RESEARCH ARTICLE

Role of platelet infiltration as independent prognostic marker for gastric adenocarcinomas

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Abstract

Background: In recent years, the relationship between malignant tumors and platelets has been paid more attention. The increase of platelets is an independent risk factor for the poor prognosis of some malignant tumors.

Methods: This study retrospectively analyzed the clinical and pathological data of 114 patients with initial gastric cancer from August 2005 to August 2018 in Shandong Provincial Hospital. Single-factor and multifactor survival analysis were used to evaluate the effect of platelet elevation on postoperative survival. The gastric cancer tissues of the Jinan Central Hospital and its matched paracancerous tissues were collected. The expression of platelets in tissues was detected by immunofluorescence technique. Different numbers of platelets were co-cultured with MKN-45 cells, CCK-8 assay and transwell assay were performed, and the expression of epithelial-mesenchymal transition-related proteins was detected.

Results: Platelet count was independent factors affecting prognosis. The stratified analysis showed that there was a statistically significant difference in the 5-year survival rate between the platelet-increase group and the normal platelet group in the TNM stages I-II. The expression of platelets in gastric cancer tissues was higher than that in adjacent tissues. The results of CCK-8 and transwell showed that platelets significantly enhanced the proliferation and metastasis capability of MKN-45 cells in a concentration-dependent manner. After co-culture, the expression level of E-cadherin protein in MKN-45 cells decreased, and the protein expression levels of N-cadherin, vimentin, and VEGFA increased.

Conclusion: Platelet elevation is closely related to the occurrence, development, and metastasis of gastric cancer, and platelet count can be used as a prognostic indicator for malignant tumors.

KEYWORDS
gastric cancer, invasion, platelet count, prognostic indicators, proliferation

1 | INTRODUCTION

Gastric cancer is one of the most common malignant tumors in China, and its therapeutic effect and prognosis are poor. Although the means of treating gastric cancer continue to emerge, due to the lack of early diagnosis, gastric cancer can often be diagnosed in the late stage.[1] In recent years, the relationship between malignant tumors and platelets has received increasing attention. Studies have shown that platelet elevation is an independent risk factor for poor prognosis of some malignant tumors such as ovarian cancer, kidney cancer, and lung cancer.[2,3] The incidence of tumor-induced platelet elevation in gastric cancer patients is 11.4%, but the effect of platelets on the invasion and proliferation of gastric cancer cells and its possible mechanisms need further study.[4] It has been reported that reducing the amount of platelets in the host's blood inhibits tumor growth and metastasis.[5] Activated platelets are known to secrete VEGF-A23-24, a key factor in promoting angiogenesis. In addition, epithelial-mesenchymal transition (EMT) refers to the phenomenon that epithelial cells transform into stromal cells under specific physiological and pathological conditions, which is defined as the loss of original polarity and cell morphology of epithelial cells and the process of cytoskeletal remodeling into interstitial cells that have the capability to migrate and invade the extracellular matrix.[5] In recent years, studies have also found that the EMT process is closely related to the growth, invasion, and metastasis of tumor cells. Research on the signaling pathways and related genes involved in the regulation of EMT processes in tumor cells has received increasing attention. Among them, N-cadherin, E-cadherin, and vimentin proteins are important for tumor invasion and metastasis, and they are also key molecules of EMT.[6,7] Studies have shown that most types of cancer cells pass through the process of transforming epithelial cells into mesenchymal cells, thereby gaining greater viability and invasiveness. The process of epithelial-mesenchymal transition includes the decreased expression of E-cadherin protein and increased expression of N-cadherin and vimentin proteins.[8,9] However, in the study of molecular mechanisms affecting the development of tumors, it has not been reported in the literature whether activated platelets induce epithelial-mesenchymal transition of gastric cancer cells and affect the invasion and metastasis of gastric cancer.

In this study, 114 patients with initial gastric cancer were selected from August 2005 to August 2018 in the Provincial Hospital Affiliated to Shandong University. The platelet elevation and prognosis were retrospectively analyzed. The prognostic significance of platelet elevation for gastric cancer patients was further explored. The purpose of evaluation is to study the effect of platelets on the proliferation and invasion of gastric cancer cells and to explore its possible molecular mechanism, and to analyze the clinical significance of platelet elevation in gastric cancer tissues and provide an experimental basis for platelet count as a prognostic indicator of malignant tumors.

2 | MATERIALS AND METHODS

2.1 | Clinical data

The clinical data indicate the clinical and pathological data of 114 patients with initial gastric cancer from August 2005 to August 2018 in the Provincial Hospital Affiliated to Shandong University. Surgery is the main treatment for gastric cancer. The indications for radical surgery are as follows: (a) stages I, II, III, and IV (except M) of primary gastric cancer, (b) the patient is in good physical condition and can tolerate surgery without severe heart, lung, liver, and kidney dysfunction, (c) those diagnosed with cancer after gastroscopy and barium meal examination, and (d) no distant metastases such as liver or lung were detected by ultrasound and CT before surgery. Exclusion criteria are as follows: preoperative acute/chronic infection or severe bleeding; association with hematological malignancies; autoimmune disease or systemic infection; and perioperative death due to surgical complications. There were 94 males and 20 females, aged from 32 to 85 years, with a median age of 61 years. Seventy-three (64%) patients had a history of smoking, and all patients did not receive neoadjuvant therapy before surgery. According to the pathological classification of WTO, there are111 cases of adenocarcinoma, 2 cases of signet ring cell carcinoma, and 1 case of stromal tumor. The postoperative clinical stage was based on the 7th edition of the UICC-AJCC TNM staging criteria, including 16 cases of stage I, 54 cases of stage II, 22 cases of stage III, and 25 cases of stage IV. The information obtained was approved by the Medical Ethics Committee of the Provincial Hospital Affiliated to Shandong University and informed consent of the patients.

2.2 | Postoperative follow-up

Follow-up methods included outpatient review and telephone follow-up. The follow-up deadline is August 31, 2018. The average follow-up time was 41.8 months. Overall survival (OS) refers to the death from the date of surgery to the date of follow-up or death.

2.3 | Collection organization

The gastric cancer tissues and their corresponding adjacent tissues of gastric cancer were removed from April 2007 to August 2018 in Jinan Central Hospital Affiliated to Shandong University. All patients were treated without any radiotherapy or chemotherapy before surgery, and the adjacent tissues were normal mucosal epithelial tissues. The specimens obtained were approved by the Medical Ethics Committee of Jinan Central Hospital and informed consent of the patients.

2.4 | Main reagents

RPMI 1640 medium was purchased from Hyclon, and fetal bovine serum (FBS) was purchased from Gibco. Human peripheral platelet fraction was purchased from Tianjin Haoyang Biological Products Technology Co., Ltd.; Matrigel was purchased from American B&D Company; transwell chamber was purchased from Corning Company; rabbit anti-human E-cadherin polyclonal antibody, rabbit anti-human vimentin polyclonal antibody, rabbit anti-human N-cadherin polyclonal antibody, and rabbit anti-human VEGFA polyclonal antibody were purchased from Proteintch; rabbit anti-human CD41 monoclonal antibody was purchased from Abcam Company; ELISA kit was purchased from Sizhengbai Co., Ltd.; and GAPDH rabbit-derived monoclonal antibody was purchased from Sigma.

2.5 | Cell culture

The human gastric cancer cell line MKN-45 was established and preserved by the Central Laboratory of Jinan Central Hospital. All cells were cultured in RPMI 1640 medium containing 10% FBS and 1% double antibody at 37°C in a 5% CO_2 incubator.

2.6 | Immunofluorescence detection of platelet expression in tissues

The tissue was baked in a 60°C incubator for 30 minutes, dewaxed, and hydrated in xylene (5 minutes) → xylene (5 minutes) → xylene (10 minutes) → absolute ethanol (5 minutes) → 95% ethanol (5 minutes) → tap water(5 minutes). The PBS was washed twice for 5 minutes each. Antigen retrieval: The tissue was repaired with PH 6.0 EDTA, fired for 13 minutes and cooled to room temperature, washed with tap water for 5 minutes, and then washed twice with PBS for 5 minutes each. Normal goat serum was blocked for 30 minutes (room temperature). The PBS was washed twice for 5 minutes each. Primary anti-CD41 (1:200 dilution) was added dropwise at 4°C overnight and rewarmed for 20 minutes. The PBS was washed twice for 5 minutes each. Fluorescent secondary antibody was added at 37°C for 60 minutes. The PBS was washed twice for 5 minutes each. DAPI was added dropwise in the dark for 5 minutes, and PBS was washed twice for 5 minutes each.

2.7 | Human peripheral blood platelet separation

The anticoagulant and the sample diluent were added according to the ratio of 1:1 by volume. The diluted blood sample was added to the liquid surface of the separation solution (the optimal ratio of anticoagulation and separation solution was 1:2) by 400-500 g and centrifuged for 25 minutes. The first layer of platelet plasma was pipetted carefully into a new 15-mL centrifuge tube, and 10 mL of

the washing solution was added, mixed, and centrifuged to be 250 g for 10 minutes. Discard the supernatant and perform subsequent experiments with 0.5ml.

2.8 | CCK-8 assay

The logarithmic growth phase cells were digested with 0.25% trypsin, and a single cell suspension was prepared from RPMI 1640 medium containing 10% FBS and seeded into a 96-well culture plate at a volume of 5×10^3 cells per well by 200 μL, with setting blank zero holes. Platelets were added to each well after the cells were attached, the numbers were 200×10^9/L, 300×10^9/L, 400×10^9/L, and 500×10^9/L, respectively, and only RPMI 1640 medium was added to the blank control group. After 24 hours of cell culture, CCK-8 was added, and the OD value of each group was detected on an automatic microplate reader. Five replicate wells were set in each group, and the experiment was repeated three times.

2.9 | Transwell assay

Corning transwell chambers with a pore size of 8 μm were coated with 50 μL of 20% Matrigel gel on the upper chamber surface and air-conditioned at room temperature for subsequent invasion experiments. Gastric cells were starved for 24 hours in a serum-free medium before invasion or migration experiments, then the cells were routinely digested and inoculated into the transwell upper chamber by about 2×10^4 cells/well, and finally, 200 μL serum-free RPMI 1640 was added to the upper chamber. Besides, in the upper and lower chambers, 500 μL of RPMI 1640 medium containing 10% FBS and different amounts of platelets were added. At 37°C, the cells were cultured for 24 hours in an incubator with 5% CO_2; then, the chamber was removed, fixed with 4% paraformaldehyde, and stained with crystal violet; finally, 5 samples were randomly selected under the microscope (×200) after the chamber was washed and decolorized with 33% acetic acid. The crystal violet on the chamber was completely eluted, and the eluate was tested for an OD value at 570 nm on a microplate reader.

2.9.1 | Detection on Western blots of EMT and VEGFA expression in cells

The total cellular protein was extracted by a conventional method, and its protein concentration was determined. 70 μg of total protein was taken and added to the 5× loading buffer with the concentration ratio of 4:1. Before electrophoresis, the protein was preheated at 95°C for 10 minutes and then was added to 6% to 10% of SDS-polyacrylamide gel pores for electrophoresis, where the electrophoresis conditions were 80 V constant voltage. After electrophoresis, the membrane was transferred into a PVDF membrane

with constant flow of 250 mA (on ice). After completion of the transfer, the membrane was washed for 5 min × 2 times with TBST. The strips were blocked for 1 hours in 5% skim milk powder, and primary antibodies (diluted with the concentration ratio of 1:750) were added at 4°C overnight. After re-warming for 30 minutes at 37°C, the primary antibodies were recycled and washed with TBST for 10 minutes × 3 times. The membrane was placed for 80 minutes in a secondary antibody incubation kit (in which the secondary antibody and the goat anti-rabbit secondary antibody were mixed with the constituent ratio of 1:10 000) on a shaker. The membrane was washed for 15 minutes × 3 times with TBST and exposed to ECL luminescent liquid.

TABLE 1 Clinicopathological features of 114 gastric cancer patients stratified by platelet count

Variable	Platelet count > 300 n (%)	Platelet count ≤ 300 n (%)	P value
Age (y)			
<65	18 (15.7)	54 (47.3)	.084
≥65	17 (14.9)	25 (21.9)	
Sex			
Male	27 (23.7)	66 (57.9)	.321
Female	8 (7.0)	12 (10.5)	
TNM stage			
I	2 (1.8)	13 (11.4)	.086
II	18 (15.8)	35 (30.7)	
III	4 (3.5)	18 (15.8)	
IV	11 (9.6)	13 (11.4)	
Grade			
Low	35 (30.7)	76 (66.7)	.243
High	0 (0.0)	3 (2.6)	

2.10 | ELISA detection of VEGFA expression in cell supernatant

According to operating instructions of the VEGFA protein assay kit, the concentration of the secreted VEGFA in the cell culture supernatant was determined after MKN-45 cells and platelets were co-cultured and the platelets were cultured in serum-free RPMI 1640 for 24 hours. The VEGFA protein concentration was calculated from the standard dose-response curve. The test was repeated 3 times.

2.11 | Statistical analysis

Data analysis was performed by using SPSS 23.0 and GraphPad statistical software. Descriptive analysis was used for general data. Chi-square test or Fisher exact probability test was used for comparing data from the above analysis. Single-factor survival analysis was performed by using Kaplan-Meier method, and significance test was performed by using log-rank method. Multivariate analysis was performed by using Cox proportional hazard model. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Relationship between preoperative platelet counts of patients with gastric cancer and clinicopathological features of patients with gastric cancer

A total of 114 patients with gastric cancer were divided into normal platelet group (PLT < 300×10^9/L) and platelet-increase group (PLT > 300×10^9/L). There was no significant difference in normal
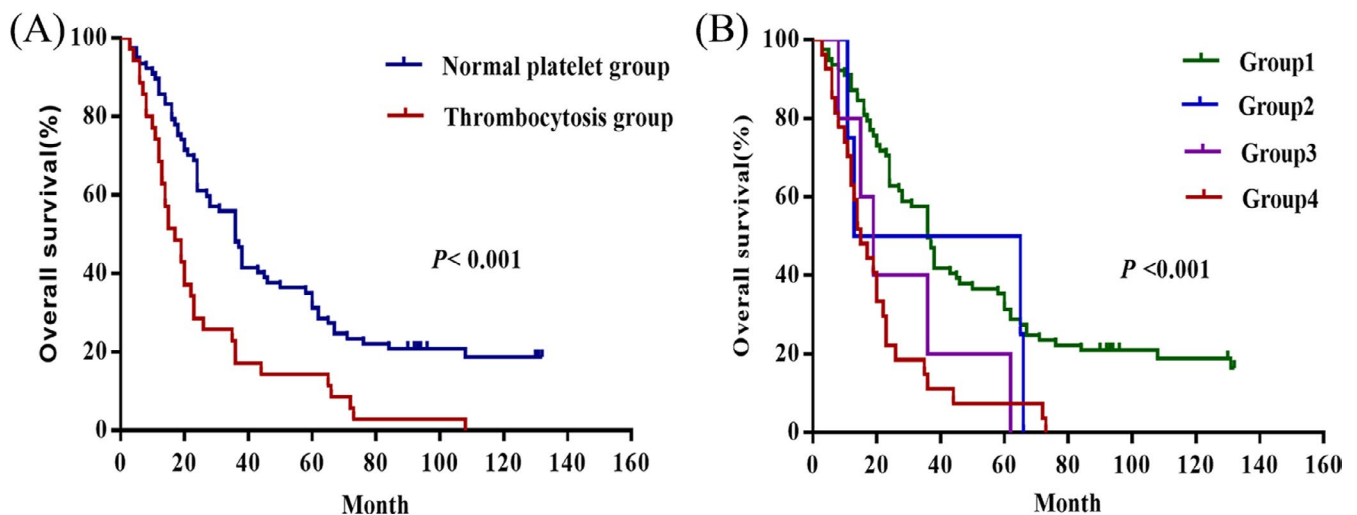
FIGURE 1 A, Overall survival curve of patients with normal platelets and patients with elevated platelets; B, Survival curves of 114 patients with different platelet counts. (group1, 100 × 10^9/L < PLT ≤ 300×10^9/L; group2, 300 × 10^9/L < PLT≤400 × 10^9/L; group3, 400 × 10^9/L < PLT≤500 × 10^9/L; and group4, PLT > 500×10^9/L)

platelet group and platelet-increase group among different age, gender, tissue differentiation, and TNM stages ($P > .05$). The results are shown in Table 1.

3.2 | Preoperative platelet counts predict the prognosis of patients with gastric cancer

The patients were followed up for 3-132 months (median follow-up time 42 months). As shown in Figure 1A, the survival rate of patients with normal platelets was superior to that of patients with increased platelets, and the difference was statistically significant ($\chi^2 = 19.431$, $P < .001$). According to 100×10^9/L < PLT≤300×10^9/L, 300×10^9/L < PLT≤400×10^9/L, 400×10^9/L < PLT≤500×10^9/L, and PLT > 500×10^9/L, 114 patients with gastric cancer were analyzed by group analysis. The survival rate of patients with PLT > 500×10^9/L was significantly lower than that of patients from other four groups. The platelet count was negatively correlated with the survival rate of gastric cancer patients ($P < .01$). The results are shown in Figure 1B. Figure 2 is stratified analysis based on TNM stage. In the TNM stages I-II, the survival rate of the normal platelet group was significantly higher than that of the platelet-increase group ($P < .001$ and $P = .0186$), but no similar results were found in the TNM stages III-IV.

3.3 | Multivariate analysis of patient survival rate

Univariate analysis of the survival rate of patients with gastric cancer showed that clinical tissue differentiation, TNM pathological stage, age, and platelet count significantly affected survival prognosis, while gender difference had no significant effect on survival prognosis. The statistically significant factors in the univariate analysis were introduced into the Cox regression model for multivariate analysis. The results showed that histopathological differentiation, age, TNM pathological stage, and platelet count were independent risk factors for prognosis (among them, $P < .05$). The results are shown in Table 2.

3.4 | Relationship between platelets of patients with gastric cancer and clinicopathological parameters of patients with gastric cancer

The immunofluorescence results were positive for red, and 10 samples were observed by a 200-fold light microscopy. The positive expression rate of platelets in gastric cancer tissues with infiltration depth of T3 and T4 was higher than that of gastric cancer tissues with infiltration depth within the range of T1-T2 (Figure 3A,B). Platelets had a higher expression rate in gastric cancer with lymph
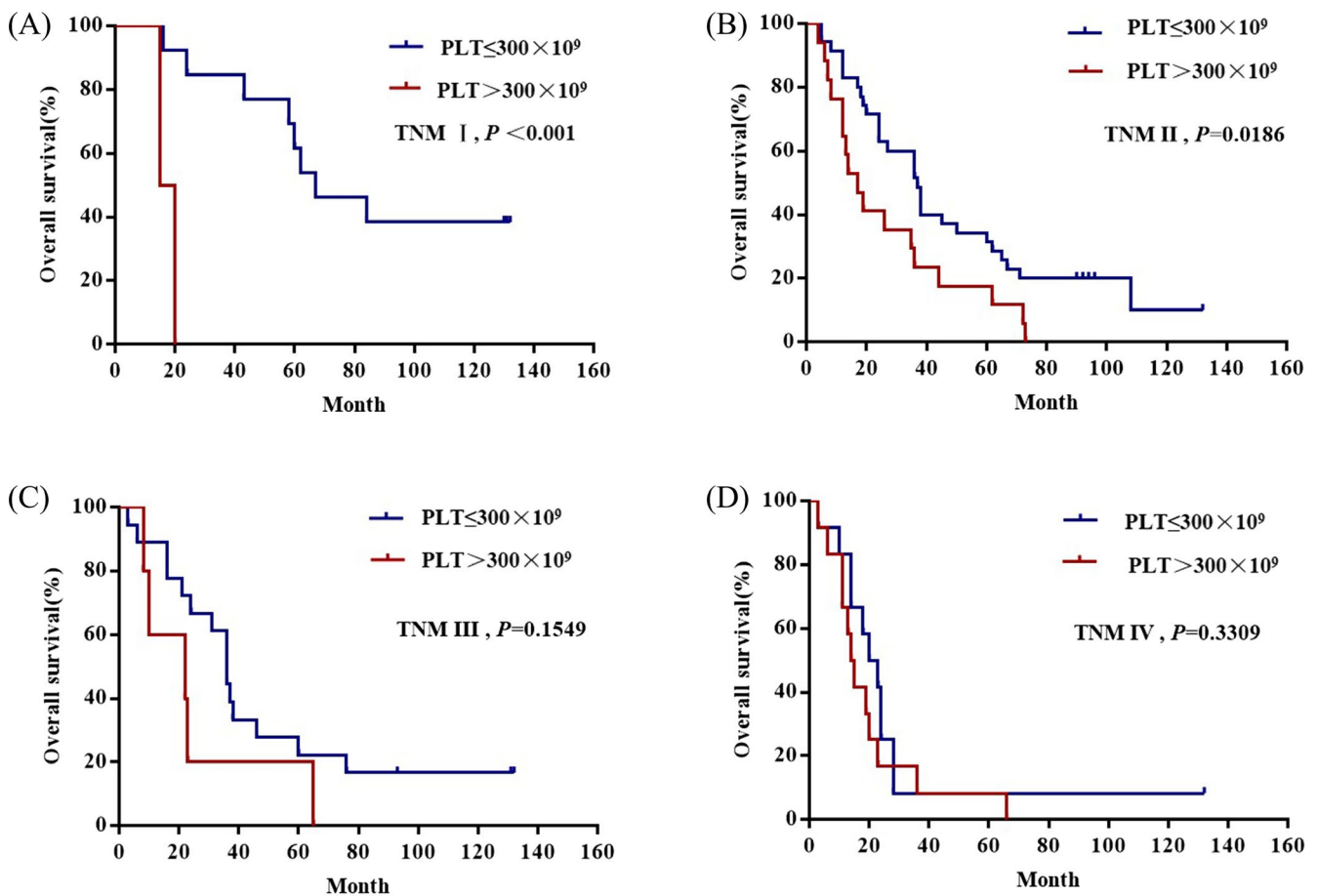

FIGURE 2 Overall survival analysis of PLT stratified by TNM I-IV stages

node metastasis (Figure 3C). No expression of platelets was found in normal gastric tissues. The above results showed that platelet aggregation was closely related to the progression of gastric cancer in gastric cancer tissues.

3.5 | Effects of platelets on the proliferation of gastric cancer cells

The results showed that activated platelets significantly enhanced the proliferation capability of MKN-45 cells when being compared with those in the control group. With the increase of the number of platelets, its promoting cell proliferation gradually increased. The number of platelets was 500×10^9/L, and thus, the platelets had the most proliferative effect when being compared with those in the control group, and their proliferation capability was in a concentration-dependent manner. The results are shown in Figure 4.

3.6 | Effect of platelets on invasion and migration of gastric cancer cells

Tanswell results showed that activated platelets significantly enhanced the invasion and migration capability of MKN-45 cells when being compared with those in the control group. As the number of platelets increased, their cell invasion and migration capability gradually increased. The platelet count was increased by 500×10^9/L. The activated platelets had the most obvious promotion effect when being compared with those in the control group, where the invasion and migration capability of the cell was in a concentration-dependent manner. The results are shown in Figure 5.

3.7 | Expression of VEGFA in cells after co-culture of MKN-45 cells with platelets

We used 500×10^9/L platelets to co-culture with MKN-45 cells for 24 hours and then detected the expression of VEGFA protein by Western blots. Figure 6A shows that VEGFA expression was up-regulated after the activated platelets were increased when being compared with those in the control group (2.04 ± 0.21 vs 15.62 ± 1.12, $P < .001$). The level of secreted VEGFR protein in the

platelet control group and the platelet-cell group culture supernatant was quantitatively analyzed by ELISA, and Figure 6B shows that the activated platelets promoted the secretion of VEGFA in MKN-45 cells ($P = .0021$).

3.8 | Activated platelets induce epithelial-mesenchymal transition (EMT) in gastric cancer cells

We used different numbers of platelets to co-culture with MKN-45 cells for 24 hours and then detected the expression of N-cadherin, E-cadherin, and vimentin proteins by Western blots. Figure 6A shows that N-cadherin and vimentin were up-regulated after the activated platelets were increased when being compared with those in the control group, and the E-cadherin protein expression was reduced.

4 | DISCUSSION

In China, the incidence and mortality of gastric cancer rank second in all tumors. With the continuous development of comprehensive treatment, the 5-year survival rate has improved, but it has not changed the current prognosis of patients with gastric cancer.[10] Many studies have confirmed that platelet elevation has a higher incidence in patients with malignant solid tumors and is an independent risk factor for poor prognosis.[11] Kwon et al[12] showed that patients with colorectal cancer with increased elevated platelets had a higher risk of lymph node metastasis. Azab et al[4] showed that breast cancer patients with secondary platelet elevation had a higher probability of distant metastasis and hypohemoglobinemia. A large number of studies have suggested that platelet counts may be used as predictors of some tumor states.[13] In this study, 300×10^9/L was selected as the cutoff point to divide the patients into the platelet-increase group and the normal platelet group. There was no significant difference in platelet increase among different tissue differentiation, pathological stages, age, and gender. The univariate analysis of this study showed that the 5-year survival rate of patients with the normal platelet group was superior to that of patients with the platelet-increase group ($P < .01$). The statistically significant factors of univariate analysis and multivariate analysis showed that the patient's age, pathological stage, platelet increase, and differentiation were independent prognostic factors for gastric cancer patients, and the risk of death from platelet-increase patients increased by about 5.58 times. The stratified analysis showed that in the TNM stages I-II, the survival rate of the normal platelet group was significantly better than that of the platelet-increase group. It can be seen that platelet increase is an independent factor for poor prognosis of gastric cancer patients. On the one hand, due to time and space constraints, the number of gastric cancer pathological specimens we collect is small, which will have an impact on the conclusion. On the other hand, there are too many factors affecting the progression of gastric cancer TNM stages III-IV. Our study found that in the TNM stages I-II, the survival rate of the normal platelet group was

TABLE 2 Multivariate analysis of clinicopathological parameters for the prediction of OS in 114 gastric cancer patients

Variables	P	Hazard ration	95.0% CI for RR	
			Lower	Upper
Age	<.0001	5.580	3.464	8.990
Platelet count	<.0001	0.422	0.268	0.665
TNM	.004	1.417	1.120	1.793
Grade	.029	0.623	0.407	0.952

significantly higher than that of the platelet-increase group, suggesting that changes in the number of platelets may be a risk factor for the development of early gastric cancer. We will expand the sample size to further study the role of platelets in TNM stage III-IV of gastric cancer.

The tumor-induced platelet increase has a high incidence in patients with malignant tumors and is also an independent risk factor for poor prognosis. Levin and Conley first reported the phenomenon of platelet hyperplasia in gastric cancer, but did not further analyze the prognosis of the platelet increase. Ikeda et al[13] first analyzed the relationship between the platelet increase of the gastric cancer patients and the prognosis of the gastric cancer patients, and believed that secondary platelet increase was an independent factor for poor prognosis of gastric cancer. Thrombocytopenia was also closely related to the progression of malignant tumors. This study showed that

the worse the tumor tissue differentiation, the more the number of thrombocytopenia and the incidence of secondary thrombocytosis in patients with lymph node metastasis much higher than that without lymph node metastasis, suggesting that thrombocytosis was related to the invasion and metastasis of gastric cancer.

Varon et al showed that platelet-derived microparticles were able to increase the expression of cell adhesion molecules, promote the release of cytokines, and activate intracellular signaling pathways, thereby changing vascular reactivity and stimulating angiogenesis, and playing an important role in tumor metastasis. This study demonstrated that the activated platelets were able to significantly enhance the invasion and migration of MKN-45 gastric cancer cells. As the number of platelets increased, their cell invasion and migration capability gradually increased. Western blot results showed that the activated platelets were able to induce epithelial-mesenchymal
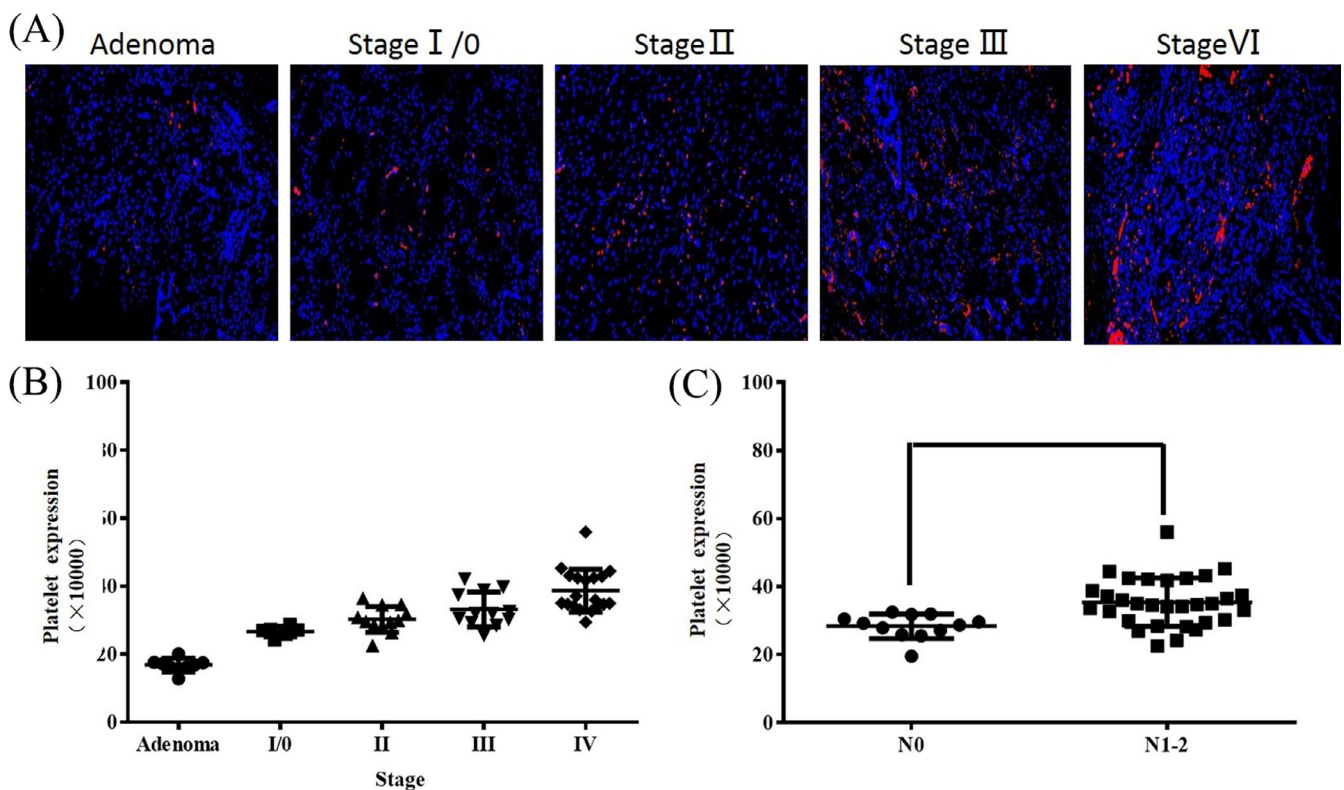
FIGURE 3 A, Expression of platelets in gastric cancer tissues (immunofluorescence × 200); B, Platelet expression in carcinoma in situ and different TNM staging gastric cancer tissues; C, platelets with lymph node metastasis (N1-2) and expression in gastric cancer tissues without lymph node metastasis (N0)

FIGURE 4 Effect of different concentrations of platelets on the viability of gastric cancer MKN-45 cells, *P < .05, **P < .01, ***P < .001
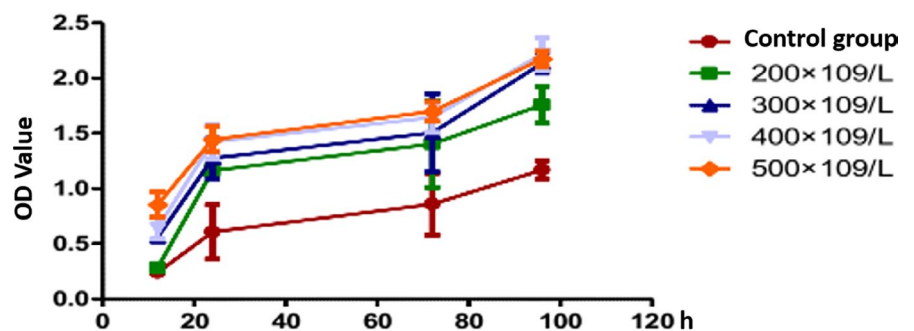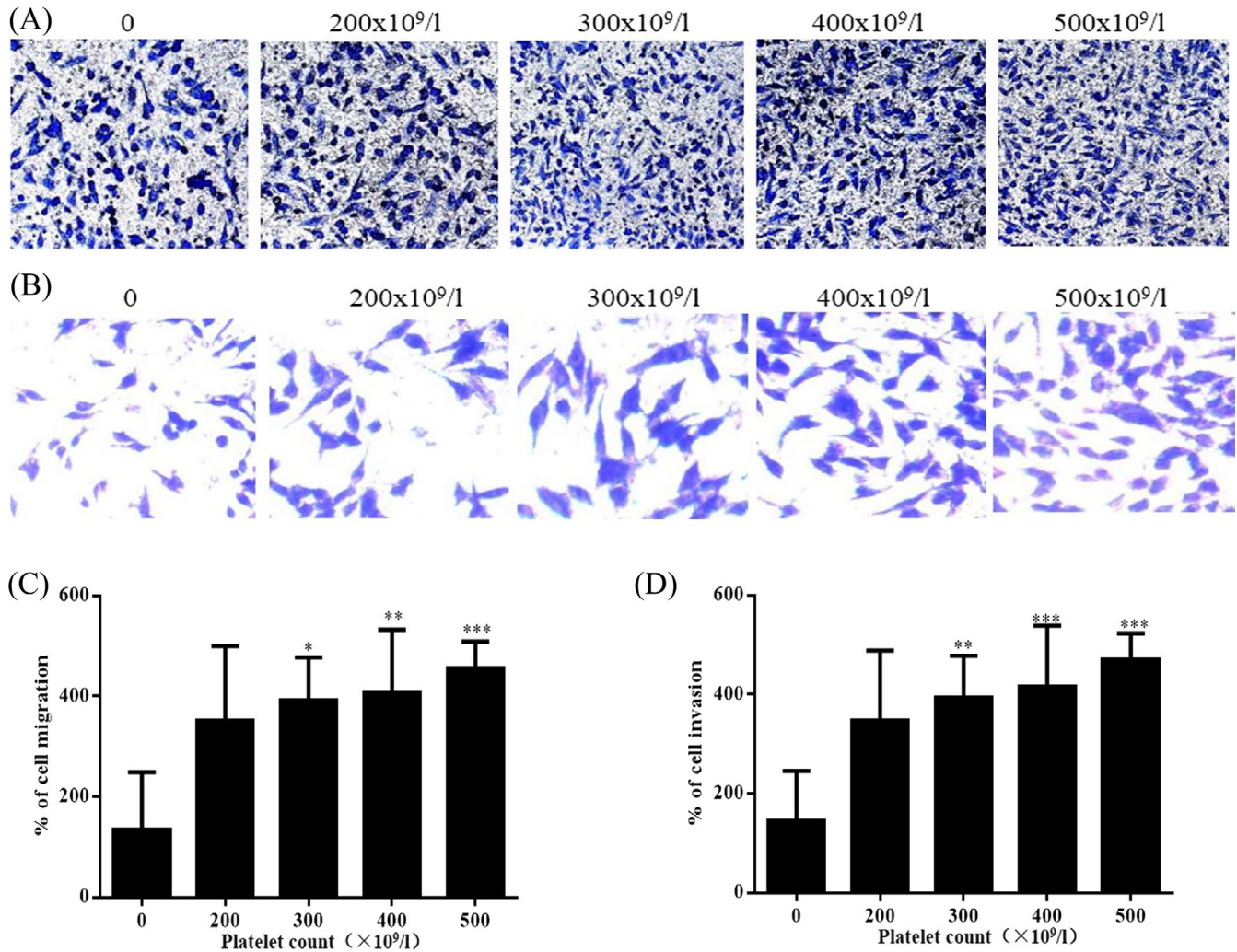
FIGURE 5 A, C, Effects of platelets on migration of gastric cancer MKN-45 cells; B, D, Effects of platelets on invasion of gastric cancer MKN-45 cells
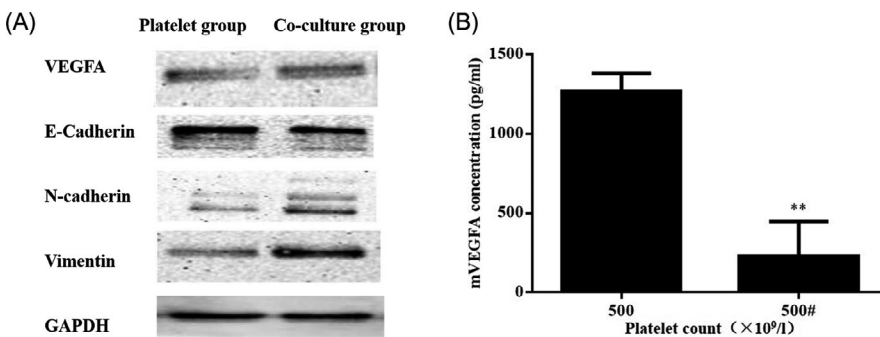

FIGURE 6 A, Expression of VEGFA, E-cadherin, N-cadherin, and vimentin in MKN-45 cells; B, secretion of VEGFA in MKN-45 cells (gastric cancer cells—platelet co-culture group [500] and platelet group [500#])

transition (EMT) of gastric cancer cells, thereby enhancing the invasion and migration capability of gastric cancer cells.

Studies have found that the platelets can promote the proliferation of tumor cells. A variety of biologically active factors, such as platelet-derived growth factors and transforming growth factors-β, can be released through platelet activation. Because these factors have the strong mitotic activity, they can directly stimulate the growth of the tumor cells,[14] stimulate the proliferation and adhesion of the tumor cells, and promote tumor growth and metastasis.[15-18]

The tumor cells can secrete some pro-inflammatory cytokines such as IL-6, which can promote the proliferation of megakaryocytes and further lead to thrombocytosis. Hyperplateletemia has been recognized as an important indicator of the prognosis of many tumors.

This study showed that the activated platelets were able to significantly promote the proliferation of MKN-45 gastric cancer cells. With the increase of the platelet count, the proliferation capability of the cell is gradually enhanced, and its proliferation effect is in a concentration-dependent manner. Western blots and ELISA

results showed that the activated platelets secreted a large amount of vascular endothelial growth factor-A (VEGFA) which directly act on tumor cells to stimulate the growth of tumor cells; besides, the platelet counts were increased with the increase of the tumor angiogenesis and metastasis. For the interaction of the platelet-tumor cell, the platelet count level can be used as a prognostic indicator for malignant tumors.

The peripheral blood cell count is a simple and economical test technique commonly used in hospitals at all levels. The platelet count can not only reflect coagulation function and immune status, but also can be used to evaluate the prognosis of cancer patients. Therefore, it is instructive for prognostic evaluation by monitoring changes in the platelet count. For gastric cancer patients with concurrent thrombocytosis, a surgery can expand the scope of radical treatment, thereby improving the patient's prognosis. The dynamic observation of platelet count changes, combined with tumor markers, may be a simple and practical marker for predicting tumor recurrence and metastasis. With regard to its deeper application value and reference value, it is still necessary to determine a large multi-center sample from clinical data. To provide new avenues for cancer therapy, it is expected to further explore a mechanism by which platelets promote tumor growth and development.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Misleh JG, Santoro P, Strasser JF, Bennett JJ. Multidisciplinary management of gastric cancer. *Surg Oncol Clin N Am.* 2013;22(2):247-264.
2. Erpenbeck L, Schfn MP. Deadly allies: the fatal interplay between platelets and metastasizing cancer cells. *Blood.* 2010;115(17):3427-3436.
3. Tomita M, Shimizu T, Hara M, Ayabe T, Onitsuka T. Prognostic impact of thrombocytosis in resectable non—small cell lung cancer. *Interact Cardiovase Thorac Surg.* 2008;7(4):613-615.
4. Azab B, Shah N, Radbel J, et al. Pretreatment neutrophil/lymphocyte ratio is superior to platelet/lymphocyte ratio as a predictor of long-term mortality in breast cancer patients. *Med Oncol.* 2013;30(1):432.
5. Varon D, Shai E. Role of platelet-derived microparticles in angiogenesis and tumor progression. *Discov Med.* 2009;8(43):237-241.
6. Cavallaro U, Christofori G. Multitasking in tumor progression: signaling functions of cell adhesion molecules. *Ann N Y Acad Sci.* 2004;1014:58-66.
7. Knudsen KA, Wheelock MJ. Cadherins and the mammary gland. *J Cell Biochem.* 2005;95(3):488-496.
8. Prasad CP, Rath G, Mathur S, Bhatnagar D, Parshad R, Ralhan R. Expression analysis of E-cadherin, Slug and GSK3beta in invasive ductal carcinoma of breast. *BMC Cancer.* 2009;9:325.
9. Nagi C, Guttman M, Jaffer S, et al. N-cadherin expression in breast cancer: correlation with an aggressive histologic variant-invasive micropapillary carcinoma. *Breast Cancer Res Treat.* 2005;94(3):225-235.
10. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell.* 2009;139(5):871-890.
11. Shimada H, Oohira G, Okazumi S, et al. Thrombocytosis associated with poor prognosis in patients with esophageal carcinoma. *J Am Coll Surg.* 2004;198(5):737-741.
12. Kwon H-C, Kim SH, Oh SY, et al. Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers.* 2012;17(3):216-222.
13. Ikeda M, Furukawa H, Imamura H, et al. Poor prognosis associated with thrombocytosis in patients with gastric cancer. *Ann Surg Ontol.* 2002;9(3):287-291.
14. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest.* 2009;119(6):1420-1428.
15. Kuo YC, Su CH, Liu CY, Chen TH, Chen CP, Wang HS. Transforming growth factor—beta induces CD44 cleavage that promotes migration of MDA-MB-435s cells through the up-regulation of membrane type 1-matrix metalloproteinase. *Int J Cancer.* 2009;124(11):2568-2576.
16. Stone RL, Nick AM, McNeish IA, et al. Paraneoplastic thrombocytosis in ovarian cancer. *N Engl J Med.* 2012;366(7):610-618.
17. Pooh RT, Fan ST, Wong J. Clinical significance of angiogenesis in gastrointestinal cancers: a target for novel prognostic and therapeutic approaches. *Ann Surg.* 2003;238(1):9-28.
18. Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin (ogen)increase metastatic potential by impeding natural killer cell–mediated elimination of tumor cells. *Blood.* 2005;105(1):178-185.