



Prognostic value of preoperative lymphocyte-monocyte ratio in elderly patients with advanced epithelial ovarian cancer

Byung Su Kwon, Hyun Joo Lee, Juseok Yang, Yong Jung Song, Dong Soo Suh, Dong Hyung Lee, Ki Hyung Kim

Department of Obstetrics and Gynecology, Pusan National University School of Medicine, and Biomedical Research Institute and Pusan Cancer Center, Pusan National University Hospital, Busan, Korea

Objective

To investigate the prognostic significance of preoperative lymphocyte-monocyte ratio (LMR) in elderly patients with advanced epithelial ovarian cancer (EOC) receiving primary cytoreductive surgery and adjuvant platinum-based chemotherapy.

Methods

A total of 42 elderly patients (≥ 65 years) diagnosed with EOC who are receiving primary cytoreductive surgery and adjuvant platinum-based chemotherapy from 2009 to 2012 was included. LMR was calculated from complete blood cell count sampled before operation. Receiver operating characteristic (ROC) curves were used to calculate optimal cut-off values for LMR. Prognostic significance with respect to overall survival (OS) and progression-free survival (PFS) were determined using log-rank test and Cox regression analysis.

Results

The optimized LMR cut-off value determined by ROC curve analysis was 3.63 for PFS and OS. The high LMR group (LMR ≥ 3.63) was found to be significantly more associated with optimal debulking ($P=0.045$) and platinum response ($P=0.018$) than the low LMR group. In addition, Kaplan-Meier analysis revealed the LMR-high group was significantly more associated with high PFS and OS rates ($P=0.023$ and $P=0.033$, respectively), and univariate analysis revealed that a high LMR, histology type, and optimal debulking and platinum responses were significantly associated with prolonged PFS and OS. However, subsequent Cox multivariate analysis showed only optimal debulking and platinum response were independent prognostic factors of PFS or OS.

Conclusion

This study suggests that LMR might be associated with treatment and survival outcomes in elderly patients with EOC receiving standard oncology treatment.

Keywords: Aged; Ovarian epithelial cancer; Lymphocyte-monocyte ratio; Survival

Introduction

Epithelial ovarian cancer (EOC) remains the most lethal gynecologic cancer and a leading cause of cancer-related death in women worldwide [1]. Epidemiologic analysis has shown the incidence of EOC increases with age and peaks in the seventh decade of life [2], and that approximately 85% of elderly patients present with stage III or IV disease [3,4]. Patients older than 65 years with advanced stage disease have poor 5-year survival rates which are only one half of those observed for women under 65 years [5]. Elderly patients usually have comorbid diseases, functional disabilities, subsequent increase of the susceptibility to treatment related toxicity, and limitations of standard-of-care treatments. Therefore, effective biomark-

Received: 2017.05.27. Revised: 2017.07.03. Accepted: 2017.08.18.

Corresponding author: Ki Hyung Kim

Department of Obstetrics and Gynecology, Pusan National University School of Medicine, and Biomedical Research Institute and Pusan Cancer Center, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 49241, Korea

E-mail: ghkim@pusan.ac.kr

<http://orcid.org/0000-0003-2364-5875>

Dong Hyung Lee

Department of Obstetrics and Gynecology, Pusan National University School of Medicine, and Biomedical Research Institute and Pusan Cancer Center, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 49241, Korea

E-mail: ldh0707@hanmail.net

<http://orcid.org/0000-0001-8406-2695>

Articles published in Obstet Gynecol Sci are open-access, distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2017 Korean Society of Obstetrics and Gynecology

ers are required that accurately predict prognosis in elderly patients with EOC.

Accumulating evidence indicates inflammation plays critical roles in the initiation and progression of various cancers. Several prognostic indicators from peripheral blood have been widely investigated as potentially useful prognostic markers in different cancers. Of these potentially useful predictors, neutrophil-to-lymphocyte ratio (NLR), the ratio of absolute neutrophil count to absolute lymphocyte count (ALC), has been shown to be associated with poorer pathologic features, such as, an advanced tumor stage, poor progression-free survival (PFS), and poor overall survival (OS) in ovarian cancer [6,7]. Furthermore, elevated preoperative platelet-to-lymphocyte ratio (PLR), that is, the ratio between platelet count and ALC, has been demonstrated to be associated with advanced stage and decreased survival in ovarian carcinoma [8,9]. Preoperative lymphocyte-monocyte ratio (LMR) is another valuable predictor of survival in patients with solid tumors of, for example, the head and neck, breast, lung, and cervix [10-13].

The prognostic and predictive values of several preoperative hematologic parameters have been investigated in EOC, but evidence regarding the use of LMR as a predictor of outcome in elderly patients with EOC is lacking. Therefore, the aim of the current study was to determine the prognostic significance of preoperative LMR in elderly patients with EOC

receiving standard oncology treatment.

Materials and methods

The archived medical records of 42 elderly patients (≥ 65 years) that underwent primary staging operation and received at least 3 courses of adjuvant platinum-based chemotherapy for EOC between April 2009 and June 2012 were retrospectively investigated. Clinical, pathological, and preoperative complete blood count variables were subjected to analysis. None of the patients received neoadjuvant chemotherapy. Histological diagnoses were based on the World Health Organization criteria, and all microscope slides were reviewed by 2 experienced gynecologic pathologists. Clinicopathologic characteristics including age, performance status, the International Federation of Gynecology and Obstetrics (FIGO) stage, tumor grade, preoperative cancer antigen 125 (CA-125) level, presence of ascites, surgical outcome, and platinum response were collected (Table 1). The study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital (E-2016087).

LMR was defined as the ratio of ALC and absolute monocyte count (AMC). Preoperative blood samples were drawn 1 to 2 weeks prior to surgery. Receiver operating characteristic

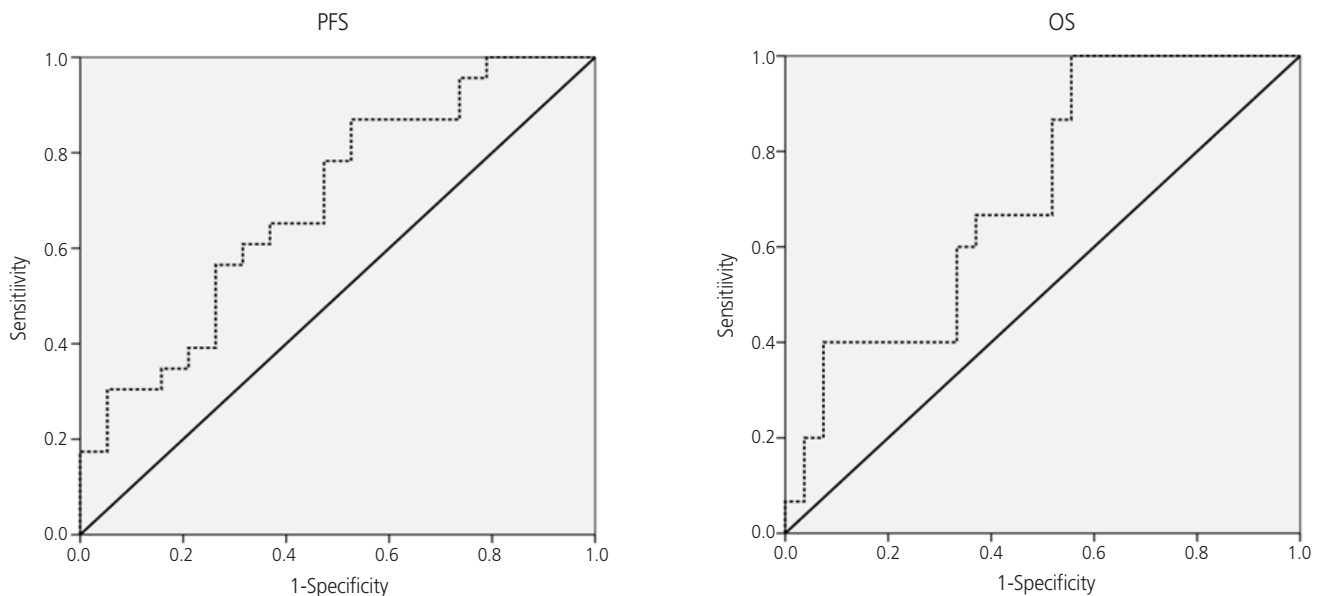


Fig. 1. Receiver operating characteristic curve analyses showing cut-off points of lymphocyte-monocyte ratio for progression-free survival (PFS) and overall survival (OS) analyses among patients older than 65 years of age with advanced-stage serous epithelial ovarian cancer. The area under the curve for PFS was 0.696 (95% confidence interval [CI], 0.536 to 0.856; $P=0.031$) and for OS was 0.711 (95% CI, 0.555 to 0.868; $P=0.025$).

(ROC) curve analysis was used to obtain optimal LMR cut-off values for predicting survival outcomes. The optimal LMR was 3.63, and this value was used as the cut-off for the allocations to LMR-low and LMR-high groups (Fig. 1). The impacts of intergroup LMR differences were evaluated for PFS and OS. The χ^2 test was used to analyze differences in proportions, and the differences in survival rates between positive and negative groups were analyzed by means of log-rank test and Cox regression analysis. Data were analyzed using Statistical Package for the Social Science (SPSS) statistical software, version 18.0 (SPSS Inc., Chicago, IL, USA). All presented *P*-values were 2-sided, and statistical significance was considered at *P*<0.05.

Results

Patient baseline characteristics are presented in Table 1. Median patient age was 70 years (range 65 to 85), and 40.5% were older than 70 years. Serous adenocarcinoma was the most common subtype (78.6%), and 83.3% of patients had a histologic grade of 2 or 3. All patients had an advanced disease stage (III to IV), at initial diagnosis 35 (83.3%) were of stage III and 3 (16.7%) were of IV. Optimal debulking was performed in 30 (71.4%) patients. All 42 patients received at least 3 courses of platinum-based adjuvant chemotherapy after primary surgery, and seven (16.7%) received more than 7 courses. At 6-month postoperative follow-ups after completing platinum-based chemotherapy, 14 (33.3%) patients were classified as platinum resistant and 28 (66.7%) as platinum sensitive.

Regarding the stratification of patients according to the LMR, 29 (69.0%) patients were allocated to the low LMR group and 13 (31.0%) to the high LMR group (Table 2). The 2 groups differed significantly in terms of optimal debulking (*P*=0.045) and platinum response (*P*=0.018).

According to Kaplan-Meier analysis, median PFS and OS for all 42 patients were 42.5 and 68.4 months, respectively. Patients in the high LMR group experienced significant more improvement in PFS (55.2 vs. 33.7 months, *P*=0.023) and OS (70.0 vs. 59.0 months, *P*=0.033) than patients in the low LMR group (Fig. 2).

Age, Eastern Cooperative Oncology Group performance status, clinical stage, histology type, tumor grade, CA-125, optimal debulking, cycles of chemotherapy, platinum response, and LMR were included in both univariate and multivariate

Table 1. Clinicopathologic characteristics of epithelial ovarian cancer

Characteristics	No. of patients (%)
Age (yr)	
<70	25 (59.5)
≥70	17 (40.5)
Performance status (ECOG)	
<2	27 (64.3)
≥2	15 (35.7)
Clinical stage (FIGO)	
III	35 (83.3)
IV	7 (16.7)
Histology type	
Serous	33 (78.6)
Non-serous	9 (21.4)
Tumor grade	
1	7 (16.7)
2/3	35 (83.3)
CA-125 (unit/mL)	
<417	21 (50.0)
≥417	21 (50.0)
Optimal debulking	
Yes	30 (71.4)
No	12 (28.6)
Cycles of chemotherapy	
≤6	35 (83.3)
>6	7 (16.7)
Platinum response	
Sensitive	28 (66.7)
Resistant	14 (33.3)

ECOG, Eastern Cooperative Oncology Group; FIGO, The International Federation of Gynecology and Obstetrics; CA-125, cancer antigen 125.

analyses. Univariate analysis showed histology type, optimal debulking and platinum response were significantly associated with PFS and OS (all *P*<0.01), and that LMR was also significantly associated with PFS (*P*=0.032) and OS (*P*=0.045). Cox multivariate analysis showed only optimal debulking (PFS, *P*=0.040; OS, *P*=0.001) and platinum response (PFS, *P*=0.001; OS, *P*=0.003) independently predicted PFS and OS (Tables 3 and 4).

Table 2. Clinical and pathologic characteristics according to the lymphocyte-monocyte ratio (LMR) in epithelial ovarian cancer

Characteristics	LMR-low (<3.63)	LMR-high (≥3.63)	P-value
Total	29 (69.0)	13 (31.0)	-
Age (yr)			0.616
<70	18 (62.1)	7 (53.8)	
≥70	11 (37.9)	6 (46.2)	
Performance status (ECOG)			0.804
<2	19 (65.5)	8 (61.5)	
≥2	10 (34.5)	5 (38.5)	
Clinical stage (FIGO)			0.881
III	24 (82.8)	11 (84.6)	
IV	5 (17.2)	2 (15.4)	
Histology type			0.146
Serous	21 (72.4)	12 (92.3)	
Non-serous	8 (27.6)	1 (7.7)	
Tumor grade			0.455
1	4 (13.8)	3 (23.1)	
2/3	25 (86.2)	10 (76.9)	
CA-125 (unit/mL)			0.113
<417	13 (44.8)	8 (61.5)	
≥417	16 (55.2)	5 (38.5)	
Optimal debulking			0.045
Yes	18 (62.1)	12 (92.3)	
No	11 (37.9)	1 (7.7)	
Cycles of chemotherapy			0.881
≤6	24 (82.8)	11 (84.6)	
>6	5 (17.2)	2 (15.4)	
Platinum response			0.018
Sensitive	16 (55.2)	12 (92.3)	
Resistant	13 (44.8)	1 (7.7)	

Values are presented as number (%).

ECOG, Eastern Cooperative Oncology Group; FIGO, The International Federation of Gynecology and Obstetrics; CA-125, cancer antigen 125.

Discussion

EOC is the most lethal gynecologic cancer and a major cause of cancer-related death in women worldwide [1]. This high mortality is mainly due to difficulties associated with early diagnosis and the development of resistance to chemotherapeutic agents and subsequent recurrence. Factors known to influence treatment outcomes in EOC include age, race, FIGO stage, tumor marker, histologic type, tumor grade, and residual tumor after surgery [14]. Age is an important risk factor in the development of ovarian cancer, and for this reason,

elderly patients comprise a large proportion of ovarian cancer cases at diagnosis. Several authors have suggested elderly patients have poorer prognoses [15,16], but as yet no definitive evaluation has been conducted on the impacts of biologic and therapeutic features on the relation between survival and age. In order to evaluate the prognostic significance of LMR in elderly ovarian patients, we retrospectively investigated 42 elderly (≥65 years) patients of median age 70 years (range 65 to 85), 17 (40.5%) of whom were older than 70. Although the number of patients included in the present study was relatively small, all patients had advanced stage (III to IV) disease

Table 3. Relationship of cancer- and host-related characteristics with progression-free survival in epithelial ovarian cancer

Characteristics	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (yr) (≥ 70 vs. < 70)	1.131 (0.328 to 3.898)	0.845	-	-
Performance status (ECOG) (≥ 2 vs. < 2)	1.393 (0.387 to 5.007)	0.621	-	-
Clinical stage (FIGO) (IV vs. III)	2.361 (0.403 to 13.844)	0.152	-	-
Histology type (non-serous vs. serous)	2.593 (0.803 to 9.844)	0.008	0.992 (0.283 to 3.477)	0.990
Tumor grade (2/3 vs. 1)	1.778 (0.345 to 9.163)	0.216	-	-
CA-125 (unit/mL) (≥ 417 vs. < 417)	1.312 (0.359 to 4.092)	0.490	-	-
Optimal debulking (no vs. yes)	3.302 (1.089 to 10.460)	< 0.001	2.284 (1.058 to 8.197)	0.040
Cycles of chemotherapy (≤ 6 vs. > 6)	1.212 (0.359 to 4.092)	0.757	-	-
Platinum response (resistant vs. sensitive)	6.797 (2.073 to 21.626)	< 0.001	4.814 (1.435 to 18.505)	0.001
LMR (< 3.63 vs. ≥ 3.63)	2.331 (1.110 to 8.997)	0.032	1.333 (0.365 to 4.860)	0.664

HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; FIGO, The International Federation of Gynecology and Obstetrics; CA-125, cancer antigen 125; LMR, lymphocyte-monocyte ratio.

Table 4. Relationship of cancer- and host-related characteristics with overall survival in epithelial ovarian cancer

Characteristics	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (yr) (≥ 70 vs. < 70)	3.100 (1.010 to 9.513)	0.048	2.587 (0.984 to 5.786)	0.161
Performance status (ECOG) (≥ 2 vs. < 2)	1.348 (0.465 to 3.905)	0.465	-	-
Clinical stage (FIGO) (IV vs. III)	1.954 (0.527 to 7.252)	0.317	-	-
Histology type (non-serous vs. serous)	3.443 (1.100 to 10.767)	0.001	0.312 (0.064 to 1.531)	0.151
Tumor grade (2/3 vs. 1)	1.928 (0.451 to 3.426)	0.911	-	-
CA-125 (unit/mL) (≥ 417 vs. < 417)	1.635 (0.534 to 5.006)	0.490	-	-
Optimal debulking (no vs. yes)	7.441 (3.433 to 15.131)	< 0.001	4.949 (2.521 to 11.882)	0.001
Cycles of chemotherapy (≤ 6 vs. > 6)	0.970 (0.215 to 3.381)	0.969	-	-
Platinum response (resistant vs. sensitive)	6.335 (2.715 to 14.803)	< 0.001	9.737 (4.421 to 31.384)	0.003
LMR (< 3.63 vs. ≥ 3.63)	1.700 (0.569 to 4.664)	0.045	0.334 (0.180 to 5.082)	0.459

HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; FIGO, The International Federation of Gynecology and Obstetrics; CA-125, cancer antigen 125; LMR, lymphocyte-monocyte ratio.

and received at least 3 courses of platinum-based adjuvant chemotherapy after primary staging surgery.

Inflammation is considered a hallmark of cancer, and tumor-associated inflammatory microenvironments are widely regarded to facilitate tumor growth and metastasis. Previous studies have reported hematological markers of systemic inflammation response, such as, NLR, PLR, and LMR, might serve as independent prognostic markers of survival in various cancers. Of these 3 parameters, LMR has been suggested to be associated with survival in malignant lymphoma [17] and in numerous solid tumors [10-13].

Lymphocytes represent host anti-tumor immune response, inducing cytotoxic cell death, suppressing tumor cell proliferation and migration, and inhibiting cancer dissemination

[18]. Monocytes are another type of leucocyte and it has been shown peripheral blood monocyte number is negatively associated with prognosis for different cancer types, which suggests monocytes might independently predict prognoses [19]. Thus, elevated peripheral blood LMR predicts a favorable prognosis in cancer patients. In a previous study, we assessed the prognostic value of LMR in a cohort of 234 EOC patients that underwent surgical resection, and found an elevated LMR was strongly correlated with age, serum CA-125 level, FIGO stage, and the presence of malignant ascites, and that the 5-year PFS and OS rates were better for those with an elevated LMR (PFS, 62.5% vs. 40.0%; $P < 0.001$ and OS, 67.2%

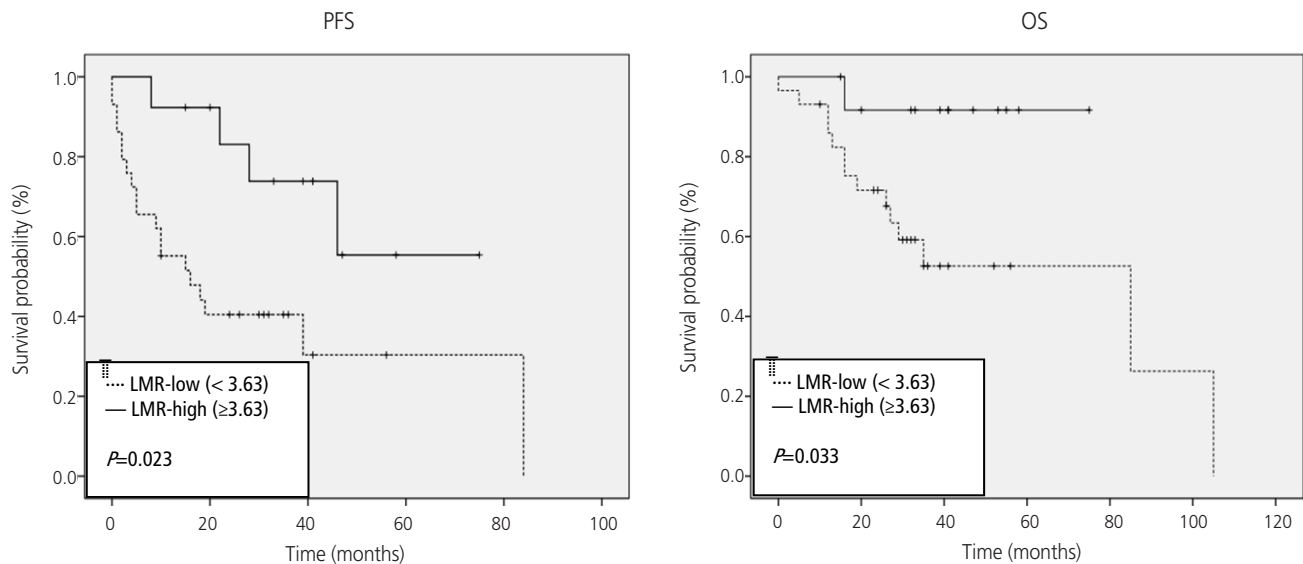


Fig. 2. Progression-free survival (PFS) and overall survival (OS) analysis according to the lymphocyte-monocyte ratio (LMR) in epithelial ovarian cancer.

vs. 42.2%; $P < 0.001$, respectively) [20]. In a previous multivariate analysis, elevated LMR was found to be strongly correlated with longer survival and to be an independent prognostic factor of survival in EOC patients [8]. Moreover, the present study shows elderly patients in the high LMR group had significantly better PFS (55.2 vs. 33.7 months, $P = 0.023$) and OS (70.0 vs. 59.0 months, $P = 0.033$) than patients in the low LMR group (Fig. 2).

Recent studies demonstrated elderly patients that receive optimal oncologic management have better PFS and OS than those receiving sub-optimal management (median PFS, 18 vs. 11 months; $P = 0.05$ and median OS, 31 vs. 20 months; $P < 0.001$, respectively) [21,22]. The fear of increasing complication rates and the absence of validated selection criteria for treatment decision making in elderly patients are major concerns. In the present study, a high LMR was found to be significantly associated with optimal cytoreduction (92.3% vs. 62.1%, $P = 0.045$) and platinum response (92.3% vs. 55.2%, $P = 0.018$). Furthermore, we found preoperative LMR might predict treatment outcomes in elderly patients with advanced EOC undergoing cytoreductive surgery and adjuvant platinum-based chemotherapy. This study had some limitations that should be addressed, including its retrospective nature and the relatively small number of patients. Further large-scale studies are needed to clarify the prognostic value of preoperative LMR in elderly patients with advanced EOC.

In conclusion, this study is first study to assess the prognos-

tic value of LMR in elderly patients with advanced EOC receiving standard oncology treatment based on LMR. Our results suggest the high LMR is predictive of optimal cytoreduction and sensitive platinum response and longer PFS and OS.

Acknowledgments

This work was supported by a two-year Research Grant of Pusan National University.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013;63:11-30.
2. Yancik R. Ovarian cancer. Age contrasts in incidence, histology, disease stage at diagnosis, and mortality. *Cancer* 1993;71:517-23.
3. Cloven NG, Manetta A, Berman ML, Kohler MF, DiSaia PJ. Management of ovarian cancer in patients older than

- 80 years of age. *Gynecol Oncol* 1999;73:137-9.
4. Uyar D, Frasure HE, Markman M, von Gruenigen VE. Treatment patterns by decade of life in elderly women (> or =70 years of age) with ovarian cancer. *Gynecol Oncol* 2005;98:403-8.
 5. Ries LA, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, et al. SEER cancer statistics review, 1975–2001 [Internet]. Bethesda (MD): National Cancer Institute; 2014 [cited 2017 Mar 1]. Available from: http://www.seer.cancer.gov/csr/1975_2001/.
 6. Wang Y, Liu P, Xu Y, Zhang W, Tong L, Guo Z, et al. Preoperative neutrophil-to-lymphocyte ratio predicts response to first-line platinum-based chemotherapy and prognosis in serous ovarian cancer. *Cancer Chemother Pharmacol* 2015;75:255-62.
 7. Williams KA, Labidi-Galy SI, Terry KL, Vitonis AF, Welch WR, Goodman A, et al. Prognostic significance and predictors of the neutrophil-to-lymphocyte ratio in ovarian cancer. *Gynecol Oncol* 2014;132:542-50.
 8. Supoken A, Kleebkao P, Chumworathayi B, Luanratanakorn S, Kietpeerakool C. Elevated preoperative platelet to lymphocyte ratio associated with decreased survival of women with ovarian clear cell carcinoma. *Asian Pac J Cancer Prev* 2014;15:10831-6.
 9. Asher V, Lee J, Innamaa A, Bali A. Preoperative platelet lymphocyte ratio as an independent prognostic marker in ovarian cancer. *Clin Transl Oncol* 2011;13:499-503.
 10. Jiang R, Cai XY, Yang ZH, Yan Y, Zou X, Guo L, et al. Elevated peripheral blood lymphocyte-to-monocyte ratio predicts a favorable prognosis in the patients with metastatic nasopharyngeal carcinoma. *Chin J Cancer* 2015;34:237-46.
 11. Ni XJ, Zhang XL, Ou-Yang QW, Qian GW, Wang L, Chen S, et al. An elevated peripheral blood lymphocyte-to-monocyte ratio predicts favorable response and prognosis in locally advanced breast cancer following neoadjuvant chemotherapy. *PLoS One* 2014;9:e111886.
 12. Hu P, Shen H, Wang G, Zhang P, Liu Q, Du J. Prognostic significance of systemic inflammation-based lymphocyte-monocyte ratio in patients with lung cancer: based on a large cohort study. *PLoS One* 2014;9:e108062.
 13. Chen L, Zhang F, Sheng XG, Zhang SQ. Decreased pretreatment lymphocyte/monocyte ratio is associated with poor prognosis in stage Ib1-IIa cervical cancer patients who undergo radical surgery. *Onco Targets Ther* 2015;8:1355-62.
 14. Winter WE 3rd, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, et al. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2007;25:3621-7.
 15. Chan JK, Urban R, Cheung MK, Osann K, Shin JY, Husain A, et al. Ovarian cancer in younger vs older women: a population-based analysis. *Br J Cancer* 2006;95:1314-20.
 16. Pectasides D, Fountzilas G, Aravantinos G, Bamias A, Kalofonos HP, Skarlos D, et al. Epithelial ovarian carcinoma in younger vs older women: is age an independent prognostic factor? The Hellenic Oncology Cooperative Group experience. *Int J Gynecol Cancer* 2007;17:1003-10.
 17. Li YL, Gu KS, Pan YY, Jiao Y, Zhai ZM. Peripheral blood lymphocyte/monocyte ratio at the time of first relapse predicts outcome for patients with relapsed or primary refractory diffuse large B-cell lymphoma. *BMC Cancer* 2014;14:341.
 18. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436-44.
 19. Qi Q, Geng Y, Sun M, Wang P, Chen Z. Clinical implications of systemic inflammatory response markers as independent prognostic factors for advanced pancreatic cancer. *Pancreatol* 2015;15:145-50.
 20. Eo WK, Chang HJ, Kwon SH, Koh SB, Kim YO, Ji YI, et al. The lymphocyte-monocyte ratio predicts patient survival and aggressiveness of ovarian cancer. *J Cancer* 2016;7:289-96.
 21. Trillsch F, Woelber L, Eulenburg C, Braicu I, Lambrechts S, Chekerov R, et al. Treatment reality in elderly patients with advanced ovarian cancer: a prospective analysis of the OVCAD consortium. *J Ovarian Res* 2013;6:42.
 22. Alphas HH, Zahurak ML, Bristow RE, Díaz-Montes TP. Predictors of surgical outcome and survival among elderly women diagnosed with ovarian and primary peritoneal cancer. *Gynecol Oncol* 2006;103:1048-53.