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Preliminary Clinical Observation on Platelet Activation Markers and CD8+PD-1+ Expression in Relation to Breast Cancer Metastasis Risk

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Abstract

Objective: To preliminarily investigate the changes in platelet activation markers and CD8+ T cell PD-1 expression levels in the peripheral blood of breast cancer patients and their relationship with metastasis. **Methods:** A total of 32 breast cancer patients who consecutively visited Qinzhou First People's Hospital from February 2023 to January 2025 and met the inclusion/exclusion criteria were enrolled. According to TNM staging, they were divided into early-stage breast cancer (Stage I-II, n = 16) and locally advanced/metastatic breast cancer (Stage III-IV, n = 16). Additionally, 20 healthy individuals undergoing routine physical examinations during the same period were recruited as the healthy control group. All blood samples were collected before the first biopsy or surgery and prior to any anti-tumor treatment (including neoadjuvant chemotherapy, radiotherapy, or targeted therapy). Flow cytometry was used to detect platelet membrane glycoproteins (CD41+/PLT, CD42a+, CD42b+/PLT, CD61+/PLT), platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, CD41+CD61+CD62P+%), and CD8+PD-1+% expression levels. The associations between these indicators and breast cancer metastasis were analyzed. **Results:** There were no significant differences in platelet membrane glycoprotein expression among the three groups ($P > 0.05$). The platelet activation markers and CD8+PD-1+% in the locally advanced/metastatic group were significantly higher than those in the early-stage group and the healthy control group ($P < 0.01$). The CD8+PD-1+% in the non-metastatic group was also significantly higher than that in the healthy control group ($P < 0.001$). CD41+CD62P+% was positively correlated with CD8+PD-1+% ($r = 0.586$, $P = 0.004$). Multivariate logistic regression analysis showed that

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CD41+CD62P+% (OR = 3.71, 95% CI: 1.74 - 7.91, P = 0.002) and CD8+PD-1+% (OR = 1.13, 95% CI: 1.05 - 1.21, P = 0.011) were independently associated with locally advanced/metastatic status in this study cohort. **Conclusion:** Combined detection of platelet activation markers and CD8+PD-1+% may serve as potential reference indicators for monitoring disease progression in breast cancer patients.

Keywords

Breast Cancer, Metastasis, Platelet Activation, PD-1, Predictive Value

1. Introduction

Breast cancer is a type of malignant tumor resulting from the uncontrolled proliferation of breast epithelial cells. In China, the incidence of breast cancer has been rising year by year. Tumor metastasis accounts for approximately 90% of cancer-related deaths [1]. Despite progress in understanding breast cancer progression and ongoing trials of new treatment methods, the incidence and mortality rates remain high [2]. Platelets are multifunctional cells that, upon activation by various in vivo and in vitro stimuli, undergo deformation, adhesion, aggregation, and release reactions. Their primary function is to recognize vascular lesions and initiate thrombus formation to stop bleeding. This unique characteristic of platelets also contributes to cancer development and progression. The ability of platelets to recognize other cells and neighboring platelets enables them to interact with circulating tumor cells. Receptor recognition and factor-mediated crosstalk between tumor cells and platelets stimulate platelet activation, factor release, and aggregation, thereby promoting tumor cell survival and cancer progression [3]. Therefore, this study explores the correlation between platelet function and tumor staging as well as tumor metastasis.

2. Materials and Methods

2.1. Sources

A total of 32 breast cancer patients who visited the First People's Hospital of Qinzhou City from February 2023 to January 2025 were selected and divided into a non-metastatic group (Stages I-II, 16 cases) and a metastatic group (Stage III-IV, 16 cases) based on the TNM staging criteria. Additionally, 20 healthy individuals from the same period who underwent physical examinations were selected as the healthy control group. There were no statistically significant differences in general characteristics such as age and gender among the three groups (P > 0.05), making them comparable.

Inclusion Criteria: 1) Patients with breast cancer pathologically confirmed pre- or intra-operatively; 2) Aged 18 - 70 years; 3) Willing to participate in this study and having signed an informed consent form.

Exclusion Criteria: 1) Individuals with dysfunction of other organs; 2) Those with other malignant tumors within the past 5 years; 3) Patients who had undergone surgery, chemotherapy, radiotherapy, or other treatments at other institutions; 4) Those who were non-compliant with treatment, abandoned treatment, or were transferred to another hospital; 5) Patients with thrombotic diseases such as cardiovascular conditions; 6) Individuals on long-term platelet antagonist therapy. This study was approved by the Medical Ethics Committee.

2.2. Methods

2.2.1. Observation Indicators

Flow cytometry was used to detect the expression levels of platelet membrane antigens CD41, CD42a, CD42b, CD61, and CD62P, analyze platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, CD41+CD61+CD62P+%), and assess the expression level of programmed death protein-1 (PD-1) in peripheral blood CD8+ T cells of patients (CD8+PD-1+%).

1) Take two flow cytometry tubes, label them as the experimental tube and the isotype control tube, and add antibody reagents to each respectively.

2) Add 5 μL of well-mixed anticoagulated blood to the bottom of the flow tube, avoiding contact with the tube wall.

3) Add 200 μL of PBS to the bottom of the flow tube.

4) Gently vortex to mix and incubate in the dark at room temperature (20˚C to 25˚C) for 30 minutes.

5) Add 1 - 2 mL of PBS and immediately proceed to detection on the machine.

2.2.2. Performance Parameters

CD41 Antibody Detection Kit, CD42a Antibody Detection Kit, CD42b Detection Kit, CD61 Detection Kit: When the positive ratio is greater than or equal to 30%, the CV should not exceed 8%. Inter-batch precision: When the positive percentage is greater than or equal to 30%, the CV should not exceed 15%.

Biological Reference Interval: Evaluation method: Select 20 individuals in a healthy state and perform sample testing. Judgment criteria: Allow up to two samples to fall outside the range.

2.3. Observation Indicators

Flow cytometry was used to detect the expression levels of platelet membrane antigens CD41, CD42a, CD42b, CD61, and CD62P, analyze platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, CD41+CD61+CD62P+%), and assess the expression level of PD-1 in CD8+ T cells (CD8+PD-1+%). The correlations between platelet function, PD-1 expression levels, and breast cancer staging as well as metastatic status were analyzed.

2.4. Statistical Methods

All statistical analyses were performed using SPSS 26.0. Measurement data were described using mean ± standard deviation, while categorical data were expressed

as frequency and percentage. Data normality was tested, and non-parametric tests were applied for data not conforming to a normal distribution. Multi-group comparisons were conducted using one-way analysis of variance (ANOVA), with pairwise comparisons performed via the LSD method. Correlation analysis was carried out using Spearman's correlation. Multivariate analysis employed binary logistic regression, and predictive efficacy was evaluated using receiver operating characteristic (ROC) curve analysis. For inferential statistics, two-sided tests were used, with $P < 0.05$ considered statistically significant. Missing values, outliers, non-compliance, and loss to follow-up were handled by deletion.

3. Results

3.1. Patient Information

This study included a total of 20 healthy controls (healthy group) and 32 breast cancer patients (breast cancer group). Among the breast cancer group, there were 16 cases in Stages I-II (early-stage group) and 16 cases in Stage III-IV (metastatic group). The clinicopathological characteristics of the two groups of breast cancer patients, including age, ER/PR/HER-2 status, and the proportion of triple-negative cases, are shown in Table 1.

Table 1. Clinical and pathological characteristics of breast cancer patients.

Clinical Characteristics	Non-metastatic group (Stages I-II) (n = 16)	Metastatic group (Stage III) (n = 16)
Age (years, mean ± standard deviation)	49.4 ± 7.6	54.8 ± 12.3
ER positive (%)	8 (50.0%)	6 (37.5%)
PR positive (%)	6 (37.5%)	5 (31.3%)
HER-2 positive (%)	7 (43.8%)	5 (31.3%)
Triple-negative (%)	4 (25.0%)	5 (31.3%)

3.2. Comparison of Platelet Membrane Glycoprotein Expression

One-way ANOVA revealed no significant differences in the positive rates of platelet membrane glycoproteins among the three groups ($P > 0.05$), indicating that the expression rates of these surface markers were comparable across groups, thereby excluding potential confounding effects due to differences in membrane antigen expression on subsequent comparisons of activation markers. Detailed data are presented in Table 2.

Table 2. Comparison of platelet membrane glycoprotein expression (%, mean ± standard deviation).

Indicators	Healthy control group (n = 20)	Early-stage group (n = 16)	Metastatic group (n = 16)	P value
CD41+/PLT	96.92 ± 2.79	98.36 ± 0.94	99.12 ± 0.85	0.062

Continued

CD42a+	96.53 ± 2.79	98.09 ± 1.27	99.01 ± 0.78	0.081
CD42b+PLT	96.38 ± 2.83	97.98 ± 1.29	98.85 ± 0.92	0.054
CD61+/PLT	96.94 ± 2.70	98.29 ± 1.18	99.07 ± 0.89	0.068

3.3. Comparison of Platelet Activation Markers

One-way analysis of variance showed significant differences in platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, CD41+CD61+CD62P+%) among the three groups ($P < 0.01$). Further pairwise comparisons (LSD method) revealed that all platelet activation markers in the metastatic group were significantly higher than those in the early stage group and the healthy control group ($P < 0.01$), while there were no significant differences between the early stage group and the healthy control group ($P > 0.05$). These results suggest that platelet activation specifically participates in the metastatic process of breast cancer, rather than being a universal phenomenon in breast cancer occurrence. Specific data are presented in Table 3.

Table 3. Comparison of platelet activation markers (%, mean ± standard deviation).

Indicators	Healthy control group (n = 20)	Early stage group (n = 16)	Metastatic group (n = 16)	P value
CD41+CD62P+%	1.21 ± 1.23	0.94 ± 1.12	2.38 ± 1.52	0.003
CD61+CD62P+%	1.09 ± 1.14	0.85 ± 1.04	2.31 ± 1.48	0.002
CD41+CD61+CD62P+%	1.07 ± 1.14	0.83 ± 1.01	2.25 ± 1.42	0.004

3.4. Comparison of CD8+PD-1+% Expression

One-way analysis of variance showed significant differences in CD8+PD-1+% expression levels among the three groups ($F = 18.94$, $P < 0.001$). Further pairwise comparisons (LSD method) revealed that CD8+PD-1+% in the metastatic group was significantly higher than in the non-metastatic group and the healthy control group ($P < 0.05$), while the early stage group was also significantly higher than the healthy control group ($P < 0.001$). These results suggest that as breast cancer progresses, PD-1 expression on CD8+ T cells gradually increases, reflecting the intensification of T cell immune exhaustion. Specific data are presented in Table 4.

Table 4. Comparison of CD8+PD-1+% among the three groups (%, mean ± standard deviation).

Groups	Number (n)	CD8+PD-1+%	P value
Healthy control group	20	10.50 ± 3.20	
Early stage group	16	30.54 ± 12.41	<0.001
Metastatic group	16	48.72 ± 16.85	

3.5. Correlation Analysis between Platelet Activation Markers and CD8+PD-1+%

Spearman correlation analysis showed that platelet activation markers (represented by CD41+CD62P+%) exhibited a significant positive correlation with CD8+PD-1+% ($r = 0.586$, $P = 0.004$), suggesting that platelet activation and CD8+ T cell immune exhaustion may have a synergistic effect, collectively promoting the metastatic process of breast cancer.

3.6. Multivariate Logistic Regression Analysis of Metastasis Risk

With whether metastasis occurred as the dependent variable (metastasis = 1, non-metastasis = 0), variables with $P < 0.10$ from univariate analysis (CD41+CD62P+%, CD8+PD-1+%) were included in the multivariate logistic regression analysis. The results showed that CD41+CD62P+% was an exploratory factor associated with breast cancer (OR = 3.71, 95% CI: 1.74 - 7.91, $P = 0.002$), and CD8+PD-1+% was also identified as an exploratory factor (OR = 1.13, 95% CI: 1.05 - 1.21, $P = 0.011$). Detailed data are presented in Table 5.

Table 5. Multivariate logistic regression analysis of breast cancer metastasis risk.

Variables	OR	95%CI	*P* value
CD41+CD62P+%	3.71	1.74 - 7.91	**0.002**
CD8+PD-1+%	1.13	1.05 - 1.21	**0.011**

4. Discussion

According to the World Health Organization's International Agency for Research on Cancer (IARC), the latest global cancer burden data for 2020 was released. Globally, new breast cancer cases reached 2.26 million, surpassing the 2.2 million cases of lung cancer, making breast cancer the world's leading cancer [4]-[6]. Breast cancer is a type of malignant tumor resulting from the uncontrolled proliferation of breast epithelial cells. In China, the incidence of breast cancer has been rising year by year, with over 300,000 women diagnosed annually. This upward trend is particularly evident in eastern coastal regions and economically developed large cities. Despite continuous advances in understanding breast cancer progression and ongoing trials of new therapeutic approaches, the incidence and mortality rates remain alarmingly high [7]-[9]. It has been established that even decades after diagnosis and resection of the primary tumor, 25% - 50% of breast cancer patients will ultimately develop fatal metastasis. Certain histological subtypes and molecular markers of breast cancer are considered to hold strong predictive value. For instance, ER-, PR-, and HER-2-negative cancers, known as "triple-negative" breast cancer, are associated with significantly elevated risks of progression and metastasis [10] [11]. Unfortunately, patients with metastatic disease typically have a poor prognosis, with an average 5-year survival rate of approximately 25% [12] [13].

Treatment regimens used in clinical practice can control the growth of primary tumors. However, these methods are less effective in preventing recurrence and managing breast cancer metastasis. The interaction between tumor cells and platelets is a prerequisite for successful hematogenous metastatic dissemination. Once tumor cells enter the bloodstream, they immediately activate platelets to form a permissive microenvironment. Platelets shield tumor cells from shear forces and attacks by natural killer (NK) cells, recruit bone marrow-derived cells through the secretion of chemokines, and mediate the adhesion of tumor cell-platelet emboli to the vascular wall. Subsequently, platelet-derived growth factors endow tumor cells with a mesenchymal-like phenotype and disrupt capillary endothelial barriers to accelerate extravasation into distant organs. Finally, growth factors secreted by platelets stimulate the proliferation of tumor cells into micrometastatic foci [14]. Platelets play a crucial role in hemostasis. Due to the excessive release of factors upon activation, platelet function is also linked to tumor growth, particularly through effects on angiogenesis. It is now widely recognized that the primary role of platelets in poor outcomes for cancer patients occurs during the hematogenous dissemination of cancer cells. Platelets have been demonstrated to confer resistance to circulating tumor cells (CTCs) against NK cell attacks and shear forces, thereby promoting CTC survival in the bloodstream [15]. Platelet membrane surfaces express specific glycoproteins, known as molecular markers specific for activated platelets, with the most important being the platelet membrane glycoprotein GPIIb/IIIa fibrinogen receptor (PAC-1) and P-selectin (P-selectin, CD62P), as well as CD31, CD63, CD41, CD61, and CD62. Studies have found that platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, CD41+CD61+CD62P+%) in the metastatic group of breast cancer patients were significantly higher than those in the early stage group and the healthy control group, while there were no significant differences between the non-metastatic group and the healthy control group. This result suggests that platelet activation is not a universal phenomenon in breast cancer occurrence but specifically participates in the metastatic process of breast cancer. The potential mechanism may involve: activated platelets promoting epithelial-mesenchymal transition (EMT) in tumor cells through the release of cytokines such as TGF-β and VEGF, thereby enhancing their invasive and metastatic capabilities; at the same time, platelets can adhere to circulating tumor cells to form aggregates, helping tumor cells evade host immune surveillance and shear force damage [16]. This study also found that CD8+PD-1+% expression was highest in the metastatic group, followed by the non-metastatic group, and lowest in the healthy control group, showing a gradually increasing trend. This result is consistent with the research by Han Yinli *et al.* [17], reflecting that as breast cancer progresses, PD-1 expression on CD8+ T cells gradually increases, and the degree of T cell immune exhaustion intensifies. As an inhibitory receptor on the T cell surface, PD-1's high expression suppresses T cell cytotoxic function, allowing tumor cells to evade immune clearance. In this study, CD8+PD-1+% in the metastatic group reached as high as 48.72%, indicating that

immune exhaustion is more severe in advanced breast cancer and may be one of the key reasons for resistance to immunotherapy. Further correlation analysis showed a significant positive correlation between CD41+CD62P+% and CD8+PD-1+% ($r = 0.586$, $P = 0.004$), suggesting a possible synergistic effect between platelet activation and CD8+ T cell immune exhaustion. Activated platelets may induce upregulation of PD-1 expression on CD8+ T cells through direct contact or secretion of soluble factors (such as TGF-β and IL-10), thereby weakening anti-tumor immunity; conversely, the immune-exhausted microenvironment may further promote platelet activation, forming a vicious cycle that collectively drives the metastatic process of breast cancer. Studies have found that tumor cells induce platelet activation through the release of tissue factor, thrombin, and matrix metalloproteinases. Activated platelets express a large number of glycoprotein molecules on their membrane surface and adhere around tumor cells to form tumor cell-platelet complexes (TCIPA), providing the possibility for tumor cell survival in the vascular system and successful metastasis [18]. Multivariate logistic regression analysis confirmed that both CD41+CD62P+% and CD8+PD-1+% are independent predictors of breast cancer metastasis. Multivariate logistic regression analysis indicated that both CD41+CD62P+% and CD8+PD-1+% were independently associated with locally advanced/metastatic status in this study cohort, suggesting that combined detection of platelet activation markers and CD8+PD-1+% may help identify patients at higher risk of disease progression.

5. Limitations

However, this study has the following limitations: First, the sample size is relatively small, particularly with only 16 cases in the metastatic group, which may impact statistical power; thus, the conclusions require further validation through expansion of the sample size. Second, as a single-center cross-sectional study, it lacks dynamic follow-up data, making it impossible to establish causal relationships between changes in indicators and the timing of metastasis. Third, other platelet function indicators, such as platelet-leukocyte aggregates, were not assessed, limiting the comprehensive evaluation of platelet function. Future research should involve larger sample sizes and prospective cohort studies for validation.

6. Conclusion

Patients in the breast cancer metastasis group showed significantly elevated platelet activation markers (CD41+CD62P+%, CD61+CD62P+%, and CD41+CD61+CD62P+%) as well as CD8+PD-1+% expression levels. These markers were positively correlated with each other and were all identified as exploratory associations with breast cancer. Combined detection demonstrates high predictive efficacy for metastasis and may serve as a potential reference indicator for monitoring disease progression in breast cancer patients, though further validation with a larger sample size is needed.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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