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Correlation Between Natural Killer Cell Activity and Systemic Inflammatory Markers for Heterogeneous Cancer Patients Treated With Wheel Balance Cancer Therapy

Hwi-Joong Kang, KMD, PhD[1], Kyeore Bae, KMD, MS[1], Jee-Hye Kim, KMD, MS[1], Chong-Kwan Cho, KMD, PhD[1], and Hwa-Seung Yoo, KMD, PhD[1]

Abstract

Background and Objective: Natural killer (NK) cells are known to have an effect on the prevention of tumorigenesis for the initial cancer, as well as the metastatic cancer. For the past several years, the relationship between cancer and inflammation has been actively studied in preclinical and clinical settings, but there are no reports on alterations in and correlation for NK cell activity (NKA) and systemic inflammatory markers. Accordingly, this study aimed to measure correlation between NKA and the levels of other systemic inflammatory markers in patients with gastric, breast, and pancreatic cancer who received Wheel Balance Cancer Therapy (WBCT). **Methods:** Forty-two electronic charts of patients with gastric, breast, and pancreatic cancer treated with WBCT from February 1, 2015 to September 30, 2015, were reviewed retrospectively. These charts were statistically analyzed, looking for alterations of and correlation for NKA and the expressions of systemic inflammatory markers. **Results:** Patients with a NKA of under 300 pg/mL at admission showed significantly higher erythrocyte sedimentation rate (ESR) and neutrophil-to-lymphocyte ratio (NLR) values and decreasing NLR values due to WBCT than patients with an NKA greater than 300 pg/mL. As a result of the correlation analysis between NKA and the levels of the systemic inflammatory markers, NKA showed significant negative correlation with NLR, ESR, and fibrinogen values. **Conclusions:** Negative correlation was identified between NKA and NLR, NKA and ESR, and NKA and fibrinogen in patients with heterogeneous cancer patients.

Keywords

natural killer cell activity, systemic inflammatory marker, neutrophil-to-lymphocyte ratio, erythrocyte sedimentation rate, fibrinogen

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Introduction

Natural killer (NK) cells are a type of lymphocyte with the distinct morphological features of large granular lymphocytes, and they were first identified in 1975 through an experiment using a mouse model.[1,2] NK cells are distinguished from T or B lymphocytes in that they are capable of spontaneously showing cytotoxicity against various target cells, including tumor cells and virus-infected cells, without sensitization to antigens, the cell surface phenotype, or the cytokine profile[3-5]; in addition, they are known to be associated with the initial defense against infection and with tumor immunity.[6] The defense mechanism by which NK cells protect the body not only involves helping other immune cells (adaptive immune system) but also includes removal of target cells through the release of chemokines and cytokines (innate immune system).[6]

NK cells play an important role in preventing the initial, as well as metastatic cancer, and as such, a deficiency in NK cell–mediated cytotoxicity is known to have an influence

[1]Dunsan Korean Medical Hospital of Daejeon University, Daejeon, South Korea

Corresponding Author:
Hwa-Seung Yoo, East West Cancer Center, Dunsan Korean Medical Hospital of Daejeon University, 75, Daedeok-daero 176beon-gil, Seo-gu, Daejeon 35235, South Korea.
Email: altyhs@dju.ac.kr

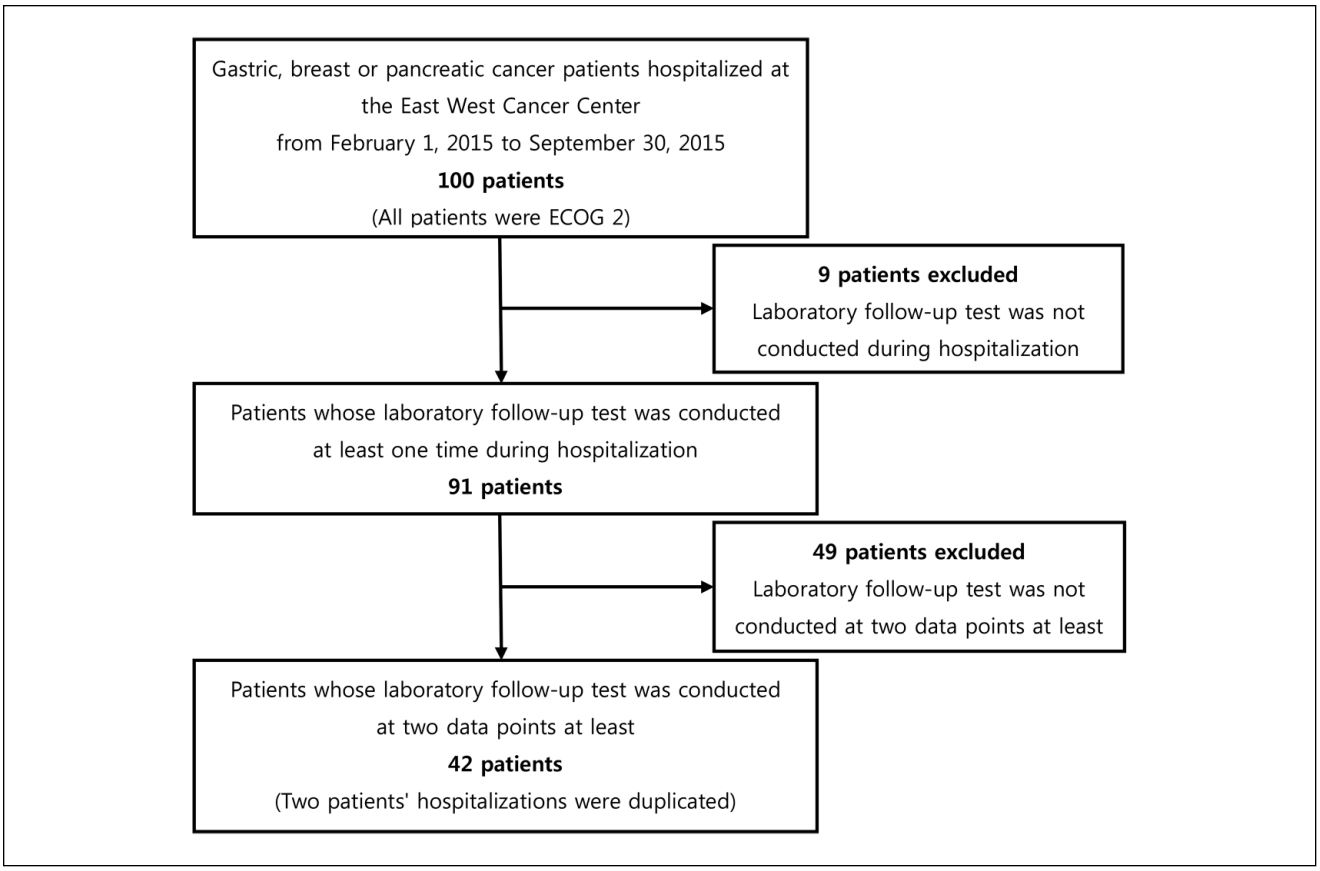

Figure 1. Selection of patients for the analysis. ECOG, Eastern Cooperative Oncology Group.

on the initial stage of human tumorigenesis.[7] In support of this, various studies have found reduced NK cell activity (NKA) in patients with esophageal and gastric,[8,9] breast,[10,11] pancreatic,[12] prostate,[13] colorectal,[14] lung,[15] bronchogenic,[16] hepatocellular,[17] and head and neck[18] cancer.

The relationship between cancer and inflammation has been actively studied in preclinical and clinical settings for the past several years.[19] Only a few decades have passed since clear evidence of inflammation playing an important role in tumorigenesis was gathered, during which time the molecular mechanisms in a few types of basal cells have also been identified.[20] Currently, the role of inflammation in tumorigenesis is well accepted, and though a direct causal relationship with inflammation has not been fully proven, the inflammatory microenvironment is certainly a vital element.[21] A wide variety of systemic inflammatory markers has been examined over the past 10 plus years for patient treatment and prognosis and for predicting the survival period. Items that can be measured from the blood and that reflect a systemic inflammatory response include fibrinogen, the erythrocyte sedimentation rate (ESR), and elevated cytokines, as well as elevated white blood cells (WBC), and factors related to those subtypes (neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and so on.[22-25]

The effects of herbal medicine on the immune function in patients with cancer have been studied. Herbal medicine combined with endocrine therapy was shown to improve the quality of life for patients with advanced prostate cancer, reduce the adverse side effects of Western medicine, improve the immune function, and enhance the therapeutic effects of endocrine therapy.[26] Another study showed that the administration of a Chinese medicinal herb complex to patients with breast cancer who were receiving chemotherapy and/or radiotherapy might have the capacity to delay, or ease, the reductions in the levels of leucocytes and neutrophils.[27] A double-blind, placebo-controlled, randomized trial showed that traditional Chinese medicine (TCM) might have an effect on maintaining the immune function in patients with ovarian cancer.[28]

Wheel Balance Cancer Therapy (WBCT) is an inpatient multimodality complementary and alternative medicine (CAM) cancer program at the East West Cancer Center (EWCC), Daejeon University Dunsan Korean Medicine Hospital (DUDKMH). It consists of (1) herbal medicine therapy, (2) an anticancer nutrition diet, (3)

metabolism activation therapy, and (4) a mind-body therapy program. Although many studies have reported that inpatient treatment with WBCT reduces the fibrinogen level and improves the quality of life in patients with cancer[29] and that the use of WBCT with Korean medicine for 21 or more days maintains C-reactive protein (CRP) and ESR and has a favorable effect on the survival rate of patients with stage IV cancer,[30] no reports have addressed alterations in either NKA or the systemic inflammatory markers. Accordingly, this study aimed to measure the correlation between NKA and the levels of other systemic inflammatory markers in patients with gastric, breast, and pancreatic cancer who received WBCT.

Patients and Methods

Chart Review

A retrospective chart review was conducted for 100 patients who had been diagnosed with gastric, breast, or pancreatic cancer. Data were from a single collection site and were obtained under a protocol approved by the institutional review board (IRB) (of DUDKMH, Daejeon, Republic of Korea (IRB approval number: DJDSKH-15-BM-E-3).

Patient Eligibility

The following inclusion and exclusion criteria were used to select participants for this study: Patients with gastric, breast, or pancreatic cancer hospitalized at the EWCC, DUDKMH, from February 1, 2015, to September 30, 2015, were considered. Then, patients on whom laboratory follow-up tests had not been conducted during hospitalization were excluded, as were patients whose laboratory follow-up tests had not been conducted at 2 data points at least, and patients whose Eastern Cooperative Oncology Group (ECOG) performance status was 3 or more (Figure 1).

WBCT, the multimodality cancer program for EWCC's inpatients, which includes the following 4 subprograms, was provided daily to all inpatients at the center[29]: The first subprogram, herbal medicine therapy, is composed of anti-angiogenic, immune-activating agents and/or herbal decoctions according to the Korean medicine differential diagnosis and was provided daily. The second subprogram, anticancer nutrition diet, is composed of constitution-specific foods, including vegetable or fruit juice with antioxidative activity, and was provided once or twice a day. The third subprogram, metabolism activation therapy, is composed of acupuncture, pharmacopuncture, moxibustion, massage, and thermotherapy and was provided to inpatients. The fourth subprogram, the mind-body therapy program, is composed of muscle relaxation therapy, meditation, yoga, and mountain climbing and was routinely provided.

Hematological Index

NK Cell Activity. NK cell activation–induced interferon-gamma (enzyme-linked immunosorbent assay [ELISA]), which has been designated as a safe and effective test for checking the condition of patients with gastric, breast, prostate, and pancreatic cancer, as well as the progress of their treatment, was used to measure the activity of NK cells. This particular test was recognized as a new medical technology in June 2014 when it passed the new medical technology safety and efficacy assessment in accordance with Korea Ministry of Health and Welfare Notification No. 2014-89. In comparison with other existing tests that use radioactive isotopes, this test has a lower cost and the results can be obtained within 24 hours. This kit defined NKA ≥300 pg/mL as normal and NKA <300 pg/mL as borderline or abnormal based on a clinical study of cancer patients and patients without cancer.[31]

Fibrinogen Test. The fibrinogen test is used to evaluate fibrinogen, a protein that is essential for blood-clot formation. When an injury and bleeding occurs, the body forms a blood clot through a series of steps. In one of the last steps, soluble fibrinogen is converted into insoluble fibrin threads that crosslink together to form a net that stabilizes the injury site and adheres to it until the area has healed. The test is done with the ACL 100 system (Instrumentation Laboratory Inc, Bedford, MA, USA).

Erythrocyte Sedimentation Rate Test. The erythrocyte sedimentation rate (ESR) test is a relatively simple, inexpensive, nonspecific test that has been used for many years to help detect inflammation associated with conditions such as infections, cancer, and autoimmune diseases. For the test, anticoagulated blood is traditionally placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells sediment is measured.

Absolute Neutrophil Count to Absolute Lymphocyte Count Ratio. The absolute neutrophil count to absolute lymphocyte count, NLR, reflects the presence of neutrophilic leukocytosis and relative lymphopenia and is recognized as an indicator of poor prognosis in patients with various cancers. The leukocyte differential count was measured by using the Celltec F system (Nihon Kohden, Tokyo, Japan).

Absolute Lymphocyte Count to Absolute Monocyte Count Ratio. The absolute lymphocyte count to absolute monocyte count ratio, LMR, reflects both lymphopenia as a surrogate marker of weakened immune response and increased number of monocytes as a surrogate marker for the microenvironment of elevated tumor burden.[32] The leukocyte differential count was measured by using the Celltec F system (Nihon Kohden).

Statistical Analysis

The level of significance was set at .05 for all the hypothetical tests. All the analyses were conducted by using SPSS version 22 for Windows. A paired *t* test was used to analyze differences in the results between the preliminary test and the secondary test. The 2-sample *t* test and 1-way analysis of variance were used to determine whether the mean NKA and the systemic inflammatory markers differed according to NKA at admission. The 2-sample *t* test was preformed to analyze the preliminary test, the secondary test, and the difference between the tests (secondary – preliminary). A correlation analysis was performed to verify the linear associations between NKA and the systemic inflammatory markers from the preliminary test, the secondary test, and the difference between the 2 tests (secondary – preliminary).

Results

Patient Characteristics

The total number of subjects in the study was 42, and their clinical characteristics are summarized in Table 1.

Table 1. Clinical Characteristics of Patients.

Variables	n (%) or Mean ± SD
Sex	
Male	8 (19.0)
Female	34 (81.0)
Age, y	54.05 ± 8.95
Age	
<60 y	32 (76.2)
≥60 y	10 (23.8)
Follow-up days	11.00 ± 2.95
Cancer type	
Gastric cancer	15 (35.7)
Breast cancer	21 (50.0)
Pancreatic cancer	6 (14.3)
Metastasis status	
Existence (stage IV)	26 (61.9)
Nonexistence	16 (38.1)
Resection status	
Operated	33 (78.6)
Inoperable	9 (21.4)
Chemotherapy status	
Undergone	31 (73.8)
Not undergone	11 (26.2)
ECOG	
0	0 (0.0)
1	0 (0.0)
2	42 (100.0)
NKA (pg/mL) at admission	
<300	23 (54.8)
≥300	19 (45.2)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NKA, natural killer cell activity.

Laboratory Changes Between Before and After the WBCT Treatment

As a result of analyzing the results from the preliminary test taken prior to and the secondary test taken during WBCT treatment, the changes in NKA and the systemic inflammatory markers showed no significance (Supplementary Table S1, reported in supplementary material).

Mean Values of NKA and the Systemic Inflammatory Markers Before and After the WBCT Treatment

In the groups with NKA <300 and ≥300 pg/mL, the mean ESR values at admission were 28.96 ± 14.77 and 18.89 ± 12.15 mm/h, respectively, showing a significant difference ($P = .022$). In addition, the mean NLR values were 3.60 ± 2.83 and 1.70 ± 0.61, respectively, showing a significant difference ($P = .004$), and the differences in the mean NLR values were −1.53 ± 3.31 and 0.20 ± 0.81, respectively, also showing a significant difference ($P = .023$) (Table 2).

Correlation Analysis Between NKA and Systemic Inflammatory Markers

A correlation analysis was performed to determine whether linear associations existed among systemic inflammatory markers from the preliminary test. At a significance level of 5%, significant linear associations were identified between NKA and ESR ($r = −0.359$; $P = .020$) and between NKA and NLR ($r = −0.318$; $P = .040$) (Figure 2). In addition, a correlation analysis was performed to determine whether linear associations existed among the systemic inflammatory markers from the secondary test. At a significance level of 5%, significant linear associations were identified between NKA and fibrinogen ($r = −0.408$; $P = .009$), NKA and ESR ($r = −0.384$; $P = .012$), and NKA and NLR ($r = −0.343$; $P = .026$) (Figure 3). Finally, a correlation analysis was performed to determine whether linear associations existed among the systemic inflammatory markers in the difference of the test results (secondary − preliminary test). At a significance level of 5%, differences in NKA showed no significant correlation with any systemic inflammatory markers (Figure 4).

Discussion

Human NK cells can be divided into 2 types according to CD56 expression on the cell's surface. One type, CD56dim cells, has natural cytotoxicity, and antibody-dependent cellular cytotoxicity accounts for the majority of NK cells whereas the other type, CD56bright cells, is involved in

Table 2. Mean Values of NKA, Fibrinogen, ESR, NLR, and LMR From the Preliminary and the Secondary Tests and the Differences Between the Tests According to NKA at Admission.

	NKA at Admission		
	<300 pg/mL (n = 23)	≥300 pg/mL (n = 19)	*P*
NKA (pg/mL)			
Preliminary	185.68 ± 99.30	973.60 ± 525.74	.000**
Secondary	312.10 ± 251.57	912.81 ± 626.64	.001**
Difference	126.42 ± 242.27	–60.80 ± 622.26	.229
Fibrinogen (mg/dL)			
Preliminary	635.09 ± 288.34	548.44 ± 211.29	.292
Secondary	708.00 ± 305.82	543.06 ± 183.03	.056
Difference	72.91 ± 248.76	–6.53 ± 97.26	.173
ESR (mm/h)			
Preliminary	28.96 ± 14.77	18.89 ± 12.15	.022*
Secondary	28.78 ± 15.31	19.74 ± 13.62	.052
Difference	–0.17 ± 11.25	0.84 ± 4.66	.715
NLR			
Preliminary	3.60 ± 2.83	1.70 ± 0.61	.004**
Secondary	2.07 ± 1.13	1.90 ± 0.82	.593
Difference	–1.53 ± 3.31	0.20 ± 0.81	.023*
LMR			
Preliminary	8.29 ± 13.27	4.47 ± 1.50	.184
Secondary	10.15 ± 28.75	4.88 ± 1.86	.431
Difference	1.86 ± 31.06	0.41 ± 1.80	.840

Abbreviations: NKA, natural killer cell activity; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.
*$P < .05$, **$P < .01$.

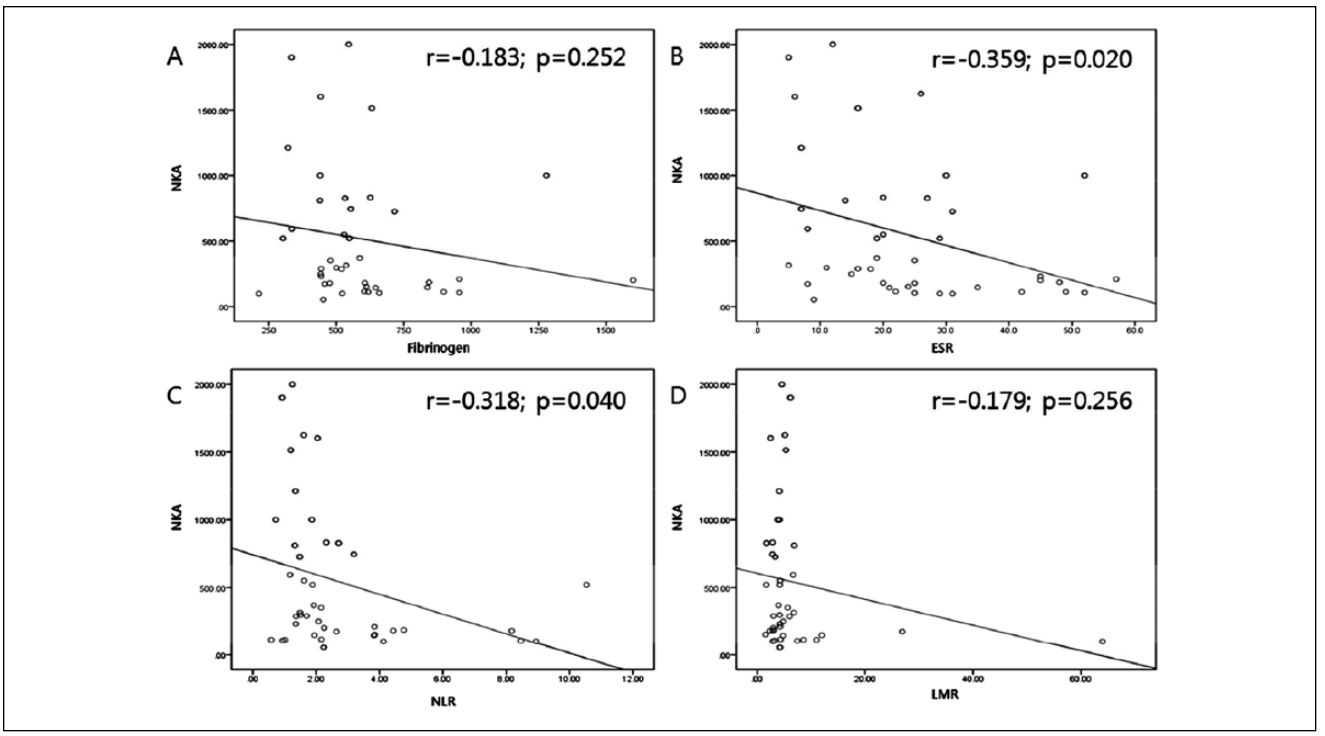

Figure 2. Correlation Analyses between NKA and fibrinogen (A), ESR (B), NLR (C), and LMR (D) at the preliminary test. NKA, natural killer cell activity; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

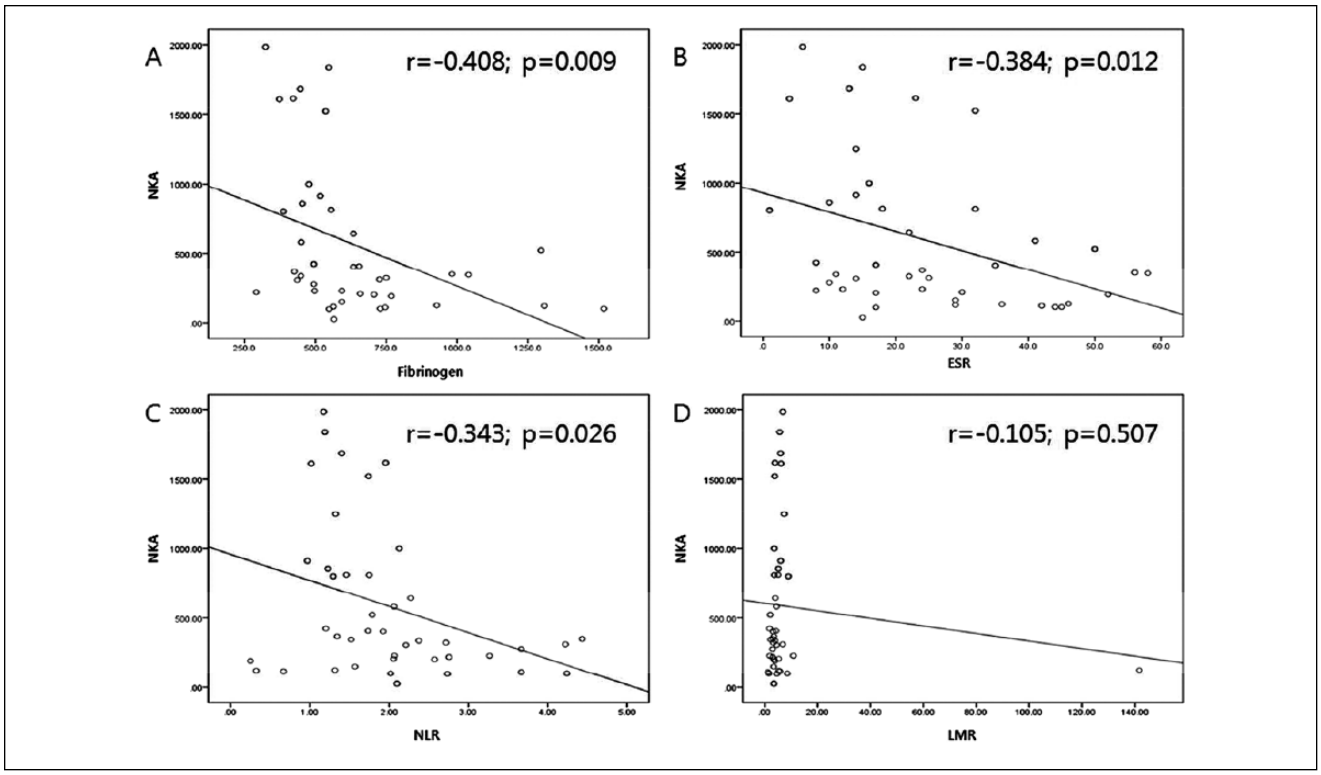

Figure 3. Correlation analyses between NKA and fibrinogen (A), ESR (B), NLR (C), and LMR (D) at the secondary test. NKA, natural killer cell activity; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

immunoregulatory functions through the release of cytokines.[33] NK cells are activated through bonding of their activation receptors with the activation ligands of target cells and with proinflammatory cytokines, such as interleukin (IL)-2, IL-12, IL-15, and IL-18, which can activate NK cells.[34] Activated NK cells induce the death of target cells by releasing perforin and granzyme, which are involved as death receptors of the targeted cells, and by secreting interferon (IFN)-γ.[35] Cytokines promote proliferation and maturation of NK cells and are involved in increasing the production of other cytokines and in enhancing the cytotoxicity in NK cells.[36] Tumor necrosis factor (TNF)-α, in addition to IFN-γ, as primary cytokines secreted from activated NK cells, shows an immune responses against cancer and virus-infected cells.[37]

NKA is associated with the prognoses for patients with various forms of cancer, for which a study reported that NKA was correlated with hematogenous metastasis of head and neck cancer,[18] and a follow-up study showed that it was associated with increased risk of death from uncontrolled regional and distant metastases.[38] Patients with colorectal cancer with low preoperative NKA had a high risk of local recurrence.[39] NKA was found to be low in most patients with a hepatocellular carcinoma (HCC), and the preoperative NKA measurement was found to be of some use in forming a posthepatectomy prognosis and in follow-up management for HCC patients.[40] Reduced NKA has been suggested to be one of the risk factors that can facilitate the progression to HCC in cirrhotic patients.[41] In a retrospective observation study on 156 patients, patients who had received a gastrectomy and had low NKA were shown to have a greater tendency of having lymphatic and vascular involvement than those with moderate to high NKA, thereby showing a significant association with a poorer survival.[42] In a general population study that spanned 11 years, low NKA was suggested as being related to increased cancer risk.[43]

Fibrinogen is a type of protein that is produced not only in liver cells but also in tumor cells.[44] It plays an important role in hemostasis[45] and is one of the acute phase reactants that become elevated with systemic inflammation or tissue injury.[46] Fibrinogen is also known to play important roles in inflammation related to proliferation, survival, migration, invasion and metastasis of tumor cells, to have a functional relationship with tumorigenesis[47,48] and to become attached to NK cells to prolong the NK cell–mediated removal of tumor cells.[49] Through recent clinical studies, a correlation between plasma fibrinogen and tumor progression has been shown in patients with esophageal,[50] gastric,[51] non–small cell lung,[52] colorectal,[53] ovarian,[54] cervical,[55] endometrial,[56] and vulvar[57] cancer.

ESR, which is referred to as the sedimentation rate, is a type of blood test that measures how quickly red blood

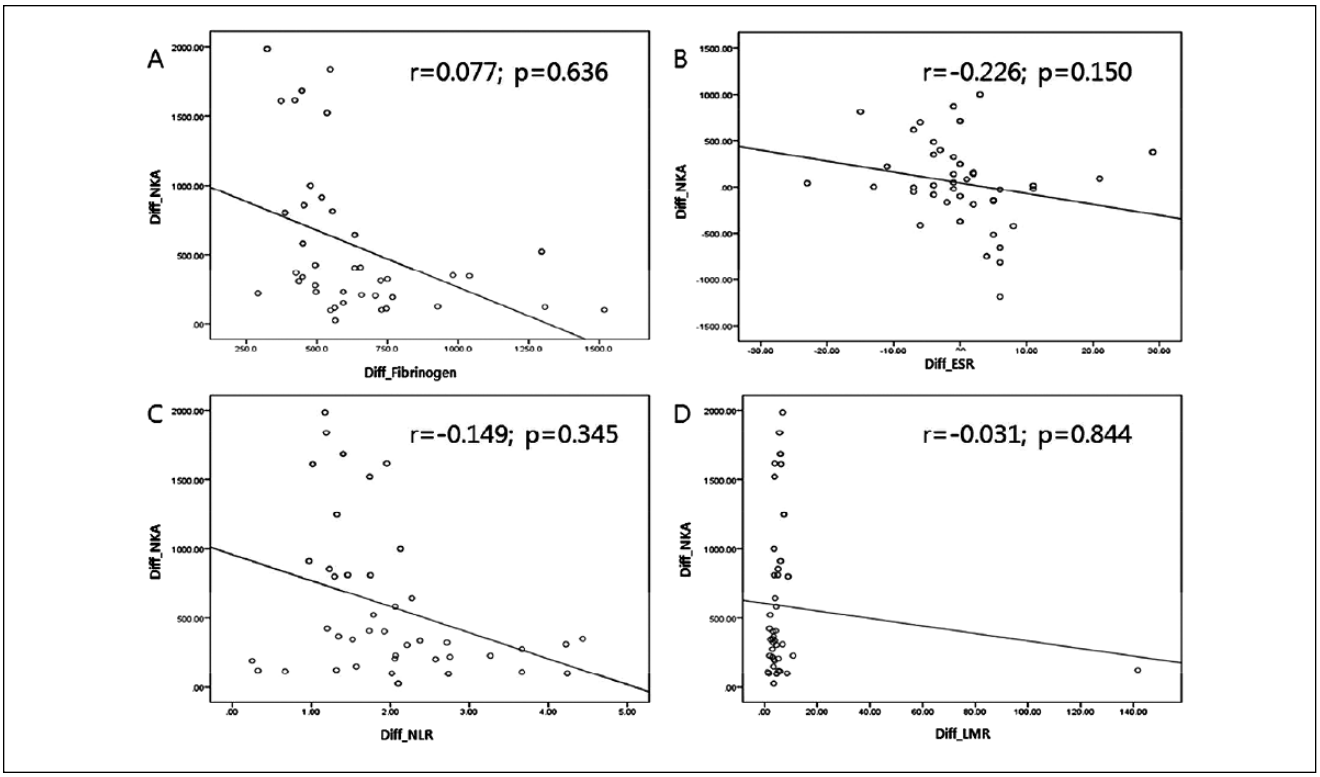

Figure 4. Correlation analyses between the difference of NKA and the differences of fibrinogen (A), ESR (B), NLR (C), and LMR (D). NKA, natural killer cell activity; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio, LMR, lymphocyte-to-monocyte ratio (Diff_NKA, difference of NKA; Diff_Fibrinogen, difference of fibrinogen; Diff_ESR, difference of ESR; Diff_NLR, difference of NLR; Diff_LMR, difference of LMR).

cells (RBCs) settle to the bottom of a test tube. ESR values measure higher as more RBCs fall to the bottom over time. When inflammation is present in the body, specific proteins force RBCs to coagulate more rapidly than under normal conditions, causing them to settle more rapidly to the bottom of the tube. These proteins are produced by the liver and the immune system under various abnormal conditions, including infection, autoimmune disease, and/or cancer.[58] ESR and the number of involved nodes were found to have major significance in predicting the length of disease-free survival in patients with primary breast cancer.[59]An elevated ESR is associated with shorter survival for patients with non–small cell cancer[60] and with progression and death in patients with prostate cancer.[61]An elevated ESR has also been shown to have a significant unfavorable association with tumor thrombus, large tumor size, advanced stage, lymph-node involvement, and distant metastasis in patients with a renal cell carcinoma.[23]

Systemic inflammatory response is well known to be related to changes in circulating WBCs, especially the presence of neutrophilic leukocytosis and relative lymphopenia.[62,63] Recently, an elevated NLR was recognized as being an indicator of poor prognosis in patients with various forms cancer,[64] as well as a cost-effective prognostic factor.[65] An elevated NLR was found to be significantly correlated with a shortening of the recurrence-free survival of HCC patients after a living-donor liver transplant.[66] In patients with advanced rectal cancer, a low NLR was associated with increased overall survival and disease-free survival, as compared with a high NLR, showing that it can be a useful prognostic factor.[67]

In this study, in patients with NKA <300 pg/mL, the systemic inflammatory markers of ESR and NLR tended to be high in the preliminary test, showing significant differences from those in patients with NKA ≥300 pg/mL. Because such differences based on treatment effects were significant for NLR, the WBCT treatment can be viewed as being more effective in patients with NKA <300 pg/mL.

The pairs of indicators that showed linear associations in the preliminary and the secondary tests were NKA-ESR and NKA-NLR pairs, both of which showed negative correlation (Figures 2 and 3). The pair of indicators that showed a linear association in the secondary test was NKA-fibrinogen pair, which also showed a negative correlation (Figure 3). These data suggest the possibility of a negative correlation between NK and systemic inflammatory markers such as NLR, ESR, and fibrinogen.

The present study was conducted with a relatively small number of charts (n = 42), therefore the statistical analysis was limited. A greater number of cases are needed to be secured so that a more specific statistical analysis can be performed according to the hypothesis generated by these preliminary results. The effects of various chemotherapies on the WBC differential must also be considered according to their mechanisms and degree of effect on neutrophils and lymphocytes. In addition, analyses should be performed to take into account underlying diseases, such as diabetes and hyperlipidemia, given the chronic inflammatory metabolic and cellular environments which accompany such metabolic disorders. In some patients, inflammation may be a precursor that induces cancer, whereas in other patients, cancer can promote an inflammatory microenvironment. Also, both processes could go on in the same patient. An inflammatory microenvironment affects proliferation, infiltration, and angiogenesis, as well as the survival of tumor cells, and future treatments with Korean medicine should be administered with the goal of targeting not only the tumor cells themselves but also the major factors that contribute to progressive growth and survival of metastatic cancer cells.

Conclusion

Negative correlations were identified between NKA and NLR, NKA and ESR, and NKA and fibrinogen in patients with heterogeneous cancer types. Further prospective data should be accumulated to confirm these negative correlations.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol*. 1975;5:112-117.
2. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer*. 1975;16:216-229.
3. Ritz J, Schmidt RE, Michon J, Hercend T, Schlossman SF. Characterization of functional surface structures on human natural killer cells. *Adv Immunol*. 1988;42:181-211.
4. Trinchieri G. Biology of natural killer cells. *Adv Immunol*. 1989;47:187-376.
5. Whiteside TL, Herberman R. Role of human natural killer cells in health and disease. *Clin Diagn Lab Immunol*. 1994;1:125-133.
6. Bryceson YT, Chiang SC, Darmanin S, et al. Molecular mechanisms of natural killer cell activation. *J Innate Immun*. 2011;3:216-226.
7. Standish LJ, Sweet ES, Novack J, et al. Breast cancer and the immune system. *J Soc Integr Oncol*. 2008;6:158-168.
8. Tsutsui S, Morita M, Kuwano H, et al. Influence of preoperative treatment and surgical operation on immune function of patients with esophageal carcinoma. *J Surg Oncol*. 1992;49:176-181.
9. Oka M, Mitsunaga H, Hazama S, Yoshino S, Suzuki T. Natural killer activity and serum immunosuppressive acidic protein levels in esophageal and gastric cancers. *Surg Today*. 1993;23:669-674.
10. Cunningham-Rundles S, Filippa DA, Braun DW Jr, Antonelli P, Ashikari H. Natural cytotoxicity of peripheral blood lymphocytes and regional lymph node cells in breast cancer in women. *J Natl Cancer Inst*. 1981;67:585-590.
11. Konjevic G, Radenkovic S, Srdic T, Jurisic V, Stamatovic Lj, Milovic M. Association of decreased NK cell activity and IFNγ expression with pSTAT dysregulation in breast cancer patients. *J BUON*. 2010;16:219-226.
12. Funa K, Nilsson B, Jacobsson G, Alm GV. Decreased natural killer cell activity and interferon production by leucocytes in patients with adenocarcinoma of the pancreas. *Br J Cancer*. 1984;50:231-233.
13. Lahat N, Alexander B, Levin RD, Moskovitz B. The relationship between clinical stage, natural killer activity and related immunological parameters in adenocarcinoma of the prostate. *Cancer Immunol Immunother*. 1989;28:208-212.
14. Shen Y, Lu C, Tian W, et al. Possible association of decreased NKG2D expression levels and suppression of the activity of natural killer cells in patients with colorectal cancer. *Int J Oncol*. 2012;40:1285-1290.
15. Sibbitt WL Jr, Bankhurst AD, Jumonville AJ, Saiki JH, Saiers JH, Doberneck RC. Defects in natural killer cell activity and interferon response in human lung carcinoma and malignant melanoma. *Cancer Res*. 1984;44:852-856.
16. LeFever AV, Funahashi A. Phenotype and function of natural killer cells in patients with bronchogenic carcinoma. *Cancer Res*. 1991;51:5596-5601.
17. Chuang WL, Liu HW, Chang WY. Natural killer cell activity in patients with hepatocellular carcinoma relative to early development and tumor invasion. *Cancer*. 1990;65:926-930.
18. Schantz SP, Shillitoe EJ, Brown B, Campbell B. Natural killer cell activity and head and neck cancer: a clinical assessment. *J Natl Cancer Inst*. 1986;77:869-875.
19. Grivennikov SI, Karin M. Inflammation and oncogenesis: a vicious connection. *Curr Opin Genet Dev*. 2010;20:65-71.
20. Karin M. Nuclear factor-κB in cancer development and progression. *Nature*. 2006;441:431-436.

21. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454:436-444.
22. Palumbo JS, Kombrinck KW, Drew AF, et al. Fibrinogen is an important determinant of the metastatic potential of circulating tumor cells. *Blood*. 2000;96:3302-3309.
23. Sengupta S, Lohse CM, Cheville JC, et al. The preoperative erythrocyte sedimentation rate is an independent prognostic factor in renal cell carcinoma. *Cancer*. 2006;106:304-312.
24. Viganó A, Bruera E, Jhangri GS, Newman SC, Fields AL, Suarez-Almazor ME. Clinical survival predictors in patients with advanced cancer. *Arch Intern Med*. 2000;160:861-868.
25. Roxburgh CS, McMillan DC. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. *Future Oncol*. 2010;6:149-163.
26. Jia Y, Li X, Li C, Zhao C. Clinical efficacy analysis of treating advanced prostate cancer by yiqi jiedu quyu recipe combined endocrine therapy [in Chinese]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2013;33:448-451.
27. Zhuang S, Chiu H, Chen S, et al. Effects of a Chinese medical herb complex on cellular immunity and toxicity-related conditions of breast cancer patients. *Br J Nutr*. 2012;107:712-718.
28. Chan KK, Yao TJ, Jones B, et al. The use of Chinese herbal medicine to improve quality of life in women undergoing chemotherapy for ovarian cancer: a double-blind placebo-controlled randomized trial with immunological monitoring. *Ann Oncol*. 2011;22:2241-2249.
29. Yoon J, Cho CK, Shin JE, Yoo HS. Changes in the laboratory data for cancer patients treated with Korean-medicine-based inpatient care. *J Pharmacopuncture*. 2014;17(1):20-26.
30. Jeon HJ, Kim JM, Cho CK, Lee YW, Yoo HS. Wheel Balanced Cancer Therapy for longer than 21 days can have a positive effect on the survival of patients with stage IV cancer. *J Pharmacopuncture*. 2015;18(3):19-31.
31. Lee SB, Cha J, Kim IK, et al. A high-throughput assay of NK cell activity in whole blood and its clinical application. *Biochem Biophys Res Commun*. 2014:445:584-590.
32. Stotz M, Pichler M, Absenger G, et al. The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer. *Br J Cancer*. 2014:110:435-440.
33. Farag SS, VanDeusen JB, Fehniger TA, Caligiuri MA. Biology and clinical impact of human natural killer cells. *Int J Hematol*. 2003;78:7-17.
34. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. *Nat Rev Immunol*. 2007;7:329-339.
35. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer*. 2002;2:850-861.
36. Yu TK, Caudell EG, Smid C, Grimm EA. IL-2 activation of NK cells: involvement of MKK1/2/ERK but not p38 kinase pathway. *J Immunol*. 2000;164:6244-6251.
37. Martín-Fontecha A, Thomsen LL, Brett S, et al. Induced recruitment of NK cells to lymph nodes provides IFN-γ for T_H1 priming. *Nat Immunol*. 2004;5:1260-1265.
38. Schantz SP, Savage HE, Racz T, Taylor DL, Sacks PG. Natural killer cells and metastases from pharyngeal carcinoma. *Am J Surg*. 1989;158:361-366.
39. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. *Arch Surg*. 1987;122:1264-1268.
40. Taketomi A, Shimada M, Shirabe K, Kajiyama K, Gion T, Sugimachi K. Natural killer cell activity in patients with hepatocellular carcinoma: a new prognostic indicator after hepatectomy. *Cancer*. 1998;83:58-63.
41. Kawarabayashi N, Seki S, Hatsuse K, et al. Decrease of CD56+ T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology*. 2000;32:962-969.
42. Takeuchi H, Maehara Y, Tokunaga E, Koga T, Kakeji Y, Sugimachi K. Prognostic significance of natural killer cell activity in patients with gastric carcinoma: a multivariate analysis. *Am J Gastroenterol*. 2001;96:574-578.
43. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet*. 2000;356:1795-1799.
44. Sahni A, Simpson-Haidaris PJ, Sahni SK, Vaday GG, Francis CW. Fibrinogen synthesized by cancer cells augments the proliferative effect of fibroblast growth factor-2 (FGF-2). *J Thromb Haemost*. 2008;6:176-183.
45. Hantgan RR, Rocco M, Nagaswami C, Weisel JW. Binding of a fibrinogen mimetic stabilizes integrin αIIbβ3's open conformation. *Protein Sci*. 2001;10:1614-1626.
46. Dvorak HF. Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing. *N Engl J Med*. 1986;315:1650-1659.
47. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357:539-545.
48. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420:860-867.
49. 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;105:178-185.
50. Takeuchi H, Ikeuchi S, Kitagawa Y, et al. Pretreatment plasma fibrinogen level correlates with tumor progression and metastasis in patients with squamous cell carcinoma of the esophagus. *J Gastroenterol Hepatol*. 2007;22:2222-2227.
51. Yamashita H, Kitayama J, Nagawa H. Hyperfibrinogenemia is a useful predictor for lymphatic metastasis in human gastric cancer. *Jpn J Clin Oncol*. 2005;35:595-600.
52. Jones JM, McGonigle NC, McAnespie M, Cran GW, Graham AN. Plasma fibrinogen and serum C-reactive protein are associated with non-small cell lung cancer. *Lung Cancer*. 2006;53:97-101.
53. Tang L, Liu K, Wang J, Wang C, Zhao P, Liu J. High preoperative plasma fibrinogen levels are associated with distant metastases and impaired prognosis after curative resection in patients with colorectal cancer. *J Gastroenterol Hepatol*. 2010;102:428-432.
54. Polterauer S, Grimm C, Seebacher V, et al. Plasma fibrinogen levels and prognosis in patients with ovarian cancer: a multicenter study. *Oncologist*. 2009;14:979-985.
55. Polterauer S, Seebacher V, Hefler-Frischmuth K, et al. Fibrinogen plasma levels are an independent prognostic

parameter in patients with cervical cancer. *Am J Obstet Gynecol*. 2009;200:647.e1-647.e7.
56. Seebacher V, Polterauer S, Grimm C, et al. The prognostic value of plasma fibrinogen levels in patients with endometrial cancer: a multi-centre trial. *Br J Cancer*. 2010;102:952-956.
57. Seebacher V, Polterauer S, Grimm C, et al. The impact of plasma fibrinogen levels on patients with vulvar cancer. *Eur J Obstet Gynecol Reprod Biol*. 2012;161:88-91.
58. Fischbach FT, Dunning MB. *A Manual of Laboratory and Diagnostic Tests*. Philadelphia, PA: Lippincott Williams & Wilkins; 2009.
59. Hacene K, Desplaces A, Brunet M, Lidereau R, Bourguignat A, Oglobine J. Competitive prognostic value of clinicopathologic and bioimmunologic factors in primary breast cancer. *Cancer*. 1986;57:245-250.
60. Encan T, Hannisdal E. Blood analyses as prognostic factors in primary lung cancer. *Acta Oncol*. 1990;29:151-154.
61. Johansson JE, Sigurdsson T, Holmberg L, Bergström R. Erythrocyte sedimentation rate as a tumor marker in human prostatic cancer. An analysis of prognostic factors in 300 population-based consecutive cases. *Cancer*. 1992;70: 1556-1563.
62. Epstein FH, Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448-454.
63. Zahorec R. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy*. 2001;102:5-14.
64. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol*. 2013;88:218-230.
65. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2014;106:dju124.
66. Motomura T, Shirabe K, Mano Y, et al. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol*. 2013;58:58-64.
67. Shen L, Zhang H, Liang L, et al. Baseline neutrophil-lymphocyte ratio (≥2.8) as a prognostic factor for patients with locally advanced rectal cancer undergoing neoadjuvant chemoradiation. *Radiat Oncol*. 2014;9:295.