Platelets and Breast Cancer

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ABSTRACT **Background:** An association was shown between thrombocytosis and future development of several cancers.

Objectives: To investigate whether pre-treatment platelet counts correlated with clinical outcomes of patients with breast cancer.

Methods: This retrospective study included 22 patients who had been diagnosed with stage I breast cancer (66.8 ± 13.2 years of age), 22 with stage II (61.6 ± 12.3 years old), and 9 with stage III and IV (64.4 ± 15.3 years old). Clinical and hematological data from the first visit to the oncology clinic were collected. The follow-up period was 12 months to 5 years.

Results: A significant difference in platelet counts was found between patients who died (187,000 ± 4000 µ/L) and those who were disease free for 5 years (248,000 ± 83,000 µ/L, *P* = 0.0001). A significant difference in platelet-to-lymphocyte ratio was found between patients who died and those with recurrence (192 ± 81 vs. 124 ± 71, *P* = 0.01). A negative correlation was found between age and lymph nodes (*Ps* = -0.305, *P* = 0.02) and staging and white blood cells count (*Ps* = -0.280, *P* = 0.04). A positive correlation was found between clinical staging and lymph nodes (*Ps* = 0.443, *P* = 0.001) and clinical staging and metastases (*P* = 0.308, P = 0.02).

Conclusions: Platelet counts may be a prognostic marker for breast cancer. Patients who died within 1 year had lower pre-treatment platelet count, which could represent an insidious disseminated intravascular coagulopathy cancer related consumption process.

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An association exists between thrombocytosis and future development of lung, renal, uterine, and colorectal cancer [1]. A prospective study that enrolled 50,000 patients found that among males, 1098 of 9435 male patients were diagnosed with cancer within 1 year after enrolling in the study out with thrombocytosis (11.6%), and 1355 out of 21,826 females were diagnosed within 1 year with thrombocytosis (6.2%) [2]. Of the patients with normal platelet counts, 106 males (4.1%) and 119 females (2.2%) were diagnosed with cancer. The study showed that the risk of cancer increases with higher platelet counts. In this study lung and colorectal cancers were the most commonly diagnosed cancers, while breast and prostate cancers were much less commonly diagnosed.

The population of the Galilee (northern part of Israel) is quite different from the population of the central part of Israel (metropolitan Tel Aviv). The northern population is composed of Jews, Arabs, and Druze.

Our aim was to study the correlation between platelet counts on the first visit to the oncology clinic and the clinical staging and outcome of patients with breast cancer in the Padeh Medical Center, which is a regional medical center in the eastern Galilee. We also studied whether platelet counts, white blood cells count, or the relationship between platelets and white blood cells were associated with the clinical outcome and predicted survival and disease-free survival of patients with breast cancer.

PATIENTS AND METHODS

This retrospective study included patients with a diagnosis of breast cancer who were treated from the first visit in our oncology clinic and had a follow-up period of at least 12 months.

The cohort comprised 22 patients with stage I breast cancer (no macroscopic involvement of lymph nodes or metastases) aged 66.8 ± 13.2 years; 22 with stage II (characterized by a larger breast mass or by involvement of axillary lymph nodes) aged 61.6 ± 12.3 years; and 9 with stage III or stage IV breast cancer (characterized by more aggressive involvement of axillary lymph nodes and distant metastases) aged 64.4 ± 15.3 years. Because of the small number of patients diagnosed with stages III and IV we grouped them together and defined them as stage III. We collected clinical and hematological data from their first visit to the oncology clinic. The study was approved by the internal review board of the Padeh Medical Center.

We recorded the blood cell counts on the patient's first visit to the oncologic clinic in our medical center. The follow-up period was at least 12 months and up to 5 years. We collected the

clinical data on disease-free survival (DFS), the histopathological findings (estrogen and progesterone receptors, and HER2 receptors), and regional lymph nodes involvement, metastases, and tumor dimensions. We used Student's *t*-test and Spearman correlation to explore correlations and differences between groups of patients at different stages of disease. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 18 (SPSS Inc., Chicago, IL, USA). A *P* value of < 0.05 was considered significant.

RESULTS

Fifty-three patients who were diagnosed with breast cancer were eligible to participate in the study. All the patients had blood analysis performed on their first visit to the oncology clinic, and all had a clinical follow-up visits for at least 12 months.

We grouped the patients into three stages according to the routine clinical assessment of patients with breast cancer. Stage I included 22 patients (66.8 ± 13.2 years old), stage II included 22 patients (61.6 ± 12.3 years old), and stage III included 9 patients (64.4 ± 15.3 years old). No significant difference was observed in relation to age. Patients in stage I had more neutrophils (9000 ± 3000 μ/L) compared with patients at stage II (7500 ± 2000 μ/L, $P = 0.04$) [Table 1]. No difference was observed in neutrophil counts between stage II and III; however there was a significant difference between stage I and III, $P = 0.02$ [Table 1]. No difference was observed in platelet and lymphocyte counts, the platelets-to-neutrophils ratios (PNR), and platelets-to-lymphocytes ratio (PLR) between patients in all stages [Table 1].

A significant difference in platelet counts between patients who died (187,000 ± 4000 μ/L) and those who were disease free for 5 years (248,000 ± 83,000 μ/L, $P = 0.0001$) was shown. A significant difference in PLR was found between patients who died and those with recurrence (192 ± 81 vs. 124 ± 71, $P = 0.01$) [Table 2].

A significant difference was observed in the PNR in patients who died (31 ± 4), compared to patients who were disease free (52 ± 25, $P = 0.001$) [Table 2]. Moreover, a significant difference in platelets count (187,000 ± 4000 μ/L vs. 248,000 ± 83,000 μ/L, $P = 0.0001$) and in PNR (31 ± 4 vs. 52 ± 25, $P = 0.0001$) was found between patients who died (3 patients) and all the others (50 patients) [Table 3].

Using the Spearman correlation, we found a negative correlation between age and regional lymph nodes ($Ps = -0.305$, $P = 0.02$, and between clinical staging and white blood cells count ($Ps = -0.280$, $P = 0.04$). A positive correlation was found between clinical staging and the existence of regional lymph nodes ($Ps = 0.443$, $P = 0.001$), between clinical staging and metastases ($Ps = 0.308$, $P = 0.02$), and between platelet count and regional lymph nodes involvement ($Ps = 0.300$, $P = 0.034$).

DISCUSSION

Our study demonstrated that younger patients were at a higher risk of developing axillary lymph node involvement and that higher staging was accompanied by lower white blood cell counts. We also showed that when a patient has more platelets on the first visit, there is a higher likelihood that she will have axillary lymph nodes involvement. However, patients who died within 1 year of follow-up had significantly lower platelet counts compared with all other patients (those who were disease free and those who had recurrence but survived) during the follow-up period.

This study showed that platelet counts may be used as a prognostic marker of survival in patients with breast cancer. Thrombocytosis was associated with more aggressive disease and lymph nodes involvement; however, patients who died within 1 year had lower platelets counts on their first visit to the oncology clinic.

We found that platelet counts could be used as a marker of prognosis. A platelet count lower than 200,000 μ/L on the first visit predicted a worse outcome, while platelets count around 250,000 μ/L predicted a good clinical outcome.

Table 1. Clinical stages of breast cancer and blood cells characteristics on the first visit. Clinical staging found a difference only in neutrophils between stage I and II and between stage I and III. No difference was observed in platelet counts or platelets-to-lymphocytes ratio or platelets-to-neutrophils ratio

Stage	Age	Platelets	Neutrophils	Lymphocytes	PNR	PLR
I	66.8 ± 13.2	236 ± 81	9 ± 3 ($P = 0.04$)	1.8 ± 0.8	44 ± 23	150 ± 60
II	61.6 ± 12.3	242 ± 80	7.5 ± 2	1.8 ± 0.5	53 ± 20	151 ± 70
III	64.4 ± 15.3)	261 ± 91	7.1 ± 1.4 ($P = 0.02$)*	1.5 ± 0.6	62 ± 31	200 ± 132

All *P* values were NS unless otherwise indicated
**P* value between stage I and III
Platelet, neutrophils, and lymphocyte values should be multiplied by 1000
The units are cells per microliter
PLR = platelets-to-lymphocytes ratio, PNR = platelets-to-neutrophils ratio

Table 2. Comparing patients who died, patients who had recurrence, and patients without recurrence. Patients who died had a significant difference in PLR compared to patients with recurrence. Comparison of patients who died to those who were disease free found a significant difference in platelets and in the PNR

	Number	Age	Platelets	Neutrophils	Lymphocytes	PNR	PLR
Died	3	65 ± 21	187 ± 4	6 ± 1	1.1 ± 0.5	31 ± 4	192 ± 81 (P = 0.01)
Recurrence	10	66 ± 11	239 ± 81	4.8 ± 1.7	2.2 ± 0.9	52 ± 18	124 ± 71
Disease-free group	40	63 ± 12	248 ± 83 (P = 0.0001)	5.7 ± 2.7	1.6 ± 0	52 ± 25	163 ± 8.2

All P values were NS unless otherwise indicated
PLR = platelets-to-lymphocytes ratio, PNR = platelets-to-neutrophils

PRE-TREATMENT PLATELET COUNTS AND PROGNOSIS

Thrombocytosis, defined as a platelet count of > 400,000 μ/L, was first described and was associated with cancer progression in the year 1872, and remains a marker of poor prognosis in patients with solid tumors [3]. A study that followed 1756 patients with pancreatic cancer found that patients with pre-treatment elevated platelet counts had a poor overall survival [4]. In patients with breast cancer, thrombocytosis was associated with a poor prognosis, suggesting a key role for platelets in the pathogenesis and the natural history of breast cancer development and progression [5,6]. The association of platelets and breast cancer was demonstrated in 1968 [7] when a 50% reduction in tumor metastases was observed in an animal model after induced experimental thrombocytopenia using neuraminidase and antiplatelet serum. The anti-metastatic effect was blocked with infusion of platelet rich plasma [7,8]. High platelet count was shown to be associated with improved survival of cancer cells in the circulation, a better adhesion of tumor cells to endothelial cells, penetration of cancer cells into the parenchyma of distant tissues, and increase of metastases spread to distant locations [8,9]. A study that screened 165 patients with breast cancer found that pre-treatment thrombocytosis was a significant adverse prognostic factor in multivariate analysis [9]. Patients with thrombocytosis were more likely to have metastases at diagnosis with breast cancer. On multivariate analysis, older age, negative hormone receptors, and higher grade staging were associated with thrombocytosis with a decreased overall survival [9].

Once activated, platelets undergo morphological changes to their shape and membranes and release small molecules and proteins. β-thromboglobulin and P-selectin, which are the hallmarks of platelet activation, are both increased in patients with breast cancer [10]. Several mechanisms are involved in platelets activation by tumor cells. Tumor cell-induced platelet aggregation (TCIPA) is an important mechanism that occurs via direct contact with tumor cells or by mediators such as adenosine diphosphate (ADP), thromboxane A2, and serine proteinases [11]. TCIPA enhances expression of glycoprotein (GP) Ib and IIb/IIIa receptors, which increases aggregation and adhesiveness of platelets leading to form thrombi and clots [11]. Other mechanisms that activate platelets include generation of matrix metalloproteinase and activation of the coagulation system [12]. Activated platelets shed microvesicles that enhance the adhesion, proliferation, chemotaxis, and invasiveness of breast cancer cells [13].

Platelets have been demonstrated to support cancer cells by different mechanisms, including direct shielding of tumor cells, protecting them from tumor necrosis factor α mediated cytotoxicity, and down regulation of the immune-receptor natural killer groups 2-member D (NKG2D), through activation of platelet derived transforming growth factor beta (TGF-β) [14, 15]. The TGF-β signaling pathway promotes invasion and spread of breast cancer cells by enhancing the epithelial-to-mesenchymal transition and immunosuppression [15]. Platelets transfer their major histocompatibility class I antigens to tumor cells and create a pseudo normal phenotype to embolic tumor cells, evading natural killer cells and natural killer cells' mediated destruction (the immune system does not recognize the tumor cells as non-self-cells) [15].

The levels of vascular endothelial growth factor (VEGF) are increased in patients with breast cancer, and serum VEGF levels are determined by platelet counts and not by tumor burden [16]. Platelets also contain other growth factors and inhibitors including platelet derived endothelial cell growth factor, transforming growth factor-β, hepatocyte growth factor, thrombospondin, endostatin, and thrombopoietin [17]. Thrombopoietin is a cytokine that stimulates megakaryocytes in the bone marrow to generate platelets. It is released from platelets on activation, and it could be that the tumor vascular bed secretes thrombopoietin, which stimulates the bone marrow to generate new platelets, inducing thrombocytosis [17].

Our findings of worse clinical outcome in patients with relative low platelets count may suggest another mechanism, that to the best of our knowledge has not been suggested so far, and that is the consumption theory. Cancer often activates the coagulation system, and could be manifested as disseminated intravascular coagulation (DIC). The mechanism for this process is

Table 3. Comparing patients who died to all other patients. Patients who died had a significant difference in platelets and in PNR compared with all other patients

	Number	Age	Platelets	Neutrophils	Lymphocytes	PNR	PLR
Died	3	65 ± 21	187 ± 4	6 ±1	1.1 ± 0.5	31 ± 4	192 ±81
Rest*	50	63 ± 12	248 ± 83	5.7 ± 3.6	1.6 ± 0.6	52 ± 25	163 ± 82

*Rest = all patients who had recurrence (8) and those who were disease free (39)
PLR = platelets-to-lymphocytes ratio, PNR = platelets-to-neutrophils ratio

deposition of platelets and fibrin within the blood vessels, with peripheral blood manifestations of relative thrombocytopenia. The clinical course of DIC in malignancy is less aggressive compared to other clinical settings. A more slowly progressive disease may remain asymptomatic. The ongoing consumption may result in low levels of platelets [18,19]. As long as the function of the liver is intact, synthesis of coagulation factors may disguise the ongoing chronic consumption of clotting proteins, and in most patients' thrombocytopenia is the most significant sign of the ongoing DIC [19].

In our study, patients with breast cancer who died had the lowest platelets counts. Low platelets count was the only marker that could suggest DIC, and indeed, those patients died within 1 year of follow-up.

PLATELET-TO-LYMPHOCYTES RATIO

A significantly higher PLR was found in patients who died compared to those who survived (P= 0.01). Other studies have demonstrated the same phenomenon. Studies have shown the interaction between tumor cells and the host microenvironment, including inflammation and the immune response, that together play an important role in tumor progression and clinical prognosis [20]. PLR has become a cost-effective, inflammation dependent and immune-related prognostic marker that has been shown to predict the prognosis of several solid tumors. High PLR has been shown to predict poor prognosis in gastric and lung cancers. Interestingly, in our study, patients who died had a PLR level of 192 ± 81, which could predict (according to the aforementioned study) a poor prognosis. Another cohort study with 2374 patients found that a PLR > 300 was an independent prognostic marker that was associated with the worst overall survival of breast cancer patients [21]. Inflammation could be an important mechanistic pathway leading to the development of cancer. Another study supported these findings, showing that PLR was an independent prognostic marker of survival in breast cancer patients [22].

LIMITATIONS

This study was retrospective; however, we are planning to continue our research in a prospective study. Another issue is the small number of patients, and we are planning a large multicenter study that will enroll thousands of patients and will examine our preliminary findings. Larger studies with diverse populations should be conducted to validate our preliminary data.

CONCLUSIONS

We found that patients with low platelet counts had the worst prognosis and died within 1 year. We surmise that this result is due to a chronic consumption coagulopathy process with relative thrombocytopenia in patients who are at the highest risk.

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Capsule

The gut microbiome switches mutant p53 from tumor-suppressive to oncogenic

Somatic mutations in p53, which inactivate the tumor-suppressor function of p53 and often confer oncogenic gain-of-function properties, are very common in cancer. **Kadosh** and colleagues studied the effects of hotspot gain-of-function mutations in *Trp53* (the gene that encodes p53 in mice) in mouse models of WNT-driven intestinal cancer caused by *Csnk1a1* deletion or Apc^{Min} mutation. Cancer in these models is known to be facilitated by loss of p53. The authors found that mutant versions of p53 had contrasting effects in different segments of the gut: in the distal gut, mutant p53 had the expected oncogenic effect; however, in the proximal gut and in tumor organoids it had a pronounced tumor-suppressive effect. In the tumor-suppressive mode, mutant p53 eliminated dysplasia and tumorigenesis in *Csnk1a1*-deficient and $Apc^{Min/+}$ mice, and promoted normal growth and differentiation of tumor organoids derived from these mice. In these settings, mutant p53 was more effective than wild-type p53 at inhibiting tumor formation. Mechanistically, the tumor-suppressive effects of mutant p53 were driven by disruption of the WNT pathway, through preventing the binding of TCF4 to chromatin. Notably, this tumor-suppressive effect was completely abolished by the gut microbiome. Moreover, a single metabolite derived from the gut microbiota, gallic acid, could reproduce the entire effect of the microbiome. Supplementing gut-sterilized p53-mutant mice and p53-mutant organoids with gallic acid reinstated the TCF4-chromatin interaction and the hyperactivation of WNT, thus conferring a malignant phenotype to the organoids and throughout the gut. This study demonstrates the substantial plasticity of a cancer mutation and highlights the role of the microenvironment in determining its functional outcome.

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Capsule

A dynamic viral spike of SARS-CoV-2

Efforts to protect human cells against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have focused on the trimeric spike (S) protein. Several structures have shown a stabilized ectodomain of the spike in its prefusion conformation. **Cai** et al. provided insight into the structural changes in the S protein that result in the fusion of the viral and host cell membranes. They purified full-length S protein and determined cryo-electron microscopy structures of both the prefusion and postfusion conformations. These structures add to the understanding of S protein function and could inform vaccine design. The trimeric viral spike (S) protein catalyzes fusion between viral and target cell membranes to initiate infection. The authors reports two cryo-electron microscopy structures derived from a preparation of the full-length S protein, representing its prefusion (2.9-angstrom resolution) and postfusion (3.0-angstrom resolution) conformations, respectively. The spontaneous transition to the postfusion state is independent of target cells. The prefusion trimer has three receptor-binding domains clamped down by a segment adjacent to the fusion peptide. The postfusion structure is strategically decorated by N-linked glycans, suggesting possible protective roles against host immune responses and harsh external conditions. These findings advance our understanding of SARS-CoV-2 entry and may guide the development of vaccines and therapeutics

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