cell profiles.

This research has potential for improving TNBC patient care. By identifying patients at higher risk, treatment plans can be tailored, leading to the optimization of resource allocation. Furthermore, our findings contribute to the growing body of knowledge on the tumor microenvironment and immune response in TNBC. While this study provides valuable insights, further research with larger cohorts and diverse patient populations is necessary to validate our findings and translate them into clinical practice.

List of Abbreviations

CD4+ T cells: Cluster of Differentiated 4+ T-cells **CD8+ T cells**: Cluster of Differentiated 8+ T-cells **HER2**: Human epidermal growth factor receptor 2 **OS**: Overall survival **PCA**: Principal component analysis **Tregs**: Regulatory T cells **TH1**: T-Helper 1 cells **TH2**: T-helper 2 cells **TILs**: Tumor-Infiltrating Lymphocytes **TNBC**: Triple-negative breast cancer

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data analyzed during this study are included in this published article and supplementary material.

Conflicts of Interest

To the author's knowledge there is no conflict of interest involved.

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Authors' Contributions

PT conceived the study, wrote the manuscript, performed the data analysis and collection. SM assisted with data analysis and supervision of techniques. DL contributed to the data analysis and edited the manuscript. BL validated the statistical models. JI supervised the project and revised the manuscript. All the authors contributed to manuscript writing and revision.

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