

Neutrophil and lymphocyte counts at diagnosis are associated with overall survival of pancreatic cancer

A retrospective cohort study

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Abstract

Neutrophil to lymphocyte ratio (NLR) has been found to be significantly associated with pancreatic cancer (PC) survival. However, no existing studies discussed the association between neutrophil count, lymphocyte count, and PC survival jointly. In this study, we aimed to analyze the influence of neutrophil and lymphocyte counts measured at disease diagnosis on the overall survival (OS) of PC. A total of 288 PC patients diagnosed between January 1, 2012, and December 31, 2013, were retrospectively selected from a population-based electronic inpatients database. Multivariate Cox model and restricted cubic spline (RCS) were used to estimate the associations between neutrophil count, lymphocyte count, and OS of PC. We found that a decreased lymphocyte count at diagnosis was significantly associated with OS of PC: for PC patients whose lymphocyte counts were less than $1.5 \times 10^9/L$, the hazard ratio (HR) was 1.82 (95% confidence interval: 1.37–2.40). Although abnormally increased baseline neutrophil count in general was not associated with OS of PC, RCS found a prominently deteriorated survival for PC patients whose baseline neutrophil counts were close to the cutoff point ($7.0 \times 10^9/L$). Our study results indicate that neutrophil and lymphocyte counts at diagnosis may have prognostic relevance in PC survival, especially lymphocyte count. The clinical significance of neutrophil inhibition and lymphocyte promotion treatments in PC patients should be further discussed.

Abbreviations: HR = hazard ratio, NLR = neutrophil to lymphocyte ratio, OS = overall survival, PC = pancreatic cancer, RCS = restricted cubic spline.

Keywords: lymphocyte count, neutrophil count, overall survival, pancreatic cancer

1. Introduction

Pancreatic cancer (PC) is a common type of cancer. It was ranked 12th when measured by incidence among all malignant tumors in the year 2012, with 338,000 newly diagnosed patients.^[1] Because

that usually no specific symptoms or signs will present in the early phase of PC, most patients cannot be diagnosed until the disease reached an advanced stage.^[2] It has been estimated that, among all diagnosed PC patients, only 20% can receive curative resection.^[3] For PC patients who are not suitable for surgery, chemotherapy is the only option for treatment. However, during the past 2 decades, the progress in chemotherapy of PC was almost stagnant.^[4] These factors with others collectively contribute to the extremely dismal survival of PC. Data from the United States revealed that, during the year 2002 to 2008, the 5-year survival rate of PC was only 6%, and over 70% of patients died within the first year after diagnosis.^[5]

Neutrophil to lymphocyte ratio (NLR) is a commonly used and very important systemic inflammation biomarker; it is defined as the absolute neutrophil count divided by the absolute lymphocyte count.^[6] An elevated NLR has been repeatedly identified to be associated with compromised survival of many types of cancer, such as gastric cancer,^[7] rectal cancer,^[8] breast cancer,^[9] and oral cancer.^[10] Several published studies also reported a hazardous role of elevated NLR in PC survival.^[11,12] As a composite index, either the increase of neutrophil count or the decrease of lymphocyte count will drive up the value of NLR. Thus, when discussing the association between NLR and PC, it will be very necessary to go one step further to explore whether these two indicators play equally important roles in this association. By doing so, we may effectively determine the intervention focus for clinical treatment of PC. Two previous studies concluded that decreased lymphocyte count was related to less optimistic

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survival of PC,^[13,14] while another study revealed that neutrophil count showed an insignificant influence on PC survival.^[15] This limited literature seems to suggest that lymphocyte count was more closely associated with PC survival than neutrophil count. However, all 3 studies were based on a small number of PC patients, and reported the effect estimate of 1 indicator without adjusting for its possible correlation with the other; thus, their findings need to be corroborated further.

The aim of this study was to simultaneously discuss the influence of neutrophil count and lymphocyte count measured at disease diagnosis on the overall survival (OS) of PC, in a large retrospective cohort of patients.

2. Methods

2.1. Study population

After institutional research ethics board of Fudan University approved, we performed a retrospective review in a mega electronic inpatients database. This database contains relevant information on disease diagnosis, clinical tests, examinations, and treatments during the whole hospitalization period for every inpatient who was admitted into county-level or above hospital within Shanghai metropolitan area, China. This database is still accumulating on a daily basis, and new information of a specific patient will be automatically merged into the existing database by pre-assigned unique personal ID.

We screened for histologically confirmed exocrine PC patients who were diagnosed between January 1, 2012, and December 31, 2013, from this database. Patients with incomplete vital information, such as age at diagnosis, whether curative operation was performed, whether chemotherapy was administered, were stepwise deleted.

By applying aforementioned criteria, a total of 676 PC patients were identified. We further selected patients who were tested for baseline neutrophil and lymphocyte counts (N=312); “baseline” was defined as “within 30 days after PC diagnosis.” Furthermore, considering that current physical performance status is an important confounder, patients who died within 30 days after diagnosis were excluded (N=24). In the end, we included 288 PC patients to analyze.

Because of retrospective nature, and no individually identifiable or sensitive information being involved, informed consents from patients had been waived.

2.2. Variables and definitions

Other potential confounders that need to be controlled for, such as age at diagnosis, sex, whether curative operation was performed, whether chemotherapy was administered, were also

extracted from this inpatients database. The administration of chemotherapy was defined as any combination of the following common drugs for treatment of PC: gemcitabine, nab-Paclitaxel, 5-fluorouracil, Irinotecan, and oxaliplatin.

The outcome of interest was OS. Date of death for PC patients was ascertained through external matching with death registration database, and the deadline for matching was set as January 31, 2015. The baseline neutrophil and lymphocyte counts were dichotomized into 2 groups by most commonly used cutoffs: $7.0 \times 10^9/L$ and $1.5 \times 10^9/L$, respectively. Baseline NLR was defined as baseline neutrophil count divided by baseline lymphocyte count. Normally used cutoff for NLR ranges from 2.3 to 5^[12]; in this study, we chose 4 integer cutoff points to discuss the influence of baseline NLR on PC survival: 2, 3, 4, 5.

2.3. Statistical analysis

Descriptive statistics were used to illustrate the characteristics of analyzed PC patients. Distributional differences between groups were checked by using *t* test, Fisher exact test, and log-rank test. Univariate Cox proportional hazards model was used to perform preliminary screening; variables with *P* values less than 0.1 were further included into multivariate Cox model to calculate adjusted hazard ratios (HRs). We further applied restricted cubic spline (RCS) to explore dose–response associations between baseline neutrophil count, lymphocyte count, and OS of PC patients. When fitting dose–response curves, we chose 3 knots for both indicators, which were the 5th, the 50th, and the 95th percentiles.

All statistical analyses were executed by SAS (version 9.2; SAS Institute Inc., Cary, NC). The significance level was set as 2-tailed probability less than 0.05.

This study has been reported in line with the STROBE criteria.^[16]

3. Results

3.1. General characteristics of PC patients

The general characteristics of 288 included PC patients are described in Table 1. The age mean at diagnosis for all patients was 64.97 years, the proportions of males and females were comparable, 21.18% of patients received curative operation, and nearly a half of the patients accepted chemotherapy. The overall median of survival was 251 days, with the maximum and the minimum of 967 and 32 days, respectively. In total, 235 (81.25%) patients died before deadline of the study.

Fifty-four patients (18.75%) filed a neutrophil count above $7.0 \times 10^9/L$ at diagnosis, whereas over 60% of patients were

Table 1

General characteristics of 288 included PC patients.

| Characteristics | Overall (N=288) | Baseline neutrophil count | | <i>P</i> |
|---|-----------------|----------------------------------|------------------------------|----------|
| | | $\leq 7.0 \times 10^9/L$ (N=234) | $> 7.0 \times 10^9/L$ (N=54) | |
| Age at diagnosis (Mean, Std.) | 64.97 (10.44) | 65.44 (10.38) | 62.89 (10.56) | 0.11* |
| Sex (Male, %) | 150 (52.08) | 119 (50.85) | 31 (57.41) | 0.45† |
| Curative operation (Yes, %) | 61 (21.18) | 49 (20.94) | 12 (20.22) | 0.85† |
| Any chemotherapy (Yes, %) | 143 (49.65) | 116 (49.57) | 27 (50.00) | 1.00† |
| Survival length (Median, d) | 251 | 258 | 198 | 0.26‡ |
| Baseline lymphocyte count ($\leq 1.5 \times 10^9/L$, %) | 177 (61.46) | 135 (57.69) | 42 (77.78) | 0.01† |

* By *t* test.

† By Fisher exact test.

‡ By log-rank test.

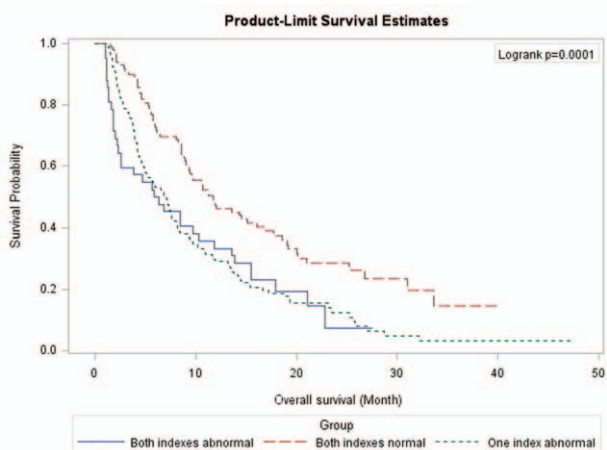


Figure 1. Survival curves of PC patients with different combinations of baseline neutrophil and lymphocyte counts.

observed a baseline lymphocyte count below $1.5 \times 10^9/L$. The survival lengths for PC patients with different baseline neutrophil count levels were not statistically different (Table 1).

3.2. OS of PC patients with different neutrophil and lymphocyte counts

We divided PC patients into the following 3 subgroups on the basis of combinations of their baseline neutrophil and lymphocyte counts: both indexes were normal (defined as neutrophil count $\leq 7.0 \times 10^9/L$ and lymphocyte count $> 1.5 \times 10^9/L$), 1 index was abnormal (defined as only neutrophil count $> 7.0 \times 10^9/L$, or only lymphocyte count $\leq 1.5 \times 10^9/L$), and both indexes were abnormal (defined as neutrophil count $> 7.0 \times 10^9/L$ and lymphocyte count $\leq 1.5 \times 10^9/L$). The survival curves for 3 subgroups were distinctively different: patients with both indexes normal observed a significantly optimistic survival compared with patients from the other 2 subgroups (Fig. 1).

3.3. Baseline neutrophil count, lymphocyte count, and OS of PC

On the basis of univariate model, other than baseline neutrophil count and lymphocyte count, age at diagnosis and whether curative operation was performed were included into multivariate Cox model. Interaction between baseline neutrophil count and lymphocyte count had also been checked in advance; however, it was insignificant ($P=0.14$). After adjustment, every

5-year increase in age was related to 12% increase in death hazard; for PC patients who received curative operation, the death hazard was 60% lower. Baseline lymphocyte count was associated with OS of PC in adjusted model: compared with patients whose lymphocyte counts were higher than $1.5 \times 10^9/L$, a decreased lymphocyte count was associated with 80% increase in death hazard. An increased baseline neutrophil count was not significantly associated with OS of PC (Table 2).

After RCS transformation, we can see that a baseline lymphocyte count less than about $0.5 \times 10^9/L$ and a baseline neutrophil count range between 5 and $10 \times 10^9/L$ were associated with elevated death hazard among PC patients. Moreover, an apparent dose-response relationship between baseline lymphocyte count and OS of PC has been identified: along with the increase of baseline lymphocyte count, the hazard of death decreased (Fig. 2).

3.4. Baseline NLR and OS of PC

As to the influence of baseline NLR on PC survival, we found that an elevated NLR determined by all 4 chosen cutoffs was associated with deteriorated OS of PC patients. All calculated HRs ranged within a narrow spectrum, from 1.71 [95% confidence interval (95% CI): 1.28–2.28] to 2.42 (95% CI: 1.75–3.36) (Fig. 3).

4. Discussion

As a commonly used and very important biomarker of systemic inflammation, NLR and its influence on PC survival had been discussed previously. In this study, we found that an elevated NLR measured at diagnosis was generally associated with significantly deteriorated survival of PC. This result was in accordance with the published studies.^[12] Our study further confirmed the hazardous effect of increased systemic inflammation burden on PC survival.

Different from 2 previous studies, which found that the prognostic role of NLR in patients with colorectal cancer was derived almost entirely from neutrophil count,^[17,18] in this study, based on multivariate Cox regression model, other than neutrophil count, we found that a decreased baseline lymphocyte count was prominently related to OS of PC. Further analysis by using RCS revealed that there was an apparent declining trend in death hazard along with the increase of baseline lymphocyte count. This finding probably suggests that compared with neutrophil count, lymphocyte count might be a more sensitive indicator in association with PC survival.

Pretreatment lymphocytopenia has been identified as an unfavorable factor in survival of various types of cancer, such

Table 2
Crude HRs and adjusted HRs for OS of PC related to multiple factors.

| Variables | Univariate Cox model | | Multivariate Cox model | |
|--|----------------------|-------|------------------------|-------|
| | Crude HR (95% CI) | P | Adjusted HR (95% CI) | P |
| Age at diagnosis (+5 y) | 1.14 (1.07–1.22) | <0.01 | 1.12 (1.05–1.19) | <0.01 |
| Sex (Male) | 0.86 (0.66–1.11) | 0.25 | — | — |
| Curative operation (Yes) | 0.38 (0.26–0.54) | <0.01 | 0.39 (0.27–0.57) | <0.01 |
| Any chemotherapy (Yes) | 0.81 (0.63–1.05) | 0.12 | — | — |
| Baseline lymphocyte count ($\leq 1.5 \times 10^9/L$) | 1.82 (1.38–2.39) | <0.01 | 1.82 (1.37–2.40) | <0.01 |
| Baseline neutrophil count ($> 7.0 \times 10^9/L$) | 1.21 (0.87–1.69) | 0.26 | 1.24 (0.88–1.74) | 0.22 |

95% CI=95% confidence interval, HR=hazard ratio.

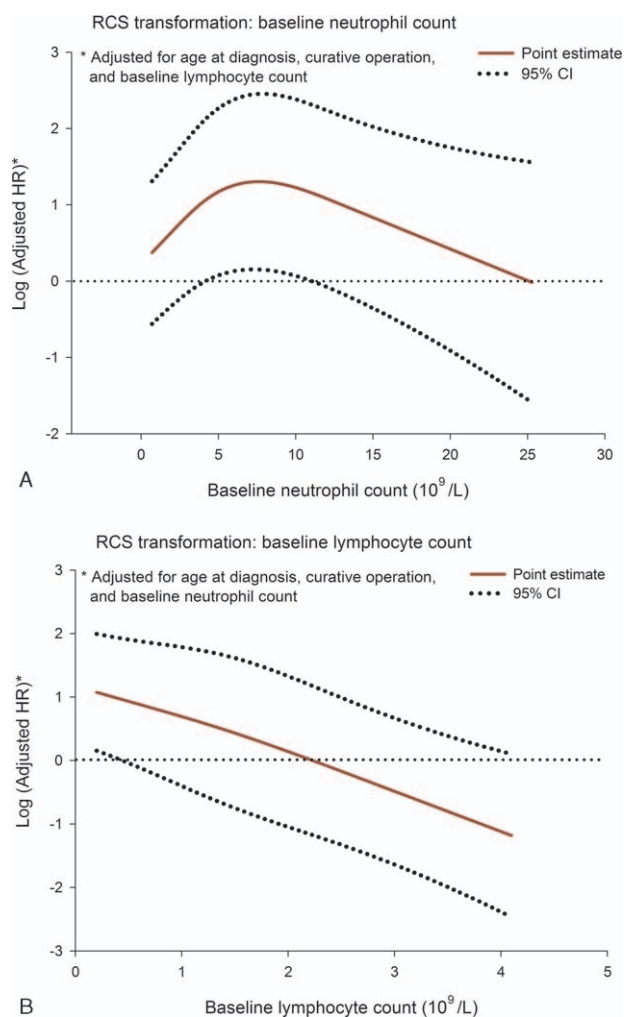


Figure 2. Dose-response associations between baseline neutrophil count, lymphocyte count, and OS of PC.

as nonsmall-cell lung cancer (NSCLC), renal cell carcinoma, ovarian cancer, as well as PC.^[13,14,19-21] Other than the explanation of systemic inflammation, which always causes concurrent neutrophilia and lymphocytopenia, it has been confirmed that lymphocytes are essential to cell-mediated immune response against cancer^[22]; thus, a decreased lymphocyte count can cause weakened antitumor capacity. Besides, decreased lymphocyte count can promote lymphatic invasion, which can further lead to a compromised survival, as suggested by a previous study in NSCLC patients.^[19]

Although neutrophilia at diagnosis in general was not significantly associated with OS of PC, RCS revealed a notably increased hazard for PC patients whose baseline neutrophil counts were between 5 and $10 \times 10^9/L$, close to the mostly used cutoff point of $7 \times 10^9/L$. Some previously published studies supported the harmful effect of neutrophilia in cancer progression and survival. For example, Li et al^[23] reported that elevated neutrophil count was associated with poor pathological differentiation and more advanced stage in gastric cancer. Other than this, it has been verified that circulating neutrophils can secrete various cytokines, such as vascular endothelial growth factor (VEGF) and tumor necrosis factor- α , all of which directly promote the progression of cancer.^[24,25] Moreover, some in vitro

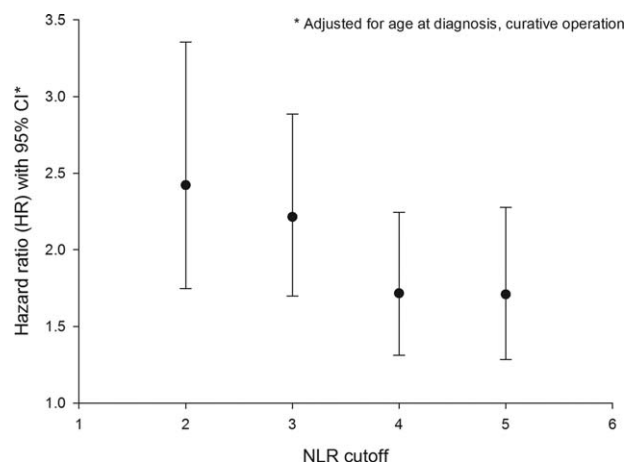


Figure 3. Association between baseline NLR and OS of PC by different NLR cutoffs.

studies also suggested that neutrophils suppressed host cellular immunity against cancer, and the level of suppression was proportional to the extent of neutrophil count increase.^[26,27] Nevertheless, why in this study, the significant association only existed in PC patients whose baseline neutrophil counts were near to the cutoff point is intriguing, and need to be further investigated.

Our study results probably suggest that, for PC patients, normally ranged neutrophil and lymphocyte counts at diagnosis in general foretell a better survival in the end. However, as the central mechanisms behind these identified associations are still elusive, the clinical relevance, and hopefully, therapeutic implication of our findings require intensive discussions. Currently, no existing studies have investigated the efficacy of neutrophils inhibition treatment in cancer patients. Although 2 studies found that temporal lymphocytosis caused by administration of interleukin 2 (IL-2) was correlated with tumor response in metastatic melanoma and renal cancer,^[20,28] another study reported that the extent of absolute lymphocyte count recovery after treatment had no significant influence on prognosis of ovarian cancer.^[21]

The strength of our study can be consolidated either through the comparatively large sample size of PC patients or through the application of RCS to thoroughly discuss the dose-response association between the 2 blood indicators and PC survival. However, our study also had several limitations. First, when estimating the adjusted associations between baseline neutrophil count, lymphocyte count, and PC survival, we could not fully adjust for the confounding caused by tumor classification of PC patients because detailed histological information was not available. However, we did control for whether the patient received curative operation, which should be an ideal surrogate of tumor classification in PC patients. Second, because of data incompleteness, our analysis was based on 288 PC patients out of the originally determined population of 676. If major characteristics of excluded patients were not comparable to included patients, selection bias can be introduced. Finally, all patients we studied were selected from a localized area in China; thus, the generalization of study results should be drawn with prudence.

In conclusion, our findings suggest that neutrophil and lymphocyte counts at diagnosis may have prognostic significance in PC survival, especially lymphocyte count. More attention

should be allocated to PC patients who presented abnormality in these 2 blood indicators upon diagnosis. The clinical significance of neutrophil inhibition and lymphocyte promotion treatments in PC patients should be discussed by future studies.

References

- [1] Ferlay J, Soerjomataram I, Ervik M, et al. *Cancer Incidence and Mortality Worldwide: IARC Cancer Base No 11*. Lyon, France: International Agency for Research on Cancer; 2013.
- [2] Ryan DP, Hong T S, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014;371:1039–49.
- [3] Bond-Smith G, Banga N, Hammond TM, et al. Pancreatic adenocarcinoma. *BMJ* 2012;344:e2476.
- [4] Thota R, Pauff JM, Berlin JD. Treatment of metastatic pancreatic adenocarcinoma: a review. *Oncology (Williston Park)* 2014;28:70–4.
- [5] American Cancer Society. *Cancer Facts & Figures 2014*. Atlanta: American Cancer Society; 2014.
- [6] Wang X, Zhang G, Jiang X, et al. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: a meta-analysis of observational studies. *Atherosclerosis* 2014;234:206–13.
- [7] Ishizuka M, Oyama Y, Abe A, et al. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients undergoing surgery for gastric cancer. *J Surg Oncol* 2014;110:935–41.
- [8] Kim IY, You SH, Kim YW. Neutrophil-lymphocyte ratio predicts pathologic tumor response and survival after preoperative chemoradiation for rectal cancer. *BMC Surg* 2014;14:94.
- [9] Noh H, Eomm M, Han A. Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients. *J Breast Cancer* 2013;16:55–9.
- [10] Perisanidis C, Kornek G, Pöschl PW, et al. High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. *Med Oncol* 2013;30:334.
- [11] Luo G, Guo M, Liu Z, et al. Blood neutrophil-lymphocyte ratio predicts survival in patients with advanced pancreatic cancer treated with chemotherapy. *Ann Surg Oncol* 2015;22:670–6.
- [12] Yang JJ, Hu ZG, Shi WX, et al. Prognostic significance of neutrophil to lymphocyte ratio in pancreatic cancer: a meta-analysis. *World J Gastroenterol* 2015;21:2807–15.
- [13] Schueneman AJ, Sugar EA, Uram J, et al. Low total lymphocyte count is associated with poor survival in patients with resected pancreatic adenocarcinoma receiving a GM-CSF secreting pancreatic tumor vaccine. *Ann Surg Oncol* 2013;20:S725–30.
- [14] Fogar P, Sperti C, Basso D, et al. Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. *Pancreas* 2006;32:22–8.
- [15] Aliustaoglu M, Bilici A, Seker M, et al. The association of pre-treatment peripheral blood markers with survival in patients with pancreatic cancer. *Hepatogastroenterology* 2010;57:640–5.
- [16] von Elm E, Altman DG, Egger M, et al. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806–8.
- [17] Neal CP, Mann CD, Sutton CD, et al. Evaluation of the prognostic value of systemic inflammation and socioeconomic deprivation in patients with resectable colorectal liver metastases. *Eur J Cancer* 2009;45:56–64.
- [18] Leitch EF, Chakrabarti M, Crozier JE, et al. Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer. *Br J Cancer* 2007;97:1266–70.
- [19] Zhang J, Huang SH, Li H, et al. Preoperative lymphocyte count is a favorable prognostic factor of disease-free survival in non-small-cell lung cancer. *Med Oncol* 2013;30:352.
- [20] Fumagalli LA, Vinke J, Hoff W, et al. Lymphocyte counts independently predict overall survival in advanced cancer patients: a biomarker for IL-2 immunotherapy. *J Immunother* 2003;26:394–402.
- [21] Milne K, Alexander C, Webb JR, et al. Absolute lymphocyte count is associated with survival in ovarian cancer independent of tumor infiltrating lymphocytes. *J Transl Med* 2012;10:33.
- [22] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–45.
- [23] Li QQ, Lu ZH, Yang L, et al. Neutrophil count and the inflammation-based Glasgow prognostic score predict survival in patients with advanced gastric cancer receiving first-line chemotherapy. *Asian Pac J Cancer Prev* 2014;15:945–50.
- [24] Kusumanto YH, Dam WA, Hospers GA, et al. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003;6:283–7.
- [25] Ulich TR, del Castillo J, Keys M, et al. Kinetics and mechanisms of recombinant human interleukin 1 and tumor necrosis factor-alpha-induced changes in circulating numbers of neutrophils and lymphocytes. *J Immunol* 1987;139:3406–15.
- [26] Petrie HT, Klassen LW, Kay HD. Inhibition of human cytotoxic T lymphocyte activity in vitro by autologous peripheral blood granulocytes. *J Immunol* 1985;134:230–4.
- [27] el-Hag A, Clark RA. Immunosuppression by activated human neutrophils. Dependence on the myeloperoxidase system. *J Immunol* 1987;139:2406–13.
- [28] Phan GQ, Attia P, Steinberg SM, et al. Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. *J Clin Oncol* 2001;19:3477–82.