



DOI: 10.32768/abc.202293346-353



Platelet Activation and Platelet Indices as Markers for Disease Progression in Women with Breast Cancer

Yousra Tera^{a,b} , Hanan Azzam^a , Nashwa Abousamra^a , Marwa Zaki^c , Ahmad Eltantawy^d, Mohammad Awad^d , Hayam Ghoneim^a, Maha Othman^{b,e}

^aClinical Pathology Department, Faculty of Medicine, Mansoura, Egypt

^bBiomedical and Molecular Sciences, Faculty of Medicine, Queen's University, Kingston, Ontario, Canada

^cPathology Department, Faculty of Medicine, Mansoura, Egypt

^dOncology Department, Faculty of Medicine, Mansoura, Egypt

^eSchool of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada

ARTICLE INFO

Received:
9 February 2022
Revised:
1 May 2022
Accepted:
3 May 2022

Keywords:

Breast cancer, Platelet activation, Platelet indices, Metastasis, Cancer, Prognosis

ABSTRACT

Background: Several studies have reported the role of platelet activation, platelet volume (MPV) and other indices in breast cancer but the data is inconsistent and diverse. The aim of this study was to systematically evaluate the role of platelet activation and platelet volume indices in women with BC as predictors of cancer progression and poor prognosis.

Methods: The patients were recruited from our local oncology center from 2019 to 2020 following ethics approval. In total, 80 patients with locally invasive BC, 20 metastatic, and 100 controls were recruited. ADP-induced platelet activation was assessed by light-transmission aggregometry. Platelet P-selectin (CD62P) expression with and without ADP stimulation was assessed by flowcytometry. The comprehensive analysis of platelet count and platelet volume indices (PVI) (MPV, PDW, MPV/P and PDW/P) was conducted. Data was analyzed in relation to tumor pathology, hormone receptors (ER, PR, HER-2) and proliferation index Ki-67. Regression analyses were conducted to predict poor prognosis, tumor aggression and metastatic potential.

Results: We found a significant increase in platelet aggregation (MA), CD62P expression, CD62P+ADP, MPV, PDW, MPV/P and PDW/P in the metastatic group compared to the locally invasive group. Univariate regression analysis showed significance for ADP MA, CD62P+ADP, MPV and PDW/P.

Conclusion: MPV/P and PDW/P can be used as simple low-cost predictors of cancer progression and poor prognosis. We conclude platelet activation and specific platelet indices can help predict prognosis in females with BC.

Copyright © 2022. This is an open-access article distributed under the terms of the [Creative Commons Attribution-Non-Commercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/) International License, which permits copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, except for commercial purposes.

INTRODUCTION

Breast cancer (BC) is the most common malignancy in females worldwide. It constitutes ~

*Address for correspondence:

Maha Othman, M.D., PhD,
 Department of Biomedical and Molecular Sciences, School
 of Medicine, Queen's University, Bottrell Hall, 18 Stuart
 Street, Kingston, Ontario, Canada
 Tel: +1 (613) 533-2600
 E-mail: othman@queensu.ca

38.8% of all malignant tumors among Egyptian female individuals,¹ with 400,000 new cases being diagnosed annually worldwide.² According to the conventional TNM classification, breast cancer is divided into stages from 0 through IV with stage 0 being non-advanced cancers that remain within their original location and stage IV being the advanced cancers that have spread outside the breast to other body parts.³ The host hemostatic system represents



one of several patient factors that are responsible for the control and progression of breast cancer, the others being the host tumor microenvironment and immune response. Platelets, coagulation, and fibrinolysis, can affect cancer hallmarks such as proliferation, evasion from immune-mediated destruction, apoptosis prevention, angiogenesis, tumor invasion and metastasis.⁴

The role of platelets extends beyond haemostasis to include inflammation and immune response, a role established in cancer patients. Platelets secrete various growth factors and pro-inflammatory cytokines that promote angiogenesis, tumor growth, invasion and metastasis of cancer cells either directly or indirectly.^{5,6} One mechanism is tumor cell-induced platelet activation and aggregation which can occur via direct contact with tumor cells or by various mediators like ADP, thromboxane A₂, or serine proteinases, including thrombin.⁷ Another mechanism is the activation of the TGF- β signaling pathway, which promotes metastasis, and invasion by inducing epithelial-to-mesenchymal transition and immunosuppression.⁷

In response to stimuli, platelets undergo a process of activation that leads to membrane-based changes resulting in the expression of markers such as P-selectin, shape change, small-molecule, and protein release. P-selectin binds its main receptor, P-selectin glycoprotein ligand-1 (PSGL-1) -expressed on most leucocytes- facilitating platelet-leucocyte aggregate and leading to a hypercoagulable state. This can explain at least in part cancer associated thrombosis.⁸ To facilitate adhesion to platelets, some cancer cells can upregulate PSGL-1.⁹ Thrombocytosis has also been described in relation to cancer metastasis and poor cancer prognosis including breast cancer.¹⁰ However, details of platelet count changes and activities in various breast cancer stages are not well-defined and are diverse.⁹

Besides surgical removal of tumor tissue, chemotherapy and radiotherapy are efficient treatment modalities of breast cancer. These treatments are known to activate coagulation and fibrinolysis. Chemotherapy stimulates platelet activation and release of platelet-derived microvesicles. Microvesicles transfer several platelet-endothelium cell adhesion receptors such as IIb/IIIa, Ib, and P-selectin and thus facilitate the attachment of platelets to the endothelium at the site of a future metastasis.¹¹ Chemotherapy can also cause thrombocytopenia and bleeding in cancer patients, which adds to the challenge of patient management, making platelet count an essential part of patients' follow up.¹²

In addition to platelet count, platelet indices are part of the automated full blood counts. These

include: Mean Platelet Volume (MPV), which is a measurement of average platelets size and Platelet Distribution Width (PDW), which is a measure of variability in platelet sizes. Plateletcrit (PCT) is the volume occupied by platelets in the blood. Other indices can be calculated as MPV/P by dividing MPV by platelet count and PDW/P by dividing PDW by platelet count. The evidence of the role of MPV as a marker of disease activity in inflammation and cancer including breast cancer has been established,¹³⁻¹⁵ but the utilization of this marker and other indices in cancer progression is not clearly defined and is not routinely applied for patients' follow up.

Despite the reported role of platelets in cancer pathology,¹⁶ there are currently no guidelines or approved indications for antiplatelet drugs in cancer therapy. Oral anti-platelet agents including ADP receptor antagonists, such as clopidogrel may seem attractive to improve the survival of patients with metastatic disease. However, these drugs (commonly combined with aspirin), are only used in cancer patients as part of therapeutic protocols for cardiovascular, pulmonary vascular, cerebrovascular and peripheral vascular diseases. Their rational use in BC patients with cardiovascular co-morbidities might be life-saving. Solid evidence for their use in cancer treatment remains to be generated.

This study was designed to comprehensively evaluate platelet activation and indices in chemo-naïve non-metastatic and metastatic breast cancer in female patients to determine whether these markers are of value in assessing tumor aggression and metastatic potential and in routine patients' follow up and assessing cancer prognosis.

METHODS

Patient recruitment and pathological assessment of cancer

This is a case-control study. The patients diagnosed with breast cancer were recruited from the Oncology unit of Oncology Center Mansoura University (OCMU) from 2019 to 2020. They were divided into two groups: non-metastatic (locally advanced) cancer,⁸ and metastatic cancer.²⁰ All patients were enrolled prior to any surgery or taking any neo-adjuvant cancer therapy. A control group of 100 age matched healthy females were also enrolled. These were recruited from the patients' relatives and also those who underwent routine lab tests for simple elective surgeries.

The patients were excluded if they were taking aspirin, non-steroidal anti-inflammatory drugs, anti-platelet drugs or any other medications that can influence platelet function (14 days prior to blood sample collection). All of the patients had to be chemo-naïve (have not received any neo-adjuvant for



at least 6 months before blood sampling). The patients with any diagnosed hematological disorders or other malignancies were excluded. Fasting for 12 hours with no fatty and dairy foods was recommended.

Pathological data were reviewed to determine the tumor size, tumor grade and lymph node status. Then, the numbers of patients in total BC patients at each stage was calculated for each parameter. Cancer aggression was evaluated according to the tumor size, histological grade and LNs involvement. With respect to tumor size, the patients were divided into two groups (less than and more than the 20mm cut-off). According to the Nottingham tumor histological grade, the patients were divided into three groups (1, 2 and 3). LNs involvement divided the patients into two groups (Neg. and Pos.).

Hormone receptor statuses ER, PR, HER-2 were evaluated via immunohistochemistry (IHC) staining and tumors were divided into ER (Neg. and Pos.), PR (Neg. and Pos.), HER-2 (Neg. (score 0,1) Equivocal (score 2) and Pos. (score 3)). The analysis of Ki-67, was based on the detection of the nuclear antigen Ki-67 using the anti-human Ki-67 monoclonal antibody MIB1. This was evaluated by quantitative analysis using a special imaging software. The Ki-67 was assessed based on the percentage of positively marked malignant cells and the percentage scores were defined as the percentage of positively stained tumor cells among the total number of malignant cells assessed. A Ki-67 cut-off point of 15 % was defined according to the experience of different pathologists as well as national and international recommendations.²⁵

Complete blood count

Blood samples were drawn from all of the participants in the study as part of the routine blood work, via 21-gauge needles into vacutainers containing tri-sodium citrate (3.2%) anti-coagulant. The blood picture was obtained using Sysmex XP-300™ Automated Hematology Analyzer. The following parameters were assessed: RBCs, WBCs, Hgb, platelet count (P). Platelet volume indices (PVIs); MPV, PDW were measured as follows: MPV/P and PDW/P. Mean platelet volume (MPV) is the average volume of platelets and PDW reflects the variation and heterogeneity in platelet size.

Platelet light-transmission aggregometry

Platelet rich plasma (PRP) was prepared by the centrifugation of the whole blood at 500g for 10min and then platelet activity was assessed using PAP-8E brand aggregometer Catalog No. 106075. (ADP); working concentration of 200 μ M was added to PRP in the pre-warmed cuvette for each patient

and tested and primary and secondary waves of aggregation were recorded. Testing was completed within 4 hours of specimen collection

Flowcytometry assessment of platelet activation

Flowcytometry (BD FACScanto II flowcytometer-Becton, Dickinson, BD Bioscience, San Jose, CA 95131, USA) was used to assess platelet activation. CD62-P was used to assess P-selectin expression with and without ADP stimulation. First, CD-41a FITC-Mouse Anti-Human Clone HIP8 was used for gating platelets, and then the percentage of positive CD62-P PE-Mouse Anti-Human Clone AK-4 was determined and compared to the control.

Statistical analysis

Data analysis was conducted using IBM SPSS (IBM Corp. 2011 Version 20.0. Armonk, NY: IBM Corp.). Descriptive statistics like the mean and standard deviation (\pm SD) were used for all the parametric numerical data and frequency and percentages were used for non-numerical data.

Shapiro test was done to test the normality of data distribution. One Way ANOVA was used for assessing the difference between the groups. Pearson correlation was used to assess the strength of association between any two quantitative variables. The Receiver Operating C (ROC) was used to evaluate the sensitivity and specificity of quantitative diagnostic measures to categorize the cases into the two groups (local BC group and metastatic BC group). The optimum cut-off point was defined as what maximized the AUC value. The area under the ROC curve (AUC) was considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and very poor for AUC values between 0.5-0.6. Regression analysis (logistic and ordinal regression) was used for the prediction of risk factors as platelet count, platelet indices, ADP aggregation and CD62-P expression, using generalized linear models.

RESULTS

Overall, the average age (mean \pm SD) of the control and the BC patient group was 54 \pm 9.6 and 52 \pm 11.9, respectively. The age of the metastatic group was significantly less than that of the locally advanced group (45.7 \pm 9.2 to 53.7 \pm 12, respectively) (P=0.007). The left side was more frequently affected in the two groups compared to the right side (54 vs 26 in the locally invasive group and 15 vs 5 in the metastatic group). The patients' characteristics according to the TNM staging system comparing locally invasive cancer (three stages) to metastatic group are also provided in Table 1.



Regarding the hematological changes, WBCs, Hgb and Platelet count was significantly less in metastatic and locally advanced BC group compared to the control but only Hgb showed a significant difference in the metastatic BC group when compared to the locally advanced group. The platelet volume indices (PVIs); MPV, PDW, MPV/P and PDW/P showed a significant increase in the metastatic BC group compared to the locally advanced group and these values in both groups were significantly higher compared to the control group (Table 1).

There was a significant increase in platelet activation either by ADP aggregation (MA) or by flowcytometry; CD-62P expression studies in the metastatic group when compared to the locally advanced BC group showed that in both groups, these were significantly higher than in the control group (Figure 1 and Table 2). Additionally, comparing the three TNM stages of locally advanced BC patient group showed that there was a significant increase in

platelet ADP aggregation and ADP-induced CD-62P expression in stage III compared to stage II. In both stage II and III groups, these were significantly higher compared to stage I group. However, the basal level of CD-62P showed no significant difference in various stages (Table 3).

Pearson correlations showed a significant positive correlation between tumor stage and grade and each of MPV, PDW, MPV/P, PDW/P, MA, CD-62P, CD-62P+ADP, CD-62P diff., but not with platelet count. There was a significant positive correlation between TNM staging and each of PDW, MPV/P, PDW/P, MA, CD-62P, CD-62P+ADP, CD-62P diff., but not with platelet count or MPV. The tumor proliferation index Ki-67 was used to assess tumor aggression and a significant positive correlation was found with MPV/P, ADP MA, CD62 P (with and without ADP stimulation) in BC patients, and a significant negative correlation with platelet count was also seen (Table 2).

Table 1. Complete blood picture and platelet volume indices in locally invasive BC, metastatic BC patient and control groups.

Groups/ Parameters	Control group (1)	locally invasive BC group (2)	Metastatic BC group (3)	P In groups	P Total
WBCs ($10^3/\text{cm}^3$) Mean \pm SD	6.5 \pm 1.5	1.2 \pm 0.15	1.1 \pm 0.09	P1=0.02 P2 \leq 0.001 P3=1.00	\leq 0.001
RBCs ($10^6/\text{cm}^3$) Mean \pm SD	5.17 \pm 0.5	4.8 \pm 2.2	4.75 \pm 1.4	P1=0.06 P2=0.05 P3=1.00	0.3
Hgb. (g/dl) Mean \pm SD	13.95 \pm 0.9	7.7 \pm 0.5	4.1 \pm 0.2	P1=0.03 P2 \leq 0.001 P3=0.001	\leq 0.001
Platelets ($10^9/\text{L}$) Mean \pm SD	23.4 \pm 4.3	11.8 \pm 1.6	10.5 \pm 1.4	P1 \leq 0.001 P2=0.01 P3=1.00	\leq 0.001
MPV (fL) Mean \pm SD	10.9 \pm 0.9	14.6 \pm 1.5	16.7 \pm 1.1	P1=0.015 P2 \leq 0.001 P=0.04	0.02
PDW (fL) Mean \pm SD	13.9 \pm 2.17	16.1 \pm 3.9	18.4 \pm 5.2	P1=0.03 P2 \leq 0.001 P3=0.03	0.044
MPV/P ($\text{fL}/10^9/\text{L}$) Mean \pm SD	0.46 \pm 0.01	1.3 \pm 0.25	1.6 \pm 0.16	P1=0.02 P2 \leq 0.001 P3=0.02	\leq 0.001
PDW/P ($\text{fL}/10^9/\text{L}$) Mean \pm SD	0.59 \pm 0.01	1.36 \pm 0.03	1.75 \pm 0.2	P1=0.023 P2 \leq 0.001 P3=0.01	0.04

Significant P-value \leq 0.05, MPV: mean platelet volume, PDW: platelet distribution width, PDW/P: PDW divided by platelet count, MPV/P: MPV divided by platelet count. P1 is the significance between the groups 1, 2. P2 is the significance between the groups 1, 3. P3 is the significance between the groups 2, 3.

The ROC showed that these parameters have excellent AUCs for discrimination between BC and control groups: ADP MA, CD-62P+ADP, CD-62P Diff., CD-62P with AUCs of 0.98, 0.97, 0.93, 0.9, respectively.

These parameters were either good: MPV/P or fair: PDW, PDW/P with AUCs of 0.8, 0.73, 0.7, respectively. The cut-off value, sensitivity and

specificity of all parameters are shown in Table 4. Given the relatively small sample size, this data need to be interpreted with caution.

Regression analysis was conducted for the prediction of poor prognosis, tumor aggression and metastatic potential of BC disease to late stages.

**Table 2.** Comparison of platelet aggregation and flow cytometry data in locally invasive/ metastatic BC patient & control groups.

Groups/ Parameters	Control group (1)	Locally invasive BC group (2)	Metastatic BC group (3)	P In groups	P Total
ADP MA (%)				P1=0.024	≤0.001
Mean ±SD	54.7±12.1	73.7±19	115±5	P2≤0.001	
CD-62P (%)				P3≤0.001	
Mean ±SD	2.7±1.3	3.5±1.4	6.9±1.9	P1=0.17	0.02
CD-62P + ADP (%)				P2≤0.001	
Mean ±SD	23.4±10	46.8±17.7	81.5±5	P3=0.02	
CD-62P Diff.				P1≤0.001	≤0.001
Mean ±SD	20.5±10	43.5±18.5	74.5±5.5	P2≤0.001	
				P3=0.015	
				P1=0.03	0.001
				P2≤0.001	
				P3≤0.001	

Significant P value ≤0.05. P1 is the significance between the groups 1, 2. P2 is the significance between the groups 1, 3. P3 is the significance between the groups 2, 3. ADP MA: maximum platelet aggregation with ADP agonist by aggregometer. CD-62P is P-selectin flow cytometry expression at basal level. CD-62P+ADP is P-selectin flow cytometry expression after incubation with ADP for 15mins. CD-62P Diff.: the difference between the flow cytometric expression of CD-62P at basal level and activated with ADP agonist

The parameters showing significance in univariable binary logistic regression analysis were MA, CD-62P, CD-62P+ADP, CD-62P diff., PLTs, MPV, PDW, MPV/P, and PDW/P (Table 5). So, these parameters can be regarded as predictors of poor prognosis, tumor aggression and metastatic potential of BC disease to late stages.

DISCUSSION

The conventional TNM staging system can predict the prognosis of breast cancer; however, patients with similar stages may show variable clinical outcomes. Recently, more attention has been focused on the clinical significance of platelet activation and platelet indices in several malignancies including breast cancer. Studies have also reported that platelet indices can predict cancer prognosis. However, to the best of our knowledge, platelet activation studies combined with PVIs

assessments have not been previously conducted in breast cancer patients.

In this study, platelet volume indices MPV, PDW, MPV/P and PDW/P were significantly higher in metastatic breast cancer compared to the locally advanced cancer patients and these figures in both groups were significantly higher than the control. Moreover, these indices were correlated with tumor characteristics including TNM stage and tumor grade. Out of all the indices, MPV/P was also correlated with tumor proliferation as evidenced by Ki-67. Platelet count showed a significant decrease in metastatic and locally advanced BC group when compared to the control but did not significantly differ when the metastatic group was compared to the locally advanced group. However, counts were negatively correlated with tumor proliferation and were correlated with the patients' age.

Table 3. Comparison of aggregation and flow cytometry data between the various TNM stages of locally invasive BC patient groups.

Groups/ Parameters	STAGE I N=10	STAGE II N=36	STAGE III N=34	P In groups	P Total
ADP MA (%)				P1=0.017	
Mean ±SD	46.8±5	66.9±12.8	88.8±14.4	P2≤0.001	≤0.001
CD-62P (%)				P3=0.019	
Mean ±SD	3.3±0.7	3.6±1.5	4±1.3	P1=1.00	1
CD-62P + ADP (%)				P2=1.00	
Mean ±SD	26±4.7	40.6±15.9	59±12.1	P3=1.00	
CD-62P Diff.				P1=0.008	≤0.001
Mean ±SD	22±4	37.8±16	55.8±12	P2≤0.001	≤0.001
				P3≤0.001	
				P1=0.003	
				P2≤0.001	≤0.001
				P3≤0.001	

Significant P-value ≤0.05. P1 is the significance between STAGES 1, 2. P2 is the significance between STAGES 1, 3. P3 is the significance between STAGES 2, 3. ADP MA: maximum platelet aggregation with ADP agonist by aggregometer. CD-62P is P-selectin flow cytometry expression at basal level. CD-62P+ADP is P-selectin flow cytometry expression after incubation with ADP for 15 minutes. CD-62P Diff.: the difference between flow cytometric expression of CD-62P at basal level and activated with ADP agonist.

*We have multiple locations of LAP in some of the patients



There was a significant positive correlation between PDW/P and TNM staging system, tumor size, tumor grade and lymph node. Both PDW and MPV/P are correlated with patient age, TNM staging system, tumor size and tumor grade. MPV showed a correlation with patient age, tumor size and tumor grade. Similarly, Gu *et al.*,¹⁷ documented inverse relations between MPV and prognosis in various cancers and found that elevated MPV levels

significantly correlated with unfavorable prognosis in breast cancer. Takeuchi *et al.*¹⁸ observed that DFS (DFS is defined as the interval between the date of the initial treatment and the first observation of the disease relapse) rate was significantly lower in the elevated PDW/P group than in the low PDW/P group (5-year survival, 81.3% vs. 89.9%, respectively; $P < 0.05$). The reason for the poor prognosis with high PDW/P was unclear.

Table 4. The area under ROC curve and performance criteria of each of the study parameters individually to determine tumor aggression and metastatic potential of disease in BC patient group.

Variables	ADP MA	D6 2P	CD-62P+ADP	CD62P Diff.	MPV	PDW	PV/ P	W/ P
AUC	0.98	0.9	0.97	0.93	0.66	0.7	0.8	0.73
	0.955	0.84	0.94	0.89	0.54	0.63	0.7	0.6
95% CI	1.000	0.96	0.998	0.98	0.77	0.77	0.9	0.84
Cut-off	97.5	4.7	74	70	15.6	17.2	1.48	1.6
Sensitivity (%)	98.8	75	95	98.7	62.5	66	75	75
Specificity (%)	97.5	75	90	90	50	54	85	53
PPV (%)	98	73	93	90	55	67	88	56
NPV (%)	99	76	96	99	65	72	76	76
Accuracy (%)	98.5	74.5	94.5	94.5	60	70	81	66

AUC, area under ROC curve; CI, confidence interval, PPV, positive predictive value; NPV, negative predictive value, MPV: mean platelet volume, PDW/P: PDW divided by platelet count, MPV/P: MPV divided by platelet count. ADP MA: maximum platelet aggregation with ADP agonist by aggregometer. CD-62P is P-selectin flowcytometry expression at basal level. CD-62P+ADP: P- selectin flowcytometry expression after incubation with ADP for 15minutes. CD-62P Diff: the difference between the flowcytometric expression of CD-62P at basal level and activated with ADP agonist.

On the other hand, these researchers¹⁸ also found that the value of MPV as a prognostic factor was inconclusive. As supported by our study, PDW may therefore be a more reliable and accurate prognostic marker than MPV in cancer patients.

Table 5. Univariable regression analysis for the prediction of tumor aggression and metastatic potential of BC.

Univariable regression analysis				
Parameters	P	OR	95% CI	
ADP MA	<0.001	1.3	1.2	1.5
CD-62P	<0.001	2.0	1.5	2.7
CD-62P +ADP	<0.001	1.3	1.1	1.5
CD-62P Diff.	<0.001	1.21	1.1	1.3
MPV	0.044	1.4	0.995	1.996
PDW	<0.001	0.978	0.967	0.989
MPV/P	<0.001	143.5	9.8	210
PDW/P	0.003	0.04	0.01	0.09

OR, odds ratio; CI, confidence interval; ordinal regression analysis is used, Significant P value ≤ 0.05 . ADP MA: maximum platelet aggregation with ADP agonist by aggregometer. CD-62P is P-selectin flow cytometry expression at basal level. CD-62P+ADP: P-selectin flow cytometry expression after incubation with ADP for 15 minutes. CD-62P Diff: the difference between the flow cytometric expression of CD-62P at basal level and activated with ADP agonist. MPV: mean platelet volume, PDW: platelet distribution width, PDW/P: PDW divided by platelet count, MPV/P: MPV divided by platelet count.

MPV and PDW are measures of size distribution and variability of platelets; in general, PDW is an indicator of variation and heterogeneity in platelet

volume; high values of this index indicate the presence of mature and immature cells simultaneously in circulation. Regarding the AUCs of the PVIs, the best cut off value, sensitivity and specificity of MPV were 15.5, 62.5% and 50%, respectively; for PDW these values were 17.2, 66% and 54%, respectively, those of MPV/P were 1.48, 75% and 85%, respectively and for PDW/P they were 1.6, 75% and 53%, respectively. In comparison with Takeuchi *et al.*¹⁸ the cut-offs for the same markers were lower, i.e., 9, 15.3, 0.59, 0.35, respectively. This may be related to the low platelet count seen in our patients especially in the advanced cancer stages.

Our data also showed a significant increase in platelet activation (either by aggregation or by ADP induced CD-62P expression studies) in the metastatic group than the locally advanced BC group, and both patient groups showed significantly higher values compared to the control group. Furthermore, platelet activation significantly differed by cancer stage; higher in stage III compared to stage II, and both groups had significantly higher activations than stage I group. PA was also correlated with patient age.

Platelet activation showed excellent AUCs curves for discrimination between local and metastatic groups with AUCs > 0.9. These findings were in agreement with Cooke, N.M., *et al.*,¹⁹ who found that patients with metastatic breast cancer displayed



significantly increased platelet aggregation responses to ADP compared with healthy controls and platelets were significantly more reactive to lower concentrations of ADP. Similarly, Toth *et al.*²⁰ found that CD62P+ PMP (platelet-derived micro-particles) were highest in patients with advanced breast cancer and significant differences were observed in patients with benign breast tumor as well as within the group of breast cancer patients. CD62P-positive PMP correlated significantly with the presence of metastases. Zhang, *et al.*²¹ studied ADP platelet aggregation and P-selectin expression on hepatocellular carcinoma patients, who were divided according to cell differentiation status.

The patients with moderate and poor differentiation displayed high percentages of P-selectin positive platelets compared to those with well-differentiated tumors or healthy control. Plasma levels of ADP in moderately/poorly differentiated patients increased by 2-fold compared with the other two groups. Several investigators,^{19,20,22,23} reported platelet activation as an important predicting factor for poor prognosis and thromboembolic risk events in BC patients.

Despite the evidence of the role of platelet in cancer progression, only few studies have evaluated antiplatelet drugs. For example, aspirin use has been most extensively studied in colorectal and breast cancer, with proven efficacy in the prevention of colorectal cancer. Aspirin is a known inhibitor of platelet aggregation and was recently shown to also attenuate platelet protein release.²⁴

These previous findings made us exclude the patients on anti-platelet therapy from our study population but this issue is worth evaluation in future studies.

REFERENCES

1. Dawoud MM, Abdelaziz KK-E, Alhanafy AM, Ali MS-d, Elkhouly EAB. Clinical Significance of Immunohistochemical Expression of Neuropeptide Y1 Receptor in Patients With Breast Cancer in Egypt. *Applied Immunohistochemistry & Molecular Morphology*. 2021;29(4):277-86. doi: 10.1097/pai.0000000000000877.
2. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, et al. Cancer treatment and survivorship statistics, 2014. *CA: a cancer journal for clinicians*. 2014;64(4):252-71. doi: 10.3322/caac.21235.
3. Kraeima J, Siesling S, Vliegen I, Klaase J, IJzerman MJ. Individual risk profiling for breast cancer recurrence: towards tailored follow-up schemes. *British journal of cancer*. 2013;109(4):866-71. doi: 10.1038/bjc.2013.401.
4. Cantrell R, Palumbo JS. The thrombin–inflammation axis in cancer progression. *Thrombosis Research*. 2020;191:S117-S22. doi: 10.1016/S0049-3848(20)30408-4.
5. Yan M, Jurasz P. The role of platelets in the tumor microenvironment: from solid tumors to leukemia. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2016;1863(3):392-400. doi: 10.1016/j.bbamcr.2015.07.008.
6. Radziwon-Balicka A, Moncada de la Rosa C, Jurasz P. Platelet-associated angiogenesis regulating factors: a pharmacological perspective. *Canadian journal of physiology and pharmacology*. 2012;90(6):679-88. doi: 10.1139/y2012-036.
7. Lal I, Dittus K, Holmes CE. Platelets, coagulation and fibrinolysis in breast cancer progression. *Breast Cancer Research*. 2013;15(4):1-11. doi: 10.1186/bcr3425.
8. Stoiber D, Assinger A. Platelet-leukocyte interplay in cancer development and progression. *Cells*. 2020;9(4):855. doi: 10.3390/cells9040855.

CONCLUSION

In summary, based on two methods for platelet activation together with the analysis of 4 different platelet indices, patients with metastatic breast cancer have platelet hyperactivity and higher PVIs that are correlated with the tumor stage and grade. These parameters (ADP MA, CD-62P+ADP, MPV, PDW/P) are useful predictors of severity and metastatic potential of cancer.

PVIs with the highest predicting value are MPV and PDW/P, and thus can be used as simple, easily available and low-cost predictors for poor prognosis, tumor aggression and metastatic potential.

Our study provides evidence in support of previous findings in the literature and adds new information that can inform treatment decisions and predict treatment outcomes. The data also highlights the potential role of antiplatelet drugs in attenuating tumor metastasis. Future studies are needed to generate solid evidence regarding the use of antiplatelet drugs in cancer therapy protocols.

ETHICAL CONSIDERATIONS

All the participants consented to take part in the study and the study was conducted in accordance with local ethics regulations and the Institutional Review Board.

FUNDING

This research used no fund from governmental or any other sources.

CONFLICT OF INTEREST

Nothing to declare.



9. Gong L, Cai Y, Zhou X, Yang H. Activated platelets interact with lung cancer cells through P-selectin glycoprotein ligand-1. *Pathology & Oncology Research*. 2012;18(4):989-96. doi:10.1007/s12253-012-9531-y.
10. Stone RL, Nick AM, McNeish IA, Balkwill F, Han HD, Bottsford-Miller J, et al. Paraneoplastic thrombocytosis in ovarian cancer. *New England Journal of Medicine*. 2012;366(7):610-8. doi: 10.1056/NEJMoa1110352.
11. Varon D, Shai E. Role of platelet-derived microparticles in angiogenesis and tumor progression. *Discovery medicine*. 2009;8(43):237-41.
12. Kuter DJ. Managing thrombocytopenia associated with cancer chemotherapy. *Oncology*. 2015;29(4):282-94.
13. Lu F, Pan S, Qi Y, Li X, Wang J. The Clinical Application Value of RDW, CA153, and MPV in Breast Cancer. *Clinical Laboratory*. 2021;67(2). doi: 10.7754/Clin.Lab.2020.200507.
14. Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR. The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashback and traumatic memories, and the Yerkes-Dodson law. *Neural plasticity*. 2007;2007. doi: 10.1155/2007/60803.
15. Shen X-B, Wang Y, Shan B-J, Lin L, Hao L, Liu Y, et al. Prognostic significance of platelet-to-lymphocyte ratio (PLR) and mean platelet volume (MPV) during etoposide-based first-line treatment in small cell lung cancer patients. *Cancer management and research*. 2019;11:8965. doi: 10.2147/CMAR.S215361.
16. Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, Honn KV. Platelets and cancer: a casual or causal relationship: revisited. *Cancer and Metastasis Reviews*. 2014;33(1):231-69. doi: 10.1007/s10555-014-9498-0.
17. Gu M, Zhai Z, Huang L, Zheng W, Zhou Y, Zhu R, et al. Pre-treatment mean platelet volume associates with worse clinicopathologic features and prognosis of patients with invasive breast cancer. *Breast cancer*. 2016;23(5):752-60. doi: 10.1007/s12282-015-0635-6.
18. Takeuchi H, Abe M, Takumi Y, Hashimoto T, Kobayashi R, Osoegawa A, et al. The prognostic impact of the platelet distribution width-to-platelet count ratio in patients with breast cancer. *PloS one*. 2017;12(12):e0189166. doi: 10.1371/journal.pone.0189166.
19. Cooke NM, Egan K, McFadden S, Grogan L, Breathnach OS, O'Leary J, et al. Increased platelet reactivity in patients with late-stage metastatic cancer. *Cancer medicine*. 2013;2(4):564-70. doi: 10.1002/cam4.86.
20. Toth B, Liebhardt S, Steinig K, Ditsch N, Rank A, Bauerfeind I, et al. Platelet-derived microparticles and coagulation activation in breast cancer patients. *Thrombosis and haemostasis*. 2008;100(10):663-9.
21. Zhang R, Guo H, Xu J, Li B, Liu Y-J, Cheng C, et al. Activated platelets inhibit hepatocellular carcinoma cell differentiation and promote tumor progression via platelet-tumor cell binding. *Oncotarget*. 2016;7(37):60609. doi: 10.18632/oncotarget.11300.
22. Kedzierska M, Czernek U, Szydłowska-Pazera K, Potemski P, Piekarski J, Jeziorski A, et al. The changes of blood platelet activation in breast cancer patients before surgery, after surgery, and in various phases of the chemotherapy. *Platelets*. 2013;24(6):462-8. doi: 10.3109/09537104.2012.711866.
23. Holmes CE, Levis JE, Schneider DJ, Bambace NM, Sharma D, Lal I, et al. Platelet phenotype changes associated with breast cancer and its treatment. *Platelets*. 2016;27(7):703-11. doi: 10.3109/09537104.2016.1171302.
24. Singla T, Singla G, Ranga S, Singla S, Arora R. Role of platelet aggregation in metastatic breast cancer patients. *Indian Journal of Pathology and Microbiology*. 2020;63(4):564. doi: 10.4103/IJPM.IJPM_817_19.
25. Inwald, E., Klinkhammer-Schalke, M., Hofstädter, F., Zeman, F., Koller, M., Gerstenhauer, M., & Ortmann, O. (2013). Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. *Breast cancer research and treatment*, 139(2), 539-552. doi: 10.1007/s10549-013-2560-8.

How to Cite This Article

Tera Y, Azzam H, Abousamra N, Zakic M, Eltantawy A, Awad M, et al. Platelet Activation and Platelet Indices as Markers for Disease Progression in Women with Breast Cancer. *Arch Breast Cancer*. 2022; 9(3): 346-53.

Available from: <https://www.archbreastcancer.com/index.php/abc/article/view/530>