RESEARCH ARTICLE

# Pre-treatment Elevated Platelet Count Associates with HER2 Overexpression and Prognosis in Patients with Breast Cancer

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## Abstract

**Purpose: To research the association between pre-treatment elevated platelet count and clinicopathologic characteristics in breast cancer (BC), as well as explore the relationship between pre-treatment elevated platelet count and HER2 status and prognosis of BC patients. Materials and Methods: A retrospective cohort of BC patients who were newly diagnosed or treated by surgery only and had pathological detection results and platelet values in the Department of Oncology, the First Affiliated Hospital of Liaoning Medical College were enrolled from 1/1/2008 until 31/12/2009, and followed up until 31/12/2014. Age, thrombocyte parameters before chemotherapy and/or radiotherapy, immunohistochemical (IHM) indexes, and regional lymph node (LN) involvement and progression-free survival (PFS) were recorded. Results: A total of 447 eligible subjects were included in this research. As we analyzed, for HER2, positive and negative, the incidence rates of elevated platelet count were 25.8% and 14.7% ($P$<0.05). In the Cox proportional hazards model both variables were independent risk factors for BC (for HER2, OR, 0.592, 95% confidence interval, CI, 0.355 to 0.985, $P$=0.044;f or PLT, OR, 0.998, 95% CI, 0.996 to 1.000, $P$=0.042). For ER, PR, Ki67 and LN involvement, the differences were not statistically significant ($P$>0.05). Conclusions: In this research, pre-treatment elevated level of platelet count demostrated a significantrelationship with HER2 amplification/overexpression, and both variables significantly influenced the prognosis of BC. However, elevated platelet count did not exhibit any association with ER, PR, Ki67 and LN involvement.**

**Keywords:** Breast cancer - platelet count - HER2 - correlation - prognosis

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## Introduction

As a adverse prognostic factor,elevated level of platelet count has certain incidence in patients of a variety of solid cancers (Monreal et al., 1998; O'Byrne et al., 1999; Buergy et al., 2012; Stravodimou et al., 2013; Fang-Xuan Li et al., 2014; Yan Li et al., 2014; Ying Chen et al., 2015), whereas the reasons were not exactly clear (Taucher et al., 2003; Buergy et al., 2012). But evidence of the prognostic role of elevated platelet count is inadequate. Previous studies (Gerdes et al., 1983; Colleoni et al., 2006; Gown, 2008; Davies et al., 2011; Pathmanathan et al., 2014; Ramirez et al., 2014) also reported that pathological indexes including HER2, ER, PR, Ki67 and LN involvement play a key role in the progress of diagnosis, treatment and prognosis of BC. And in the process of clinical practice, patients had different expression status of HER2 observed a concomitant of different incidence rates of elevated platelet count, and had different survival time, but the mechanisms remain to be further studied. We therefore performed a multivariate, retrospective cohort analysis in an attempt to assess the correlations between elevated platelet count with HER2 and BC prognosis.

## Materials and Methods

*Study subjects*

The study was approved by The First Affiliated Hospital of Liaoning Medical University ethical committee, and all patients signed a written informed consent.We conducted this multivariate, retrospective cohort analysis by filtrating 447 eligible subjects from 1372 BC patients from 1/1/2008 until 31/12/2009,and following up 6 years until 31/12/2014.These 447 cases were all newly diagnosed,and have detection results of IHM indexes and LN involvement,with the tissue samples were obtained by surgery or puncture,and had platelet parameters record detected before adjuvant therapy.Then large amounts of data statistics was taken to collect information such as: age,platelet counts, HER2, ER, PR, Ki67, LN involvement and PFS.

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**Table 1. Cohort Characteristics**

| Patient Variable | Percent of patients(%) |
|---|---|
| Female | 99.55 |
| Male | 0.45 |
| Operation--surgery | 86.35 |
| Operation--puncture | 13.65 |
| lymphatic metastasis | 47.87 |
| Radiotherapy and/or Chemotherapy | 100 |
| Progression-free survival | 62.42 |
| Progressive Disease | 34.23 |
| Death | 11.41 |
| Lost to follow-up | 3.36 |
| HER2 subgroup positive rate | 16.8 |
| positive rate of ER subgroup | 58.92 |
| positive rate of PR subgroup | 51.47 |
| positive rate of Ki67 subgroup | 78.23 |
| positive rate of LN subgroup | 55.32 |

BC,breast cancer;SD, standard deviation;HER2,human epidermal growth factor receptor type 2;ER,estrogen receptors;PR,progestrone receptor;Ki67, Ki67 proliferation index; LN, regional lymph node involvement

**Table 2. The Analysis Result of the Correlations between Elevated Platelet Count and Clinicopathologic Characteristics**

| Clinicopathologic characteristics | | Percent of patients(%) | | P |
|---|---|---|---|---|
| | | PLT≤350 | PLT>350 | |
| HER2 | Negative | 85.3 | 14.7 | 0.03 |
| | Positive | 74.2 | 25.8 | |
| ER | Negative | 84.1 | 15.9 | 0.69 |
| | Positive | 85.4 | 14.6 | |
| PR | Negative | 86.9 | 13.1 | 0.2 |
| | Positive | 82.4 | 17.6 | |
| Ki67 | <10 | 84.4 | 15.6 | 0.47 |
| | ≥10% | 87.8 | 12.2 | |
| LN | Negative | 86 | 14 | 0.36 |
| | Positive | 82.6 | 17.4 | |

*Classification of subgroups*

On account of the normal reference ranges of platelet count was 100-300*$10^9$/L, the standard of the elevated was defined as a platelet count exceeding 350*$10^9$/L in this research,for purpose of minimizing the false positive rate.And in order to ensure the accuracy of the HER2 amplification/overexpression detection results,we selected those the IHM results were "3+"or those the FISH detection results were amplified or positive to be the HER2-positive group.In spite of the cut-off values to distinguish Ki67-high from Ki67-low in BC were uncertain,on the base of the highest Youden's index (0.346) (Bewick et al., 2004), <10 and ≥10 were chosen to divide the Ki67 subgroups.The outcome factor was the untreated elevated platelet count (≥350*$10^9$/L) while the study factors were HER2 (positive or negative), ER (positive or negative), PR(positive or negative), LN (positive or negative), Ki67 (<10 or ≥10; or continuous).

*Follow-up visits*

All patients were followed up according to the National Comprehensive Cancer Network (NCCN) guidelines recommendations since the first visit to our department to 12/31/2014.

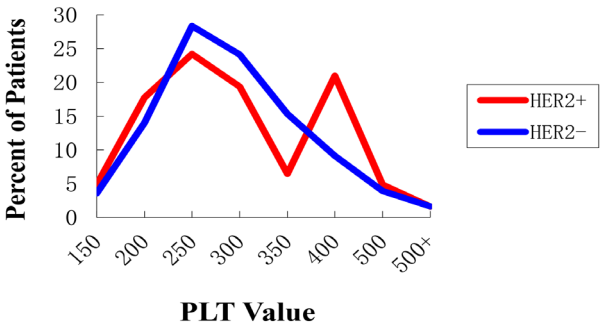


**Figure 1. Proportions of Patients in Different Platelet Count Value Intervals in the HER2 Cohort.** The HER2+ group has a higher incidence rate of elevated platelet count than the HER2- group (*P*=0.031)

*Statistical analysis*

Chi-square tests was performed to assess the relationship between elevated platelet count and subgroups of every variable, significance inferred at *P*<0.05. And Cox proportional hazards regression analysis was used to estimate or confirm hazard ratios of the related variables, choosing 95 percent as confidence intervals. Kaplan-Meier curves were presented, and log-rank tests for the comparison of survival curves. Furthermore, to minimize the risk of identifying a chance retrospective statistical correlation, all computations throughout the total cohort were repeated.

## Results

*The analysis of demographic characteristics of the cohort*

A total of 447 untreated patients with BC during the past 72 months were analyzed. These patients has a mean (SD) age of 52.7(10.0),range from 20 to 84 years old. Among the total cohort (Table 1), 99.6% patients were female, 86.4% patients were treated with surgery, and the rest 13.6% were diagnosed by puncture. All the 447 patients were treated by radiotherapy and/or chemotherapy. Until the end of follow-up, 62.4% patients were alive without progressive disease, 25.3% patients were alive with recurrence or metastasis and 11.4% patients were dead,3.7% patients lost to follow-up.The positive rates of HER2 expression and LN involvement were 16.8% (62/369) and 55.3% (213/385), respectively.

*Correlations between pre-treatment elevated platelet count and clinicopathologic characteristics*

The analysis results of platelet count in terms of five variables were shown in Table 2. In the HER2-positive group, the incidence rates of elevated platelet count was higher than that in the HER2-negative group (25.8% *vs* 14.7%, p=0.031, Figure 1). However, the associations between elevated platelet count and ER, PE, Ki67 and LN did not reach statistical significance (*P*=0.691, *P*=0.197, *P*=0.467, *P*=0.361, respectively, Figure 2-5).

*Influence of platelet count and HER2 status on prognosis of BC*

As we analyzed, both the two variables were identified as the independent risk factors for poor prognosis of

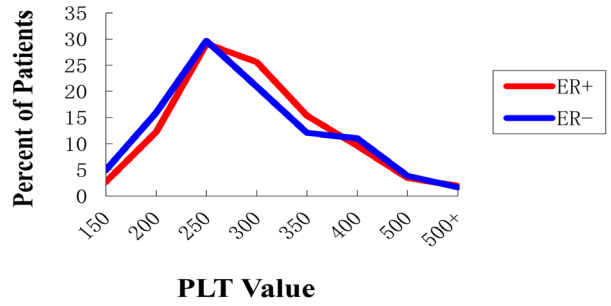


**Figure 2. Proportions of Patients in Different Platelet Count Value Intervals in the ER Cohort.** The incidence rates of elevated platelet count in the ER+ group and the ER- group did not demonstrate any remarkable difference (*P*=0.691)

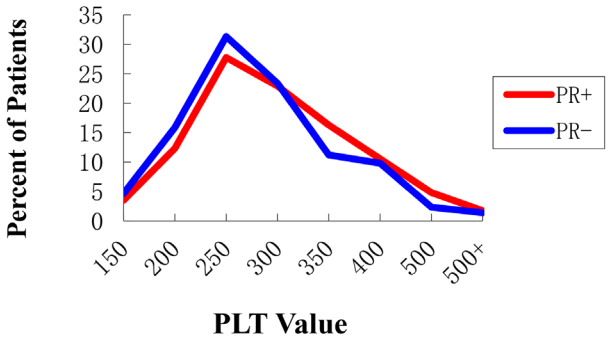


**Figure 3. Proportions of Patients in Different Platelet Count Value Intervals in the PR Cohort.** The difference between incidence rates of elevated platelet count in the PR+ group and the PR- group is not significant (*P*=0.197)

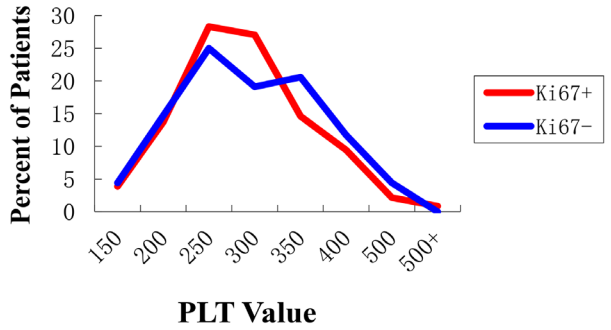


**Figure 4. Proportions of Patients in Different Platelet Count Value Intervals in the Ki67 Cohort.** The difference between incidence rates of elevated platelet count in the Ki67-low group and the Ki67-high group is not significant (*P*=0.467)

BC (for HER2, OR:0.592,95% confidence intervals, CI:0.355 to 0.985, *P*=0.044; for PLT, OR:0.998,95% confidence intervals, CI:0.996 to 1.000, *P*=0.042),which was in accord with previous research (Taucher et al., 2003; Burstein, 2005; Pathmanathan et al., 2014). Figure 6, 7 showed the survival functions.

## Discussion

From this retrospective research,the central insight is that the elevated platelet count had a statistical significant positive correlation with HER2 status.As the identification of the correlation,the author considered it has important implications to further study.

The percentage of elevated platelet value in the total cohort appeared to be 15.21%, comparable to 10-63% in other solid cancers (Olesen et al., 1988; Gastl et al.,1993;

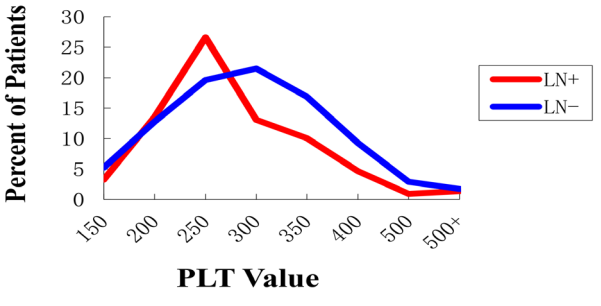


**Figure 5. Proportions of Patients in Different Platelet Count Value Intervals in the LN Cohort.** The difference between incidence rates of elevated platelet count in the LN+ group and the LN- group is not significant (*P*=0.361)

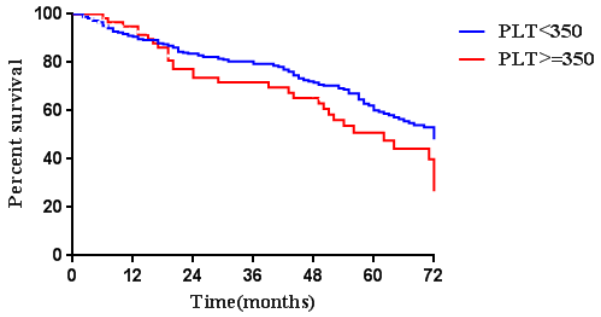


**Figure 6. Association between Pre-treatment Platelet Count and Survival (PFS) of BC Patients**

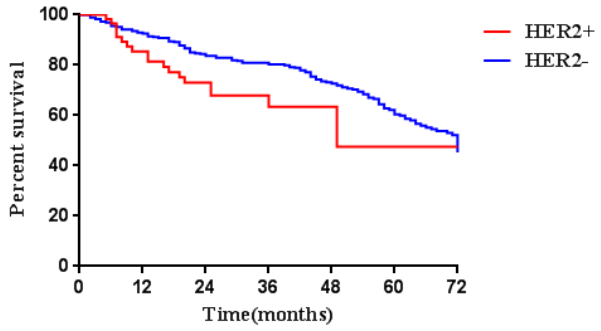


**Figure 7. Association between HER2 and Survival (PFS) of BC Patients**

Pedersen et al.,1996; Nakano et al.,1998; Eltabbakh et al., 1999; O'Byrn et al., 1999; Ikedaet al., 2002). The positive finding of the study was that the elevated platelet count and the HER2 status were statistically significant correlated, and both these two factors were significantly related to the prognosis of BC.Elevated platelet count remarkably influences the OS and PFS of BC patients, by affecting the metastatic potential of tumor cells. At the level of molecular biology, interaction between high platelet count and malignancy was a complicated network of signaling pathways via cytokines like vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), interleukin 6(IL-6), and others that have been implicated in phases of tumor growth and progression. Among these biochemical markers, VEGF played a critical role to connect elevated platelet value and HER2 together (Verheul et al., 1997; Laughner et al., 2001). As expected,our research demonstrated the

significant relevant between elevated platelet value and HER2 upregulation.But question remains unresolved as to how exactly the elevated platelet count is correlated with HER2 amplification/overexpression in BC patients. One explanation is that the HER2-positive BC triggers a upregulation of VEGF,therefore lead to the elevated level of platelet count. Other possible perspectives are HER2-positive BC promotes a high level of platelet, platelets therefore release large doses of VEGF in the plasma.

In this research, due to the elimination of patients whose IHM results of HER2 were"2+", the positive percentage of HER2 was 16.80%,which was not comparable to the 20-30% in previous study(Foy et al.,2012).However,the lower HER2 positive percentage minimized the false positive rate of elevated platelet count in the HER2-positive subgroup. HER2 was reported overexpressed in gastric cancer, and especially in BC. The upregulation of HER2 in BC promotes elevated risk of recurrence, poor prognosis,and even do harm to the efficacy of hormonal therapy(Puhalla et al.,2013),so the identification of its relevance to high platelet values in our research may help to provide synergism to the diagnose and adjuvant therapy targeting biochemical markers of BC.

Advantages of this research were a multivariate cohort analysis and a relatively big sample size.But some limitations should be mentioned. Firstly, our research was a hospital-based study in China,the subjects may not well represent the general population.Secondly,the results should be cautioned because of the differences of region and race.Thirdly,we need to further study the mechanisms of the correlation between elevated platelet count and HER2 amplification/overexpression,and large sample size multi-center prospective clinical trials were needed to confirm the effect of antiplatelet treatment in HER2-positive BC therapy.

In conclusion, the positive finding of the study was that pre-treatment elevated platelet count and the HER2 status were statistically significant associated, and both these two factors were significantly related to the prognosis of BC. Therefore, antiplatelet treatment maybe useful to improve therapeutic efficacy and prognosis of HER2-positive BC patients.

## References


Bewick V, Cheek L, Ball J (2004). Statistics review 13: receiver operating characteristic curves. *Crit Care*, **8**, 508-12.

Buergy D, Wenz F, Groden C, Brockmann MA (2012). Tumor-platelet interaction in solid tumors. *Int J Cancer*, **130**, 2747-60.

Burstein HJ (2005). The distinctive nature of HER2-positive breast cancers. *N Engl J Med*, **353**, 1652-4.

Chen Y, Zhang L, Liu WX, Liu XY. (2015). Prognostic signifiance of preoperative anemia, leukocytosis and thrombocytosis in Chinese women with epithelial ovarian cancer. *Asian Pac J Cancer Prev*, **16**, 933-9.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG), C Davies, J Godwin, R Gray, et al (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*, **378**, 771-84.

Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME (1999). Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. *J Surg Oncol*, **70**, 6-12.

Fang-Xuan Li, Li-Juan Wei, Huan Zhang, Shi-Xia Li, Jun-Tian Liu (2014). Significance of thrombocytosis in clinicopathologic characteristics and prognosis of gastric cancer. *Asian Pac J Cancer Prev*, **15**, 6511-7.

Foy KC, Miller MJ, Moldovan N (2012). Immunotherapy with HER-2 and VEGF peptide mimics plus metronomic paclitaxel causes superior antineoplastic effects in transplantable and transgenic mouse models of human breast cancer. *Oncoimmunology*, **1**, 1004-16.

Gastl G, Plante M, Finstad CL, et al (1993). High IL-6 levels in ascitic fluid correlate with reactive thrombocytosis in patients with epithelial ovarian cancer. *Br J Haematol*, **83**, 433-41.

Gerdes J, Schwab U, Lemke H, Stein H (1983). Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer*, **31**, 13-20.

Gown AM (2008). Current issues in ER and HER-2 testing by IHC in breast cancer. *Mod Pathol*, **21**, 8-15.

Ikeda M, Furukawa H, Imamura H, et al (2002). Poor prognosis associated with thrombocytosis in patients with gastric cancer. *Ann Surg Oncol*, **9**, 287-91.

International Breast Cancer Study Group. Colleoni M, Gelber S, Goldhirsch A, et al (2006). Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: international breast cancer study group trial 13-93. *J Clin Oncol*, **24**, 1332-41.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (2001). HER2 (neu) signaling increases the rate ofhypoxia-inducible factor 1 (HIF-1) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol*, **21**, 3995-4004.

Li Y, Miao LY, Xiao YL, Cai HR, Zhang DP (2014). Elevated platelets enhance cancer cell migration,promote hematogenous metastasis and associate with a poor prognosis in advanced non-small cell lung cancer cases. *Asian Pac J Cancer Prev*, **15**, 139-43.

Monreal M, Fernandez-Llamazares J, Pinol M, et al (1998). Platelet count and survival in patients with colorectal cancer-a preliminary study. *Thromb Haemost*, **79**, 916-8.

Nakano T, Chahinian AP, Shinjo M, et al (1998). Interleukin 6 and its relationship to clinical parameters in patients with malignant pleural mesothelioma. *Br J Cancer*, **77**, 907-12.

O'Byrne KJ, Dobbs N, Propper D, Smith K, Harris AL (1999). Vascular endothelial growth factor platelet counts, and prognosis in renal cancer. *Lancet*, **353**, 1494-5.

O'Byrne KJ, Dobbs N, Propper D, Smith K, Harris AL (1999). Vascular endothelial growth factor platelet counts, and prognosis in renal cancer. *Lancet*, **353**, 1494-5.

Olesen LL, Thorshauge H (1988). Thrombocytosis in patients with malignant pleural mesothelioma. *Cancer*, **62**, 1194-6.

Pathmanathan N, Balleine RL, Jayasinghe UW, et al (2014). The prognostic value ofKi67 in systemically untreated patients with node-negative breast cancer. *J Clin Pathol*, **67**, 222-8.

Pedersen LM, Milman N (1996). Prognostic significance of thrombocytosis in patients with primary lung cancer. *Eur Respir J*, **9**, 1826-30.

Puhalla S, Brufsky A (2013). Treatment of HER2-positive breast cancer:looking backwards briefly. *Lancet Oncol*, **14**, 1250-1.

Ramirez A, Boulaiz H, Morata-Tarifa C, et al (2014). HER2-signaling pathway, JNK and ERKs kinases, and cancer stem-like cells are targets of Bozepinib. *Onco Target*, **5**, 3590-606.

Stravodimou A,Voutsadakis IA (2013). Pretreatment Thrombocytosis As A Prognostic Factor In Metastatic Breast Cancer. *Int J Breast Cancer*, **2013**, 289563-8.

Taucher S, Salat A, Gnant M, et al (2003). Impact of pretreatment thrombocytosis on survival in primary breast cancer. *Thromb Haemost*, **89**, 1098-106.

Verheul HM, Hoekman K, Luykx-de Bakker S, et al (1997). Platelet:transporter ofvascular endothelial growth factor. *Clin Cancer Res*, **3**, 2187-90.